Accelerating the Development and Uptake of Rapid Diagnostics to Address Antibiotic Resistance

A Workshop

October 13-14, 2022

NAS Lecture Room/East Court
2101 Constitution Avenue NW
Washington, DC 20418

Accelerating the Development and Uptake of Rapid Diagnostics to Address Antibiotic Resistance

October 13-14, 2022

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Forum on Drug Discovery, Development, and Translation
Forum on Medical and Public Health Preparedness for Disasters and Emergencies
Forum on Microbial Threats

Accelerating the Development and Uptake of Rapid Diagnostics to Address Antibiotic Resistance

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October 13-14, 2022 ▪ Washington, DC

The use and misuse of antibiotics contributes to the rise in drug-resistant bacteria – a serious and worsening threat to human health. Addressing the problem of antibiotic resistance requires measures to spur innovation and ensure the prudent use of existing drugs. Rapid point-of-care diagnostics can play an important role in avoiding unnecessary use of antimicrobials by providing clinicians with the right information at the right time to help them make decisions about appropriate drug treatment for patients. Diagnostics also have the capacity to support early detection and diagnosis of drug-resistant bacterial infections, enable disease surveillance, and help prevent disease spread.

An ad hoc planning committee under the auspices of the National Academies of Sciences, Engineering, and Medicine, will organize a two-day public workshop to discuss the current landscape of rapid diagnostics to address antibiotic resistance, consider challenges and opportunities for spurring innovation, and discuss practical next steps for accelerating the development of new diagnostic tools.

The public workshop will feature invited presentations and discussions to:

- Examine the current state of rapid diagnostic development, including examples of successes and limitations of current approaches.
- Consider the unique challenges for the development and use/uptake of rapid diagnostics in health care settings (e.g., feasibility of clinical utility studies)
- Consider gaps that rapid diagnostics may be best-suited to address (e.g. tools to support targeted treatment decisions in the healthcare setting, tools to enable real-time surveillance based on routine hospital data).
- Discuss practical short- and long-term opportunities for spurring the development and uptake of new diagnostics that can help address antibiotic resistance.

The planning committee will organize the workshop, develop the agenda, select and invite speakers and discussants, and moderate or identify moderators for the discussions. A proceedings of the presentations and discussions at the workshop will be prepared by a designated rapporteur in accordance with institutional guidelines.

The National Academies of Sciences, Engineering, and Medicine are private, nonprofit institutions that provide expert advice on some of the most pressing challenges facing the nation and the world. Our work helps shape sound policies, inform public opinion, and advance the pursuit of science, engineering, and medicine. For more information about this workshop, contact Carolyn Shore (cshore@nas.edu).

Planning Committee

Kent E. Kester (co-chair), International AIDS Vaccine Initiative
Betsy Wonderly Trainor (co-chair), CARB-X
Carey-Ann Burnham, Pattern Bioscience
Paul Eder, National Institute of Allergy and Infectious Diseases, NIH
Deborah Hung, Harvard Medical School
Robin Patel, Mayo Clinic
Susan Van Meter, The American Clinical Laboratory Association
Ribhi Shawar, Center for Devices and Radiological Health, FDA
John Billington, GSK
Amanda Jezek, Infectious Diseases Society of America

Accelerating the Development & Uptake of Rapid Diagnostics to Address Antibiotic Resistance – A Workshop

October 13, 2022, 8:30 am – 5:00 pm (ET)

October 14, 2022, 8:30 am – 1:00 pm (ET)

NAS Lecture Room/East Court
2101 Constitution Avenue NW Washington, DC 20418

To watch a zoom livestream, please visit the workshop page here.

PURPOSE

This workshop, convened by the National Academies' Forum on Drug Discovery, Development, and Translation; the Forum on Medical and Public Health Preparedness for Disasters and Emergencies; and the Forum on Microbial Threats; and will provide a venue for stakeholders to discuss the current landscape of rapid diagnostics to address antibiotic resistance, consider challenges and opportunities for spurring innovation, and discuss practical next steps for accelerating the development of new diagnostic tools.

The public workshop will feature invited presentations and discussions to:

- Examine the current state of rapid diagnostic development, including examples of successes and limitations of current approaches.
- Consider the unique challenges for the development and use/uptake of rapid diagnostics in health care settings (e.g., feasibility of clinical utility studies)
- Consider gaps that rapid diagnostics may be best-suited to address (e.g. tools to support targeted treatment decisions in the healthcare setting, tools to enable real-time surveillance based on routine hospital data).
- Discuss practical short- and long-term opportunities for spurring the development and uptake of new diagnostics that can help address antibiotic resistance.

DAY 1: THURSDAY, OCTOBER 13, 2022

8:30 am WELCOME AND OPENING REMARKS (20 MIN)

Kent E. Kester, *Workshop Co-chair*
Vice President, Translational Medicine
International AIDS Vaccine Initiative

Betsy Wonderly Trainor, *Workshop Co-chair*
Alliance Director
CARB-X

8:50 am SESSION I – Defining the Need for Rapid Diagnostics: Where do We Go From Here?

Session Objectives:

- Examine the current state of rapid diagnostic development, including examples of successes and limitations of current approaches;
- Consider gaps that rapid diagnostics may be best-suited to address (e.g. tools to support targeted treatment decisions in the healthcare setting, tools to enable real-time surveillance based on routine hospital data);
- Discuss what "success" might look like in future.

8:50 am Fireside Chat (30 mins)

KAREN C. CARROLL, *Keynote Speaker*
Director, Division Medical Microbiology & Professor of Pathology
Johns Hopkins University School of Medicine

9:20 am Panel Discussion (40 mins)

Moderator: Karen Carroll, Johns Hopkins University School of Medicine

Patient Perspective
BRADLEY BURNAM
Founder & AMR Survivor
Turn Therapeutics

Developer Perspective
CRAIG WHITEFORD
Head of AMR Program
Becton Dickinson

Bioethics Perspective
TRACEY L. COHEN
Distinguished Visiting Scholar
Institute for Bioethics & Health Policy, University of Miami Miller School of Medicine

Reimbursement Perspective
SUSAN VAN METER
President
American Clinical Laboratory Association

10:00 am Q&A/Audience Discussion (30 mins)

10:30 am COFFEE BREAK (*30 minutes*)

11:00 am SESSION II – Challenges in Development and Use of Rapid Diagnostics in Healthcare Settings

Session Objectives:

- Consider the unique challenges for the development of rapid point-of-care diagnostics to address antibiotic resistance;

- Discuss lessons learned from other diseases, including COVID-19, for rapid diagnostics development and use; and
- Consider generalizable applications and practical approaches to overcome barriers to the development and use/uptake of rapid diagnostics for drug-resistant bacterial infections.

11:00 am **Presentation 1 (15 mins)**
Lessons Learned from COVID-19 in the U.S. that are applicable to AMR
JOSEPH LUTGRING
Medical Officer, Division of Healthcare Quality Promotion, National Center for Emerging and Zoonotic Infectious Diseases
Centers for Disease Control and Prevention

11:15 am **Presentation 2 (15 mins)**
Lessons Learned from abroad through the WHO's Access to COVID-19 Tools Accelerator Program
WILLIAM "BILL" RODRIGUEZ
Chief Executive Officer
FIND, the Global Alliance for Diagnostics

11:30 am **Presentation 3 (15 mins)**
Lessons Learned from Programs focused on Accelerating the Development of Rapid Dx's to date
PAUL EDER
Senior Scientific Officer
Concept Acceleration Program – Diagnostics
National Institute of Allergy and Infectious Disease

11:45 am **Panel Discussion (30 mins)**

Moderator: Paul Eder, National Institute of Allergy and Infectious Disease

Clinician Perspective
ALEX GRENINGER
Assistant Professor and Assistant Director of the Clinical Virology Laboratories
University of Washington Medical Center

Regulatory Perspective
KRISTIAN ROTH
Deputy Director
Division of Microbiology Devices, FDA

Industry/Economic Perspective
DAVID PERSING
Head of R+D
Cepheid

12:15 pm **Q&A/Audience Discussion (45 mins)**

1:00 pm **LUNCH BREAK (*1 hour*)**

2:00 pm SESSION III – INCENTIVES AT THE INTERSECTION OF DIAGNOSTICS AND DRUG DEVELOPMENT

Session Objectives:

- Consider common incentives/disincentives for the development of rapid diagnostics and new antibiotics; and
- Discuss innovative ways to foster innovation at the intersection of complementary diagnostics and drug development.

2:00 pm **Setting the Stage (10 mins)**
JOHN BILLINGTON
Head of Commercial Pipeline & Health Security, Policy & Advocacy
GSK

2:10 pm **Panel Discussion (40 mins)**
A Case Study: A new joint venture between a drug and dx developer -- Aurobac

Moderator: John Billington, GSK

Developer Perspective
VALÉRIE RAYMOND-SCHWARTZMANN
Companion Diagnostics Program Director
bioMerieux

Therapeutics Perspective
ALITA MILLER
Chief Scientific Officer
Entasis Therapeutics

Biotechnology Perspective
GREGORY FRANK
Director, Global Public Policy
Merck

HHS Perspective
KIM SCIARRETTA
Interdisciplinary Scientist/Project Officer
Biomedical Advanced Research and Development Authority (BARDA)

2:50 pm **Audience Q&A (10 mins)**

3:00 pm **COFFEE BREAK (30 mins)**

3:30 pm SESSION IV – POLICY LEVERS: A MENU OF OPTIONS

Session objectives:
- Lay out a “menu” of policy options for incentivizing the development and use of rapid diagnostics.

3:30 pm **Panel Discussion (1 hour)**

Moderator: Mark Mcclellan, Duke Margolis Center for Health Policy

Industry Perspective
PHYLLIS ARTHUR
Vice President, Infectious Diseases & Diagnostics Policy
BIO

PACCARB Research Perspective
SARAH MCCLELLAND
Health Policy Analyst
U.S. Department of Health and Human Services

Analyst Perspective
JACLYN LEVY
Director, U.S. Policy
AMR Action Fund

Law & Economics Perspective
KEVIN OUTTERSON
Professor of Law, Boston University
Executive Director, CARB-X

4:30 pm **Q&A/Audience Discussion (30 mins)**

5:00 pm **ADJOURN WORKSHOP DAY 1**

DAY 2: FRIDAY, OCTOBER 14, 2022

8:30 am SESSION V – HEALTH EQUITY CONSIDERATIONS FOR DIAGNOSTIC DEVELOPMENT AND USE

Session Objectives:

- Consider the health equity implications for the development and use of rapid point-of-care diagnostics in health care settings; and
- Discuss practical approaches for incorporating diversity, equity, inclusivity, and access into push and pull incentives for spurring diagnostic development.

8:30 am **Keynote (30 mins)**

DIANE SHADER SMITH (Mother of the Late Mallory Smith)
Patient Advocate
"Salt in my soul" Co-author/Producer

9:00 am **Roundtable Discussion (60 mins)**

Moderator: Amanda Jezek, Infectious Diseases Society of America

Bioethics Perspective
NICHOLAS EVANS
Chair, Associate Professor
University of Massachusetts, Lowell

Research Perspective
DAN BAUSCH
Director of Emerging Threats & Global Health Security
FIND, The Global Alliance for Diagnostics

Public Health Perspective
MELINDA PETTIGREW
Professor of Epidemiology and Deputy Dean
Yale School of Public Health

Access and Innovation Perspective
ANTHONY SO
Professor of the Practice; Director, Innovation+Design Enabling Access (IDEA) Initiative
Johns Hopkins Bloomberg School of Public Health

10:00 am **Discussion (30 mins)**

10:30 am **COFFEE BREAK (*30 minutes*)**

11:00 am SESSION VI – INTEGRATING RAPID DIAGNOSTICS AND ANTIBIOTIC STEWARDSHIP

Session Objectives:

- Discuss practical and actionable policy options to integrate rapid point-of-care diagnostics and antibiotic stewardship in healthcare settings;

- Consider the role of reimbursement, incentives, and guideline development for rapid point-of-care diagnostics in healthcare settings.

11:00 am **Panel Discussion (30 mins)**

Moderator: Robin Patel, Mayo Clinic

Stewardship Program Perspective
RITU BANERJEE
Medical Director, Pediatric Antimicrobial Stewardship Program
Vanderbilt University

Lab Clinician Perspective
CAREY-ANN BURNHAM
Professor of Pathology & Immunology, Molecular Microbiology, Pediatrics, and Medicine
Washington University School of Medicine in St. Louis

Industry/Developer Perspective
DIANE FLAYHART
Global Program Leader
Becton, Dickinson and Company

11:30 am **Q&A/Audience Discussion (30 mins)**

12:00 pm SESSION VII – A PATH FORWARD

Session Objectives:

- Based on a menu of policy options, consider practical and actionable next steps that would be most impactful for spurring the development and use of rapid diagnostics in healthcare settings; and
- Discuss practical short- and long-term opportunities for specific stakeholders to take action.

12:00 pm **Roundtable Discussion (50 mins)**

Moderator: Betsy Wonderly Trainor, CARB-X

Dx Developer/Public Health Equity Perspective
JEAN PATEL
Principal Scientific Affairs, Microbiology
Beckman Coulter Diagnostics

Pharma Perspective
JOSEPH LARSEN
Vice President, Clinical Development
Locus Biosciences, Inc.

Clinician Perspective
ROBIN PATEL
ID Physician, Laboratory Director
Mayo Clinic

Regulatory/Policy Perspective
RIBHI SHAWAR

Branch Chief, Division of Microbiology Devices, Office of In Vitro Diagnostic and Radiological Health
Center for Devices and Radiological Health, FDA

Health Equity Perspective
SUSAN VAN METER
President
American Clinical Laboratory Association

12:50 pm WRAP UP DISCUSSION AND CLOSING REMARKS

Kent E. Kester, *Workshop Co-chair*
Vice President, Translational Medicine
International AIDS Vaccine Initiative

Betsy Wonderly Trainor, *Workshop Co-chair*
Alliance Director
CARB-X

1:00 pm ADJOURN DAY 2

COVID-19 Policies for Non-Staff Access to National Academies Facilities

Current Operating Status:

Effective April 8, 2022

All facilities of the National Academies of Sciences, Engineering, and Medicine are **open**.

To prevent infection and spread of the COVID-19 virus, and as an integral measure towards the safety and health of everyone in our buildings, **the National Academies require that all visitors to NASEM facilities be up-to-date on their vaccinations against COVID-19 per CDC guidance. Additionally, do not enter the building if you have flu-like symptoms.**

Visitors must show their official COVID-19 Vaccination Record Card (or a digital photo of the card) before entering any National Academies building. Anyone who fails to present a vaccination card (or its copy) will not be allowed access to our facility; no exemptions or exceptions will be accommodated. For more details regarding access to NASEM facilities and expectations for visitors, please visit our operating status webpage.

If you test positive for COVID-19 recently after attending the workshop in-person, please contact Andrew March (amarch@nas.edu) so that the National Academies can contact other workshop participants who may have been exposed.

All workshop participants are strongly encouraged to wear a mask while indoors at the Keck Center unless eating, drinking, or speaking into a microphone. Please consider using an at-home rapid COVID test the night before or morning of the workshop. The HVAC system in the Keck Center is equipped with air purifiers, and additional air purifiers will be running in the room while the workshop is taking place. If you would prefer to eat outdoors, you are welcome to take provided meals across the street to the National Building Museum lawn, National Law Enforcement Officers Memorial, or Judiciary Park.

Volunteers and invited guests should not feel obligated to travel to participate in a meeting being held at one of our facilities during this time. We encourage remote attendance to the meeting for anyone who is not comfortable traveling to or participating in an in-person meeting. The National Academies have made investments in new equipment in our meeting rooms to accommodate interactive, hybrid meetings so that the experience for those not in the room will be as engaging as possible. In certain circumstances, such as for meetings involving classified or controlled information or events of significant importance, a request for participants to attend in-person may be extended. Please reach out to the meeting organizer to discuss needed accommodations for hybrid meetings if you are unable to attend in-person.

The National Academies' leadership is closely monitoring the evolving situation related to the COVID-19 pandemic, and is basing their approach to National Academies' business on the current scientific evidence on COVID-19 and the best public health advice. The priority of the National Academies is the safety of our staff and our larger community of volunteers, sponsors, and members. Please be mindful that this may require unanticipated adjustments to events associated with National Academies projects.

- **Nearest drugstore:**
  - CVS: 400 Massachusetts Ave, approx. 7-minute walk from Keck
  - Walgreens: 801 7th St NW, approx. 8-minute walk from Keck
- **Nearest COVID-19 testing site:** CVS at 655 K St NW (appointment required)

Accelerating the Development and Uptake of Rapid Diagnostics to Address Antibiotic Resistance – A Workshop
Planning Committee Biosketches

Co-chairs

KENT KESTER, MD is currently Vice President, Translational Medicine, at IAVI. He was previously Vice President and Head, Translational Science and Biomarkers at Sanofi Pasteur, the vaccine business unit of the Sanofi Group. Prior to this, in the context of a 24-year career in the US Army, he worked extensively in clinical vaccine development and led multiple research platforms at the Walter Reed Army Institute of Research, the U.S. Department of Defense's largest and most diverse biomedical research laboratory an institution he later led as its Commander. His final military assignment was as the Associate Dean for Clinical Research in the School of Medicine at the Uniformed Services University of the Health Sciences (USUHS). During his military service, Dr. Kester was appointed as the lead policy advisor to the US Army Surgeon General in both Infectious Diseases and in Medical Research & Development. In these capacities, he worked extensively in the interagency environment and developed a variety of Army and DoD medical policies related to infectious diseases.

Dr. Kester holds an undergraduate degree from Bucknell University and an M.D. from the Sidney Kimmel Medical College, Thomas Jefferson University, completing his internship and residency in internal medicine at the University of Maryland and a research fellowship in infectious diseases at the Walter Reed Army Medical Center. Currently a member of the US Government Presidential Advisory Council on Combating Antibiotic-Resistant Bacteria (PACCARB), the Department of Veterans Affairs Health Services Research & Development Service Merit Review Board, the National Academy Standing Committee on Emerging Infectious Diseases and 21st Century Health Threats, and the CEPI Scientific Advisory Committee, he previously chaired the Steering Committee of the NIAID/USUHS Infectious Disease Clinical Research Program, and has served as a member of the FDA Vaccines & Related Biologics Products Advisory Committee (VRBPAC), the NIAID Advisory Council, and the CDC Office of Infectious Diseases Board of Scientific Counselors. He is a Vice Chair of the National Academy of Medicine Forum on Microbial Threats. Board-certified in both internal medicine and infectious diseases, Dr. Kester holds faculty appointments at USUHS and the University of Maryland; and is a fellow of the American College of Physicians, the Royal College of Physicians of Edinburgh, the Infectious Disease Society of America, and the American Society of Tropical Medicine and Hygiene where is also serves as the Society's Secretary-Treasurer. He is a member of the clinical faculty at the University of Maryland Shock Trauma Center in Baltimore and the Wilkes-Barre VA Medical Center in Wilkes-Barre, PA.

BETSY WONDERLY TRAINOR brings over a decade of experience in the diagnostics industry, focused mainly on the validation and implementation of infectious disease diagnostics. Betsy has a background in clinical and behavioral research that started during her undergraduate studies at Cornell University. Betsy's work initially focused on HIV in Africa and then on infectious diseases, globally, including HCV, TB, and Ebola. In addition to leading diagnostic validation studies, she has led the commercialization of diagnostic product lines and a variety of global partnerships on behalf of both private and public entities. She has supported the development of global guidelines and negotiated deals with large donor organizations as well as private entities. Betsy has recently led business development efforts at multiple innovative diagnostic companies, including Daktari

Diagnostics and Aldatu Biosciences, in addition to leading product validation and implementation efforts at the Foundation for Innovative New Diagnostics (FIND) and the World Health Organization (WHO). Betsy is looking forward to putting her experience to good use in supporting the CARB-X mission.

Members

JOHN BILLINGTON, JD, MPH is Head of Health Security and Infectious Diseases Policy and Advocacy in the global corporate government affairs department of GSK. In this role, he is responsible for the company's policy and advocacy strategy for infectious diseases at the enterprise level, with a focus on pandemic preparedness and antimicrobial resistance (AMR). Prior to this role, John was director of science policy for the global vaccines business and director of U.S. vaccines policy before that. Before joining GSK, John was director of health policy at the Infectious Diseases Society of America (IDSA). At IDSA, John served as lead subject matter expert on vaccines, antibiotics, and medical countermeasures policy. He also launched and coordinated the U.S. Stakeholder Forum on Antimicrobial Resistance (S-FAR), a national stakeholder partnership to advance U.S. and global policies and practices in response to AMR. Earlier in his career, John was a manager in the health reform practice at Avalere Health, a health policy advisory firm. He was also a legislative fellow for health policy in the office of U.S. Senator Sherrod Brown of Ohio. John received his Juris Doctor and Masters of Public Health from the Ohio State University in Columbus, Ohio. He received his undergraduate degree from Colgate University in Hamilton, New York. He is originally from Cleveland, Ohio and lives in Washington, DC with his wife Eleanor, daughter Frances, and son Sam.

Carey-Ann Burnham, PhD, D(ABMM), FIDSA, F(AMM) is currently Chief Clinical Officer, Pattern Bioscience, and Professor of Pathology & Immunology, Washington University in St. Louis School of Medicine. She was previously Professor of Pathology & Immunology, Pediatrics, Molecular Microbiology, and Medicine at Washington University in St. Louis School of Medicine and Vice Chair for Faculty Mentoring & Advancement in Pathology & Immunology. In addition, for 11 years she served as Medical Director of the system clinical microbiology laboratory at Barnes-Jewish Hospital in St. Louis, and the program director for the Medical and Public Health Microbiology Fellowship at Washington University. At Washington University, her research program focused on the development of new diagnostics for infectious diseases, antimicrobial resistance, and transmission and epidemiology of antimicrobial resistant microorganisms. Burnham is actively involved in several editorial roles, including serving as an editor for the *Journal of Clinical Microbiology*, *Clinical Microbiology Newsletter*, and *Clinical Microbiology Procedures Handbook, 5th ed*. Burnham has held leadership roles in professional societies, including the American Society for Microbiology, Clinical and Laboratory Standards Institute, the American Academy of Microbiology, and the Academy of Clinical Laboratory Physicians and Scientists. In 2020, Burnham received the prestigious American Society for Microbiology Award for Research and Leadership in Microbiology, and Burnham has published more than 200 articles in the area of diagnostic and clinical microbiology.

PAUL EDER, PHD is a Senior Scientific Officer and leads the Concept Acceleration Program for Diagnostics in the Division of Microbiology and Infectious Diseases (DMID) at NIAID. Previously he served for eight years as a Senior Medical Diagnostics Advisor at the Biomedical Advanced Research and Development Authority (BARDA) inside the US DHHS. For seven years prior he served as the Director of Assay Development at Qiagen, where he used a Bill and Melinda Gates Foundation grant to create the first WHO pre-qualified HPV screening test for utility in resource-limited communities worldwide. For that he won the Qiagen Sydney Brenner R&D Award for outstanding accomplishment in global R&D. He completed postdoctoral fellowships in nuclear tRNA transport at the Howard Hughes Medical Institute at the University of Pennsylvania and in

catalytic RNA with Nobel laureate Sidney Altman at the Department of Biology at Yale University. His Ph.D. is in biochemistry from the University of Iowa. He holds ten issued patents and has authored 25 publications and reviews.

DEBORAH HUNG, MD works at the interface of chemical biology, genomics, and bacterial pathogenesis to establish new paradigms for an antibiotic based on the essential biology required for a pathogen to cause disease within the host. Using her training as a synthetic chemist, bacterial geneticist, and clinical physician, she explores approaches to disrupting the pathogen-host interaction. Dr. Hung is a physician-scientist at the Broad Institute of MIT and Harvard, the Department of Molecular Biology at the Massachusetts General Hospital, and the Department of Genetics at Harvard Medical School, and is the Co-Director of the Infectious Disease and Microbiome Program at the Broad Institute. She is an attending physician at the Brigham and Women's Hospital in Boston in infectious diseases and critical care medicine.

AMANDA JEZEK is currently the Senior Vice President for Public Policy and Government Relations at the Infectious Diseases Society of America (IDSA), which represents over 12,000 ID physicians and scientists. Amanda oversees IDSA's public policy and government relations department, with responsibility for policy development and advocacy on IDSA priority issues, including antimicrobial resistance, the infectious diseases workforce, pandemic preparedness and response, immunizations, federal funding, and other issues relating to public health and biomedical research. Amanda has been with IDSA since 2011, previously serving as IDSA's Government Relations Director. Prior to joining IDSA, Amanda was the Deputy Director for Federal Affairs at the March of Dimes Foundation. In this capacity, Amanda led the March of Dimes' policy development and lobbying efforts on all issues related to access to healthcare for women of childbearing age, infants, and children, including the Foundation's work on the Affordable Care Act. Amanda also lobbied for Mental Health America, and worked as a legislative assistant and press secretary for U.S. Representative Grace Napolitano (D-CA). Amanda holds a B.A. in Political Science from Dartmouth College.

ROBIN PATEL MD(CM), D(ABMM), FIDSA, FACP, F(AAM) graduated from Princeton University with a BA in Chemistry and from McGill University with an MD. She completed a residency in Internal Medicine and fellowships in Infectious Diseases and Microbiology at Mayo Clinic. She is the Elizabeth P. and Robert E. Allen Professor of Individualized Medicine, Professor of Medicine, Professor of Microbiology, Director of the Infectious Diseases Research Laboratory, and Co-Director of the Clinical Bacteriology Laboratory at Mayo Clinic. Dr. Patel is a Fellow of American Academy of Microbiology, Past President of the American Society for Microbiology, an associate editor for Clinical Infectious Diseases, and course director for the Mayo Clinic Alix School of Medicine microbiology course. She is also the Laboratory Center Director for the NIH's Antibacterial Resistance Leadership Group. Her research focuses on clinical bacteriology diagnostic testing, antimicrobial resistance, and microbial biofilms.

RIBHI SHAWAR, PHD currently serves as the branch chief at the Division of Microbiology in the Office of In vitro Diagnostic and Radiological Health (OIR) at the Center for Devices and Radiological Health (CDRH) at FDA where he specializes in the area of antimicrobial susceptibility testing and detection of resistance. Dr. Shawar has a Ph.D. in Medical Microbiology and an M.Sc. in Medical Parasitology. He is a certified diplomate by the American Board of Medical Microbiology (ABMM) for 25 years and was recently elected as a Fellow of the American Academy of Microbiology (F-AAM). Dr. Shawar is a recognized clinical microbiologist with diverse experience in hospital settings, diagnostic and pharmaceutical microbiology and anti-infective drug development with strong knowledge in regulatory aspects both in antimicrobial drug products and infectious disease diagnostics. Dr. Shawar has authored multiple publications in peer reviewed journals, served or currently serves as advisor to several CLSI Sub-committees and is the recipient of multiple awards including

GSK Gold Award and most recently FDA's Outstanding Service Award for his pioneering role in the creation of the FDA-CDC AR Isolate Bank. He served for two terms as Chair of the ABMM Standards and Examinations Committee (Parasitology) and as member of the editorial board of the Journal of Clinical Microbiology. Prior to joining FDA, Dr. Shawar served in multiple roles at various institutions including Baylor College of Medicine, VA Medical Center in Houston, PathoGenesis Corporation, Chiron (Novartis) in Seattle, and GlaxoSmithKline in Philadelphia.

SUSAN VAN METER was named ACLA President in March 2022. She previously served as Executive Director of AdvaMedDx where she directed the policy, advocacy, communications, regulatory, payment and legislative strategy and operations of the association, which represents manufacturers of in vitro diagnostic (IVD) clinical tests in the U.S. and abroad. In this role, she also developed the association's robust response to the COVID-19 pandemic and ongoing advocacy efforts. Prior to her role at AdvaMedDx, Van Meter served as the Senior Vice President of Federal Relations at the Healthcare Association of New York State (HANYS), where she was responsible for developing their priorities and strategies to further the interests of their hospital and health system members. She also previously worked in the Centers for Medicare & Medicaid Services' (CMS) Office of Legislation. Van Meter holds undergraduate and graduate degrees from Villanova University and Boston University, respectively.

Accelerating the Development and Uptake of Rapid Diagnostics to Address Antibiotic Resistance – A Workshop

Speaker Biographies

PHYLLIS ARTHUR is Vice President for Infectious Diseases and Emerging Science Policy at the Biotechnology Innovation Organization (BIO). In this role Ms. Arthur is responsible for working with member companies in vaccines, antimicrobial resistance, molecular diagnostics and biodefense on policy, legislative and regulatory issues. Ms Arthur joined BIO in July 2009 as the Director of Healthcare Regulatory Affairs. Prior to joining BIO, she worked in numerous marketing and sales positions for Merck & Co Inc in their Vaccine Division. Over her 16-year career at Merck, Ms Arthur launched several exciting new vaccines in the United States and internationally, including the first HPV vaccine, GARDASIL. During her years in Marketing, she worked closely with clinical and academic thought leaders in infectious diseases, oncology and public health. In addition, Ms Arthur also led a large vaccine sales organization of over 75 representatives and managers covering 14 states.
Before graduate school, Ms Arthur worked as a research assistant for two economists at the Brookings Institution in Washington, DC. There she conducted economic analyses related to savings and investment policies for the OECD countries. Ms Arthur received her B.A. in 1987 in Economics and International Politics from Goucher College and her M.B.A. in 1991 from the Wharton School of Business at the University of Pennsylvania.

RITU BANERJEE, MD, PHD is Professor in the Division of Pediatric Infectious Diseases at Vanderbilt University Medical Center. She is the Director of the Antimicrobial Stewardship Program and Director of Clinical Services for Pediatric Infectious Diseases at Vanderbilt's Children's Hospital. She received her MD and Ph.D degrees from Washington University in St. Louis and then completed Pediatrics residency and Pediatric Infectious Disease fellowship at the University of California, San Francisco. She is a member of many national committees through the Pediatric Infectious Diseases Society, the American Academy of Pediatrics, the Infectious Diseases Society of America, and the Antibacterial Resistance Leadership Group. Dr. Banerjee conducts federally - funded clinical research about antibiotic stewardship and implementation and outcomes of rapid diagnostics for infectious diseases.

DANIEL BAUSCH, MD, MPH&TM, FASTMH is the Senior Director of Emerging Threats and Global Health Security at FIND, the global alliance for diagnostics (www.FINDdx.org), in Geneva, Switzerland, leading FIND`s efforts on pandemic preparedness and response. He is trained in internal medicine, infectious diseases, tropical medicine, and public health.
Dr Bausch specializes in the research and control of emerging tropical viruses, with over 25 years' experience in sub-Saharan Africa, Latin America, and Asia combating viruses such as Ebola, Lassa, hantavirus, and SARS coronaviruses. Previously, he served as Director of the United Kingdom`s Public Health Rapid Support team (2017-21), a joint effort by Public Health England and the London School of Hygiene & Tropical Medicine to respond and conduct research to prevent and control outbreaks of dangerous infectious diseases around the world. He has also held

posts at the World Health Organisation in Geneva, Switzerland; U.S. Naval Medical Research Unit No. 6 in Lima, Peru; Tulane School of Public Health and Tropical Medicine in New Orleans, USA; and the U.S. Centers for Disease Control and Prevention in Atlanta, USA.
In addition to his role at FIND, Dr Bausch holds an appointment as a Professor of Tropical Medicine at the London School of Hygiene and Tropical Medicine and is the current President of the American Society of Tropical Medicine and Hygiene (www.ASTMH.org). He places a strong emphasis on capacity development in all his projects and has a keen interest in the role of the scientist in promoting health and human rights. Dr Bausch is fluent in English, French and Spanish.

John Billington, JD, MPH, is Head of Health Security and Infectious Disease Policy and Advocacy in the global corporate government affairs department of GSK. In this role, he is responsible for the company's policy and advocacy strategy for infectious diseases at the enterprise level, with a focus on pandemic preparedness and antimicrobial resistance (AMR). Prior to this role, John was director of science policy for the global vaccines business and director for U.S. vaccines policy before that. Before joining GSK, John was director of health policy at the Infectious Diseases Society of America (IDSA). At IDSA, John served as lead subject matter expert on vaccines, antibiotics, and medical countermeasures policy. He also launched and coordinated the U.S. Stakeholder Forum on Antimicrobial Resistance (S-FAR), a national stakeholder partnership to advance U.S. and global policies and practices in response to AMR. Earlier in his career, John was a manager in the health reform practice at Avalere Health, a health policy advisory firm. He was also a legislative fellow for health policy in the office of U.S. Senator Sherrod Brown of Ohio. John received his Juris Doctor and Masters of Public Health from the Ohio State University in Columbus, Ohio. He received his undergraduate degree from Colgate University in Hamilton, New York.

BRADLEY BURNAM is Founder and CEO of Turn Therapeutics, a biotechnology company addressing critical patient needs across advanced wound care, infectious disease, and dermatology. In an attempt to treat his own recurring, antibiotic-resistant skin infection, Burnam developed the first patented method for permanently and stably fusing polar, water-soluble ingredients in petrolatum without an emulsifier. This process, known commercially as PermaFusion®, enabled Burnam to stably suspend broad-spectrum, liquid antimicrobials into a petrolatum carrier. The initial commercial embodiment, which became Turn's first FDA cleared product, is a non-cytotoxic, petrolatum-based, broad-spectrum antimicrobial ointment with the physical consistency/safety profile of OTC topical antibiotics. PermaFusion® has since enabled Burnam to create Turn's growing product portfolio and therapeutic pipeline, including multiple FDA cleared advanced wound products and a lead therapeutic, non-antibiotic topical candidate intended to be indicated for the treatment of antibiotic resistant skin infections.
Burnam is a self-taught regulatory and formulation expert who singlehandedly facilitated Turn's first three FDA clearances. He is a regular guest lecturer at UCLA and was a keynote speaker at the 2019 UCLA commencement. He holds a Bachelor's from UCLA and a Master's from Stanford University.

Karen Carroll, MD is a professor of pathology at the Johns Hopkins University School of Medicine. Her areas of clinical expertise include Medical Microbiology and Infectious Diseases. Dr. Carroll serves as the Director of the Division of Medical Microbiology and the Director of the ACGM-accredited fellowship program in medical microbiology in the Department of Pathology.

Dr. Carroll earned her M.D. from the University of Maryland School of Medicine. She completed her residency at the University of Rochester Medical Center, and performed a fellowship in medical microbiology at the University of Utah and a fellowship in infectious disease at the University of Massachusetts Medical School.

Her research interests include the evaluation of novel diagnostic platforms, diagnosis of bloodstream infections/sepsis, and the diagnosis and epidemiology of healthcare-associated infections, such as MRSA and *Clostridium difficile*. More recently Dr. Carroll has collaborated with researchers on novel rapid microfluidics platforms and next generation sequencing technologies for detection of a variety of pathogens. She has participated in programs to foster appropriate test utilization and diagnostic stewardship.

Tracey L. Cohen is a Distinguished Visiting Scholar at the University of Miami Miller School of Medicine Institute for Bioethics & Health Policy. As a healthcare attorney, Ms. Cohen has represented national and Florida healthcare systems in regulatory compliance, peer-review, transactional and litigation matters. Ms. Cohen also has practiced as an intellectual property attorney, overseeing the intellectual property portfolios of major healthcare institutions, corporations, and individuals.
Ms. Cohen served as an Adjunct Professor of Law at Nova Southeastern University Broad College of Law for six years, where she created curriculum and taught courses in intellectual property law. She is also a legal writer, and has published articles for Nolo, a division of Martindale-Hubbell.

Ms. Cohen received her B.A. in Philosophy from Brandeis University, and her J.D. from the University of Florida Levin College of Law, where she was the recipient of a Book Award in Legal Research and Writing and sat on the Senior Editorial Board of the *University of Florida Journal of Law and Public Policy*. Ms. Cohen also holds a Master of Science in Bioethics from Columbia University.

Paul Eder, PhD is a Senior Scientific Officer and leads the Concept Acceleration Program for Diagnostics in the Division of Microbiology and Infectious Diseases (DMID) at NIAID. Previously he served for eight years as a Senior Medical Diagnostics Advisor at the Biomedical Advanced Research and Development Authority (BARDA) inside the US DHHS. For seven years prior he served as the Director of Assay Development at Qiagen, where he used a Bill and Melinda Gates Foundation grant to create the first WHO pre-qualified HPV screening test for utility in resource-limited communities worldwide. For that he won the Qiagen Sydney Brenner R&D Award for outstanding accomplishment in global R&D. He completed postdoctoral fellowships in nuclear tRNA transport at the Howard Hughes Medical Institute at the University of

Pennsylvania and in catalytic RNA with Nobel laureate Sidney Altman at the Department of Biology at Yale University. His Ph.D. is in biochemistry from the University of Iowa. He holds ten issued patents and has authored 25 publications and reviews.

NICHOLAS G. EVANS, PHD is Associate Professor of Philosophy at the University of Massachusetts Lowell. A 2020-2023 Greenwall Foundation Faculty Scholar, he currently conducts research on the ethics of emerging technologies, with a focus on national security issues. He is best known for his research on "dual-use research" in the life sciences and has recently begun work examining research ethics concerns arising from the performance enhancement of active military personnel, funded by the US Air Force Office of Scientific Research.

In addition to his work on emerging technologies, Evans is a recognized expert in public health ethics, writing on the ethics of social distancing, research ethics during health emergencies, and the use of force in pandemic response. His 2016 collection, Ebola's Message: Public Health and Medicine in the 21st Century received favorable reviews in Nature. In late 2021 he will publish a new, sole-authored work on pandemic preparedness with The MIT Press titled, War on All Fronts: A Theory of Just Health Security.

Prior to his appointment at the University of Massachusetts, Evans completed postdoctoral research at the Perelman School of Medicine at the University of Pennsylvania. In 2015, he held an Emerging Leaders in Biosecurity Initiative Fellowship at the UPMC Center for Health Security, Baltimore. He also previously served as a policy officer with the Australian Department of Health and Australian Therapeutic Goods Administration.

DIANE FLAYHART is the Global Program Leader AMR at the Antimicrobial Resistance Fighter Coalition/BD. Diane leads efforts for the Antimicrobial Fighter Coalition, a global organization that aspires to change behaviors across the globe that will maintain the effectiveness of antibiotics for future generations. The Coalition seeks to substantially increase awareness of drug-resistant infections and encourage action across a wide range of stakeholders, including policymakers, health agency officials, professional societies, clinicians, patients, and family members. The Coalition is mobilized by BD where Diane has served roles of increased responsibility focused on Marketing and Commercial Excellence since 2007. She started her career at Johns Hopkins Medical Institution working as a medical technologist in the Microbiology Laboratory. Diane completed her Master's Degree in Business Administration at the Johns Hopkins University.

GREG FRANK, PHD is Director, Global Public Policy with Merck, where he leads Merck's global antimicrobial resistance (AMR) policy. Previously Dr. Frank served as Senior Director, Infectious Disease Policy at the Biotechnology Innovation Organization (BIO), where he led several infectious diseases policy issues, including AMR and vaccine regulatory policy. Prior to BIO, Dr. Frank led science and diagnostics policy as Program Officer for Science and Research Policy at the Infectious Diseases Society of America (IDSA).
Dr. Frank serves on the Leadership Council of the National Institute of Antimicrobial Resistant Research and Education (NIAMREE), and the expert advisory committee for the Partnership to Fight Infectious Diseases. He is a Board member on the AMR Industry Alliance. Previously, Dr.

Frank has served on the U.S. Presidential Advisory Committee on Antibiotic Resistant Bacteria (PACCARB) and joined expert advisory committees of the Access to Medicines Foundation AMR Benchmark, the Global AMR R&D Hub, and the Duke-Margolis Center for Health Policy Antimicrobial Incentives & Payment Reform Project.

Dr. Frank received his doctorate in immunology at the University of Pittsburgh and pursued his postdoctoral training at the Laboratory of Viral Diseases at the National Institute of Allergy & Infectious Diseases.

Alex Greninger, MD, PhD, MS, MPhil is assistant director of the UW Medicine Clinical Virology Laboratory and a UW assistant professor of Laboratory Medicine. Dr. Greninger focuses on genomic and proteomic characterization of a variety of human viruses and bacteria, with a focus on respiratory viruses and human herpesviruses. He has discovered a number of new human and animal viruses. His basic science lab at South Lake Union uses genomically informed approaches to understand human infectious diseases. Dr. Greninger earned his M.D. and Ph.D., from UC San Francisco, his M.S. in Immunology from Stanford and his M.Phil. in Epidemiology from Cambridge in England. Dr. Greninger has clinical interests in facilitating clinical trial testing for respiratory viruses and human herpesviruses.

JOE LARSEN, PHD is Vice President, Clinical Development at Locus Biosciences where he leads development programs for Locus's clinical stage assets. Previously, Dr. Larsen was Vice President, Strategic Portfolio Development at Venatorx Pharmaceuticals, a biotechnology company focused on the development of novel antibiotics. Prior to that, Dr. Larsen spent ten years in the federal government, including serving as Deputy Director of Chemical, Biological, Radiological and Nuclear (CBRN) medical countermeasures at the Biomedical Advanced Research and Development Authority (BARDA), where he oversaw the $2.8B Project Bioshield fund for the late-stage development and procurement of medical countermeasures. Dr. Larsen received his Ph.D. in microbiology and immunology at the Uniformed Services University of the Health Sciences and a B.A. in microbiology from the University of Kansas.

JACLYN LEVY is the Director of U.S. Policy at the AMR Action Fund, where she works to support policy solutions and market reforms related to antimicrobial R&D, pull incentives, stewardship, and access. She joined the Fund from the Infectious Diseases Society of America (IDSA), where she served as Director of Public Policy and advanced a broad portfolio of research, diagnostics, and public health policy issues. Prior to IDSA, Jaclyn worked as a biosecurity analyst for the U.S. Department of Homeland Security and as a scientific editor and consultant. She has over a decade of experience in federal and global policy and advocacy work and has authored numerous publications on pandemic preparedness, antimicrobial resistance, molecular diagnostics, and biomedical R&D. Jaclyn holds an MS in Biohazardous Threat Agents & Emerging Infectious Diseases from Georgetown University and a BA from The George Washington University. She was a 2020 Atlantic Council Millennium Fellow.

JOSEPH LUTGRING, MD works as a medical officer for the Division of Healthcare Quality Promotion at the Centers for Disease Control and Prevention. He is a graduate of Indiana

University School of Medicine. He is board certified in internal medicine, infectious diseases, and medical microbiology. He has interests in diagnostic stewardship, antimicrobial resistance, and working on topics at the intersection of clinical infectious diseases and the microbiology laboratory.

STANLEY MARTIN, MD is a board-certified and fellowship-trained specialist in infectious diseases. His clinical interests include immunocompromised hosts, transplantation and infections related to mechanical circulatory support. His research interests include antimicrobial stewardship, population health and optimization of inpatient care. Dr. Martin earned his medical degree from the University of Tennessee. He completed his residency at Mayo Clinic, Rochester, and his fellowship in infectious diseases at Massachusetts General Hospital. Dr. Martin is certified by the American Board of Internal Medicine in infectious diseases. He is Geisinger's director of the Division of Infectious Diseases.

ALITA MILLER, PHD is the Chief Scientific Officer at Entasis Therapeutics, where she has played a key role in the discovery and development of several novel antibacterial agents, including ETX0462, sulbactam-durlobactam and zoliflodacin. She has over 20 years of experience in antibacterial research, first at Pfizer where she led both large and small molecule discovery projects and then at AstraZeneca, where she was Head of Microbial Genetics and Genomics. Dr. Miller obtained a BA in Chemistry from Kalamazoo College and a PhD in Biochemistry & Molecular Biology from the University of Chicago. Her postdoctoral training was in the DiRita lab at the University of Michigan.

KEVIN OUTTERSON, JD, LLM teaches health care law at Boston University, where he co-directs the Health Law Program. He serves as the founding Executive Director and Principal Investigator for CARB-X, an >$800M international public-private partnership to accelerate global antibacterial innovation. Key partners in CARB-X include the US Government (BARDA & NIAID), the Wellcome Trust, the German Federal Ministry of Education and Research (BMBF), the UK Government (GAMRIF), and the Bill & Melinda Gates Foundation. Professor Outterson's research work focuses on the law and economics of antimicrobial resistance, particularly push and pull incentives for antimicrobials. He served as a senior author on many key research reports on antibiotic innovation, including Chatham House, ERG, DRIVE-AB, and the Lancet Commission. Professor Outterson was given the 2015 Leadership Award by the Alliance for the Prudent Use of Antibiotics for his research and advocacy work. He has testified before Congress, Parliamentary working groups, WHO, and state legislatures. Since August 2016, he leads CARB-X, the world's most innovative antibiotic accelerator.

JEAN PATEL, PHD currently serves as the principal scientist, scientific affairs, at Beckman Coulter. Prior to her role at Beckman Coulter, Dr. Patel served as the science team lead, antibiotic resistance coordination and strategy unit, at the Centers for Disease Control (CDC), where she led implementation of its Antibiotic Resistance Laboratory Network and the CDC and FDA Antibiotic Resistance Isolate Bank.

Dr. Patel has served as chair and vice chair of the Clinical and Laboratory Standards Institute Subcommittee for Antimicrobial Susceptibility Testing and works with the World Health Organization (WHO) to develop technical guidance for detecting resistance and strengthening global surveillance of antimicrobial resistance.

ROBIN PATEL MD(CM), D(ABMM), FIDSA, FACP, F(AAM) graduated from Princeton University with a BA in Chemistry and from McGill University with an MD. She completed a residency in Internal Medicine and fellowships in Infectious Diseases and Microbiology at Mayo Clinic. She is the Elizabeth P. and Robert E. Allen Professor of Individualized Medicine, Professor of Medicine, Professor of Microbiology, Director of the Infectious Diseases Research Laboratory, and Co-Director of the Clinical Bacteriology Laboratory at Mayo Clinic. Dr. Patel is a Fellow of American Academy of Microbiology, Past President of the American Society for Microbiology, an associate editor for Clinical Infectious Diseases, and course director for the Mayo Clinic Alix School of Medicine microbiology course. She is also the Laboratory Center Director for the NIH's Antibacterial Resistance Leadership Group. Her research focuses on clinical bacteriology diagnostic testing, antimicrobial resistance, and microbial biofilms.

David (Dave) Persing, MD, Ph.D is Executive Vice President and Chief Scientific Officer at Cepheid and in 2017 was appointed Chief Scientific Officer (CSO) for the Danaher Diagnostics Platform. He has spent most of his 30-year career in biomarker discovery, translational medicine and innovation in the diagnostics space. As CSO, Danaher Diagnostics Platform, he has the responsibility for providing scientific, medical and strategic input to the Diagnostics' Operating Companies and Platform leadership. He also has the responsibility for development of new clinical processes, technologies or products that advance patient care, innovation and competitive positioning of the Danaher Diagnostics group of operating companies.

Dave joined Cepheid in 2005 and has since focused on the enablement of molecular diagnostic technology to meet global needs in infectious diseases and oncology. He conducted his scientific and medical training with Don Ganem and Nobel laureate Harold Varmus at the University of California, San Francisco. After residency training in Clinical Pathology at Yale University, he held leadership roles in academia and industry starting in the early 1990s with the design, implementation, and scaleup of the first PCR reference laboratory at the Mayo Clinic. His interest in the democratization of molecular diagnostic methods has been longstanding, starting in 1993 with his publication of the first of five widely adopted textbooks to include PCR protocols and guidelines for laboratory operations. He has published over 300 peer-reviewed articles and reviews, including multiple articles in the New England Journal of Medicine, Science, and PNAS. In 2020, he was named to the Fierce Pharma list of the 22 most influential scientists in the fight against COVID-19. To maintain a connection with the latest trends in translational medicine, Dave also serves as Consulting Professor of Pathology at Stanford University School of Medicine. He obtained his MD and PhD degrees from UCSF in 1988.

MELINDA PETTIGREW, PHD is the Anna M. R. Lauder Professor of Epidemiology and the Interim Dean at the Yale School of Public Health. Pettigrew conducts both laboratory and epidemiologic

studies. Her work focuses on how disruptions of homeostasis in the respiratory and gastrointestinal microbiota influence colonization resistance, development of antibiotic resistance, and risk of bacterial infections. Additional research seeks to identify epidemiologic and genetic factors that influence whether opportunistic pathogens asymptomatically colonize or cause diseases such as pneumonia and exacerbations of chronic obstructive pulmonary disease.

Pettigrew is nationally known for her research and leadership in her roles on the steering and executive committees for the Antibiotic Resistance Leadership Group (ARLG). The ARLG is a National Institutes of Health sponsored initiative that supports a national network of scientists to develop, implement, and manage a clinical research agenda to combat the public health crisis of antimicrobial resistance. As the associate director of the Scientific Leadership Core for the ARLG, she leads efforts to implement and integrate principles of diversity, access, equity, and inclusion throughout the network.

A graduate of Grinnell College, Pettigrew received her Ph.D. from Yale University in 1999. She conducted a postdoctoral fellowship at the University of Michigan prior to joining the Yale School of Public Health faculty in 2002. Pettigrew's research has been supported by grants from the National Institutes of Health, the Centers for Disease Control and Prevention, and private foundations. In addition to her research, she is highly regarded for her teaching. She has received the Yale School of Public Health Distinguished Teaching Award and the Inspiring Yale Award. She also serves on the editorial board of the scientific journal mBio.

Valérie Raymond Schwartzmann, PharmD has been the Director of the Companion Diagnostics Program at bioMérieux since 2014. The objective of this program is to forge close partnerships with healthcare industries (drugs, vaccines, medical and surgical equipment) in order to deliver diagnostic tests with high medical value as well as to support drug prescription and improve patient care in the context of personalized medicine.

Prior to this position, Valérie was Marketing Director "Acute & Critical Care" within the Immunoassay Unit of bioMérieux. She helped develop a marketing strategy centered on the medical value of products, for clinicians, patients and diagnostic laboratories. Valérie has acquired 14 years of experience in sales and marketing both at local and corporate level, mainly in the field of diagnostics but also in the field of medical devices and pharmaceutical products (Roche Diagnostics -- commercial organization, Fresenius HemoCare - Marketing Director and Responsible Pharmacist).

Valérie is a Doctor of Pharmacy and a former intern at the Hospitals of Lyon. She completed her training with a DEA in Analytical Chemistry, an AEU in Toxicology and a Certificate in Pharmacovigilance.

William "Bill" Rodriguez, MD is the Chief Executive Officer of FIND, the global alliance for diagnostics. Bill previously served as Chief Medical Officer between 2015 and 2017. He joined from the Draper Richards Kaplan Foundation, a global venture philanthropy firm supporting early-stage, high-impact social enterprises. A medical doctor, he has extensive experience across both

private and public sectors, founding his own diagnostics company, Daktari Diagnostics - a venture-backed start-up company developing portable diagnostics for HIV, HCV, tuberculosis, typhoid, and maternal health for use in low- and middle-income countries. He is a highly respected figure in the global health community, serving as advisor to the World Health Organization, Bill & Melinda Gates Foundation, national governments on global HIV, tuberculosis, Ebola and COVID-19, as well as numerous established and start-up for-profit and not-for-profit social enterprises focused on global health. He is a graduate of Brown University and the Yale University School of Medicine.

KRISTIAN ROTH, PHD is the Deputy Director in the FDA's Division of Microbiology Devices. He received his Ph.D. in analytical chemistry from the University of California Riverside studying surface bound porphyrin molecules then completed a postdoc at the University of California Santa Barbara studying the materials synthesis properties of large protein conjugates with Prof. Dan Morse. Afterward he moved to the Seattle area to join CombiMatrix, a startup diagnostics company targeting infectious diseases using DNA microarray detection. There, he worked on developing nucleic acid amplification assays for the detection and differentiation of influenza using a novel microfluidic cartridge coupled with electrochemical array detection. Dr. Roth then moved to the Maryland area to join Meso Scale Discovery developing platforms for the detection of influenza, hepatitis and radiation biodosimetry. In 2011 he started at FDA as a scientific reviewer and was involved in the writing of guidance documents for assay migration and multiplex infectious disease detection then later served as Branch Chief in the Multiplex Bacteriology and Medical Countermeasures branch in the Division of Microbiology (DMD). Dr. Roth has also been a stakeholder in DMD's emergency response to the recent Ebola and Zika outbreaks.

KIMBERLY SCIARRETTA, PHD is the Solving Sepsis Program Manager within the Division of Research Innovation and Ventures (DRIVe), Biomedical Advanced Research and Development Authority (BARDA), part of the Assistant Secretary for Preparedness and Response (ASPR), within the United States Department of Health and Human Services (HHS). Previously Dr. Sciarretta was a Project Officer within the BARDA CBRN Division, and prior to that, was a technical consultant to multiple US Government Agencies. Dr. Sciarretta was one of the inaugural members of DRIVe and is leading efforts towards improving patient outcomes for sepsis through strategic interagency activities and critical technology investments with external partners. Dr. Sciarretta received her PhD from the University of Chicago in Molecular Genetics and Cell Biology. Her expertise broadly spans medical countermeasure development, biochemistry, synthetic biology, advanced manufacturing and chemical and biological defense technologies.

Anthony D. So, MD, MPA is the second Professor of the Practice and Founding Director of the Innovation+Design Enabling Access (IDEA) Initiative at the Johns Hopkins Bloomberg School of Public Health. The IDEA Initiative fosters innovation and design of new technologies for greater health access and impact. As Director of the Strategic Policy Program of ReAct—Action on Antibiotic Resistance, he works with a global network dedicated to meeting the challenge of antimicrobial resistance, and his program serves as the Secretariat to the Antibiotic Resistance Coalition.

He served as one of the Co-Conveners of the UN Interagency Coordination Group on Antimicrobial Resistance that delivered recommendations to the UN Secretary General in 2019. Most recently, he was Co-Chair of the Technical Working Group aligning pharmaceutical innovation incentives to achieve fair pricing for the 2021 WHO Fair Pricing Forum and currently is a member of the Technical Advisory Group of WHO's COVID-19 Technology Access Pool. He also has served as a member of the Antibiotic Resistance Working Group of the U.S. President's Council of Advisors in Science and Technology and as part of the WHO expert Technical Consultation on In Vitro Diagnostics for Antimicrobial Resistance.

His research and policy work on collaborative R&D models and incentives for innovation has received support under a Robert Wood Johnson Investigator Award in Health Policy Research. He contributed to the Lancet Infectious Diseases Commission on Antibiotic Resistance, co-edited the Chatham House's Working Group's report, *Towards a New Global Business Model for Antibiotics: Delinking Revenues from Sales,* and co-authored "A Framework for Costing the Lowering of Antimicrobial Use in Food Animal Production" for the UK Review on Antimicrobial Resistance.

SUSAN VAN METER was named ACLA President in March 2022. She previously served as Executive Director of AdvaMedDx where she directed the policy, advocacy, communications, regulatory, payment and legislative strategy and operations of the association, which represents manufacturers of in vitro diagnostic (IVD) clinical tests in the U.S. and abroad. In this role, she also developed the association's robust response to the COVID-19 pandemic and ongoing advocacy efforts. Prior to her role at AdvaMedDx, Van Meter served as the Senior Vice President of Federal Relations at the Healthcare Association of New York State (HANYS), where she was responsible for developing their priorities and strategies to further the interests of their hospital and health system members. She also previously worked in the Centers for Medicare & Medicaid Services' (CMS) Office of Legislation. Van Meter holds undergraduate and graduate degrees from Villanova University and Boston University, respectively.

CRAIG WHITEFORD, MS, PHD leads a diverse set of R&D Scientists for BD Integrated Diagnostic Solutions (IDS), who have developed diagnostic tests for blood stream infections, Tuberculosis (ID/DST), bacterial identification & antibiotic susceptibility, enteric diseases, respiratory diseases, sexually transmitted infections, and oncology. Over the past 18 years, Craig has held both R&D and Project Management roles, from which he has driven WW product development from ideation to the launch for the BD MAX Molecular platform as well as various phenotypic platforms inclusive of the BACTEC, MGIT, Phoenix, Phoenix AP as well as KiestrA laboratory automation system, which consist of robotics and artificial intelligence (AI)-driven software that is transforming the way clinical laboratories operate. With his keen interest in infection diseases, he has also led several Technology Sensing and Development Teams.
Successfully serving BD in many different roles, his Teams have developed more than a dozen IVD devices. Working closely with Regulatory Affairs, Medical/Clinical Affairs and Quality Affairs to develop strategies for clinical trials as well as regulatory bodies' submission (FDA, China, Japan, ANVISA, CE regulations). Before coming to BD, he worked as a Lab Manager for

a Pediatric oncology lab at the National Cancer institute within the National Institutes of Health. Researching, developing, and deploying one of the largest Microarray technologies and research efforts at the National Institutes of Health.

Craig holds a Bachelor of Science in Biology & Microbiology, Master of Science, and a Ph.D. in Microbiology from Kansas State University.

ABOUT THE FORUM

The Forum on Drug Discovery, Development, and Translation (the Forum) of the National Academies of Sciences, Engineering, and Medicine (the National Academies) was created in 2005 by the National Academies Board on Health Sciences Policy to foster communication, collaboration, and action in a neutral setting on issues of mutual interest across the drug research and development lifecycle. The Forum membership includes leadership from the National Institutes of Health, the U.S. Food and Drug Administration, industry, academia, consortia, foundations, journals, and patient-focused and disease advocacy organizations.

Through the Forum's activities, participants have been better able to bring attention and visibility to important issues, explore new approaches for resolving problem areas, share information and find common ground, and work together to develop ideas into concrete actions and new collaborations.

Priority areas of emphasis for the Forum include:

Promoting Collaboration Across the Biomedical Research Lifecycle

The Forum highlights critical issues and facilitates communication and collaboration across the biomedical research lifecycle to bridge the ever-widening gap between scientific discoveries and the translation of those discoveries into life-changing medications. The Forum explores approaches to enhance evidence-based decision-making across all stages of drug R&D.

Enhancing the Workforce and Infrastructure for Drug Discovery, Development, and Translation

Considerable opportunities remain to improve and enhance the workforce and infrastructure supporting the clinical trials enterprise. The Forum has fostered the development of strategies to improve the discipline of innovative regulatory science, anticipate future workforce needs, and focus attention on building a workforce that is diverse, adaptable, and resilient.

Spurring Person-Centered Innovation in Drug R&D

Revolutionary advances in biomedical research and technology applications present new and exciting opportunities for the discovery and development (R&D) of new therapies for patients. There is increasing recognition of the need for more person-centered approaches that prioritize lived experience, equity, and justice in setting priorities for developing new therapies and accelerating evidence development. The Forum offers a neutral space for stakeholders to advance critical policy discussions on biopharmaceutical R&D nationally and globally to enable innovation in drug R&D that addresses the most pressing public health priorities.

Envisioning the Future for the Clinical Trials Enterprise

The evolution of health care is expanding the possibilities for integration of clinical research into the continuum of clinical care; new approaches are enabling the collection of data in real-world settings; and new modalities, such as digital health technologies and artificial intelligence applications, are being leveraged to advance clinical research. At the same time, the clinical research enterprise is strained by rising costs, varying global regulatory and economic landscapes, increasing complexity of clinical trials, barriers to recruitment and retention of research participants, and a clinical research workforce that is under tremendous demands. The Forum brings together patients, providers, academia, industry, federal agencies, payers, nonprofit organizations, and funders to de-risk innovation and enable a clinical trials enterprise that is more efficient, effective, person-centered, inclusive, and integrated into the health delivery system.

For more information about the Forum on Drug Discovery, Development, and Translation, please visit at:

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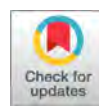
MINIREVIEW



Clinical Utility of Advanced Microbiology Testing Tools

Melissa B. Miller,[a] Faranak Atrzadeh,[b] Carey-Ann D. Burnham,[c] Stephen Cavalieri,[d] James Dunn,[e] Stephen Jones,[f] Charles Mathews,[f] Peggy McNult,[g] John Meduri,[h] Chris Newhouse,[i] Duane Newton,[j] Michael Oberholzer,[k] John Osiecki,[i] David Pedersen,[f] Nicole Sweeney,[l] Natalie Whitfield,[m] Joe Campos,[n] on behalf of the ASM Clinical and Public Health Microbiology Committee and the ASM Corporate Council

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ABSTRACT Advanced microbiology technologies are rapidly changing our ability to diagnose infections, improve patient care, and enhance clinical workflow. These tools are increasing the breadth, depth, and speed of diagnostic data generated per patient, and testing is being moved closer to the patient through rapid diagnostic technologies, including point-of-care (POC) technologies. While select stakeholders have an appreciation of the value/importance of improvements in the microbial diagnostic field, there remains a disconnect between clinicians and some payers and hospital administrators in terms of understanding the potential clinical utility of these novel technologies. Therefore, a key challenge for the clinical microbiology community is to clearly articulate the value proposition of these technologies to encourage payers to cover and hospitals to adopt advanced microbiology tests. Specific guidance on how to define and demonstrate clinical utility would be valuable. Addressing this challenge will require alignment on this topic, not just by microbiologists but also by primary care and emergency room (ER) physicians, infectious disease specialists, pharmacists, hospital administrators, and government entities with an interest in public health. In this article, we discuss how to best conduct clinical studies to demonstrate and communicate clinical utility to payers and to set reasonable expectations for what diagnostic manufacturers should be required to demonstrate to support reimbursement from commercial payers and utilization by hospital systems.

KEYWORDS clinical utility, evidence, health economics, molecular methods, outcomes research, reimbursement

Until recently, clinical microbiology diagnostic techniques have relied almost exclusively on culture and isolation of microbes from patient specimens, with biochemical and phenotypic analysis to identify pathogen(s) causing infections (1–3). While creating the infrastructure for clinical laboratory scientists to perform culture and conduct analyses was costly, once well established, these techniques are inexpensive

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and can be highly standardized. Unfortunately, these techniques are time consuming, typically requiring 2 to 5 days or more to identify pathogens. In select cases, culture techniques may fail to adequately grow certain etiologic agents (e.g., *Mycoplasma pneumoniae*) or detect a viral pathogen. While developments in this space have historically focused on automating the culture process to reduce hands-on time and overall turnaround times to diagnosis, the turnaround time remains slow, which often causes physicians to treat empirically before diagnostic confirmation. Extended empirical treatment time may lead to the inappropriate use of antimicrobials, which may further contribute to the growing burden of antibiotic resistance. Studies have estimated that 30% to 50% of prescribed antimicrobials may be overprescribed or unnecessary (4, 5), which may contribute to the spread of infections due to increased antimicrobial resistance. Use of systemic antibiotics can lead to a disruption in the microbiome that can result in diarrhea and other complications (6, 7). One study concluded that some 20% of patients receiving antibiotics experienced an adverse drug event (8). The goal, therefore, is to avoid unnecessary antibiotics in addition to getting the patient on the most appropriate antibiotics when necessary.

In recent years, the introduction of new technologies has positively impacted both the diagnosis and treatment paradigms for infections. These tools are in the process of revolutionizing clinical microbiology testing in various settings. These include technologies such as matrix-assisted laser desorption–ionization time of flight mass spectrometry (MALDI-TOF MS), multiplex molecular diagnostic panels, and innovations that bring nucleic acid amplification testing to the point of care. The proteomic-based technology MALDI-TOF MS has seen wide adoption, particularly among academic hospitals. While this method still requires isolation and culture of pathogens, MALDI-TOF MS allows for the identification of a specific microbe based upon its unique proteomic fingerprint (9, 10). MALDI-TOF MS has led to significant time and cost savings, as correct diagnoses are made more rapidly without the need for additional confirmatory tests. Multiplex molecular diagnostic panels (11, 12) are also being introduced more commonly for a variety of conditions, including sepsis and nonspecific syndromes, such as respiratory or gastrointestinal (GI) infections. Multiplex assays can combine tests for numerous pathogens and resistance markers in a single panel, which can significantly reduce the time to diagnosis and, in select situations, bypass the need for culture. Furthermore, improvements in engineering and technology have also led to the development of improved point-of-care (POC) tests (13–16), which are poised to significantly impact the future treatment paradigm for many infectious conditions, such as viral respiratory infections and sexually transmitted infections (STIs). Low-complexity POC tests allow for nonlaboratory personnel (e.g., nurses and physician assistants) to conduct tests at the initial site of care, potentially allowing physicians to administer the therapy at the initial consultation and eliminating the need for follow-up assessments.

These tools have the potential to address many key challenges in the field of infection management by reducing the time to diagnosis and informing earlier therapeutic decisions, which may improve clinical decision making, patient outcomes, workflow, and antimicrobial stewardship (5, 17–19). These types of innovations also have the potential to significantly improve both individual patient outcomes and broader public health by facilitating better tracking of pathogens and changes in/development of antibiotic resistance (2).

However, in many cases, the clinical deployment of these technologies has been restrained by skepticism from payers and hospital administrators over clinical, and ultimately cost, benefits. Select referenced studies are underpowered from a statistical perspective, which may not demonstrate a benefit as clearly as would be desired. In some cases, advanced microbiology tests provide limited improvement in accuracy over standardized laboratory culture-based tests, although they provide workflow and efficiency benefits. Therefore, it is imperative to demonstrate robust evidence of clinical utility in a timely and cost-effective manner to increase our understanding of the benefits of adoption of advanced microbiology tests across care settings. More robust

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studies of clinical utility will also improve our knowledge of the impact on clinical outcomes and operations, which can lead to enhancements in care.

OVERVIEW OF CLINICAL UTILITY AND EVIDENCE DEVELOPMENT CONSIDERATIONS

Clinical utility of a test is related to the added value it has for patient management. A test has utility if its results (positive or negative) provide information that is of value to the patient and the provider in making decisions about effective clinical care. It can take the form of improved efficiency in clinical decision making, streamlined clinical workflow, better patient outcomes, and/or cost offsets or avoidance (20–24). The level of clinical utility evidence required will likely depend upon a variety of factors, including the current standard of care (SOC), the setting of care, and potential cost offsets to mitigate the added cost of care, as well as the magnitude of the cost of the test itself.

Clinical utility, in the microbial space, is considered to include instances in which new approaches can inform treatment decisions by providing information to key stakeholders, such as the patient, physician, and payers, to diagnose, monitor, and/or predict disease progression. For example, rapid POC influenza testing has been shown in several studies to significantly reduce prescriptions of antimicrobials and increase prescriptions of oseltamivir (Tamiflu) in outpatient care clinics (25–27). Rapid POC influenza testing can improve antibiotic stewardship and positively impacts patient management via a faster resolution of flu symptoms. Better initial treatment decision making could also influence important outcomes, like morbidity and mortality, for at-risk patient populations, such as the elderly and immunocompromised.

It is important to note that a necessary first step for any new diagnostic is to demonstrate that it meets, and potentially surpasses, the bar for accuracy of the current SOC. However, accuracy alone does not, in and of itself, demonstrate clinical utility. Instead, it is a prerequisite for utilization that facilitates impact on clinical care, which can then translate into clinical utility.

It is important that microbiologists conducting clinical utility studies consider which type of trial is most appropriate for the technology and endpoint they wish to study, as well as the ultimate stakeholder audience for the diagnostic technology. Whenever possible, studies designed to generate evidence of clinical utility should consider the needs of potential patient populations. For instance, hospital administrators often look for evidence generated in a system that closely resembles their organization, to provide confidence that the clinical utility demonstrated in a trial may translate to real-world experience. Also, many hospital systems may conduct their own trials with new diagnostic technology, to provide real-world evidence for improved workflow, decision making, and patient care resulting from the adoption of new technology. Published results are impactful for commercial payers when they can demonstrate clear changes in clinical decision making for patients that are representative of their plan members that were directly facilitated by the information provided by the test. Ideally, payers would like to see data demonstrating that these decisions correlated with positive clinical outcomes. However, payers are aware that many factors go into clinical outcomes beyond the diagnostic result, and therefore it may be sufficient to simply show a connection between the changes in decision making and potential clinical outcome.

Once the audience is defined, the study designers also need to determine the appropriate level of evidence. The key considerations are the overall size of the trial and its representativeness. The size of the trial relates to the relative rarity of the events that occur. Statistical power calculations can be done to determine the number of patients needed relative to the delta in a change of endpoint. As the numbers of groups of patients and parameters to be measured increase, the number of patients needed to achieve a significant result will also increase. For instance, a study that measures only one patient group (e.g., high risk for respiratory conditions) with two diagnostic arms (e.g., SOC compared to multiplex panels) may be able to reach significance with 100 patients. A trial that was following three cohorts of patients (e.g., high risk, low risk, and the general population) and measuring three diagnostic arms (e.g., culture and phe-

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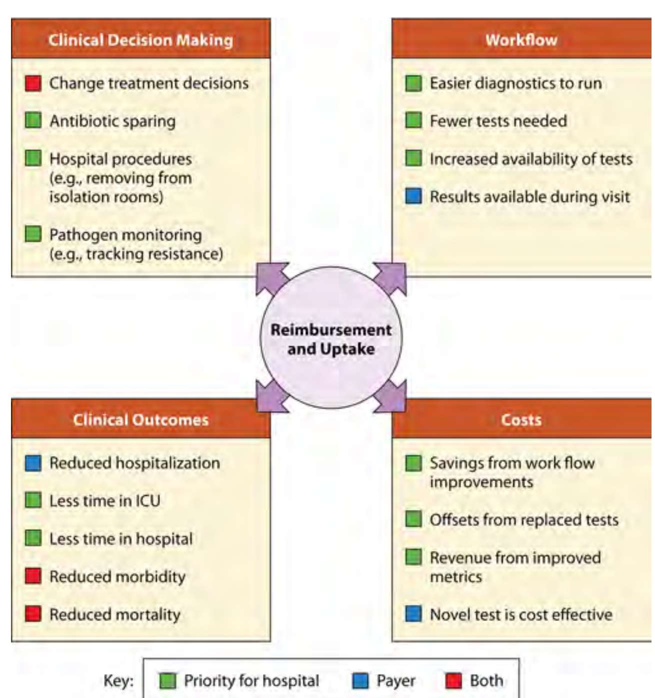

FIG 1 Endpoints for studies of clinical utility.

notype, single-gene PCR tests, and multiplex molecular panels) would likely require 800 or more patients (28, 29). A study of this complexity and size may not be feasible for many clinical labs.

To illustrate representativeness, it is important to conduct the trial at a location which is comparable to the broader clinical community. For instance, by testing MALDI-TOF MS technology in large academic hospitals with a significant volume of testing, the investigators were able to demonstrate the significant clinical utility of MALDI-TOF MS to improve workflow and decision making in central laboratories of major hospital systems (30, 31). When researchers conduct studies in hospital systems with a unique patient population or practices, it may be challenged by payers and others as being nonrepresentative.

OVERVIEW OF CLINICAL UTILITY EVIDENCE DEVELOPMENT CONCEPTS

While overall clinical utility for microbial diagnostics covers the areas defined above, the value proposition of technology may typically be demonstrated by impacting one or more of the following categories: clinical decision making, clinical workflow, patient outcomes, and clinical costs (Fig. 1).

The first step is defining the current standard of care; researchers should always strive to test clinical utility in comparison to the current SOC, with the goal of significantly improving at least one step of the diagnostic paradigm. Therefore, microbiologists should carefully study the SOC and determine where the new test can make the most significant improvement (32). If current care is well defined, an observational trial measuring changes in care could be sufficient. If the current SOC is not well defined, these types of studies will be most effective if they are large prospective studies over multiple institutions, allowing for the determination of the clinical utility of such a test in a variety of settings with different care paradigms.

Researchers can demonstrate clinical utility through a variety of trial designs (33), including randomized control trials (25, 34), in which patients are randomly assigned as they enter the health care system to a new diagnostic being tested or to the SOC (Fig. 2). At the end of the study period, the researchers can ascertain how measured endpoints varied between the two groups. Prospective interventional or observational

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Trial Design	Endpoint/Outputs	Payer and Hospital Needs Met	Issues and Limitations
Prospective Randomized Control Trial (RCT)	Treatment decision-making and outcomes uch as progression-free survival / reduced morbidity and mortality	Gold standard of clinical trial, provides data on causation of results from intervention and limits bias	Expensive and complex trials to run, more difficult to conduct over multiple centers
Prospective Observational/ Case Cohort Studies	Shows impact of changes in diagnostics or treatments for a variety of types of clinical utility	Can show results for issues not related to outcome and treatment decisions (e.g., workflow) more easily than RCT	More likely to result in bias being introduced into study not as widely used in medical sciences as RCT
Retrospective Case Study	Confirmation of anticipated changes from new tests and healthcare economics analysis	The easiest way to pool data over many centers to show subtle effects or effects hidden by significant noise in data	Requires access to extensive records often from multiple institutions, not as widely accepted as RCT

FIG 2 Select examples of types of trials that can be used to demonstrate clinical utility.

trials (17, 19, 35, 36) are conducted by measuring a specific endpoint(s) for a selected length of time before introducing the new diagnostic. After the new diagnostic has been established, the same endpoint(s) will be measured over a similar period so that changes caused by the diagnostic can be ascertained. Retrospective analysis trials (37–39) are conducted once a test has been widely deployed for an extended period of time; researchers can then gather historical data for the endpoint(s) either prior to the deployment of the test or from locations where it has not been deployed and compare these endpoint data to data from similar institutions where the test is in place. Finally, one can collect information about the impact of test on clinical decision making using a pretest/posttest survey instrument. In this type of study, a physician is asked to record their current care plan based on available information. They are then presented with the test results and asked if their decision would change. Pretest/posttest survey instruments are helpful in that they capture the actual shifts in thinking that occurs as a result of the test, but it is limited in that it does not track actual behavior or outcomes moving forward.

Although a particular trial design may be more appropriate for a select technology or setting of care, all of these types of trials can be appropriate ways to demonstrate clinical utility. Historically, randomized control trials have been considered the gold standard for evidence generation and still are in many circumstances, especially for pharmaceutical agents. Select commercial payers, likely informed by their experience with pharmaceuticals, may expect randomized control trials for advanced microbial diagnostics, although there is a growing understanding that each of the trial types has advantages in certain situations but may not be required or may have limitations in others. For example, it is difficult to execute a diagnostic trial with a “placebo” test. In particular, diagnostic tests can show their value through retrospective and prospective observational studies, in which researchers compare the care delivered by a novel technology to a historical standard of care.

Clinical decision making. Improvements in clinical decision making are often the primary endpoint of diagnostic studies, due to both feasibility and the fundamental purpose of diagnostics, which is to inform better the treatment choices physicians make. It is important to note that improved clinical decision making can create value by not just improving therapy decisions and supporting antibiotic stewardship efforts but also through conserving resources (e.g., faster emergency room [ER] triage, or removing patients who do not have communicable infections from isolation rooms).

When studying changes in clinical decision making resulting from the implementation of new technologies, common endpoints that may be examined include measuring time to initiation of appropriate therapy, time to initiation of antibiotic escalation/de-escalation protocols, degree of expedited ER triage for hospital admission or discharge, the time patients spend in isolation rooms, and effective antibiotic

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stewardship metrics. For instance, several successful studies have been completed using MALDI-TOF MS in central hospital laboratories, demonstrating changes in types of antimicrobials prescribed and reduced time to placing patients on appropriate antibiotic regimens (19, 35, 40, 41).

Technologies that impact the removal of patients from isolation rooms or critical treatment decisions (e.g., for patients with sepsis) need to show statistical impact for instances and causation for important decisions (4, 17, 19). For example, a recent study of improved decision making from new diagnostic technology in the management of sepsis showed via a randomized controlled trial that multiplex panels have hastened the diagnostic process and led to the faster placement of patients on appropriate antimicrobials (40). These changes in decision making result in, at a minimum, improvement in antibiotic stewardship, and they may over a longer time frame reduce mortalities related to sepsis (34, 42, 43).

As technologies become more widely used or are being applied to more common decision points, retrospective and prospective studies become more feasible and are easier to execute. For example, the greatest utility of POC influenza tests for changing clinical decision making is evident with studies in ERs, primary care physician offices, and outpatient clinics, by showing reductions in empirical antibiotic treatment and increases in Tamiflu prescriptions in these clinical settings through larger retrospective and prospective studies (25, 44, 45). Similarly, a study of a gastrointestinal panel showed reduction of several patient days on antibiotic and in the length of time to discharge (46). In the future, multiplex syndromic panels for outpatients may develop evidence of clinical utility by targeting specific high-risk groups, such as immunocompromised and pediatric patients and elderly patients in assisted living facilities. Hospital admission is a critical decision point for these patients and therefore a potential endpoint; expediting hospitalization of patients with severe infections may significantly improve health outcomes. Furthermore, some patients with less severe infections may benefit from avoiding hospital admission, limiting the potential of nosocomial infections and avoiding the sizable cost of hospitalization. Information about the use of these technologies can be collected through collaborative efforts in multiple institutions or from a single institution that works with high volumes of vulnerable patients.

Clinical workflow. Clinical workflow is substantially different in the outpatient and inpatient settings and typically is of higher priority for inpatient services. For hospitals, enhancing clinical workflow has the potential to significantly improve patient care and lead to lower costs, particularly as more complex and/or labor-intensive tests may be replaced with simpler and/or more efficient tests. Also, such improvements can aid hospitals in meeting important quality metrics, such as limiting hospital-acquired infections and improving antimicrobial stewardship. Improvements in the workflow can be accomplished by streamlining the diagnostic process or by more rapidly monitoring changes in antimicrobial resistance or types of pathogens present (19). Many hospital central laboratories are limited to running tests for select pathogens at certain times of the day and/or week, due to the complexity of tests which require highly trained technologists to perform the assays. Also, there may be periods of higher volumes (e.g., influenza season, disease outbreak, etc.) where central laboratories can be overwhelmed by the workload. High-volume laboratory demands can have further downstream impacts, including delaying the ability for other diagnostic tests and clinical services in the hospital to be efficiently run. New advanced microbial testing tools offer the potential to significantly improve workflow in both outpatient clinics and laboratories by decreasing the time and technical expertise required to perform each test. Also, advanced technologies may allow an increased number of patient samples to be tested simultaneously, thereby increasing efficiency and throughput of the laboratory, resulting in improved workflow and faster results. Unfortunately, clinical workflow is rarely quantified and reported in the literature, making comparisons across institutions difficult. More studies in this area would significantly benefit the field. Relevant endpoints tested for clinical workflow could include time to reaching a confirmed diagno-

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sis, number of tests that must be run for diagnosis, clinical laboratory hours worked per diagnosis, number of samples the laboratory processes per unit time, and the frequency at which backlogs develop in the laboratory. Furthermore, it is important to note that the United States is currently facing a shortage of trained clinical laboratory personnel that is not expected to improve in the immediate future. Technologies that allow fewer technicians to run more tests will likely play an essential role in overcoming this challenge (47–49).

One of the most significant ways for improvements in outpatient workflow clinical utility is to show that clinics can triage patients faster and thereby increase the volume of patients assessed and treated in a similar time frame. Increasing the volume of patients a physician can see in a select amount of time has the potential to improve patient access by allowing a physician to see more patients per day (50, 51). For example, when a POC test identifies a patient requiring immediate treatment while also identifying individuals that are safe to release, it could significantly improve workflow by diagnosing and treating patients in a single visit without the need for follow-up assessments if the diagnosis is delayed (e.g., sexually transmitted infection [STI] tests, HIV, influenza, group A *Streptococcus*, etc.). POC tests can not only identify patients with severe infections that need to be admitted to the hospital but can also identify patients with more minor conditions that are candidates for discharge and therefore reduce occupation of ER beds (50, 51). One key consideration is that often these decisions are made quickly, so to impact clinical workflow, the results must be made available within a limited time frame (e.g., during or immediately after the visit). Prospective or retrospective studies may be sufficient to demonstrate altered patient flow due to the utilization of a new diagnostic technology. Appropriate endpoints for outpatient workflow could include time to diagnosis for outpatients, number of patients seen, number of tests/diagnostic procedures performed, duration of visit per patient, and frequency for which follow up visits are needed. An example of this type of study can be found in a recent publication describing a randomized control trial in which the benefits of same-day testing for chlamydia and gonorrhea were evaluated compared to SOC testing (2- to 3-day turnaround time). In this study, there was 0% undertreatment compared with 43.8% for patients tested by the SOC. (52) A retrospective study of a multiplex molecular gastrointestinal (GI) testing panel showed not only a reduction in antibiotic prescription but also fewer endoscopic and abdominal radiology procedures (46).

Patient outcomes. Patient outcomes are a central focus of medical studies and are typically focused on reductions in morbidity and mortality. Microbial diagnostics may meaningfully improve patient outcomes, as a more rapid diagnosis will likely directly impact timely clinical decision making and improve overall patient care (19, 36, 53, 54). While these benefits may represent evidence for clinical utility, it is often difficult to demonstrate that improved outcomes are specifically due to the diagnostic test, given the multitude of factors associated with patient therapeutic response. The diagnostic tool itself should only be held to the standard of informing the correct treatment decision and should not be required to prove that the therapeutic positively impacts care, as limitations in the treatment can overwhelm improvements in diagnostics and many benefits of decision making (e.g., antibiotic sparing) will not result in morbidity and mortality improvements. It should be a given that when a pathogen is correctly identified and the optimal treatment is initiated that an improvement has occurred, whether or not this translates into a measured direct reduction in morbidity and mortality. Therefore, the most appropriate approach may be for diagnostic developers to demonstrate an improvement in clinical decision making for reimbursement.

These efforts can initially be focused on the demonstration of clinical utility in smaller patient populations that are more likely to benefit from diagnostic improvements, such as infants, immunosuppressed or compromised patients, and the elderly. For instance, multiplex molecular syndromic panels might show significant improve-

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ments for immunocompromised patients by quickly identifying those that should be treated immediately for serious infections.

Clinical outcomes are of paramount importance to both inpatient and outpatient care. However, the particular outcomes desired can be different in each setting. For inpatient care, the most common desired outcomes will be reducing the length of hospital stay, the time patients spend in the intensive care unit (ICU), readmissions, and, ultimately, infection-associated mortality. For example, MALDI-TOF MS, when conducted post positive blood culture has shown a reduction in mortality for patients with sepsis in prospective interventional trials (19). However, in the inpatient setting, patient outcomes may be dependent on multiple factors other than diagnostics, particularly for more serious infections or for patients with complex comorbidities. Studies of patient outcomes, therefore, are often assessed with prospective or retrospective clinical trials, as they will need to be conducted over several institutions or conducted for long periods of time to generate sufficient statistical power to reach conclusions on a diagnostic-specific impact on patient outcomes.

For outpatient care, critical endpoints regarding patient clinical outcome include situations in which the identification and management of infections avoid hospitalization and development of more serious conditions. In many circumstances, changes in clinical decision making that would reasonably be expected to improve outcomes along with hospital admission rates will be appropriate endpoints. However, once a diagnostic is widely used in the outpatient setting, large retrospective studies over many medical systems using the technology will likely be feasible.

Cost-benefit evaluation. Underlying each of these categories is a fundamental question about whether the additional clinical benefits can be justified from a financial perspective. In the United States, this takes the form of cost/impact and simple return on investment analysis rather than formal cost-effectiveness evaluations as seen in other markets. Advanced technologies usually come at some additional cost, but they have the potential to free up resources by reducing the use of other tests, or by avoiding additional diagnostic procedures. It is worth remembering that different stakeholders in the hospital may be motivated by different cost-benefit considerations; for instance, laboratory directors may be interested in how cost translates to efficiency, while C-suite executives will likely focus more on return on investment.

In the inpatient setting, cost will be less of a concern because inpatient care is focused on more serious infections with severe/costly outcomes. Also, there are more ways for costs to be offset in inpatient care. For instance, studies with multiplex gastrointestinal panels have shown some ability to reduce cost by removing patients from high-cost isolation rooms and moving them to general wards when the patients were shown not to have communicable infections (5). Other studies have shown cost reductions through fewer diagnostic testing/imaging studies and reduced length of stay, not only in GI patients but in those with the respiratory virus as well (46, 55). Another example is the use of MALDI-TOF MS, which has routinely demonstrated lower costs per sample than culture and phenotype assays for organism identification. However, the high upfront cost of MALDI-TOF MS instruments means these savings might only be realized by high-volume laboratories. Appropriate endpoints for measuring cost are direct spending changes in dollars, but more sophisticated health economic modeling showing differences in quality-adjusted life years/incremental cost-effectiveness ratios may be justified (4). It is worth noting that cost avoidance is often harder to quantify and track than direct costs and revenue, which makes cost savings benefits from a test more difficult to communicate to payers and users.

For outpatient care, direct costs are unlikely to show clinical utility, as tests will be compared to relatively inexpensive methods with limited ability to create offsets. In this situation, cost becomes a barrier, leading commercial payers to be less enthusiastic to reimburse a test because of the negative economic impact it may have. Of particular note, high-priced tests are more likely to be held to higher evidentiary thresholds by commercial payers, who may demand larger clinical trials or randomized control trials,

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when at a lower price point prospective observational or retrospective trials would have been accepted.

DISCUSSION OF WAYS TO FOSTER CLINICAL UTILITY EVIDENCE DEVELOPMENT AND INNOVATION

Role of evidence. To effectively maximize clinical uptake and broad payer coverage of advanced technologies, the microbiology community must collectively form an action plan involving a variety of stakeholders to collaborate and demonstrate the beneficial effects of advanced technologies in the management of infectious diseases. A key starting point for collaborative efforts in the microbial diagnostic space would be to conduct studies to determine the economic and clinical challenges and limitations of the current diagnostic paradigm. Properly conducted studies on this topic could help persuade payers of the potential room for growth in this area and will set the stage for the possible benefits of new technologies that can result in improvements in patient care.

Key stakeholders should work closely with publishers of clinical guidelines to articulate the role and best practices for the use of these tools to better inform payers on the value and practicality of new tests. The continued generation of appropriate clinical data may also lead to a willingness to include advanced technologies in published guidelines. Including new diagnostic technologies in clinical guidelines would add significant value, particularly in the eyes of payers, who refer to published guidelines to inform their decision on whether to reimburse a technology. Clear guidelines are particularly important for those technologies where the added cost of a technology is currently perceived as a limitation for wide adoption in routine practice (e.g., next-generation sequencing [NGS] technologies for microbiology).

Prioritizing clinical utility evidence generation by hands-on users in real-world settings, such as improved impact on everyday clinical decision making and individual patient outcomes, will be important to drive the future value proposition of advanced microbiology technologies. While this additional evidence will likely play a major role in facilitating broader utilization of new diagnostics among health care entities, it will also educate payers on the added value to encourage broader payer coverage and reimbursement.

It may be possible to eventually demonstrate outcomes not only at the individual patient level but also at the population level. For example, there is potential to show that outcomes are improved via public health benefits that result from better antibiotic selection and community-acquired resistance management through the regional applied use of advanced diagnostic technologies. The full benefits of these technologies will only be realized once antimicrobial stewardship and operational improvements (e.g., strain tracking, hospital infection control surveillance, etc.) are applied in aggregate. This will require a greater collaborative/coordinated effort across multiple institutions potentially coordinated by a public health entity. These types of studies will require collaboration between many stakeholder organizations, such as the American Society of Microbiology (ASM), clinical and physician societies, and government groups. When these types of studies are conducted, positive results should be utilized to encourage the Centers for Disease Control and Prevention (CDC) to publish formal guideline updates encouraging widespread adoption of a diagnostic technology, given the significant improvements in patient outcomes and potentially in public health.

Role of the microbiology and infectious disease community. As noted above, many of the forms of clinical utility require showing the impact on clinical decision making; therefore, it is important for microbiologists to understand how clinical decision making is done in the current SOC by engaging clinicians managing infections. Success in developing clinical utility information will require microbiologists building bridges to members of the clinical community. The clinical stakeholders needed include not only infectious disease specialists but also infection control practitioners and primary care and emergency room physicians to determine the impact of these tools on everyday care. Better coordinated action requires findings consensus about the key

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benefits and required evidence and jointly and clearly articulating this information to key stakeholders, such as hospital administration and payers.

A role for industry. Both the pharmaceutical and diagnostic manufacturing industries will also be required to be involved in orchestrating the generation of clinical utility evidence. Given that hospital administrators often prefer to undertake a trial period with new technologies to gain first-hand experience, diagnostic technology manufacturers may need to pursue collaborations for this to be actively achieved. Furthermore, they can work to guide not only microbiologists but also facilitate early partnership with those in clinical and financial roles about the design of studies which could help illustrate the clinical utility of these deployed tools in a fair and balanced way. They can also play a role in helping community hospitals understand where to find clinical utility information and how to share clinical utility information so that advanced care approaches are not limited to academic medical centers. This support can come in the form of research grants that are specifically for utilization reviews rather than for traditional clinical trials. Critically, the microbial diagnostic industry should recognize that efficacy and clinical utility trials conducted by industry are often viewed skeptically by clinicians and payers. A better solution may be to provide funding to institutions using the technology to support studies of clinical utility and best practices that the institutions can publish independently.

Additionally, pharmaceutical leaders in the microbiology space may be required to actively participate in data gathering and publication supporting the concept that next-generation antimicrobials may be more effective, particularly if paired with the most advanced diagnostic technologies. This will likely require active collaboration between pharmaceutical and diagnostic companies to ensure the clinical utility benefit of appropriate prescribing of next-generation treatments is influenced by novel technologies entering the space. The National Institutes of Health (NIH) and the CDC have the potential to facilitate and mediate these types of collaborations through improving communications, providing funding for important studies, promoting a strong clinical research environment, and supporting inclusion in clinical guidelines when tests are shown to be effective and economical.

A role for government and advocacy. Demonstrating the value of novel microbiology technologies will likely require a holistic approach to be undertaken by the microbiology community. Engaging key agencies, such as the CDC and NIH, to increase funding for large studies to generate large data sets of evidence will be a key strategy to articulate the message. Such studies involving a variety of stakeholders should aim to demonstrate improved antibiotic stewardship, patient outcomes, and communicate the overall economic and health benefits for the community following the adoption of novel microbiology technology in the future. For example, the CDC has responded to the U.S. National Action Plan for Combating Antibiotic-Resistant Bacteria by launching initiatives such as Antibiotic Resistance (AR) Solutions, which involves investments in national infrastructure to prevent resistant infections (https://www.cdc.gov/drugresistance/solutions-initiative/index.html). We urge the CDC to include evaluation of advanced diagnostic technologies as potential tools to improve antimicrobial stewardship, clinical decision-making/workflow, clinical outcomes, and the detection, tracking, and prevention of resistant infections.

Professional societies, such as ASM, will likely play a key role in developing close working relationships with government organizations, such as the NIH and CDC, to emphasize the value of clinical utility of advanced microbial diagnostics. Importantly, these groups should focus on supporting the development of evidence and sharing of information in areas that are not a high priority to any single stakeholder to help resolve collective action issues. Moreover, demonstrating robust clinical utility will likely require clinical microbiologists to engage with each stakeholder type, from a variety of physician groups to hospital administrators and payers, to aid understanding and communicate the potential benefit that advanced microbiology tools provide in various care settings. These improvements may be achieved by improving not only the speed

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and accuracy of disease diagnosis but also key characteristics such as workflow and cost avoidance (32).

Implications for future incorporations of technology. The advances and innovations in microbial diagnostic technologies over the last decade are beginning to have a significant impact on the way we diagnose and manage infectious diseases. In the coming years, an additional cohort of new microbial diagnostics is expected to enter the space. Technologies that include advanced genomics (56, 57), proteomics, and rapid susceptibility tests (58) are expected to cause dramatic changes by tackling some of the most important problems for microbial diagnostics. Additionally, advanced analytic tools, such as artificial intelligence and machine learning, can enhance the information extracted from the data these technologies collect (49, 59, 60).

For example, the menu of culture-independent nucleic acid amplification tests and syndromic panels is expanding. These advances will likely favor the deployment of culture-independent reporting of antimicrobial resistance (AMR) determinants, including the creation of a clear correlation of AMR genotype to antimicrobial susceptibility phenotype/MICs. Also, automated microscopy is being leveraged for early detection of sepsis by detection of morphological changes in monocytes indicative of dysregulated immune response or morphological changes in bacteria indicative of drug susceptibility (61).

Next-generation sequencing methods and proteomics (e.g., MALDI-TOF) are expected to impact key diagnostic segments in the future. In contrast to PCR panels, these methods have the potential for "hypothesis-free" detection of pathogens and host response markers. NGS-based analysis of pathogens further allows phenotypic prediction, such as detection of AMR determinants, virulence factors, and mobile genetic elements. Also, whole-genome sequencing of isolates by next-generation sequencing allows strain typing at nucleotide-level resolution for epidemiological studies and infection control. These methods have tremendous potential in the clinical microbiology lab, opening a novel paradigm for diagnostics.

However, to be deployed clinically and realize this potential, these technologies will need to build on efforts associated with more established technology that has demonstrated clinical utility. Our hope is that the concepts outlined throughout this paper will facilitate the demonstration of the clinical utility of recently launched novel methods so that the even newer tools and techniques, as described above, will be able to find a pathway to success and routine application in the clinical microbiology laboratory. Adoption of these technologies may also require hospitals and payers to place a higher priority on infection control than they do currently and to support their infection control centers.

CONCLUSION

The need for improvements in microbial diagnostics and thereby in management of infectious disease is clear and urgent. This need has the potential to be filled by a combination of new technologies that have entered the diagnostic space or will enter it shortly. However, there is a clear gap in the field that is preventing these technologies from being widely deployed to fill the current unmet clinical need for rapid and improved testing. While the necessity of deploying better microbial diagnostics is not lost on microbiologists and infectious disease specialists, other key stakeholders have lower awareness. Therefore, a collective effort is needed from microbiologists and clinicians handling infectious diseases to communicate to other stakeholders the costs and downsides of the current SOC. Demonstrating and communicating how the low cost of phenotypic methods is often offset by the high cost of preventable morbidity and mortality that comes from a slow diagnostic SOC, and how new tests can directly impact and improve clinical decision making, is needed. Clearly defining and describing these issues to commercial payers, hospital administrators, and government regulators will smooth the deployment of these technologies and benefit individual and communal health.

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Innovative and rapid antimicrobial susceptibility testing systems

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Abstract | Antimicrobial resistance (AMR) is a major threat to human health worldwide, and the rapid detection and quantification of resistance, combined with antimicrobial stewardship, are key interventions to combat the spread and emergence of AMR. Antimicrobial susceptibility testing (AST) systems are the collective set of diagnostic processes that facilitate the phenotypic and genotypic assessment of AMR and antibiotic susceptibility. Over the past 30 years, only a few high-throughput AST methods have been developed and widely implemented. By contrast, several studies have established proof of principle for various innovative AST methods, including both molecular-based and genome-based methods, which await clinical trials and regulatory review. In this Review, we discuss the current state of AST systems in the broadest technical, translational and implementation-related scope.

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Over the past few decades, the emergence of antimicrobial resistance (AMR) has outpaced the development and market entry of new antimicrobial agents[1], and we need to encourage interventions to preserve the currently available antimicrobials[2]. Antimicrobial susceptibility testing (AST) systems can play a major role in achieving this goal. These diagnostic tests inform about drug susceptibility for a particular pathogen and detect possible drug resistance, thus guiding the appropriate use of antimicrobials. The main problem with current AST methods is speed, for in most cases conducting AST requires overnight incubation, and it may require 48–72 h to complete, depending on the drug–organism combination. There are many opinions as to what constitutes 'rapid AST', but most clinical microbiologists define rapid testing as being feasible during a single working shift — that is, within 8 h or less. Rapid AST provides antibiotic susceptibility profiles that can support and facilitate antimicrobial stewardship as well as epidemiological surveillance[3]. How best to manage infectious diseases by combining stewardship, optimized rapid AST and AMR data collection is an important objective for investigation[4].

For the purpose of this Review, we define phenotypic AST as a microbiological procedure whereby a pure culture of a single organism is grown (in solid or liquid media) in the presence or absence of an antimicrobial agent. Microbial growth or inhibition is observed longitudinally. Phenotypic AST methods provide a direct indication of the susceptibility of a given microorganism to an antimicrobial agent at defined concentrations, and in some cases such methods provide a quantitative assessment of the minimal inhibitory concentration (MIC) of the antibiotic. By contrast, the term 'AMR detection methods' is used to describe methods that detect (pre-existing) proteomic or genomic signatures that predict antimicrobial resistance (FIG. 1).

Although most commercially available and frequently used AST methods have been shown to be reliable and reproducible[5], the systems are complex and have several limitations, and one of the main limitations is speed, as we mentioned above. The early stage of microbial growth (lag phase) is one time-limiting factor[6]. Monitoring cell density in suspension is different from (visually) analysing cell division and requires different technological approaches. Density can be monitored by relatively simple spectral means, whereas visualization of cells requires sophisticated microscopy. Moreover, growth rate (net cellular doubling time), the concentration of growth markers to be detected, intrinsic natural genetic mutability, fitness decrease following the acquisition of antibiotic resistance, biofilm formation and the ability to transduce, transform or conjugate all need to be taken into account when assessing antimicrobial susceptibility or resistance by AST methods[7]. Antimicrobial susceptibility may differ between individual cells, leading to heterogeneous populations; the adequate detection of heterogeneity is clinically important but is difficult with current AST methods[8]. Some bacterial species exhibit mechanisms that drive the emergence of de novo resistance or gene mutations, enabling the rapid adaptation to antibiotics, which may not be detected with current methods[9]. AST usually identifies individual cells that remain alive or in a state of suspended animation in the presence of

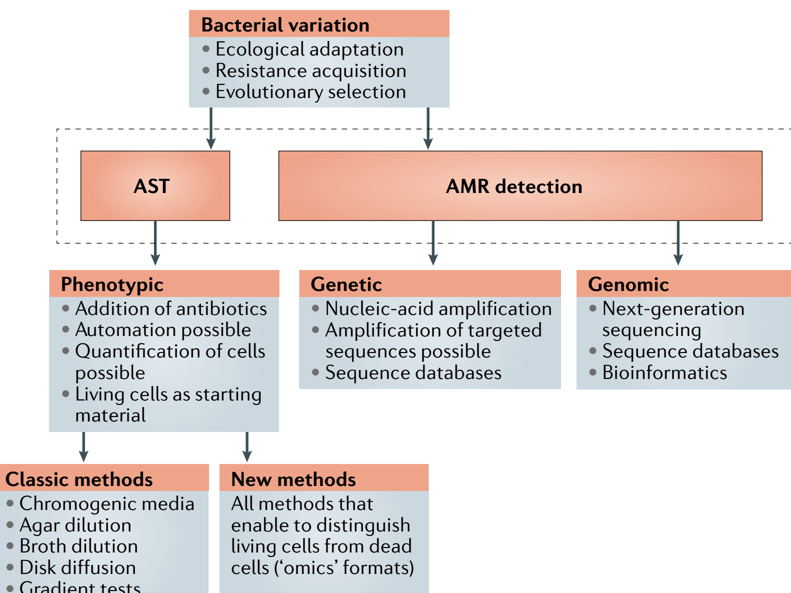

Fig. 1 | **Schematic overview of antimicrobial susceptibility testing and antimicrobial resistance detection methods.** Antimicrobial susceptibility testing (AST) and antimicrobial resistance (AMR) detection methods detect basic features of bacterial variation that result from ecological adaptation, resistance acquisition and evolutionary selection. Phenotypic AST, including the use of selective chromogenic culture media, mostly detects the arrest of growth in the presence of different concentrations of different antimicrobial agents[157]. There is a need for quantifying the cells, however, as inoculum effects may substantially affect determination of the minimal inhibitory concentration[158]. Rapid evolution of phenotypes may occur, and the new methods especially are focused towards circumventing such possible changes, by providing a more definite live–dead distinction[159]. Molecular AMR detection attempts to specify resistance genes, as well as mutations in and expression of these genes or their genomic signature, using molecular-based (both DNA and RNA) methods. This is surrogate AST and has to be validated against phenotypic data to be useful[101,106]. For this method, extensive resistance marker databases and innovative bioinformatics methodologies are mandatory requirements.

Minimal inhibitory concentration
(MIC). The lowest concentration of an antimicrobial agent that prevents visible growth of a bacterium species or isolate. The MIC is defined by combined activities of the microorganism, the affected patient and the antimicrobial agent itself.

Lag phase
The temporary period in which microorganisms are adapting to a new environment, avoiding threats and metabolizing, and increasing in cell size but not yet actively dividing and multiplying. During this period, cells are synthesizing enzymes and other factors needed for actual cell division under the new environmental conditions.

antimicrobials (tolerance)[10,11]. Distinguishing reversibly growth-inhibited as opposed to dead microbial cells may be more difficult but is relevant, as reversal of the inhibition may increase infection risk once more. Although sensitivity is often limited, some protocols that reliably distinguish growth-inhibited from dead cells have been reported[12]. In addition, AMR is also increasing in anaerobes[13], and specialized infrastructure and laboratory equipment is required in order to conduct AST in the absence of oxygen, which might not be generally available[14]. A number of the compromising aspects of phenotypic tests are unlikely to be improved, as these are of an intrinsic biological nature, such as the duration of the lag or growth phase. The development of new AST methods is slow, costly, logistically complex and riddled with regulatory issues[15]. Current key issues include the needs for improved speed, cost control and platforms that cover as many microorganism–drug combinations as possible. In addition, to enable routine use in clinical microbiology laboratories, the system platform should have a small footprint — since laboratory space is very costly — have low maintenance needs and provide high throughput.

Newer AST methods might not directly measure phenotypic cell viability, but rather detect surrogates of viability, such as the modulation of proteins, metabolites, DNA and/or mRNA, or changes in proteomes, metabolomes, genomes and/or transcriptomes, to provide indirect evidence of antibiotic susceptibility or resistance[16]. Such tests include proteomic, lipidomic or genotypic methods, which characterize proteins, lipids and fatty acids, or nucleic acids, respectively. A notable limitation is that the correlation between these newer, marker-directed methods and classic phenotypic methods may vary across specific combinations of organisms and antimicrobial agents[17,18]. For these newer methods to be used in clinical decision making, they need to be calibrated and referenced back to standard phenotypic methods that have been used for many decades. Moreover, phenotypic methods are generally known to predict susceptibility or resistance more accurately. They can also generate MICs of various antimicrobial agents, even though this usually requires extended incubation and a large inoculum (10^4–10^6 cells per unit volume) (BOX 1). In light of the many reviews on AST that have been published recently[19–22], in this Review we aim to assess new technologies in terms of developmental phase, regulatory review and commercialization. First we briefly discuss the routinely used AST methods, and then we explore current efforts to improve phenotypic AST systems, including new emerging technologies, as well as genomic and gene-based AMR detection methods. We conclude by highlighting the challenges and opportunities for new rapid AST systems at both the academic and company levels.

Automated AST methods

Routinely used traditional and automated methods. 'Traditional' AST methods include disc diffusion and broth dilution assays, which are used to compare the visible growth of microorganisms in the presence of specific concentrations of antimicrobials under defined test conditions (Supplementary Fig. 1). Analysis is based either on decreased or no visible growth within the zone of inhibition on solid agar, or on the lack of visible turbidity in broth after appropriate incubation. Traditional methods help define the MICs, which are the current reference standard in measuring antibiotic activity. These two key AST formats have been automated: during disc diffusion assays, inhibition zones can be read automatically[23], and several formats of automated broth dilution assays are commercially available[24].

The first automated AST instrument was the Autobac-1, which was presented as a prototype in 1971 (REF.[25]). Most of the current automated systems enable high throughput and extensive testing of many microorganism–antibiotic combinations, but they vary in terms of accuracy or time to result. Four automated instruments are currently cleared by the FDA for in vitro diagnostics (IVD): VITEK2 (bioMérieux), MicroScan WalkAway (Siemens Healthcare Diagnostics), BD Phoenix (BD Diagnostics) and Sensititre ARIS 2X (Trek Diagnostic Systems). Three of these systems generate rapid (3.5–16 h) results, whereas the fourth (Sensititre ARIS 2X) takes longer on average to report end points. However, it must be noted that even the so-called rapid methods require a standardized microbial inoculum, which entails culturing the specimen for 24–48 h

Tolerance
The microbial ability to resist being killed by antimicrobials. This ability is distinct from (multi)drug resistance and is not caused by mutant microorganisms, but rather by cells existing in a dormant, non-dividing state.

Zone of inhibition
If bacteria are grown as layers on solid growth media and an antibiotic stops the bacteria from growing or kills them, there will be an area around the place where the antibiotic has been positioned (usually in a well or on a paper disc) where the bacteria have not grown enough to be visible. The radius of such a region of growth inhibition is correlated with the level of antibiotic susceptibility of the strain being tested.

(sometimes longer) prior to inoculation into the AST system. All of the automated methods require dense suspensions of bacteria as the primary inoculum. The MicroScan WalkAway is a large incubator and reader that utilizes microdilution trays that are inoculated manually, incubated and examined for growth. Susceptibility test panels for Gram-negative bacteria contain fluorogenic substrates and can be read in 3.5–7 h. Separate panels for Gram-positive and Gram-negative bacteria read turbidimetric end points and are completed in 4.5–18 h. The BD Phoenix Automated Microbiology System has a large incubator with a capacity to process 99 test panels containing doubling dilutions of individual antibiotics. The system monitors each panel every 20 min using turbidimetric and colorimetric detection. The VITEK2 system uses compact plastic reagent cards that contain small quantities of antimicrobial agents and test media in a 64-well format and is based on turbidimetric monitoring of growth. The instrument can perform up to 240 simultaneous tests, and this number will increase when VITEK2 cards with a larger capacity become available. This example shows that a simple redesign of the disposables used in classic AST methods may already lead to improvements. The Sensititre ARIS 2X is an automated incubation and reading system with a 64-panel capacity. The test panels are standard 96-well microdilution plates that can be inoculated manually or automatically. Growth is determined by fluorescence measurement after 18–24 h of incubation.

These systems are linked to software used to interpret the AST results, including ‘expert systems’ for the detection of atypical patterns and unusual resistance phenotypes[26]. It remains important to be aware of the risk of overestimating susceptibility, as was recently emphasized for nontuberculous mycobacteria[27]. For more technical detail, additional reviews are available[21,28].

New automated AST systems. More recently, new entrants have penetrated the automated AST market. Two methods, both of which are real-time microscopy-based, are the most likely techniques to reduce turnaround and hands-on time and are close to launch or have been commercially launched. These technologies, developed by USA-based Accelerate Diagnostics[29,30] and Korean Quantamatrix[31,32], have undergone clinical evaluation, but whether current claims for rapid turnaround time (TAT) will translate into favourable patient outcomes has yet to be determined. Some of the tests developed by Accelerate Diagnostics have been cleared by the FDA[30]. Although the initial test array for new commercial systems is likely to be small, the use of various technologies may potentially lead to broad-based testing covering many drug–organism combinations. Still, none of the aforementioned technologies fulfils all the routine clinical needs for rapid AST. The major limitations of the methods described are test completeness, number of different offerings and cost.

Box 1 | **Quantitative versus qualitative antimicrobial susceptibility testing**

There is a need for quantitative susceptibility testing systems, and the question is how this relates to genomics[35]. With a diminishing number of fully active, broad-spectrum antimicrobial agents, it is becoming increasingly important to use the variability in phenotypic resistance levels detected by phenotypic tests. There is a difference between successful meropenem therapy for isolates with a minimal inhibitory concentration (MIC) of 16 mg/l and isolates with a MIC of 128 mg/l. Both are resistant isolates according to all available breakpoints, but many would consider it reasonable to try meropenem in the first but not the second case. Dosing would be adjusted, and meropenem would be given in combination with another agent, but differences in the expression of meropenem resistance markers are worth considering, as the number of effective antimicrobials available for resistant bacteria decreases. Such important phenotypic differences may not be reliably detectable using current molecular methods, as the relationship between molecular antimicrobial susceptibility testing systems, quantitative differences in gene copy numbers and the differential expression of genes has not yet been defined in much detail.

Improving phenotypic AST systems

Automating result interpretation for traditional phenotypic analyses. Recently, laboratory automation has become increasingly prominent, and automation is being eagerly adopted in the clinical–diagnostic setting. Information technology is an important aspect of all automation-related activities. With Total Laboratory Automation (TLA), instrumentation is utilized to barcode and inoculate culture media, streak culture plates and move the plates to specialized incubators with high-resolution cameras that are then used to image the culture plates during the incubation process[33]. The next phases of microbiology laboratory automation include enhancements such as automated disc dispensing and interpretation of disc zone sizes. With the consistent incubation temperature and atmosphere that is provided by the automation system, there is more rapid growth of microorganisms and recovery of fastidious organisms[34]. Thus, it is likely that disc diffusion testing will be able to be read and interpreted earlier, and thus the reference method of disc diffusion will likely be able to provide results more rapidly than current methods when used in the context of laboratory automation[23]. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has already developed standardized rapid lecture protocol cultures for inhibition zones, permitting disc diffusion testing in 4, 6 and 8 h directly from positive blood.

There have been interesting developments in phenotypic methods (TABLE 1), such as the use of imaging and software tools to interrogate resistance levels. Still, these methods are rarely incorporated into routine laboratory use. Analytical methods have been developed to assist clinicians with the interpretation of AST data. AntibiogramJ is a user-friendly, free, open-source, platform-independent tool that uses images of disc diffusion tests for more reliable interpretation of the size of the inhibition zone[36]. The data are 87% concordant with those generated by an experienced microbiologist. Mobile phone photos can be used for data sharing and independent verification at a distance. There are many options for expanding this format, and successful application of mobile technology ‘in the field’ has already been achieved[37].

Emerging methods. Simple detection of microbial resistance is the mainstay of selective chromogenic culture media (FIG. 1). Resistant organisms can easily be recognized by their coloured colonies while overgrowth

Table 1 | **New offers in qualitative and quantitative AST systems**

Companies[a]	Technologies used	Approach and system	Country of origin	Status
Abacus Diagnostica	PCR platform; portfolio includes methicillin-resistant *Staphylococcus aureus* and *Clostridioides difficile*	Molecular; Genomera CDX	Finland	Developed in 2012
Affinity Biosensors	Microorganism mass measurement	Phenotypic; LifeScale AST	USA	Sold since 2017 but unavailable in the USA
Arc Bio	Identification and AST based on shotgun sequencing	Genomic; Galileo pathogen solution	USA	Launched in 2018
ARCDIA International Oy Ltd	mariAST measures species-specific bacterial growth in real time and combines in-well culture and high-accuracy detection	Phenotypic; mariPOC	Finland	In approval process
ARES Genetics	Development of AST database and currently developing an associated wet laboratory approach	Genomic; bioinformatics; GEAR database	Austria	Established in 2017
Ascenion GmBH	Combination of a BacLight viability staining with automated confocal laser scanning microscopy and detailed image analysis	Phenotypic; autofocus microscopy	Germany	Patent registered in 2010; the technology is offered for in-licensing or co-development of a screening platform
AUS Diagnostics	Multiplexed tandem PCR	Molecular; Mini- and UltraPlex	Australia	Sold since 2018, European conformity marking announced
BacterioScan	Laser light-scattering instrument	Phenotypic; 216 Dx urinary tract infection (UTI) system	USA	In 2018 the FDA issued a 510K premarket notification clearance for the 216Dx UTI detection system
BioFire	Multiplexed, syndrome-oriented PCR	Molecular; FilmArray	USA	Various tests cleared for sale by the FDA
Biotrack Diagnostics	Solid-state cytometry or fluorescence in situ hybridization and specific antibodies for fluorescent micro-agglutination are used for specific detection of molecules	Phenotypic; biochemical; AquaScope and AquaPrep	The Netherlands	Not known
Click Diagnostics	Cartridge-based, hand-held thermocyclers configured to move a fluid between distinct chambers and visually read colorimetric results	Molecular; company in stealth mode	USA	Not known
Dayzero Diagnostics	High-throughput bacterial DNA sequencing and proprietary machine-learning algorithms to rapidly predict pathogen species and drug resistance profiles	Genomic; bioinformatics; epiXact	USA	In development since March 2018
EliTech Group	Triplex PCR assay	Molecular; URIFAST	France	Sold since 2018
FASTinov	Cell sorting	Cellular detection, FACS	Portugal	Patent available since 2012, no products to date
Firebird	Molecularly targeted nucleic acid testing	Molecular; next-generation DNA sequencing	US	Not known
First Light Biosciences	Offers sensitive detection of diagnostic markers directly in complex sample matrices	Molecular; MultiPath platform	USA	Presented at the American Society for Microbiology conference in 2019
GeneCapture	Non-amplified RNA detection	Molecular; CAPTURE platform	USA	Early development; POC platform estimated date of launch, 2025
GeneFluidics	Molecular-based, PCR-less identification of species-specific phenotypic markers of resistance and susceptibility, as they are clinically relevant end products of many genetic pathways	Molecular; UTImax	USA	Sold for research use only since December 2017
GFC Diagnostics Ltd	DNA hybridization technology and have developed a rapid, POC test for detection of MRSA	Molecular; SafeTube IsoScreen	UK	Product announced in October 2017
Gradientech AB	Proprietary microfluidic technology solution to create stable substance gradients for AST of positive blood culture samples in 2 h	Phenotypic; QuickMIC and CellDirector	Sweden	Product estimated date of launch, 2023

Table 1 (cont.) | **New offers in qualitative and quantitative AST systems**

Companies[a]	Technologies used	Approach and system	Country of origin	Status
Klaris Diagnostics (Pattern)	Uses deep neural networks to recognize the unique 'biometric fingerprint' produced by different bacterial species encapsulated within microfluidic droplets	Phenotypic; bioinformatic; single-cell biometric analysis	US	Not known
MicrobeDx Inc	Hybridization and capture of target ribosomal RNA, thus leveraging its natural amplification and negating the need for PCR	Molecular; UMD SelectNA	Germany	Product estimated date of launch, 2024
Nanopore Diagnostics LLC	iNDxer, which is a nanopore sensor for counting dilute amounts of nucleic acid biomarkers (for example, DNA, RNA) directly in minimally processed samples	Molecular; iNDxer	USA	Product estimated date of launch, 2024
Nexogen Inc	Programmable enrichment and real-time selective sequencing method for the rapid diagnostics for AMR	Molecular; not defined	USA	Prototype phase
Next Gen Diagnostics	Overnight whole-genome sequencing with bioinformatics assessment	Genomic; bioinformatics; proprietary bio-info dashboard	UK and USA	Not known
Orbital diagnostics	Scattered light integrated collector	Phenotypic; scattered light integration collector	UK	Not known
Phase Genomics	High-throughput chromatin conformation proximity ligation technology	Molecular; next-generation DNA sequencing	USA	Proof of concept published in 2017; product launch currently not known
PhAST Diagnostics	Single-cell imaging	Phenotypic system	USA	Not known
QSM Diagnostics, Inc.	Quantitative electrochemical measurement of bacterial colonization levels	Phenotypic system	USA	Not known
Resistell	Nanomotion detection-based antibiotic susceptibility testing	Phenotypic; atomic force microscopy and cantilevers	Switzerland	Not known
Selux Diagnostics	Europium-cryptate-diamine chelate used to universally label the bacterial surface; cryptates and cryptands form 3D structures that function as ion cages	Phenotypic; biochemical; bacterial surface area chemistry, next-generation phenotyping	USA	Product estimated date of launch, 2023
Seraph Biosciences Inc.	Field portable ultra-high sensitive Raman system	Phenotypic; SeraSpec	USA	Not known
Specific Diagnostics LLC	Small molecule sensor array responds to metabolic by-products and detects volatile organic compounds	Biochemical; reveal system	USA	Product estimated date of launch, 2022
Spectromics	Spectrometric monitoring of phenotypic changes that occur in reactions between the sample and a panel of candidate antibiotics	Phenotypic; 10-min POC test at the general practitioner	UK	Not known
Spindiag GmBH	Ultrafast and highly sensitive PCR for drug-resistant pneumonia and sepsis	Molecular; SpindiagONE	Germany	Product estimated date of launch, 2024
Symcel Sverige AB	Label-free multichannel assay that measures the specific metabolic phenotype of cells and pathogens in real time	Phenotypic; calScreener	Sweden	Product estimated date of launch, end of 2020
Talis Biomedical Corp.	Combination of SlipChips, fluorescence and bright field real-time imaging that enables unlimited test formats in a compact platform	Phenotypic; LAMP technology	USA	Not known
TheoremDx Inc	Simultaneous protein and DNA and/or RNA assays; high-tech graphene chips combining all methods; cheap, reliable, cloud-based artificial intelligence	Proteomic; molecular; graphene rapid identification platform	USA	Product estimated date of launch, 2025

This is a highly dynamic area of research and development, with companies being launched and disappearing; hence, this table provides a snapshot rather than a comprehensive list. The companies listed are mostly small or medium sized, with a focus on the translation of academic proof-of-concept or proof-of-principle findings into clinical applications, including dedicated instruments and case-specific assays for as many organism–drug combinations as possible. The diversity shown in the table reflects the fact that the gold standard genomic technology (next-generation sequencing in combination with the bioinformatics pipeline) has certainly not been defined yet, and that it is quite unlikely that this gap will be resolved within the next 5 years. A brief description of efforts to develop genomic tests, often in combination with classic PCR-based methods, is provided. AMR, antimicrobial resistance; AST, antimicrobial susceptibility testing; FACS, fluorescence-activated cell sorting; POC, point of care. [a]Listed in alphabetical order.

of non-relevant and susceptible microbiota is suppressed[38]. To enhance the sensitivity of chromogenic media, fluorescent substrates can be added to the media to facilitate the detection of microcolonies after a short incubation[39]. In addition, methods that enable the identification of microscopic changes in cell morphology[40] have improved the detection of drug susceptibility, including cumbersome resistance traits such as, for example, polymyxin resistance[41]. These procedures can be directly implemented in microbiology laboratories, without the requirement of new equipment or increased workload. Recently, it was shown that surface labelling of bacteria substantially shortened the microscopic analysis of AST results in 96-well formats using only a standard microplate reader[42]. To improve the current systems, various prototype AST methods with different approaches have been reported, including the use of mobile phones[37], laser scatter technology[43], sensing of bacterial vibrations using phase-noise measurements on resonant piezo-electric substrates[44], protein-adsorbed magnetic nanoparticle-mediated protocols[45], field-effect enzymatic detection[46], microfluidics[47–49], glucose metabolization[50], optical screening and diffusometry[51], nanometre-scale bacterial deformation measurement[52], mass spectrometry[53], nanowire sensors[54], electrochemical sensing[55], real-time laser scattering[56], atomic force microscopy[57], fluorescence-activated cell sorting (FACS)[58], Raman and infrared spectroscopy at the single-cell level[59,60], nanotube-assisted microwave electroporation[61], hydrodynamic trapping[62] and video microscopy[63]. In this Review, we do not describe these methods in detail, due to the vast differences in the protocols. FIGURE 2 summarizes some of the newer test principles. It is noteworthy that these methods all require upscaling and transition from experimental to diagnostic laboratories. Furthermore, extensive clinical and outcome studies will be needed to prove their value in the clinical decision-making process.

One emerging method that is more advanced uses matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-TOF-MS), a technique that is already being used in clinical laboratories for bacterial identification. MALDI-TOF-MS is a relatively new technology, and implementation of the technique for the rapid identification of causative bacterial organisms has been shown to be effective. For positive blood cultures, MALDI-TOF-MS can quickly identify bacterial growth, which accelerates the overall process of AST reporting[64]. Note that the MS applications mentioned above do not change the AST strategy as such. Very recently, a microdroplet-based MALDI-TOF-MS approach that enables rapid AST was presented[65]. However, clinical trials that can demonstrate direct detection of resistance factors via MALDI-TOF-MS are still lacking[66].

Phenotypic AST for mixed bacterial populations. Some AST methods have been developed that can identify resistance in complex mixtures of bacterial species[67,68]. Recently it was shown that the dynamics of kanamycin resistance could be monitored using Raman spectroscopy in artificial mixtures of different microbial strains[69]. The fraction of cells that exhibit changes in their individual Raman spectrum increases with increasing antibiotic concentrations[59]. This type of AST, sometimes referred to as 'phenotypic resistomics', would potentially enable many resistance markers in complex microbial populations to be catalogued. Currently, few software programs can translate phenotypic databases into clinically actionable advice for clinicians, but these programs are being developed for clinical use[70].

Molecular AMR detection methods

Detection of protein markers of resistance. Not all molecular methods for the detection of resistance are nucleic-acid-based. Several AST methods that involve the direct detection of protein markers of resistance (such as β-lactamases) have been described — for example, those using specific protein arrays[71]. These AST methods often involve using antibodies to capture and enrich proteins. Either the antibodies or the proteins can be labelled with fluorescent tags for visualization. The interaction between the antibodies and resistance enzymes requires optimization, but several lateral flow assays (LFAs) have been developed for the detection of β-lactamases[72]. An LFA specific to New Delhi metallo-β-lactamase (NDM) 1 was 100% sensitive and specific in a collection of 350

Microbiological parameters
- One versus many cells
- One versus more species
- Heterogeneous AMR
- Cell permeability
- Metabolic status
- Rapid versus slow growth
- Induction of resistance
- Low-level resistance
- New resistance mechanism

Toolbox	Read-out	Suited for single cells
Microfluidics	Viability, growth	✓
Droplet test	Viability, growth	
Cytometry	Viability, growth	✓
Microscopy	Morphology	✓
Mass spectrometry	Spectral change	
Light scattering	Spectral change	
Electrochemistry	Conductivity	
Cantilevers	Viability, growth	✓
NMR	Spectral change	
Microsound	Movement	
Phages	Viability, growth	
Calorimetrics	Viability, growth	
Transcriptomics	Viability, growth	✓

Fig. 2 | **New phenotypic methods: microbial characteristics along with restricted survey and description of the mechanisms of new phenotypic methods to help overcome the limitations of current methods.** The box on the left defines the microbiological features that pose a challenge to classic antimicrobial susceptibility testing methods. The box on the right summarizes some of the new technologies, the phenotypic data types they generate and whether or not the tools are suited to the analysis of single cells. The latter question is of importance for the direct analysis of clinical specimens, where the number of bacteria is a limiting factor to classic technologies. AMR, antimicrobial resistance; NMR, nuclear magnetic resonance.

isolates from Myanmar[73]. Similar performance was established for a combined LFA that detected OXA-48, IMP, NDM and VIM enzymes[74]. Moreover, an LFA for mobilized colistin resistance 1 (MCR1) also exhibited 100% sensitivity and 98% specificity[75], and an LFA that targeted OXA-48 and/or OXA-163 was 100% sensitive and specific[76].

Whereas MALDI-TOF-MS enables overall protein profiling of microorganisms for their identification, more advanced methods can detect individual proteins by peptide mapping in the selected reaction-monitoring (SRM) mode[77]. Similar approaches also work for lipids[78]. In conclusion, methods have been developed that can efficiently detect and identify resistance-associated macromolecules. However, none of these methods is fully comprehensive, the test formats are technically diverse and the applications are usually very specific for a resistance mechanism.

Assessing AMR via multiplex gene detection. Various non-phenotypic methods have recently been reported, which include niche applications of nucleic acid probe array-mediated detection of specific resistance genes, for example[79], and PCR tests for similar targets[80]. Very recently, LAMP, an isothermal molecular amplification method, was used for the detection of AMR-defining genes or mutations, leading to detection of AMR in *Escherichia coli* directly from urine[81]. Molecular detection of resistance genes can also benefit from high-resolution melting analyses of amplicons after classic PCR. A recent study described a method that combines species identification and AST based on melting curves and machine learning[82]. Despite the technological innovation and rapidity of these methods, more data are needed to evaluate the value they add, and although the first data are being presented, formal publications of the results are still largely missing. The assays described above usually detect a single gene target, or at the most a few targets. These include the methicillin resistance markers *mecA* and *mecC*, the vancomycin resistance markers *vanA* and *vanB*, and the genes encoding extended spectrum β-lactamases (ESBLs). Many resistance gene-targeting tests, usually PCR-based, have entered the market. It would be beyond the scope of this Review to summarize all currently available tests, and the reader is referred to the existing literature[83]. Several of these diagnostic tests enable the multiplexed detection of various genes, including genetic polymorphisms, in a single rapid assay[84].

Molecular AST assays based on nucleic-acid-mediated amplification of specific resistance markers have the added the potential for fast TATs to hasten the administration of empiric patient treatment or to allow early adjustment of targeted therapy[85] (FIG. 3). Here we consider all those methods that detect resistance genes to be molecular AMR detection tests, and we note that these methods are essentially indirect surrogates for classic AST methods. The molecular methods currently available have minimal hands-on time, often use a 'sample-to-answer' approach and are rapid. One of the first systems on the market, the IDI-MRSA assay (Infectio Diagnostic), used in combination with the Smart Cycler II rapid DNA amplification system (Cepheid), was used for methicillin-resistant *Staphylococcus aureus* (MRSA) screening[86]. Aside from screening for MRSA in nasal swabs[87], tests for the most common vancomycin resistance markers, *vanA* and *vanB*[88], and a screening method for the carbapenem resistance genes bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA48} and bla_{IMP} in rectal swabs have been released[89], and more such tests will be released in the future. However, most of these assays were intended for surveillance rather than for guiding treatment in the setting of infection. The positive predictive value (PPV) and negative predictive value (NPV) of a specific test will vary depending on local resistance prevalence. New multiplex amplification assays have recently become available for 'syndromic infectious disease' testing, whereby, in addition to organism identification, the detection of resistance genes can be considered another advantage. The BioFire FilmArray BCID panel (TAT ≈ 1 h) identifies 24 common causes of bacteraemia and 3 resistance genes — *mecA, vanA/B* and bla_{KPC} — from blood cultures[90]. The Curetis Unyvero system can be used for the diagnosis of pneumonia, implant and tissue infection, blood culture infection and intra-abdominal infection, and includes more comprehensive resistance panels with 19, 17, 16 and 22 resistance markers per test, respectively[91,92]. The Nanosphere VERIGENE system (recently acquired by Luminex) is a blood culture identification system with two panels: Gram-positive bacteria and Gram-negative bacteria. This system also includes a few resistance markers (*mecA*, *vanA*, *vanB*, $bla_{CTX\text{-}M}$, bla_{IMP}, bla_{KPC}, bla_{NDM}, bla_{OXA} and bla_{VIM})[93]. DiagCORE by STAT-Dx (recently acquired by Qiagen and cleared by the FDA) is intended to quickly diagnose respiratory infections. The molecular approach to AMR detection is becoming more popular, given its rapidity and broad coverage. Molecular detection of AMR will go beyond PCR testing once isothermal nucleic amplification becomes more available and is shown to be cost-effective[94].

Implementing molecular AMR detection. The molecular assays described above are the first truly rapid diagnostic methods that compete with classic phenotypic AST methods. It is likely that one method could eventually combine proteomic, immunological and nucleic acid-mediated detection[95]. One of the most important barriers preventing universal acceptance of molecular tests as compared with growth-based methods is cost. In addition, the absence of a certain target resistance marker often does not correlate with phenotypic susceptibility. Current PCR tests are not able to monitor all resistance factors comprehensively for all bacterial species in a single test. There is also a need for expertise regarding the interpretation of molecular AMR detection and for an understanding that the detection of a resistance gene or mutation does not necessarily correlate with resistance. Also, most resistance marker assays cannot assign the marker to a specific organism in a polymicrobial sample. In addition, new mutations or non-characterized resistance mechanisms will remain elusive when researchers turn exclusively to molecular AMR detection. However,

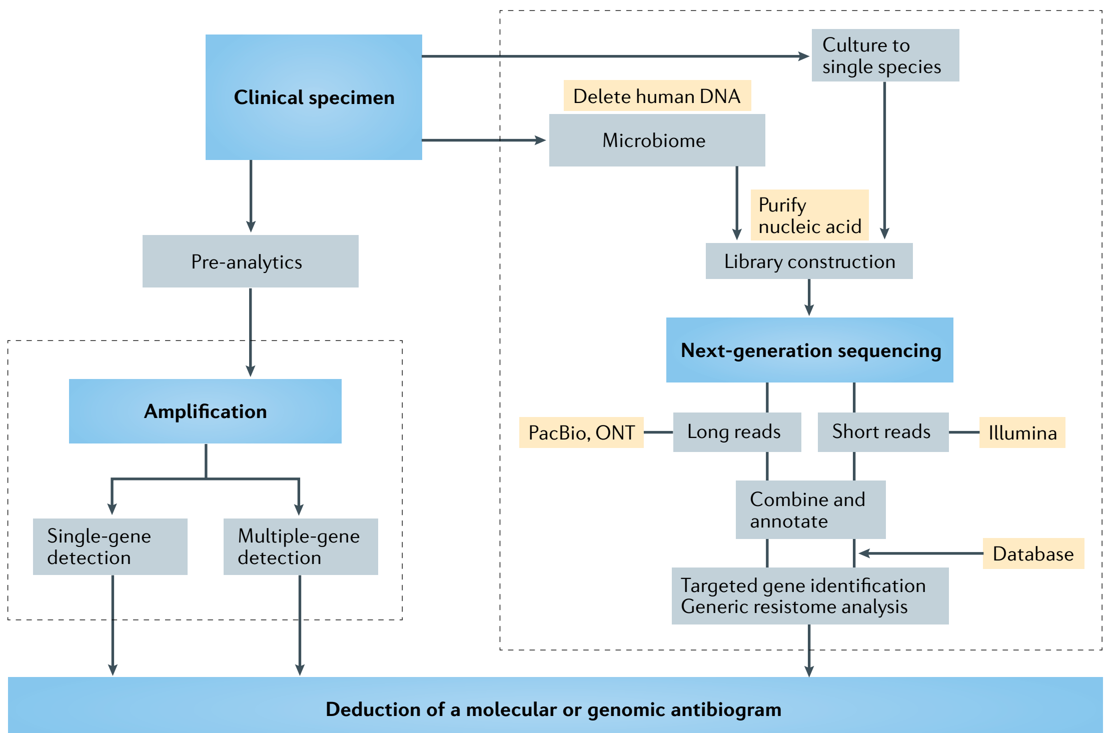

Fig. 3 | **Molecular antimicrobial susceptibility testing assays.** Concepts are shown that relate to new, mostly nucleic-acid amplification-based molecular and next-generation sequencing-based genomic methods for antibiotic susceptibility testing. The route from a clinical specimen to a diagnostic result is indicated by arrows. For the molecular test (left dashed box) this suggests a mostly three-step procedure: rapid sample preparation, amplification and amplicon characterization. The sequencing protocol (right dashed box) is more complicated, owing to the diversity of technologies available, their sequence output (long versus short reads) and the still non-standardized bioinformatic pipelines, new versions of which are continually being presented in this new diagnostic domain. ONT, Oxford Nanopore Technologies.

as experience with these tests grows, and as data are gathered on their efficacy and clinical impact, it is likely that they will be more widely adopted.

Broad-spectrum genomic AST

A trend towards the use of genomic rather than gene-based methods for the combined identification of bacterial species and antibiotic resistance is emerging in the diagnostic market. Applying whole-genome sequencing (WGS) enables essentially all genes involved in AMR to be traced. In principle, this would facilitate comprehensive genomic cataloguing of all resistance factors present in a given bacterial cell. This technique is not yet without pitfalls (see below). The switch towards WGS is being fuelled by the need for more accurate and rapid infection prevention measures[96]. In fact, once an organism has been isolated by culture, bacterial identification and antibiotic resistance gene detection via bioinformatic analysis (for example, using the Comprehensive Antibiotic Resistance Database (CARD) and ResFinder) are surprisingly easy to perform. In many cases, these pipelines have the potential to be fully automated[97]. Still, currently there is no convincing evidence that WGS outperforms multiplex PCR, despite the limitation on the number of genes that can be screened by PCR.

The most impactful and timely diagnostic innovations developed recently have used 'omics' approaches[98], and the current third-generation or fourth-generation protocols and methods may soon be available to the diagnostic community, with the first clinically validated and FDA-approved genomic tests for cystic fibrosis already being used[99]. EUCAST has created a subcommittee on AST by WGS. The WGS strategy requires databases that include sequences for all resistance genes and resistance-associated mutations, as well as sequencing instruments that are currently being sold for less than US$20,000. When a complete bacterial genome is subsequently analysed for overlap with the entries in such databases, an isolate-specific 'resistome' can be mapped[100]. Clinical microbiologists should not use these software packages without understanding how gene sequence matching occurs. There is a danger in relying on default values and presuming that all antibiotic resistance genes within a sample will be found using these. A review of the software packages and databases that are available can be found in BOX 2.

Some studies have reported an approach that is partly species independent, is compatible with MIC determination[101] and details resistance traits against families of antimicrobials[102]. In addition to genome-based resistome analyses, RNA-mediated transcriptomic approaches have been described[103,104]. Deep sequencing of amplified DNA still adds distinct value in this field, as well[105]. The omics-oriented approach is promising, but its use for AST still needs validation[106].

Box 2 | **The importance of genomic databases**

Well-known resistance factor databases include ResistoMap (also suited to microbiome research[129]), the Canadian Antibiotic Resistance Database (CARD)[130], Antibiotic Resistance Gene Annotation (ARG-ANNOT)[131], ResFinder[132], GEAR-base[133], NanoARG[134] and others, most of which are proprietary and industry-owned and industry-curated. There is a need for target-specific databases; those describing overall heterogeneity among β-lactamases[135] and all antimicrobial resistance-associated mutations in *Mycobacterium tuberculosis* (MUBII-TB-DB)[136] are just two examples. A recent review described the close correlation between such databases and the intricacies of the sequencing strategies and methods used[137]. Each of these databases is continuously updated and curated, especially when new resistance markers or mechanisms are being discovered. The databases are combined with specific software packages to facilitate searches for resistance markers; these packages all have their specific advantages and disadvantages[138,139]. Finally, clinical validation studies have been performed for large collections of bacterial strains that belong to clinically relevant species. These species include, among many others, *M. tuberculosis*[18,140,141], *Staphylococcus aureus*[142], *Klebsiella pneumoniae*[143,144], *Neisseria gonorrhoeae*[35,145], *Escherichia coli*[146], *Shigella sonnei*[147] and *Pseudomonas aeruginosa*[148–150].

Note that the use of different bioinformatic pipelines in combination with different databases may result in different outcomes[107]. The biggest barriers to routine application of species-independent approaches are the need for cheaper sequencing platforms and the urgent need for user-friendly 'sample in, data out' bioinformatic solutions. One technology that is generating a lot of interest is nanopore sequencing (for example, Oxford Nanopore Technologies (ONT)). This technique is relatively easy to use, and the bioinformatic interface has progressed substantially[108]. The sequences generated by this technology are long, enable easier assembly of complete genomes and can identify plasmids and large-scale genomic re-arrangements. However, the accuracy of ONT sequencing is still lagging behind that of other sequencing technologies. New mathematical methods (for example, machine learning) may correct these deficiencies. It has already been shown that WGS substantially enhances the quality of genotype-to-phenotype transitions[109,110]. This will require continuous phenotypic and genotypic screening for new resistance mechanisms and markers, database maturation and the development of smart software and visualization tools. BOX 3 exemplifies how these methods can be used to identify resistance genes in complex mixtures of bacterial species.

The implementation of WGS in a clinical microbiology setting is being seriously considered[111–114] (TABLE 1). Its integration into routine use will require access to clinical sites for method evaluation, strain collection for the expansion of databases, increasingly mature next-generation sequencing platforms, reduced cost and studies demonstrating its impact on patient management.

Challenges for new AST methods

The major issues restricting the successful implementation of new AST methods are the need for substantial biomass, prolonged time to result, competition in the market and complexities of the developmental process. All current high-throughput AST methods require large microbial inocula. Practical approaches towards innovation that lower the inoculum density are rare. A means of reducing the input while maintaining the TAT has not yet been identified. What has surfaced over the past decade are methods that enable the concentration and purification of microbial cells directly from clinical specimens or positive blood cultures. Direct AST of cell pellets derived from urine samples has been shown to be successful[115]. A number of protocols for the identification of microorganisms from positive blood cultures have been reported, consisting of combinations of host cell lysis with the centrifugation and/or filtration of bacterial cells, and these methods have been shown to be useful for direct AST[116]. However, the ability to purify low numbers of microorganisms from large volumes of biological samples (bacteraemia) remains a challenge in diagnostic microbiology[117].

In addition, time and cost pose further challenges to the development and implementation of a new AST method. Fortunately, a pipeline is emerging for the new methods that have been described[118–120]. Typically, the innovation process involves a novel scientific discovery (that is, defining the methodology that will be used to distinguish living from dead bacterial cells) and a verification period to improve the technological aspects of the assay, usually by limiting trials to few bacterial species and one or two key antimicrobial

Box 3 | **Clinical metagenomics and resistance gene detection**

Clinical metagenomics is the study of genetic material recovered directly from clinical samples using whole-genome sequencing technologies[151]. This so-called deep sequencing (or shotgun sequencing) produces a diverse profile for detecting, characterizing and (semi-)quantifying all species present in a sample. This approach has revealed that new resistance genes are easily identifiable[152]. This implies that clinical metagenomics can be considered an additional and complete approach for the detection of all antimicrobial resistance marker genes in a single specimen[153,154]. This is frequently referred to as 'resistome analysis' or 'resistomics'.

Most data have been generated using faecal specimens, and studies on the recovery of the gastrointestinal microbiota after antibiotic treatment have been a productive area of research[155]. Faecal resistome analysis has helped quantify the dynamics of the microbiota before, during and after antibiotic treatment and has shown that the microbiota of young adults restores well after antibiotic use. The use of machine learning has already shown that in certain cases dynamic changes of the microbiota of the gastrointestinal tract in the presence of antibiotics can be predicted. Thus, metagenomics can help in the development of personalized antibiotic treatment regimens[156]. Generating complete inventories of resistance genes in clinical specimens will be helpful in defining which antibiotics will have no effect. Longitudinal follow-up studies of patients can lead to the early detection of emerging resistance during treatment and thus to changes in treatment protocols. However, one remaining issue is the accurate association of a resistance gene with a particular species (phasing).

agents (usually of the bactericidal variety). If the proof of concept is successful, a scientific publication ensues, and potential patents are secured. At this point, one big barrier is finding a corporate partner capable of developing the test and that is willing to invest substantial resources for further improvements. The decision of whether to invest is usually based on due diligence assessment, business development discussions and the perceived competitiveness of the new AST method[15]. It is relatively easy to get to the proof-of-principle stage, but a full-blown validation of new AST technology for all possible combinations of bacterial species and antibiotics is daunting and beyond the financial capabilities of start-up companies. Defining the microbial panel width and global robustness of a new test requires huge investments. Many new methods fail to mature, since getting validation projects financed without a guarantee of success is problematic[121].

When discussing the performance of various AST technologies, it is mandatory that a non-biased approach be developed. This is not an easy task, owing to the diversity of the reported protocols, of the antibiotics to be analysed and of the species of microorganisms to be evaluated. In this context, important criteria for comparing different methods include microbiological and diagnostic value (that is, resistance mechanism versus phenotypic testing), as well as TAT[15]. Current TATs range from 20 min, for rapid PCR-based gene profiling, to days, for cell-division-based assays and genome sequencing[122,123], although the TAT for genome sequencing is likely to become shorter in the future. A second comparative approach evaluates the commercial landscape, to provide a sense of how new diagnostics will fit in the market and what competition already exists in that space. This approach is limited, since it tends to magnify the shortcomings rather than the strengths of new technologies. These two approaches can be used in combination, which is probably more realistic for predicting success. There is a tendency to overestimate the capabilities of regulatory-approved methods and to undervalue the use of new technologies.

Concluding remarks

AST is constantly evolving, with many alternative methods being ready for implementation either now or in the near future[120]. In this context, it is important to spend less time on small, incremental improvements to existing technology, but rather to strive for substantial advances so that new tests with superior performance characteristics can be approved and marketed as soon as possible. This will require the community of AST developers, manufacturers and end-users to recognize and leave the limitations of traditional AST behind. For example, debate is still ongoing regarding the actual number of bacterial colonies that should be tested for phenotypic or genotypic resistance[124]. New AST technologies may identify all of the resistance phenotypes and/or genotypes present within a clinical sample, but without isolation and species identification, the significance of the identified resistance mechanisms may not be obvious or important[125]. Perhaps it can be concluded that many options for AST could be 'mixed and matched' in a clinical laboratory, depending on the diversity of the patient population or workflow, but it seems impossible to recommend which could be the best combination or combinations. It is unlikely that a single method will completely replace the existing automated high-throughput methods. It is more likely that new methods will be supplemental, for the time being. For instance, most new AST formats test positive blood cultures — which means that a method should be capable of identifying species or be closely coupled to classic or MALDI-TOF-MS-mediated identification. Obviously, the need to identify a clinical isolate at the species level slows the diagnostic process. Additionally, EUCAST, the Clinical and Laboratory Standards Institute (CLSI) and the FDA should be integral parts of the development process and should recommend clinical MIC breakpoints to be used in diagnostic laboratories[126]. New technologies will also require optimizations of both pre-analytics (clinical sample and strain handling) and post-analytic follow-up at the clinical level. Finally, patient health management and cost are of pivotal importance. Costly tests or tests for which the clinical and financial return on investment has not been made clear will fail to be implemented[127]. Bringing the issues surrounding AMR and AST to broader public attention is a must. This will require promoting a better understanding of antimicrobial usage (stewardship) and clear explanations to the general public about drug selection and the development of multidrug resistance from overuse of antimicrobial agents[128]. The One Health principle, which integrates microbiology, the environment and human and animal interactions in one continuum, should be used to explain the scope of the problems associated with AMR. Approaches that prioritize and maximize infection prevention and antibiotic stewardship should become the standard of care. Clearly, the research agenda for AMR and AST should include translational funding for bridging the current gap between established and new technologies and for speeding the application of new technologies following regulatory approval. In this context, it is of interest to mention the new EU regulation (Regulation (EU) 2017/746 of the European Parliament and Council, published 5 April 2017, on in vitro diagnostic medical devices, repealing Directive 98/79/EC and Commission Decision 2010/227/EU), which should facilitate the rapid implementation of ground-breaking diagnostic tools. The EU and the Innovative Medicines Initiative (IMI) also fund various projects aimed at improving routine diagnostics (for example, New Diagnostics for Infectious Diseases (ND4ID), the Viral and Bacterial Adhesion Training Network (ViBrANT), Combatting Bacterial Resistance in Europe (COMBACTE), Value Dx and other projects).

As we have outlined in this Review, several promising new AST systems and protocols are being developed and implemented in the clinical setting, and such improved AST methods will help manage AMR.

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ResFinder: https://cge.cbs.dtu.dk/services/ResFinder

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NATIONAL ACTION PLAN FOR COMBATING ANTIBIOTIC-RESISTANT BACTERIA

2020-2025

October 2020

From the Federal Task Force on
Combating Antibiotic-Resistant Bacteria

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SECTION 1

INTRODUCTION & BACKGROUND

Executive Summary

The National Action Plan for Combating Antibiotic-Resistant Bacteria (CARB), 2020-2025, presents coordinated, strategic actions that the United States Government will take in the next five years to improve the health and wellbeing of all Americans by changing the course of antibiotic resistance.

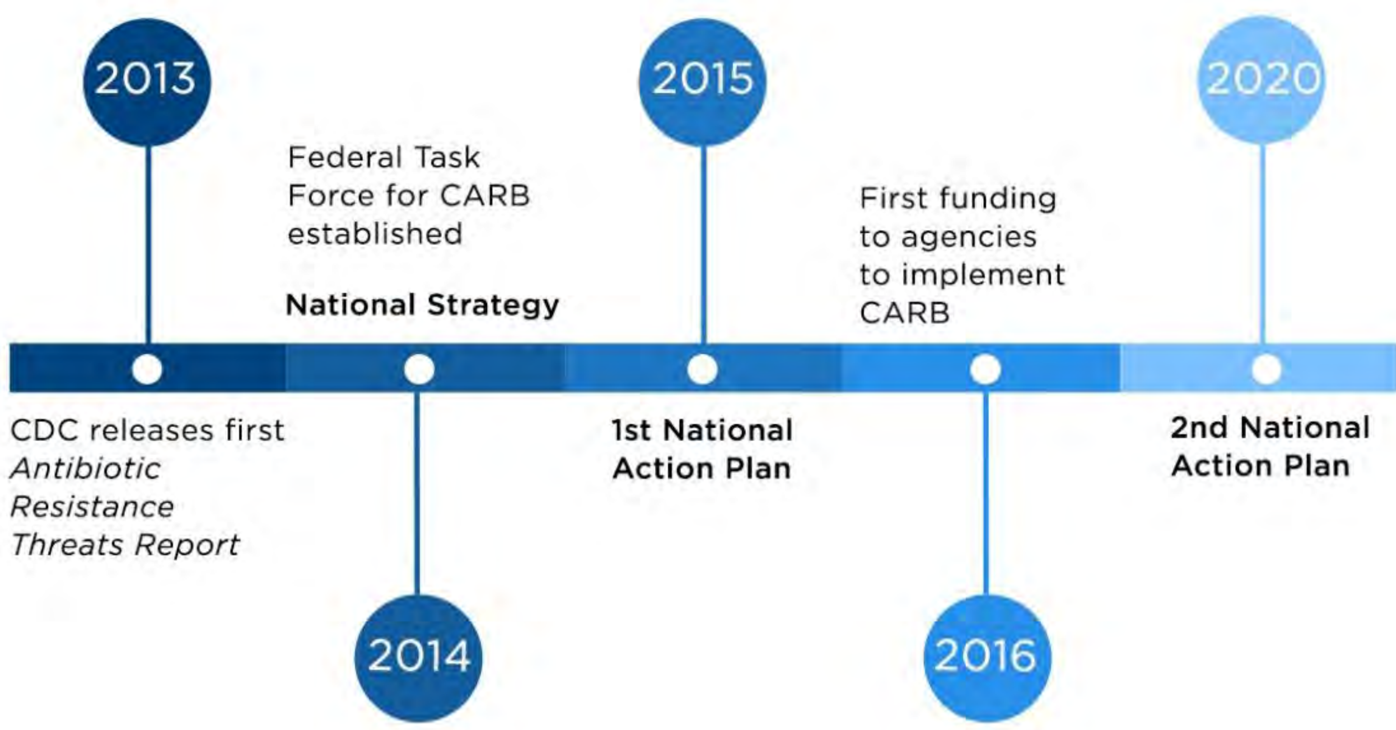
This Plan is based on the U.S. Government's 2014 National Strategy for CARB, and builds on the first National Action Plan released in 2015 by expanding evidence-based activities that have already been shown to reduce antibiotic resistance, such as optimizing the use of antibiotics in human and animal health settings.

This Plan continues to prioritize infection prevention and control to slow the spread of resistant infections and reduce the need for antibiotic use. To ensure that patients receive the right antibiotic care, the Plan supports innovative approaches to developing and deploying diagnostic tests and treatment strategies. A One Health approach, which recognizes the relationships between the health of humans, animals, plants, and the environment, is integrated throughout the Plan, with an expanded effort to understand antibiotic resistance in the environment. The Plan also focuses on collecting and using data to better understand where resistance is occurring, support the development of new diagnostics and treatment options, and advance international coordination.

The U.S. Government will report annually on progress toward the objectives set in the Plan.

About the National Action Plan for Combating Antibiotic-Resistant Bacteria, 2020-2025

This Plan describes activities that the U.S. Government will undertake from 2020 through 2025 to reduce the impact of antibiotic and antimicrobial resistance on the nation.[1]



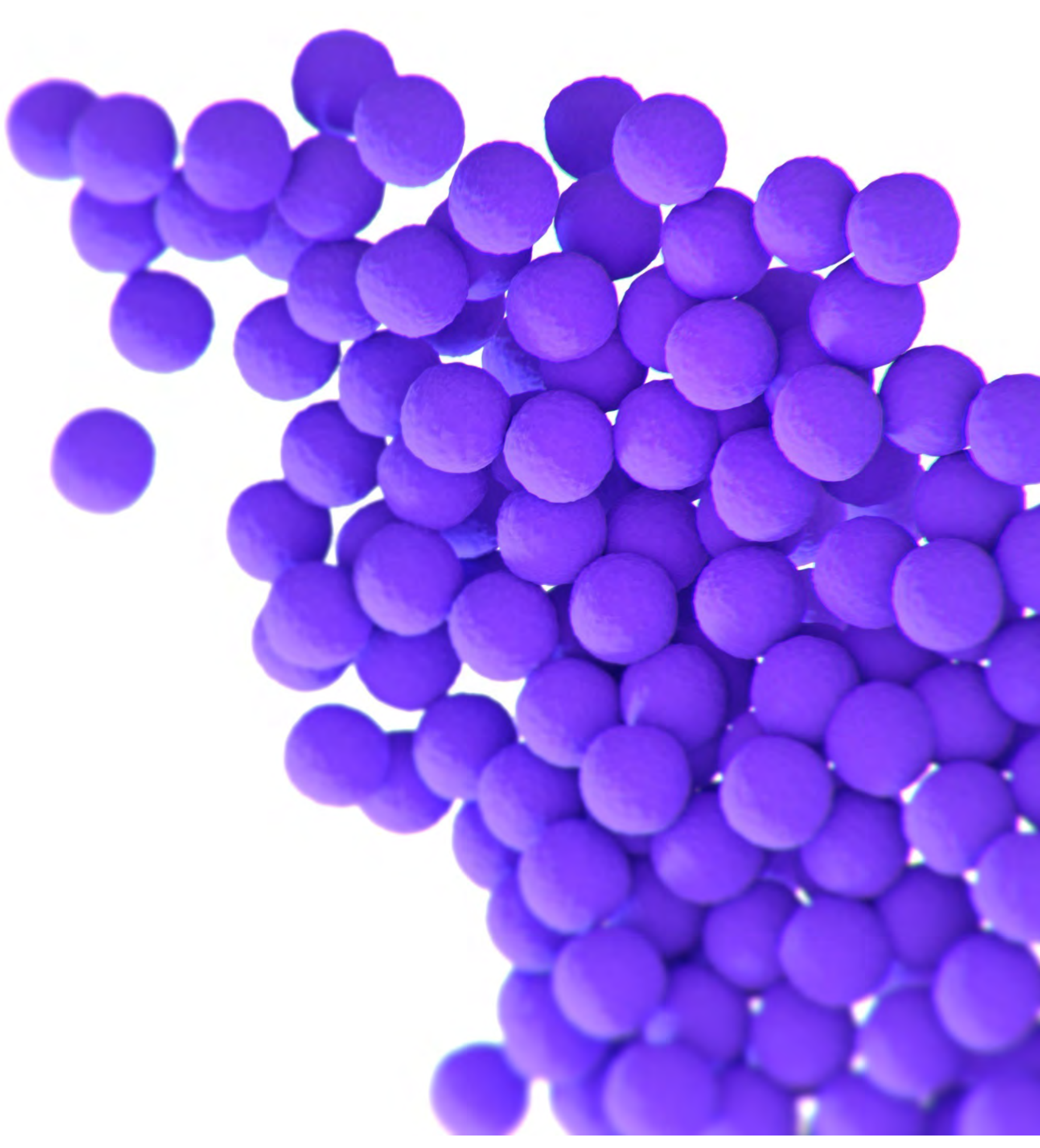
To address the growing threat of antibiotic resistance, the U.S. Government released the National Strategy for CARB in September 2014, which outlined five inter-related goals to guide Federal action. At the same time, Executive Order 13676 established the Federal Task Force for CARB to identify actions to implement the National Strategy. In March 2015, the Task Force released the first National Action Plan for CARB, aimed at moving the nation toward the goals of the National Strategy through specific objectives, strategies, and milestones to be achieved within 1, 3, and 5 years. The Task Force has issued reports on progress toward these milestones for years 1 and 2, year 3, and year 4 of the original plan. A forthcoming final report will cover year 5 and an overall assessment of progress.

The new National Action Plan for 2020-2025, presented here, maintains the original 5 goals of the National Strategy and includes new objectives and targets aimed at achieving those goals.

The Task Force is co-chaired by the Secretaries of the U.S. Departments of Health and Human Services (HHS), Agriculture (USDA), and Defense (DoD), and also includes the Departments of Interior (DoI), State (DoS), and Veterans Affairs (VA), the Environmental Protection Agency (EPA), the U.S. Agency for International Development (USAID), the National Science Foundation, and representatives from the Executive Office of the President. The HHS Office of the Assistant Secretary for Planning and Evaluation coordinates the Task Force, prepares annual progress reports, and led the development of this document.

[1] This Plan follows the framework of CDC's 2019 AR Threats Report and uses the term "antibiotic" to describe antibacterial and antifungal drugs, which kill bacteria and fungi, respectively. "Antimicrobial resistance" is a broader umbrella term that also includes resistance in other microbes not included in this Plan, such as viruses and parasites.

The Presidential Advisory Council on Combating Antibiotic-Resistant Bacteria (PACCARB), also established by Executive Order 13676, is composed of both Federal and non-Federal subject-matter experts in human and agricultural health. The PACCARB provides information and recommendations to the HHS Secretary regarding programs and policies to combat antibiotic resistance and to improve capabilities to prevent, diagnose, mitigate, or treat antibiotic-resistant infections.[2]



[2] The activities and duties of the PACCARB are governed by Public Law 92-463, the Federal Advisory Committee Act (FACA), and are assigned in section 505(b) of Public Law 116-22 (June 24, 2019), the Pandemic and All-Hazards Preparedness and Advancing Innovation Act of 2019 (PAHPAIA).

The Task Force for Combating Antibiotic-Resistant Bacteria

The Department of Health and Human Services (HHS) and its following components:

AHRQ	Agency for Healthcare Research and Quality
ASPE	Assistant Secretary for Planning and Evaluation
ASPR	Assistant Secretary for Preparedness and Response
BARDA	Biomedical Advanced Research and Development Authority within ASPR
CDC	Centers for Disease Control and Prevention
CMS	Centers for Medicare and Medicaid Services
FDA	Food and Drug Administration
NIH	National Institutes of Health
OGA	Office of Global Affairs

The United States Department of Agriculture (USDA) and its following components:

APHIS	Animal and Plant Health Inspection Service
ARS	Agricultural Research Service
FAS	Foreign Agriculture Service
FSIS	Food Safety and Inspection Service
NIFA	National Institute of Food and Agriculture
OCS	Office of the Chief Scientist

The Department of Defense (DoD) and its following components:

DHA	Defense Health Agency
GEIS	Global Emerging Infections Surveillance
IDCRP	Infectious Disease Clinical Research Program
MIDRP	Military Infectious Diseases Research Program
MRSN	Multidrug-Resistant Organism Repository and Surveillance Network
PVC	Pharmacovigilance Center
WRAIR	Walter Reed Army Institute of Research

Department of the Interior (DoI)

Department of State (DoS)

Environmental Protection Agency (EPA)

United States Agency for International Development (USAID)

Department of Veterans Affairs (VA)

Abbreviations

AMR	Antimicrobial resistance
AR	Antibiotic resistance
CARB	Combating Antibiotic-Resistant Bacteria
CARB-X	Combating Antibiotic-Resistant Bacteria Biopharmaceutical Accelerator
ESBL	Extended-spectrum beta-lactamase
FAO	Food and Agriculture Organization
GEIS	Global Emerging Infections Surveillance
GLASS	Global Antimicrobial Resistance Surveillance System
HAI	Healthcare-associated infections
IPPS	Inpatient Prospective Payment System
LTC	Long-term care
NAHMS	National Animal Health Monitoring System
NARMS	National Antimicrobial Resistance Monitoring System
NHSN	National Healthcare Safety Network
OIE	World Organization for Animal Health (formerly the Office International des Epizooties)
PACCARB	Presidential Advisory Council on Combating Antibiotic-Resistant Bacteria
TATFAR	Transatlantic Taskforce on Antimicrobial Resistance
UNEP	United Nations Environment Programme
WASH	Water, sanitation, and hygiene
WHO	World Health Organization

Background

The Threat of Antibiotic Resistance

The landmark discovery of the first modern antibiotics in the early 20th century contributed to historic improvements in human health and life expectancy. Along with improved sanitation systems, hygiene, and vaccination, antibiotics and other medicines have greatly reduced the incidence of deaths from bacterial infections. However, these advances must not be taken for granted, because microbial pathogens continually evolve new ways to evade the drugs designed to kill them. Pathogens and their drug-defeating genes can also be transferred among humans, animals, and the environment. The evolution and spread of antibiotic resistance challenge our continued ability to prevent and treat infectious diseases in humans and animals.

Antibiotic-Resistant Infections Threaten Modern Medicine

AT LEAST **1.7M**
adults develop sepsis each year.

Surgery

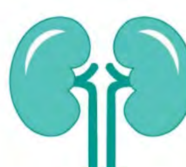
1.2M
women had a cesarean section (C-section) in 2017.

Chronic Conditions

MORE THAN **30M**
people have diabetes.

Organ Transplants

MORE THAN **33,000**
organ transplants were performed in 2016.



Dialysis for Advanced Kidney

MORE THAN **500,000**
patients received dialysis treatment in 2016.

Cancer Care

AROUND **650,000**
people receive outpatient chemotherapy each year.

(Source: CDC's 2019 AR Threats Report)

Antibiotic-resistant infections can also complicate the response to and recovery from public health emergencies. For example, during the 2009 H1N1 influenza pandemic, many patients acquired secondary bacterial infections in addition to influenza, and some of these infections were resistant to antibiotics. While the implications of antibiotic resistance are not yet clear for the ongoing response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and associated COVID-19 illness, increased use of antibiotics and other antimicrobial medicines—both appropriate and inappropriate—to address primary or secondary infections has the potential to further accelerate the emergence of antibiotic resistance.

In 2013, the U.S. Centers for Disease Control and Prevention (CDC) reported on the most worrisome antibiotic-resistant threats in the U.S., sounding the national alarm and identifying necessary actions to face these threats. In 2019, the CDC updated these national estimates and found that each year, more than 2.8 million antibiotic-resistant infections occur in the United States, resulting in the deaths of more than 35,000 Americans. Although the total economic impact of antibiotic resistance is difficult to determine, the CDC estimates that just a subset of resistant infections caused more than $4.8 billion in medical costs in 2017.[3] Similarly, antibiotic-resistant pathogens can harm animal health, though the scope of resistance in animals is less well characterized than in humans. Antibiotic resistance is a challenging threat, but aggressive actions now can prolong the effectiveness of existing antibiotics and prevent infections in the future, ultimately saving lives and money.

[3] Sum of estimated attributable healthcare costs in 2017 for carbapenem-resistant *Acinetobacter*, drug-resistant *Campylobacter*, extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, vancomycin-resistant Enterococci, multidrug-resistant *Pseudomonas aeruginosa*, and methicillin-resistant *Staphylococcus aureus*. Source: CDC 2019 AR Threats Report.

The U.S. Government Response

The U.S. Government is responding to antibiotic resistance with a comprehensive and coordinated suite of actions implemented by a diverse set of agencies using a One Health approach. The National Strategy for Combating Antibiotic-Resistant Bacteria (CARB) lays out five goals to reduce the incidence and impact of antibiotic-resistant infections:

Goal 1: Slow the Emergence of Resistant Bacteria and Prevent the Spread of Resistant Infections

Goal 2: Strengthen National One Health Surveillance Efforts to Combat Resistance

Goal 3: Advance Development and Use of Rapid and Innovative Diagnostic Tests for Identification and Characterization of Resistant Bacteria

Goal 4: Accelerate Basic and Applied Research and Development for New Antibiotics, Other Therapeutics, and Vaccines

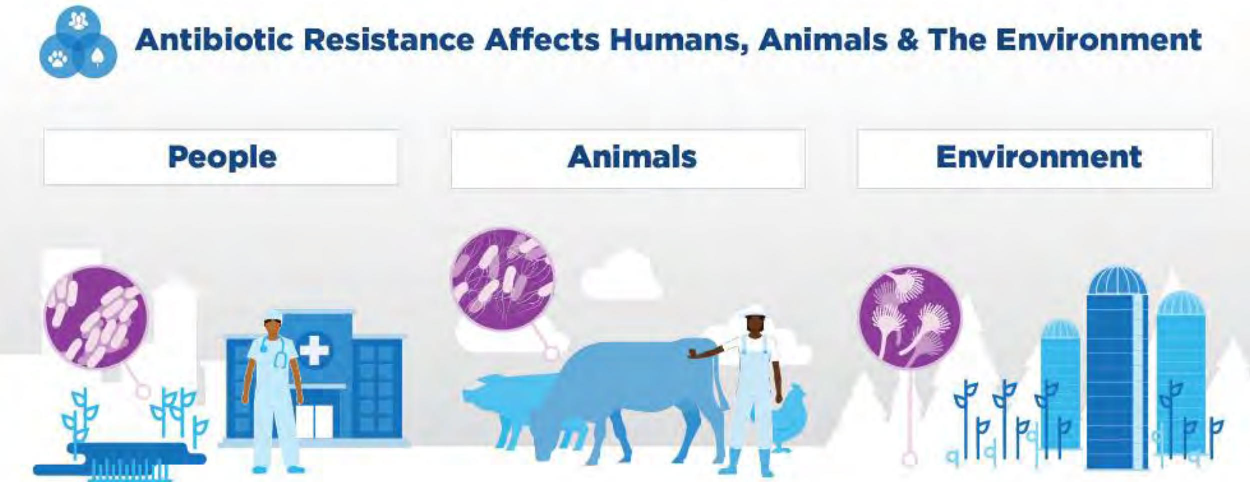
Goal 5: Improve International Collaboration and Capacities for Antibiotic-resistance Prevention, Surveillance, Control and Antibiotic Research and Development.

These goals are pursued by the Federal Departments and Agencies of the CARB Task Force, which is co-chaired by the Secretaries of Health and Human Services, Agriculture, and Defense. In 2015, the Task Force launched the first National Action Plan for CARB, and substantial progress has been made in the past five years. Working with local, State, tribal, territorial, and international partners, the U.S. Government has:

- Established a new national Antibiotic Resistance Laboratory Network (AR Lab Network);
- Launched a strategic initiative to support antibiotic stewardship in veterinary settings;
- Developed new programs to improve antibiotic use across healthcare settings;
- Launched a biopharmaceutical accelerator, CARB-X;
- Supported the development and approval of new diagnostic and treatment options;
- Pursued alternatives to antibiotics in agriculture; and
- Obtained hundreds of commitments to global action from a broad range of sectors and stakeholders.

A ONE HEALTH CHALLENGE

The Interconnected Threat of Antibiotic Resistance



(Source: CDC's 2019 AR Threats Report)

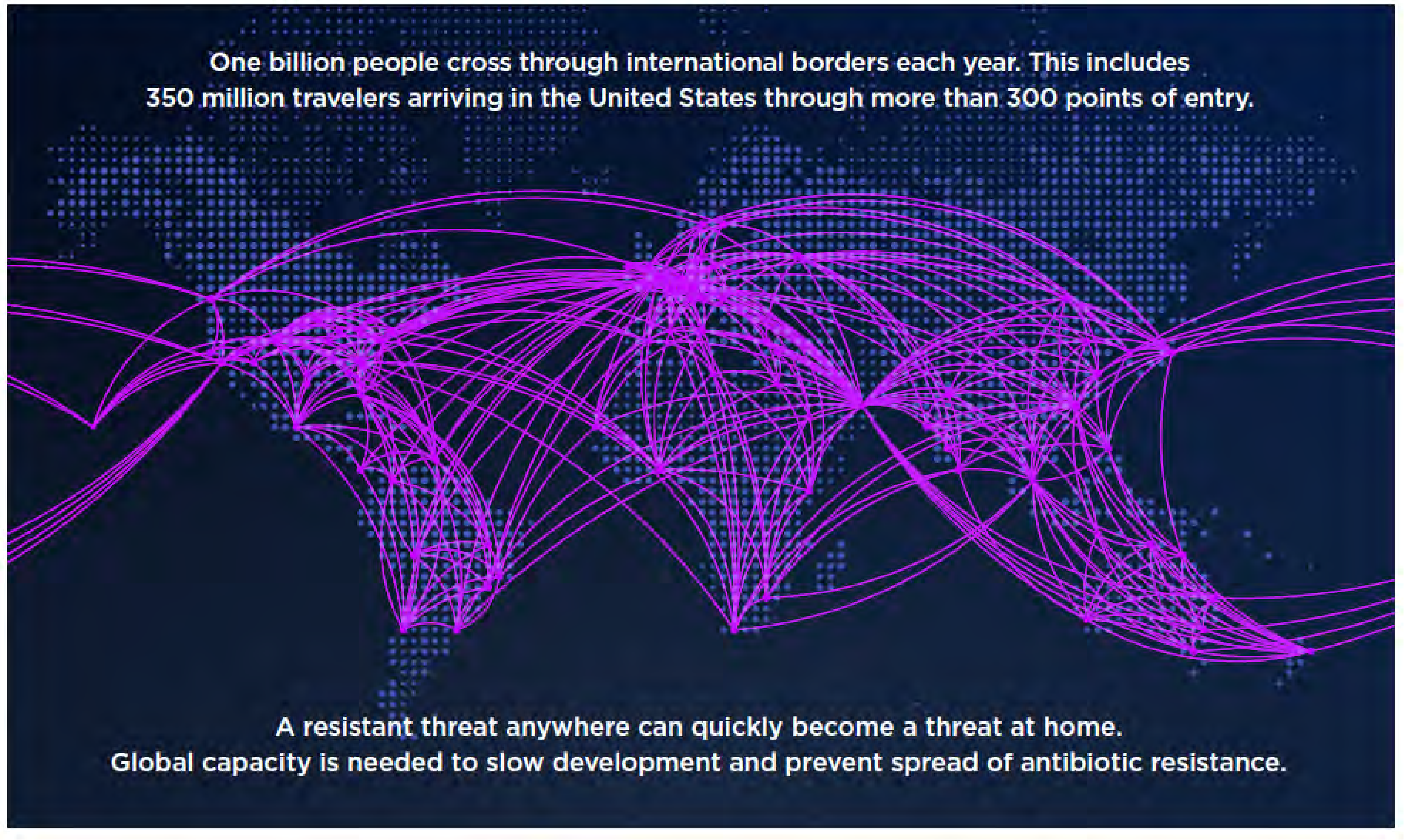
One Health is a collaborative, multisectoral, and transdisciplinary approach—working at the local, regional, national, and global levels—with the goal of achieving optimal health outcomes by recognizing the interconnection between people, animals, plants, and their shared environment.

Human, animal, plant, and environmental health are all connected**.** The antibiotics used to treat infections may be the same or similar in humans and animals; the manufacture, use, and disposal of antibiotics in all settings can potentially drive the emergence of resistance. When antibiotic-resistant bacteria arise, they may spread among humans, animals, and the environment. A One Health approach recognizes the interconnectedness between the health of people, animals, plants, and the environment and encourages a collaborative response to the threat of antibiotic resistance. The CARB Task Force employs a One Health approach by engaging U.S. Government agencies that oversee human, animal, and environmental health and by promoting collaboration and communication to address antibiotic resistance in every relevant sector.

Collaborations among U.S. Government Departments and Agencies have produced important efforts to fight antibiotic resistance. For example, the strong relationship between USDA and the Food and Drug Administration (FDA) within HHS led to an innovative strategy to help ensure that medically important antibiotic drugs (those that are important for therapeutic use in humans) fed to food-producing animals are limited to uses necessary for assuring animal health. FDA sought broad public input and engaged affected stakeholders over several years on plans to work with pharmaceutical companies to voluntarily withdraw production uses (e.g., growth promotion, increased feed efficiency) of medically important antibiotics and to require veterinary oversight of their remaining therapeutic uses. FDA and USDA jointly participated in workshops across the U.S. that brought together producers, veterinarians, and feed suppliers to create a shared understanding of these new initiatives and to discuss the management challenges to implementing them. All pharmaceutical companies with affected products agreed to adopt FDA's judicious use approach, withdrew affected drugs from the market, and fully implemented the recommended changes by the three-year target. In 2019, FDA reported a 38 percent decline between 2015 and 2018 in medically important antibiotics sold for use in food-producing animals. USDA continues to monitor antibiotic use practices on farm, as well as other practices used to address animal health challenges through national studies of animal agriculture. These studies will help experts understand the effects of FDA policy changes on producers. USDA also conducts and funds research to find effective alternatives to antibiotics and other interventions to help maintain animal health and welfare, food security, and agriculture sustainability.

Antibiotic Resistance Spreads Easily Across the Globe

Resistant bacteria and fungi can spread across countries and continents through people, animals, and goods.



(Source: CDC's 2019 AR Threats Report)

Recognizing that domestic action alone is insufficient, the U.S. Government works with multisectoral organizations, partner nations, the private sector, civil society, and other stakeholders to address the threat of antibiotic resistance. Internationally, the U.S. Government has helped to secure high-level commitments to address antibiotic resistance by national leaders, organizations, and Ministers. These commitments include the development and adoption of the World Health Organization (WHO) Global Action Plan on Antimicrobial Resistance, the Declaration of the United Nations High Level Meeting on Antimicrobial Resistance, commitments made at the G7 and G20 meetings, and additional actions by the WHO, the Food and Agriculture Organization of the United Nations (FAO), the World Organization for Animal Health (OIE), and the United Nations Environment Programme (UNEP).

The National Action Plan for Combating Antibiotic-Resistant Bacteria, 2020-2025

Efforts to reduce the effects of antibiotic resistance are working: from 2012 to 2017, the overall number of U.S. deaths from antibiotic-resistant infections fell by 18 percent, and the number of U.S. deaths from resistant infections in hospitals fell by nearly 30 percent as a result of efforts to prevent infections and control their spread. However, antibiotic resistance continues to harm too many Americans, and worrisome trends are emerging, including the discovery of new resistant pathogens, such as *Candida auris,* and an increase in resistant *Neisseria gonorrhoeae* infections. Other drug-resistant, community-acquired bacterial infections from group A *Streptococcus* and ESBL-producing Enterobacteriaceae, for example, are also increasing. The U.S. Government is therefore committed to sustained and enhanced work to combat antibiotic resistance.

In September 2018, the CARB Task Force began developing an updated National Action Plan for CARB, which would cover activities in the years 2020 through 2025. The Task Force reviewed prior efforts and anticipated future challenges and opportunities. The PACCARB solicited and reported on public input, which the Task Force considered alongside perspectives from Federal experts. The result is a set of coordinated, strategic actions aimed at changing the trajectory of antibiotic resistance and improving the health and wellbeing of all Americans, as well as the health of animals, plants, and the environment.

Many of the actions build on and expand evidence-based activities initiated under the 2015-2020 National Action Plan for CARB that have already shown impact, such as the appropriate use of antibiotics in human health, animal health, and in the environment. The Task Force continues to consider infection prevention and control, especially within healthcare facilities, to be high priorities, to both slow the spread of antibiotic-resistant infections and to reduce the need for antibiotic use. Many actions focus on collecting data and turning it into information that can be used to better understand where resistance is occurring, to support the development of new diagnostics and treatment options, and to advance international coordination.

Implementing the activities outlined in this plan will depend on the availability of resources and capacity. The new National Action Plan for CARB, 2020-2025, does not exhaustively list all Federal activities that address antibiotic resistance. Rather, it includes the continuing and new actions that are considered the highest priority for reducing antibiotic resistance in the next five years.

Measuring and Reporting Progress

The 2020 Plan maintains the original five goals of the 2014 National Strategy but establishes a new set of objectives to move the country toward those goals. Whereas the 2015 plan included 1-, 3-, and 5-year milestones meant to capture the activities of specific Federal Departments and Agencies, the 2020 plan includes targets, many of which are composites of multiple activities pursued by multiple agencies. Each listed Department and Agency will report on progress toward these targets within the relevant timeframe. Where possible, the 2020 Plan has established targets to be achieved by 2021, with some targets set for longer timeframes. Each annual report on this Plan will provide updated or added targets as relevant along with rationale for these changes. For example, the first annual report might note that a 2021 target has been achieved and establish a new 2022 target for that objective.

Task Force members reviewed the barriers faced in the past five years while implementing the 2015 Plan and anticipated challenges over the next five years. Certain challenges apply to all five goals, including the allocation of limited resources, obstacles to data gathering and sharing, and uncertainty about the participation of research and industry partners. Implementing the activities outlined in this plan will depend on the availability of resources and capacity. The COVID-19 pandemic has necessitated redirection of public-health, infection control, and antibiotic stewardship resources and will continue to affect implementation of the activities described here. In future annual progress reports, the CARB Task Force will discuss challenges encountered during the preceding year, how it addressed these challenges, and any new challenges identified. Specific challenges are also noted for each Goal below. Appendix B lists all the Challenges.

In most cases, the CARB Task Force will not have direct evidence that the activities listed in this plan cause changes in relevant outcomes. This is because important human, animal, plant, and environmental health metrics are influenced by a variety of interdependent factors, making it difficult to establish single cause-and-effect relationships between specific activities and specific outcomes. However, tracking progress toward the targets listed here should inform the Task Force's understanding of relevant changes over time, allowing agencies to change course when necessary to more effectively achieve the objectives and make progress toward the goals.

Work under the 2015 Plan has clarified the Task Force's understanding of the challenges inherent in collecting and analyzing the data needed to combat antibiotic resistance. The 2020 Plan therefore designates several "Data Development" objectives that aim to develop new or improved data infrastructure, collection, or analysis options. Appendix A lists the Data Development strategies.

The Task Force will continue to report annually on progress, including challenges identified and actions taken to address those challenges. The Task Force may make changes to objectives or targets to accommodate potential new data sources or other unforeseen but informative changes to this work. These changes and their rationale will be included in annual reports.

SECTION 2

NATIONAL GOALS

Goal 1

Slow the Emergence of Resistant Bacteria and Prevent the Spread of Resistant Infections

Bacteria and fungi have developed unique mechanisms to resist the effects of antibiotics and antifungals, and continual evolution of antibiotic resistance is inevitable. However, the development of resistance can be slowed and its effects on human and animal health can be minimized. One way to achieve this is through primary prevention of infections through infection control and other interventions. Using antibiotics only when needed in humans and animals reduces the likelihood that bacteria will develop resistance and extends the effectiveness of current antibiotics. Appropriate antibiotic use also minimizes potential healthcare-related harms to patients, such as adverse drug events and the overgrowth of harmful bacteria (e.g., *Clostridioides difficile* infection, which causes severe diarrhea, toxic colon inflammation, and sometimes death). The objectives below build on recent improvements in infection prevention and control that have saved lives, particularly in hospital settings.

Anticipated Challenges

Prevention and containment efforts, along with improved use of antibiotics, can slow the emergence and spread of antibiotic resistance genes and antibiotic-resistant pathogens and can limit their impact on humans and animals. However, many challenges to these efforts exist. Some of these challenges are related to changing behaviors to ensure optimal infection-control practices and appropriate prescribing of antibiotics. Others could be part of identifying and scaling up best practices across spectrums of care, ensuring their continuity, and coordinating these practices across One Health. Still others could be related to engaging all relevant stakeholders for buy-in and support of best practices.

Objective 1

Expand national, regional, and State capacity for detecting, containing, and preventing antibiotic-resistant infections.

Objective 1.1

Reduce the number of infections and deaths from pathogens identified as antibiotic-resistant threats by CDC.

CDC
Decrease healthcare-associated antibiotic-resistant infections by 20 percent by 2025 and community-acquired antibiotic-resistant infections by 10 percent by 2025.

Objective 1.2

Support investments in U.S. health departments (including in all States and select tribes, territories, and large cities) to detect, contain, and prevent antibiotic-resistant infections.

CDC
Award an average of $2.5 million to Epidemiology and Laboratory Capacity Cooperative Agreement-funded health departments by 2025.

Objective 1.3

Support responses to identify, prevent, and contain antibiotic-resistant pathogens.

CDC, DoD/MRSN
Increase capacity nationwide to contain antibiotic-resistant infections and control outbreaks.

Objective 1.4

Conduct consultations or assessments related to antibiotic-resistant cases, outbreaks, and transmission in healthcare and the community for prevention and containment.

CDC, DoD/MRSN
Increase collaborative efforts at national, regional, and/or state levels to assist with antibiotic resistance response and prevention efforts in the general and military populations.

Objective 1.5

Monitor and report on antibiotic resistance among selected animal pathogens to detect new resistance patterns.

APHIS
Publish one report on an animal pathogen describing emerging antibiotic resistance by 2021.

Objective 2
Engage the public and other stakeholders to develop, expand, and increase national and State education, training, and communication campaigns focused on using antibiotics responsibly, stopping the spread of antibiotic resistance, and preventing infections and life-threatening conditions like sepsis.

Objective 2.1

Expand the scope and reach of CDC's awareness campaigns, including *Be Antibiotics Aware* and *Get Ahead of Sepsis*.

CDC
Each year, increase clicks, impressions, and earned or paid media.

Objective 2.2

Develop new or expanded educational training guidelines, outreach, and awareness activities to educate stakeholders, such as consumers, healthcare providers, and industries, on best practices for using antibiotics responsibly, stopping the spread of antibiotic resistance, and preventing infections.

CDC, CMS, APHIS
Increase and expand outreach activities each year.

Objective 2.3

Expand the promotion and utility of training guidelines and other communication materials.

CDC, APHIS
Each year, increase the number of individuals trained, continuing education units earned, and reach of efforts.

Objective 3
Develop and implement policies and practices to promote the responsible use of antibiotics.

Objective 3.1
Improve national outpatient antibiotic use.

CDC, DoD
Lower the annual rate of outpatient antibiotic dispensing per 1,000 U.S. population, overall and among specified subpopulations.

CDC, DoD
Lower the annual proportion and rate of antibiotic prescriptions for outpatient visits where antibiotics are not needed (according to evidence-based guidelines) and provide descriptive statistics for trends in unnecessary prescribing patterns.

Objective 3.2
Help healthcare providers adopt recommended antibiotic use practices.

CDC, DoD
Each year, increase the number of facilities and providers that implement CDC's best practices.

Objective 3.3
Support national and State policies that improve the use of antibiotics across healthcare settings and communities.

CDC, CMS
Develop and optimize interpretative guidance for the antibiotic stewardship requirements within the conditions of participation for Medicare and Medicaid programs.

Objective 3.4
Partner with clinical societies to consider options for improving the development, speed, and harmonization of antibiotic use and diagnostic guidelines that reflect clinical and public health needs for major syndromes.

CDC
Initiate at least one coordinated effort to improve antibiotic or diagnostic guidelines by 2021.

Objective 3.5

Support research to improve the responsible use of antibiotics across settings and translate important findings into practice.

AHRQ, CDC, DoD
Increase research on the responsible use of antibiotics and translate significant findings into practice.

Objective 3.6

Evaluate data on antibiotic use and stewardship practices in production animal species, including cattle, swine, poultry, goats, and sheep.

APHIS
Publish information on relevant practices by 2021.

Objective 3.7

Engage the animal health community, crop protection community, and other relevant stakeholders to advance strategies intended to foster the responsible use of medically important antibiotics in plants and animals.

FDA, CDC, EPA
Develop and implement strategies by 2025.

Objective 4

Develop and implement evidence-based policies and practices to prevent infections and stop the spread of antibiotic resistance across One Health.

Objective 4.1

Support further prevention of healthcare-associated infections (HAIs) prioritized in the National HAI Action Plan.

AHRQ, CDC, CMS, DoD
Meet the targets identified in the National HAI Action Plan.

Objective 4.2

Support national and State policies to help prevent HAIs and stop the spread of antibiotic resistance within and between settings and communities.

CDC, CMS
Develop and optimize guidance for improving infection control standards across healthcare settings.

Objective 4.3

Promote biosecurity practices on farms and other animal care facilities to reduce the risk from antibiotic-resistant pathogens.

APHIS
Develop updated biosecurity educational materials by 2022.

Objective 4.4

Collect information about biosecurity practices on farms to optimize educational materials about biosecurity for different industries.

APHIS
Report results of biosecurity data from National Animal Health Monitoring System from 2019 (Goats) and 2021 (Feedlot, Swine) by 2022.

Objective 4.5

Increase research on infection prevention and the emergence and spread of antibiotic resistance and use this research to prevent infections and the spread of antibiotic resistance.

AHRQ, CDC, DoD
Increase research in this area and translate significant findings into practice.

Goal 2

Strengthen National One Health Surveillance Efforts to Combat Resistance

Antibiotic resistance is unquestionably a One Health issue, impacting the health of humans, animals, plants, and the environment. Efforts to identify antibiotic-resistant organisms, track the spread of resistance, and measure the effect of antibiotic use require surveillance across human, animal, and plant populations and the environment, as well as collaboration from the U.S. Government agencies and partners across each of these settings. The following objectives aim to create a stronger coordinated national One Health effort with more extensive surveillance of antibiotic use and resistance to combat this threat.

Anticipated Challenges

Challenges to strengthening the national infrastructure for surveillance of antibiotic use and resistance could arise when encouraging local, State, and private partners and stakeholders to collect and share data across the human, animal, plant, and environmental (e.g., water, soil) sectors. Enhancing training and testing capacities will require laboratories to maintain ongoing support for staff, continuously maintain their testing equipment, and advance their testing methodologies. The cost-effectiveness and quality of testing (including whole-genome sequencing) depends on appropriate assignment of laboratory roles and responsibilities in accordance with expertise and capacity. Improving electronic surveillance will be necessary for efficient, timely, and consistent submission of data from frontline sources to and across Federal Departments and Agencies. Many challenges are inherent to these activities, such as those associated with sharing electronic data on antibiotic use and resistance, developing and implementing minimum data-quality standards of measurement, and ensuring enough resources to support isolate and data repositories. Federal Departments and Agencies will need to write new policies and processes for the secure and confidential storage and sharing of data. Success in these activities will require extensive, coordinated, and comprehensive efforts by all partners across One Health.

Objectives begin on next page.

Objective 1

Strengthen testing and training capacities and capabilities, enhance integration and harmonization of testing data, and expand the reach of Federal antibiotic resistance laboratory networks across One Health.

Objective 1.1

Expand surveillance through existing systems to monitor antibiotic resistance from multiple sources across One Health.

CDC, FDA, APHIS, FSIS
Increase the amount of laboratory testing for antibiotic resistance, the number of isolates accompanied by test results and available data, and the number of different specimen sources and specimen types collected.

DoD/GEIS, MRSN
Submit all identified multidrug-resistant bacterial and fungal isolates of concern (e.g., antibiotic-resistant pathogens identified in the CDC 2019 AR Threats Report) from DoD Defense Health Agency Medical Centers for centralized and standardized genetic characterization at the Multidrug-Resistant Organism Repository and Surveillance Network (MRSN) by 2023.

Objective 1.2

Increase whole-genome sequencing and antibiotic resistance phenotypic and genotypic testing in laboratory networks for antibiotic-resistant pathogens listed in CDC's 2019 AR Threats Report and upload sequenced data to the National Institutes of Health (NIH) National Center for Biotechnology Information at the National Library of Medicine or to other approved, secure, and widely accessible databases.

CDC, APHIS, DoD/MRSN
Increase percentage of isolates with test results and uploaded sequence data.

Objective 1.3

Establish an accelerator program to advance implementation of whole-genome sequencing, metagenomics, and other molecular testing for antibiotic-resistant pathogens in humans, animals, plants, and the environment and to coordinate training guidance across agencies and among public and private organizations.

CDC, DoD
Establish at least one collaboration through this program to enhance whole-genome sequencing or metagenomics techniques by 2022.

Objective 2

Continue expanding and improving access to specimen and data repositories for research and innovation.

Objective 2.1

Expand the contents of current repositories across One Health of bacterial and fungal strains and their associated genotypic, phenotypic, and descriptive data and, where possible, improve and increase the accessibility, transparency, interoperability, security, storage, and utility of these data.

CDC, FDA, NIH, APHIS, FSIS, DoD/MRSN, EPA
Increase the number of isolates, panels, and data available and relevant publications in the scientific literature.

Objective 2.2

Support and expand efforts to provide rapid, accurate, and comprehensive access to antibiotic-resistant isolates, integrated data sources (including genomic, phenotypic, and functional data), and up-to-date computational analysis tools, and improve adherence to the "FAIR" (findability, accessibility, interoperability, and reusability) principles for scientific data management and stewardship.

NIH
Award new grants that support access to data and computational tools focused on antibiotic resistance.

NIH
Offer training opportunities and outreach for FAIR principles.

Objective 2.3

Through the National Antimicrobial Resistance Monitoring System (NARMS) and the Veterinary Laboratory Investigation and Response Network (Vet-LIRN), contribute antibiotic-resistant isolates from food and animals to the existing CDC and FDA AR Isolate Bank.

CDC, FDA, FSIS
Establish mechanisms for sharing food and animal isolates by 2021.

Objective 2.4

Migrate DoD's bacterial and fungal genome sequencing data and associated phenotypic data to a secure, cloud-based or equivalent environment, to allow authorized Federal users to access pathogen data.

DoD
Identify suitable storage solutions that will satisfy access requirements by 2021.

Objective 3

Strengthen the national infrastructure for antibiotic resistance surveillance data across One Health, by improving capacity, utility, timeliness, and the use of harmonized terminology.

Objective 3.1

Expand the number of sources for and quantity of antibiotic resistance surveillance data collected from inpatient healthcare facilities.

CDC, CMS, DoD, VA
Explore interagency collaborations to examine options for increased reporting to the CDC National Healthcare Safety Network (NHSN) Antibiotic Resistance Option.

CDC, DoD/MRSN, VA
75 percent of acute care hospitals, 100 percent of DoD hospitals, 100 percent of applicable VA hospitals that have transitioned to the VA's updated electronic health record, and 25 percent of critical access hospitals, reporting to the NHSN Antibiotic Resistance Option.

DoD/MRSN, VA
Expand DoD and VA collaborations to increase the number of VA medical centers submitting multidrug resistance data or isolates from multidrug-resistant pathogens to the MRSN

Objective 3.2

Expand the number of sources for and quantity of community-transmitted antibiotic resistance surveillance data from humans including sexually-transmitted infections, enteric diseases, respiratory illness, and other diseases caused by antibiotic-resistant pathogens.

CDC, DoD/GEIS
Each year, increase the number of human isolates collected and analyzed.

Objective 3.3

Expand the number of sources for and quantity of antibiotic resistance surveillance data from animals, farms, and production facilities.

CDC, FDA, NIH, APHIS, FSIS
Increase the number of animal, feed, or food isolates collected, analyzed, and used for prevention and response efforts.

Objective 3.4

Establish new capacities for collecting antibiotic resistance data from the environment, including water and soil.

CDC, FDA, ARS, EPA, U.S. Geological Survey (USGS) within the U.S. DoI
Establish at least two projects to expand antibiotic resistance data collection from the environment, including national-scale testing of surface waters as part of NARMS by 2022.

Objective 3.5

Establish a platform for more comprehensive understanding of the carriage of antibiotic resistance genes (also known as the resistome) present across One Health.

CDC, USGS
Establish a pilot sampling strategy to collect healthy human, animal, plant, and environmental specimens and epidemiological data by 2023.

Objective 4
Strengthen the national infrastructure for antibiotic use surveillance data across One Health, by improving capacity, utility, timeliness, and the use of harmonized terminology.

Objective 4.1
Expand the number of sources for and quantity of surveillance data on the use of antibiotics from inpatient and outpatient healthcare facilities to improve understanding and implementation of the optimal use of antibiotics.

CDC, CMS, DoD, VA
Explore interagency collaborations to examine options for increased reporting to the CDC National Healthcare Safety Network (NHSN) Antibiotic Resistance Option.

CDC, DoD
100 percent of acute care and 50 percent of critical access hospitals reporting to the CDC NHSN Antibiotic Use Option.

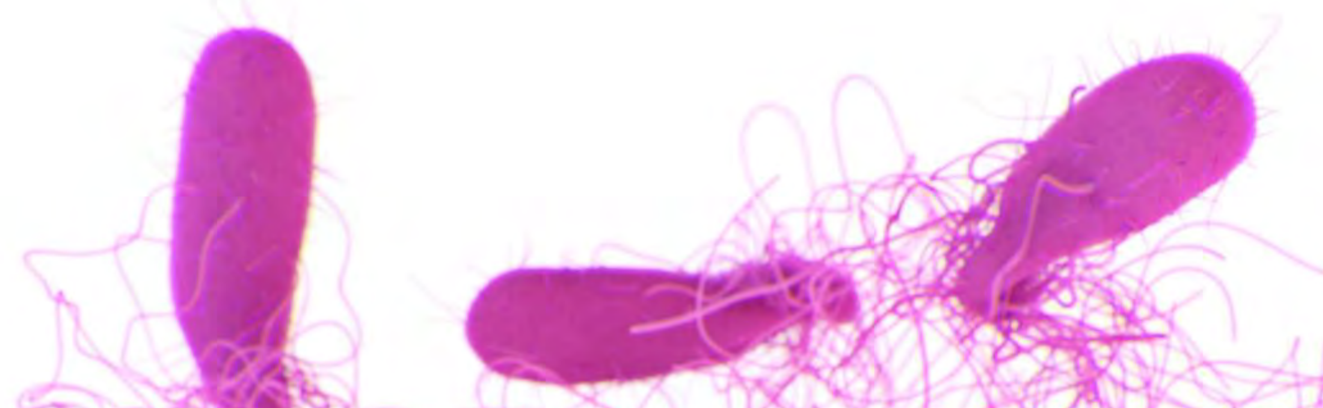
CDC
Improve timelines of annual outpatient antibiotic use tracking and reporting by 2021.

CDC, DoD/PVC
Implement tracking of antibiotic use in all DoD Military Health System facilities, using the Standardized Antimicrobial Administration Ratio (based on observed inpatient antimicrobial days of therapy), by 2021.

DoD
Increase the percentage of optimal antibiotic prescriptions in the DoD Military Health System.

Objective 4.2
Develop new or expand the number of sources for and quantity of surveillance data on the use of antibiotics collected from animals, farms, and production facilities to improve understanding and implementation of responsible use of antibiotics.

FDA, APHIS
Increase published reports and dashboards on antibiotic use in animals.



Goal 3

Advance Development and Use of Rapid and Innovative Diagnostic Tests for Identification and Characterization of Resistant Bacteria

New diagnostic tests are urgently needed to detect antibiotic resistance and to improve surveillance, the control of infections, and treatment decision-making by providers. One major impediment to introducing new diagnostics is a lack of research on their appropriate use in clinical and veterinary care and a lack of point-of-care antibiotic resistance diagnostics in outpatient settings. More information is needed to determine the impact of diagnostics on improving the use of antibiotics and related outcomes in humans and animals, to identify circumstances in which specific diagnostics improve outcomes, and to identify best practices for integrating diagnostics into relevant decision-making processes. Finally, there is a critical need to leverage existing capabilities to promote the validation, adoption, and appropriate use of new and currently available diagnostics.

Anticipated Challenges

When companies develop new diagnostic tests, they face challenges such as the high cost of some components of the tests, technical difficulties in preparing and obtaining clinical samples, and pathogen-drug interactions. Limited return on investment for new diagnostics is also a significant challenge. Determining the appropriate use of new and existing diagnostic tools requires an engaged response from the research community. Once diagnostics are developed, stimulating their appropriate adoption and use requires the creation of evidence-based guidelines and appropriate reimbursement policies, an often protracted and complex process.

Objective 1
Develop and validate new diagnostics.

Objective 1.1

Develop new or enhance existing diagnostics that use isolates and primary samples to determine the presence, severity, or antimicrobial susceptibility or resistance of bacterial or fungal infections and to identify appropriate treatment.

ASPR/BARDA, CDC, FDA, NIH, ARS, DoD
Support 10 new antibiotic resistance-related diagnostics projects across the U.S. Government by 2021, through funding or scientific or technical support.

Objective 2
Support research to determine the appropriate use of diagnostics.

Objective 2.1

Stimulate research to better understand the appropriate use of diagnostics to determine the presence, severity, or antimicrobial susceptibility or resistance of bacterial or fungal infections in human and veterinary care.

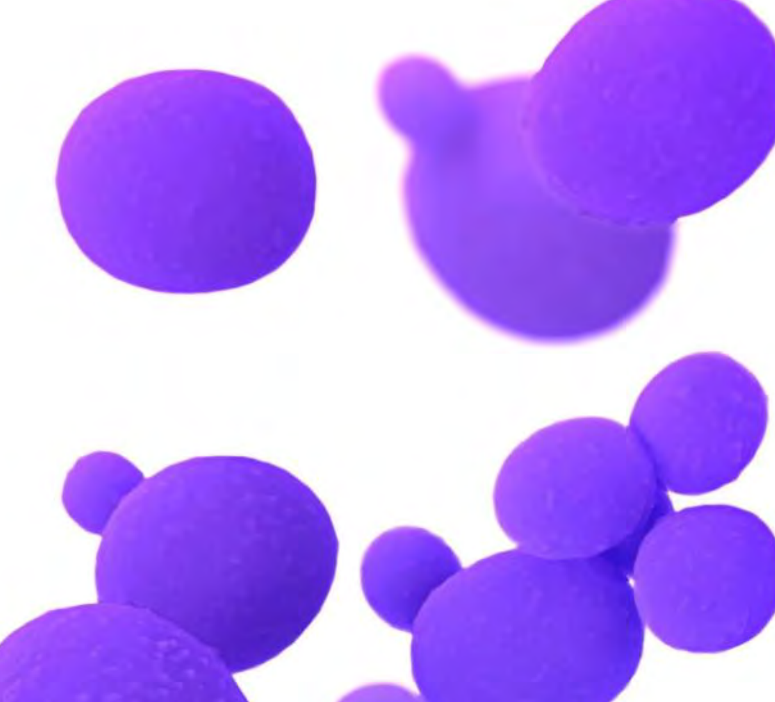
AHRQ, CDC, NIH, DoD/MIDRP
Invite research applications and support research on the appropriate use of CARB-related diagnostics in human clinical and veterinary care.

Objective 3
Stimulate the appropriate adoption and use of diagnostics.

Objective 3.1

Develop evidence-based guidance to promote the appropriate use of new diagnostics and to improve the use of existing diagnostics that determine the presence, severity, or antimicrobial susceptibility or resistance of bacterial or fungal infections in human clinical care.

CDC, FDA
Support the development of evidence-based guidelines for the use of new and existing antibiotic and antifungal resistance-related diagnostics.



Goal 4

Accelerate Basic and Applied Research and Development for New Antibiotics, Other Therapeutics, and Vaccines

Antibiotic effectiveness is continually reduced through the evolution of antibiotic resistance, requiring support for basic and applied research as part of a comprehensive One Health strategy. Research can improve our understanding of the many factors that contribute to the emergence, spread, and persistence of antibiotic resistance and can support new strategies for preventing and mitigating infections. Prolonging the effectiveness of an existing drug depends on research to determine its optimal dose, duration, regimen, and drug combinations. The pipeline of new antibiotics must be continually primed through discovery and development research. In addition, research on alternatives to antibiotics, including bacteriophages, monoclonal antibodies, immune modulators, and phytochemicals, suggests that these products can help prevent and treat infections in humans and animals without promoting antibiotic resistance. Effective vaccines that prevent infection may also reduce use of antibiotics, thereby avoiding resistance. Research on other innovative products (e.g., live biotherapeutics, including microbiome-based products, prophylactic monoclonal antibodies, and decolonizing agents) could expand the range of strategies and help reduce the impact of antibiotic resistance. Developing therapeutic and preventative products (including those directed at decolonization) requires intensified efforts to support basic research, turn discoveries into products, and facilitate clinical trials. The final objective of Goal 4 aims to promote the economic sustainability of the antibiotics market through collaboration with the private sector.

Anticipated Challenges

Across One Health, the multi-year process to develop new antibiotics, therapeutics, and vaccines includes a high rate of attrition within the discovery pipeline. Discovery of new classes of antibiotics with activity against gram-negative bacteria is also very challenging, and the development pathways for most non-antibiotic therapeutics remain uncharted. Additionally, the lag time between completing and publishing the results of basic and applied research studies can delay their real-world impact.

In addition to the targeted objectives of Goal 4 listed below, the U.S. Government will pursue several broad activities to accelerate research on antibiotic resistance.

Activity 1
Enhance basic research on antibiotic resistance mechanisms, as well as translational and clinical research on therapeutics, vaccines, and diagnostics.

NIH, ARS, NIFA
Support at least 1,000 publications focused on basic, translational, and clinical research to combat antibiotic resistance by 2021.

Activity 2
Support the training of new investigators and new entrants in the field to improve research capacity on antibiotic resistance.

NIH, ARS, NIFA
Provide support to at least 60 new or early-career investigators by 2021.

Activity 3
Enhance interagency collaborations to accelerate basic and applied research for developing new antibiotics, therapeutics, and vaccines.

ASPR/BARDA, CDC, FDA, NIH, ARS, DoD
Establish at least two new collaborations for human health and one for agriculture by 2021, through interagency agreements, collaborative programs, and interdisciplinary workshops.

Objective 1
Expand basic and applied interdisciplinary research to better understand the emergence, spread, and persistence of antibiotic resistance, and develop mitigation strategies for antibiotic resistance in human, animal, agricultural, and environmental settings.

Objective 1.1
Advance our understanding of the emergence, spread, and persistence of antibiotic resistance.

CDC, NIH, ARS, NIFA
Report success stories to disseminate new knowledge about antibiotic resistance and inform mitigation strategies in human health (at least two stories) and agriculture (at least one story) by 2021.

Objective 2
Intensify basic, translational, and clinical research to support the discovery and development of new treatments, including antibiotics, non-traditional therapeutics, and optimized treatment regimens.

Objective 2.1
Support the discovery and preclinical development of new therapeutics.

ASPR/BARDA, NIH
Award 100 new projects (e.g., grants, contracts, CARB-X awards) aimed at therapeutic discovery or development by 2024.

DoD
Identify one candidate therapeutic for bacterial infections in human medicine for further research and development by 2022.

ARS, NIFA
Identify one candidate therapeutic for bacterial infections in agriculture for further research and development by 2021.

NIH, ARS, NIFA, DoD
Report success stories about additional therapeutic options for human health (at least 5 stories) and agriculture (at least 1 story) by 2021.

Objective 2.2
Support clinical research into and development of new treatments, including antibiotics, non-traditional therapeutics, and optimized treatment regimens.

ASPR/BARDA, NIH, DoD
Facilitate development of 10 novel potential therapeutics for bacterial infections in humans by 2022.

FDA
Provide guidance on regulatory requirements, including clinical trial designs and other relevant topics.

ASPR/BARDA
Support New Drug Application (NDA) filings for three new therapeutics to treat bacterial infections in humans by 2025.

Objective 2.3

Provide specimens, testing, data, and evaluations to collaborations aimed at developing new agents or older agents for new uses and to support establishment or revision of antibiotic-susceptibility testing standards.

CDC, DoD/WRAIR
Establish at least two projects supporting the development of new agents and standards by 2021.

Objective 3

Intensify basic, translational, and clinical research to support the discovery and development of new preventative products or strategies.

Objective 3.1

Support the discovery and development of new preventative strategies.

ASPR/BARDA, NIH
Award 25 new projects aimed at discovering or developing new preventative products for use in human medicine by 2022.

ARS, NIFA
Support two candidate preventative agents for agricultural uses by 2021.

CDC, NIH, ARS, NIFA
Report success stories about improved preventative strategies for human health (at least two stories) and agriculture (at least one story) by 2021.

Objective 3.2

Clarify pathways for new pharmaceutical preventatives by defining appropriate clinical trial designs, including end points.

CDC, FDA, NIH, DoD
Convene two meetings to discuss developmental pathways and regulatory considerations, including clinical trial designs, by 2023.

Objective 3.3

Facilitate development of vaccines that prevent bacterial and fungal infections with known rates of resistance, and augment existing post-licensure evaluation systems to evaluate vaccination rates and antibiotic or antifungal use and resistant infections over time.

CDC
Establish at least two antibiotic-resistant pathogen-related projects to further vaccine development or uptake by 2022.

CDC
Further support existing active, laboratory, population-based bacterial and fungal monitoring activities to provide vital serotype distribution and resistance data to inform development of vaccine candidates for bacteria or fungi with known resistance.

Objective 4

Enhance efforts to promote sustainability of the commercial market for new antibiotic products.

Objective 4.1

Support the creation of a network of clinical trial sites to reduce barriers to research and to establish a comprehensive understanding of the safety and effectiveness of new antibiotic agents in challenging clinical settings and indications.

FDA
Provide scientific and technical support for establishing the network, including recommendations on platform trial design and other regulatory considerations.

ASPR/BARDA, NIH, DoD/WRAIR/IDCRP
Establish the network and begin enrolling patients by 2023.

Objective 4.2

Examine changes in new technology add-on payments under the CMS Inpatient Prospective Payment System (IPPS) Final Rules, starting with the FY 2020 IPPS/long-term care hospital prospective payment system final rule, to inform potential additional actions.

CMS
Report the number of applications, approvals, and renewals for new technology add-on payments and the estimated amount of those payments.

Objective 4.3

Strengthen commercial markets for antibiotic products through direct Public Health and National Security purchases.

ASPR/BARDA
Acquire antibiotics to ensure national security and to provide revenue to encourage commercialization.

Objective 4.4

Support efforts to secure U.S.-based manufacturing infrastructure.

ASPR/BARDA
Work with innovator companies to generate domestic production of critically needed products and expand U.S.-based manufacturing capabilities.

Goal 5

Improve International Collaboration and Capacities for Antibiotic-resistance Prevention, Surveillance, Control, and Antibiotic Research and Prevention

As outlined in the National Biodefense Strategy, the U.S. Government Global Health Security Strategy, and in accordance with the U.S. Government's engagement through the Global Health Security Agenda, the U.S. Government works to enhance the capacities of governments, civil society, academia, and the private sector in partner countries and the international community to address the emergence, spread, and impact of antibiotic resistance. To do this, rapidly detecting and containing antibiotic-resistant pathogens through enhanced laboratory networks is critical. Several objectives within this goal build on existing international research collaborations to combat antibiotic resistance. Concerted efforts to align resources internationally could support clinical trials to evaluate new products and provide data on the best way to use existing products. These efforts would also promote the translational development of diagnostics, treatments, and vaccines, support research to understand the development and spread of antibiotic resistance, identify risk factors linked to human health outcomes, and generate mitigation strategies.

Anticipated Challenges

Developing global consensus around updates to international guidance can be a difficult and protracted effort. Also difficult is the process of supporting the efforts of partner countries to better identify the emergence and spread of antibiotic resistance. Establishing a well-functioning international network that can detect and respond to antibiotic resistance requires substantial and well-aligned resources, including the ability to tap experts to help with the containment of resistance. Alternatively, a global network could focus on high-value locations, such as those with a high risk for developing outbreaks of new high-threat pathogens and with frequent embarkation points for travel to the U.S.

Objective 1

Enhance U.S. leadership in the global fight against antibiotic resistance.

Objective 1.1

Examine mechanisms for appointing a U.S. Federal Champion for International CARB, who would support the Secretaries of HHS, USDA, DoS, and the Administrator of USAID by advocating for U.S. policy positions on antibiotic resistance at international fora and organizations using a One Health approach, and who would report to the CARB Task Force to inform international engagements.

OGA, USDA, DoD, USAID

Convene a working group of the CARB Task Force to define interagency needs and develop options for appointing a Federal Champion for International CARB by 2021.

Objective 1.2

Enhance engagements with multilateral organizations to support progress on U.S. priorities to combat antibiotic resistance.

OGA, DoS, USDA, USAID
Support international antibiotic resistance policy efforts to prioritize and coordinate antibiotic resistance efforts within and across international partner organizations (e.g., FAO, the G7 and G20, the Asia-Pacific Economic Cooperation Forum, the Global Health Security Initiative, and the UN One Health Global Leaders Group on AMR) by 2022.

OGA
Chair the Global Health Security Agenda AMR Action Package by 2022.

ASPR/BARDA, CDC, FDA, OGA, USDA, NIH
Complete and implement the Work Plan of the Transatlantic Taskforce on Antimicrobial Resistance (TATFAR) for 2021-2025 and develop a new Scope of Work for TATFAR by 2021.

FDA, USDA
Work with international partners through the Codex Alimentarius Commission's Task Force on Antimicrobial Resistance to develop global, science- and risk-based guidance on managing foodborne antimicrobial resistance and surveillance, including revising the Codex Code of Practice to Minimize and Contain Foodborne Antibiotic Resistance and developing new Guidelines for Integrated Surveillance of Antimicrobial Resistance.

CDC, USAID
Continue to support member governments' sharing of antibiotic-resistant pathogen information to the relevant collaborating centers, including to the WHO Global Antimicrobial Resistance Surveillance System (GLASS).

Objective 1.3

Provide additional financial or technical support to public and private organizations to further U.S. priorities to combat antibiotic resistance.

OGA, USAID
Support international policy efforts to reduce antibiotic resistance beyond the current mandates of U.S. Government Departments and Agencies by 2022.

Objective 1.4

Increase the U.S. Government's presence in international organizations and other multilateral efforts to combat antibiotic resistance.

CDC, OGA, USDA, DoD, DoS, USAID
Provide at least one AMR expert either by secondment or appointment to a multilateral organization to enhance the U.S. Government's programmatic collaborations and provide high-level technical and policy guidance by 2022.

Objective 1.5

Enhance domestic and international communications about the U.S. Government's activities to combat antibiotic resistance and increase the coordination of Federal Departments and Agencies on the CARB Task Force around large-scale efforts and announcements.

All Departments and Agencies on the CARB Task Force
Increase coordination among the CARB Task Force on communication strategies by instituting regular calls by 2021.

CDC, OGA, NIH, USDA, DoD, DoS, USAID
Increase high-level social-media promotion of antibiotic-resistance activities among the Departments and Agencies on the CARB Task Force.

Objective 2

Promote increased awareness and capacity in countries to address the emergence and slow the spread of antibiotic resistance.

Objective 2.1

Improve capacity in partner countries to implement effective practices to prevent and control infection, including through the availability and proper use of water, sanitation, and hygiene (WASH).

CDC, FAS, DoS, USAID
Assist governments, civil society, and the private sector in a total of 10-15 low- or middle-income countries to develop national plans or capacity for preventing and controlling infections in both animals and humans by 2022.

CDC, USAID
Assist governments, civil society, and the private sector in 10 to 15 low- or middle-income countries to improve the monitoring of WASH in healthcare facilities or to create and/or implement standards for environmental health in healthcare settings.

Objective 2.2

Optimize the use of antibiotics in humans, animals, and agriculture outside of the U.S.

CDC, USAID
Assist governments, civil society, and the private sector in at least four low- or middle-income countries with capacity-building for antibiotic stewardship and regulation to address the appropriate use and availability of quality-assured antibiotics in humans and animals by 2022.

Objective 2.3

Promote the use of existing and new vaccines, including pneumococcal and typhoid-conjugate vaccines, to reduce the unnecessary use of antibiotics.

CDC, USAID
Promote prevention and vaccine use in low- and middle-income countries, including through the U.S. Government's partnership with Gavi, the Vaccine Alliance, supported by funding and technical assistance from USAID and CDC worldwide.

Objective 2.4

Conduct surveillance that identifies the presence and movement of antibiotic resistance genes of concern within partner nations as part of DoD/GEIS-funded surveillance to protect military force health.

DoD
Submit isolates of multi-drug-resistant pathogens to the MRSN for advanced characterization and provide reports to the labs that can also inform surveillance of antibiotic resistance, by 2021.

Objective 3

Generate consistent and actionable global data on antibiotic resistance, including by extending CDC's AR Lab Network to global sites to address the identification, emergence, spread, and effects of antibiotic resistance.

Objective 3.1

Expand the AR Lab Network and other networks (e.g., PulseNet International) internationally to implement networks for detection and containment that can rapidly test and respond to high-threat antibiotic-resistant pathogens in key regions.

CDC
Launch at least one international AR Lab Network project and make operational at least one international AR Lab Network laboratory by 2022. Incorporate five additional laboratories by 2026.

Objective 3.2

Charge the global AR Lab Network with detecting and containing new and critical antibiotic-resistance threats.

CDC, DoD
Establish the capacity of the global AR Lab Network to receive and test isolates and deploy rapid responses to control and contain infections.

Objective 3.3

Identify innovative and effective strategies for stopping the spread of antibiotic-resistant pathogens in low- and middle-income countries.

CDC
Establish "learning laboratories" through the AR Lab Network to develop or test innovative, cost-effective solutions for containing critical-threat antibiotic-resistant pathogens by 2021.

Objective 3.4

Improve the standardization of laboratory methodologies and data collection to improve the quality, reliability, and utility of data to facilitate global comparisons of antibiotic resistance.

CDC, DoD
Implement standardized or harmonized laboratory methods and data collection in AR Lab Network facilities to facilitate comparison of antibiotic-resistance trends when appropriate. Initiate data-reporting efforts with trusted partner nations by 2021.

Objective 3.5

Expand overseas screening of long-term visitors to the U.S. (e.g., international workers and students) from high-risk countries to prevent the importation of cases of multidrug-resistant tuberculosis.

CDC, DoS
Pilot screening in five countries by 2021. Expand to 45 countries by 2025.

Objective 4
Increase international collaborations to facilitate basic, translational, and clinical research into understanding the causes of antibiotic resistance and developing countermeasures.

Objective 4.1

Collaborate with international scientists and organizations to better understand the development, spread, and health risks of antibiotic resistance and resistance sources present in animals, the environment, the community, and healthcare settings.

CDC, ARS, DoD/GEIS/WRAIR, USAID
Conduct research and/or surveillance projects to evaluate sources of antibiotic resistance and mechanisms of persistence, with a focus on animal and environmental systems by 2023.

Objective 4.2

Promote the alignment of U.S. and international translational and clinical research activities to facilitate the development of new products to better diagnose, prevent, and treat infections or to provide data on the best use of existing products.

ASPR/BARDA, NIH
Report one success story about products or regimens undergoing preclinical or clinical testing by 2021.

FDA
Convene a meeting with international regulators to seek alignment on clinical trial designs for new products by 2023.

SECTION 3
APPENDICES

Appendix A: Data Development Objectives

These objectives aim to develop new or improved data infrastructure, collection, or analysis techniques.

Goal 1	• Objective 3.4: Partner with clinical societies to consider options for improving the development, speed, and harmonization of antibiotic use and diagnostic guidelines that reflect clinical and public health needs for major syndromes.
Goal 2	• Objective 1.3: Establish an accelerator program to advance implementation of whole-genome sequencing, metagenomics, and other molecular testing for antibiotic-resistant pathogens in humans, animals, plants, and the environment and to coordinate training guidance across agencies and among public and private organizations. • Objective 2.1: Expand the contents of current repositories across One Health of bacterial and fungal strains and their associated genotypic, phenotypic, and descriptive data and, where possible, improve and increase the accessibility, transparency, interoperability, security, storage, and utility of these data. • Objective 2.3: Through the National Antimicrobial Resistance Monitoring System (NARMS) and the Veterinary Laboratory Investigation and Response Network (Vet-LIRN), contribute antibiotic-resistant isolates from food and animals to the existing CDC and FDA AR Isolate Bank. • Objective 3.4: Establish new capacities for collecting antibiotic resistance data from the environment, including water and soil. • Objective 3.5: Establish a platform for more comprehensive understanding of the carriage of antibiotic resistance genes (also known as the resistome) present across One Health. • Objective 4.2: Develop new or expand the number of sources for and quantity of surveillance data on the use of antibiotics collected from animals, farms, and production facilities to improve understanding and responsible use of antibiotics.
Goal 3	• No Data Development strategies for Goal 3.
Goal 4	• Objective 3.3: Facilitate development of vaccines that prevent bacterial and fungal infections with known rates of resistance, and augment existing post-licensure evaluation systems to evaluate vaccination rates and antibiotic or antifungal use and resistant infections over time.
Goal 5	• Objective 3.1: Expand the AR Lab Network and other networks (e.g., PulseNet International) internationally to implement detection and containment networks that can rapidly test and respond to high-threat antibiotic-resistant pathogens in key regions.

Appendix B: Challenges

Anticipated Challenges to Implementing the National Action Plan for CARB, 2020-2025

As the Task Force drafted the 2020 Plan, its members reviewed the barriers faced in the past five years while implementing the 2015 Plan and anticipated challenges over the next five years. Certain challenges apply to all five goals, including the allocation of limited resources, obstacles to data gathering and sharing, and uncertainty about the participation of research and industry partners. Implementing the activities outlined in this plan will depend on the availability of resources and capacity. The COVID-19 pandemic has necessitated redirection of public-health, infection-control, and antibiotic stewardship resources and will continue to affect implementation of the activities described here. In future annual progress reports, the CARB Task Force will discuss challenges encountered during the preceding year, how it addressed these challenges, and any new challenges identified.

Goal 1: Slow the Emergence of Antibiotic-Resistant Bacteria and Prevent the Spread of Resistant Infections

Prevention and containment efforts, along with improved use of antibiotics, can slow the emergence and spread of antibiotic resistance genes and antibiotic-resistant pathogens and can limit their impact on humans and animals. However, many challenges to these efforts exist. Some of these challenges are related to changing behaviors to ensure optimal infection-control practices and appropriate prescribing of antibiotics. Others could be part of identifying and scaling up best practices across spectrums of care, ensuring their continuity, and coordinating these practices across One Health. Still others could be related to engaging all relevant stakeholders for buy-in and support of best practices.

Goal 2: Strengthen National One Health Capacity and Surveillance Efforts to Combat Antibiotic Resistance

Challenges to strengthening the national infrastructure for surveillance of antibiotic use and resistance could arise when encouraging local, State, and private partners and stakeholders to collect and share data across the human, animal, plant, and environmental (e.g., water, soil) sectors. Enhancing training and testing capacities will require laboratories to maintain ongoing support for staff, continuously maintain their testing equipment, and advance their testing methodologies. The cost-effectiveness and quality of testing (including whole-genome sequencing) depends on appropriate assignment of laboratory roles and responsibilities in accordance with expertise and capacity. Improving electronic surveillance will be necessary for efficient, timely, and consistent submission of data from frontline sources to and across Federal Departments and Agencies. Many challenges are inherent to these activities, such as those associated with sharing electronic data on antibiotic use and resistance, developing and implementing minimum data-quality standards of measurement, and ensuring enough resources to support isolate and data repositories. Federal Departments and Agencies will need to write new policies and processes for the secure and confidential storage and sharing of data. Success in these activities will require extensive, coordinated, and comprehensive efforts by all partners across One Health.

Goal 3: Advance the Development and Use of Rapid and Innovative Diagnostic Tests for Identifying and Characterizing Antibiotic-Resistant Bacteria

When companies develop new diagnostic tests, they face challenges such as the high cost of some components of the tests, technical difficulties in preparing and obtaining clinical samples, and pathogen-drug interactions. Limited return on investment for new diagnostics is also a significant challenge. Determining the appropriate use of new and existing diagnostic tools requires an engaged response from the research community. Once diagnostics are developed, stimulating their appropriate adoption and use requires the creation of evidence-based guidelines and appropriate reimbursement policies, an often protracted and complex process.

Goal 4: Accelerate Basic and Applied Research to Develop New Antibiotics, Therapeutics, and Vaccines

Across One Health, the multi-year process to develop new antibiotics, therapeutics, and vaccines includes a high rate of attrition within the discovery pipeline. Discovery of new classes of antibiotics with activity against gram-negative bacteria is also very challenging, and the development pathways for most non-antibiotic therapeutics remain uncharted. Additionally, the lag time between completing and publishing the results of basic and applied research studies can delay their real-world impact.

Goal 5: Improve International Collaboration and Capacities for Preventing, Tracking, and Controlling Antibiotic Resistance and for Antibiotic Research and Development

Developing global consensus around updates to international guidance can be a difficult and protracted effort. Also difficult is the process of supporting the efforts of partner countries to better identify the emergence and spread of antibiotic resistance. Establishing a well-functioning international network that can detect and respond to antibiotic resistance requires substantial and well-aligned resources, including the ability to tap experts to help with the containment of resistance. Alternatively, a global network could focus on high-value locations, such as those with a high risk for developing outbreaks of new high-threat pathogens and with frequent embarkation points for travel to the U.S.

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PLOS GLOBAL PUBLIC HEALTH

OPINION

Diagnostic tests to mitigate the antimicrobial resistance pandemic—Still the problem child

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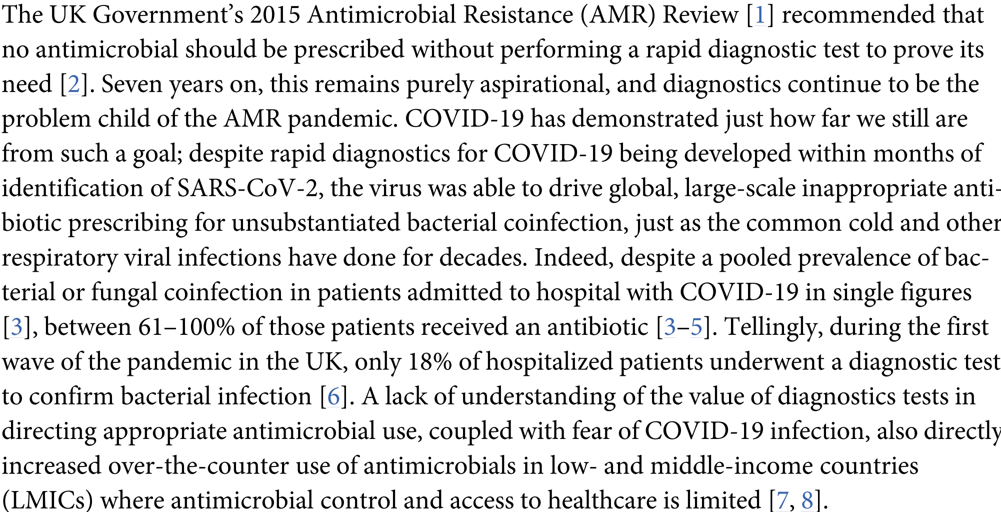

The UK Government's 2015 Antimicrobial Resistance (AMR) Review [1] recommended that no antimicrobial should be prescribed without performing a rapid diagnostic test to prove its need [2]. Seven years on, this remains purely aspirational, and diagnostics continue to be the problem child of the AMR pandemic. COVID-19 has demonstrated just how far we still are from such a goal; despite rapid diagnostics for COVID-19 being developed within months of identification of SARS-CoV-2, the virus was able to drive global, large-scale inappropriate antibiotic prescribing for unsubstantiated bacterial coinfection, just as the common cold and other respiratory viral infections have done for decades. Indeed, despite a pooled prevalence of bacterial or fungal coinfection in patients admitted to hospital with COVID-19 in single figures [3], between 61–100% of those patients received an antibiotic [3–5]. Tellingly, during the first wave of the pandemic in the UK, only 18% of hospitalized patients underwent a diagnostic test to confirm bacterial infection [6]. A lack of understanding of the value of diagnostics tests in directing appropriate antimicrobial use, coupled with fear of COVID-19 infection, also directly increased over-the-counter use of antimicrobials in low- and middle-income countries (LMICs) where antimicrobial control and access to healthcare is limited [7, 8].

Lack of diagnostics, rapid or otherwise, also continues to challenge our understanding of the true global burden of AMR. A recent report from the Global Research on Antimicrobial Resistance (GRAM) project in *The Lancet* estimated that 4.95 million deaths were associated globally with bacterial AMR in 2019. Of these, 1.27 million deaths–more than HIV/AIDS and malaria combined–were directly attributable to resistance [9]. This estimate, based on statistical modelling of over 470 million pieces of global data, has been hailed as the most robust and comprehensive estimate of the global burden of AMR to date, but is likely just the tip of the iceberg. As acknowledged by the GRAM report's authors, AMR surveillance data from many LMICs are extremely limited; although the statistical model attempted to account for this, it is probable that the burden of death from AMR in LMICs was vastly underestimated, similar to the underestimation of COVID-19 deaths. Furthermore, AMR deaths are projected to increase over time [10], and the ever-present challenge of delivering a health service without effective antimicrobials speaks to the enormous morbidity that AMR is capable of reaping, along with high mortality.

To date, investment in diagnostics to aid in mitigating AMR has largely focused on increasing laboratory surveillance capacity. While AMR surveillance data from LMICs remains limited, the Fleming Fund and other investors in laboratory capacity have helped to improve data availability [11]. The effect of this on appropriate prescribing at the front line, however, remains undetermined. In regions where most prescribing is empiric, such as South East Asia

Table 1. Examples of commercially available automated platforms for identification of bloodstream infections, antimicrobial resistance, or susceptibility testing.

Company	Product name	Purpose	Reason for unsuitability to LMICs
Accelerate Dx	Accelerate Pheno	ID & AST	High cost, low throughput
Abacus Diagnostica	GenomEra CDX system	Only MRSA/SA & *Streptococcus pneumoniae*	Limited panel
bioMérieux	BioFire FilmArray	ID & AMR	High cost
Cepheid, a subsidiary of Danaher	GeneXpert	Only MRSA/SA	Limited panel
Curetis	Unyvero System	ID & AMR	High cost, multiple instruments
GenMark Diagnostics	ePlex System	ID & AMR	High cost
GENOMICA S.A.U.	CLART technology	ID & AMR	High complexity
iCubate	iC System	ID & AMR	Limited panel
Luminex/Nanosphere	Verigene	ID & AMR	High cost, separate sample preparation
Master Diagnóstica	Sepsis Flow Chip	ID & AMR	Not integrated, large instrument
OpGen	AdvanDx PNA FISH	ID	ID only, manual workflow, limited panel
Q-linea	ASTar	AST	High cost, AST only
QuantaMatrix	QMAC dRAST	AST	High cost, AST only
Vela Diagnostics	Great Basin Analyser System	*Staphylococcus* species ID & AMR	Limited panel

AMR, antimicrobial resistance (genotypic); AST, antimicrobial susceptibility testing (phenotypic); ID, identification; MRSA, methicillin-resistant *Staphylococcus aureus*; SA, *Staphylococcus aureus*.

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and Sub-Saharan Africa, there remains an urgent need for point-of-care rapid diagnostics if we are to have an impact on misuse of antimicrobials in human health [12]. A case in point is the appropriate use of antimicrobials for children presenting with undifferentiated fever in low-resource settings where bacterial, viral, and malarial infections predominate. C-reactive protein (CRP), the most commonly used diagnostic test to differentiate bacterial from viral infections, is non-specific and may be elevated in a number of non-bacterial infections including malaria and severe dengue fever [13, 14], and with particular relevance to the current global pandemic, COVID-19 [15, 16]. Eight years since the launch of the Longitude Prize, which aims to reward the first innovators to develop a point-of-care diagnostic test that will conserve antimicrobials and is accurate, rapid, affordable and easy to use anywhere in the world, we are still waiting for a winner [17]. Current commercially available diagnostics (Table 1) are not fit-for-purpose in low-resource settings, where diagnostics to drive antimicrobial stewardship is most needed. Although COVID-19 has led to a dramatic increase in capacity for molecular testing in many countries, which can be repurposed to improve access to diagnostics for AMR as well as other common diseases as the pandemic wanes, cost and logistical considerations limit use of such tests at the primary and community healthcare levels.

Investment in diagnostic development for AMR remains substantially below that for new antimicrobials [18]. Furthermore, access to diagnostic tests at the primary healthcare level in LMICs is poor [19], and tests for infections other than HIV, tuberculosis and malaria must often be paid for by the user, incurring out-of-pocket expenses that are rarely affordable. The Access to COVID-19 Tools (ACT)-Accelerator, a global collaboration to accelerate development, production, and equitable access to COVID-19 tests, treatments and vaccines, was able to reduce the cost of COVID-19 rapid tests to LMICs to less than US$3 (and in some instances less than US$1 [20]), and has provided funding for procurement of at least 128 million diagnostic tests [21]. As the cost of taking global action against AMR will be high (up to $40 billion US dollars per decade as estimated by the O'Neill report [1]), programmes similar to the ACT-Accelerator are likely to be required to support mitigating AMR in LMICs.

Distinguishing between bacterial and viral infections is just one enabler of antimicrobial stewardship. Another is identifying the causative pathogen and understanding its resistance profile. Nucleic acid-based tests have revolutionized the field of diagnostics, enabling clinicians to identify pathogens and their resistance genes in a 'plug and play' system within one hour [22]. Next-generation sequencing (NGS), which has proven to be a powerful tool for identification of SARS-CoV-2 mutations that predict 'resistance' to vaccines or therapies during the COVID-19 pandemic, could provide equally rapid access to information that would change antibiotic management both at the individual and population level. However, the current prerequisite for a positive bacterial culture prior to NGS is a limiting factor that will require a solution, and the perceived lack of commercial incentives and evidence supporting the market potential for AMR diagnostics in LMICs means that current molecular technologies are not designed to withstand the environmental and funding constraints of low-resource settings. Furthermore, interpretation of resistance mutations continues to be a bottleneck, and advancements will require a concerted effort similar to the cataloguing work performed for *Mycobacterium tuberculosis* mutations [23]. Developments in phenotypic automation and microfluidics are progressing but the potential of these technologies for tackling AMR has not yet been fully achieved in clinical settings. Without progress on these fronts, laborious phenotypic determination of antimicrobial susceptibilities will remain the main work effort of most microbiology laboratories.

AMR may not be as palpable a pandemic as COVID-19, but with an annual death toll in the same order of magnitude, it represents a clear and present danger. The same impetus and enthusiasm for investment and prioritization of diagnostics to help end the COVID-19 pandemic needs to be applied to AMR. Without this, Jim O'Neill's ambition [2] of using diagnostics to direct all antimicrobial therapy will remain merely an aspiration, and we will still be counting the cost of the lack of diagnostics at the point-of-care and escalating deaths from AMR into the next decade.

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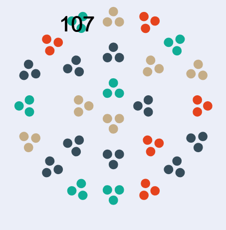

Policy Brief #02: August 2021

Ensuring AMR Diagnostic Innovation & Uptake

Based on the study

Estimating global patient needs and market potential for priority health technologies addressing antimicrobial resistance

Priority Needs for New AMR Diagnostics

The pivotal role that improved diagnoses can play as part of our global response in tackling the growing burden of antimicrobial resistance (AMR) has been repeatedly emphasised[1], yet progress so far remains limited[2].

DX ROLES IN THE AMR RESPONSE

- Improve when & how we use antibiotics
- Lower antibiotic demand – preserving antibiotic efficacy
- Reduce the urgency to develop new antibiotics
- Improve & speed-up development of new antibiotics
- Improve surveillance & knowledge of AMR

The patent-based system links diagnostic development to its commercialization in one chain. Similar to antibiotics, AMR diagnostics face post-launch challenges in: a) how much successful development will be rewarded and b) securing health system uptake. The priority diagnostic needs that were identified and quantified in this study[3] are summarised in '**EAG's Prioritised Diagnostics**'.

Modelling AMR Markets & Needs

A multidisciplinary, global, Expert Advisory Group (EAG) was formed to identify – initially through a human patient-need led approach – some of the highest AMR patient needs for new health technologies. The four profiles (2 x diagnostics [Dx]; 2 x antibiotics [Tx]) were then quantified in terms of the need (patient numbers) and market potential (peak revenues) to generate global (80% of the world) forecasts to 2040[3].

The Dx workstream adopted a bottom-up, static model combining patient estimates with 'eligibility' (consideration of the diagnostic context) prior to conversion to revenues. Alternative scenarios were used to explore the high uncertainty of outputs given the weak data availability & hypothetical need profiles.

EAG'S PRIORITISED DIAGNOSTICS

Rapid near-patient globally-applicable culture-independent tests from a single sample to:

- **Dx1 (Bac. vs other):** Determine if an infection is bacterial or not. *To inform whether an antibiotic should be prescribed.*
- **Dx2 (ID/susceptibility):** Rapidly identify the pathogen/s and susceptibility (lack of resistance) for priority MDR Gram negative bacteria. *To inform the use of targeted treatment at initiation.*

- Recent work by the World Health Organisation (WHO)[4,5] & other actors was taken into consideration in determining these need profiles
- The profiles here enabled the quantification to be performed & are not full target product profiles (TPPs)[6] intended for developers

Meeting Priority Dx Needs will be Modestly Rewarded

Global revenue is determined by the total amount all market buyers (healthcare payors) are able, or willing, to pay. Under current market conditions, revenue forecasts for these two aspirational diagnostics are similarly modest at around **$400m** by 2040 (i.e., 15 years after launch), with development costs in the range of $20–150m[7].

Forecasted global market potentials of ~$400m, indicate a private value far below their social value

The modest revenues and forecasted uptake of these critical new devices is largely due to a: 1) competitive market landscape and 2) weak rationale (use case) for physicians to use them over empirical antibiotic use. The unfavourable cost/benefit balance grows outside high income countries (HICs).

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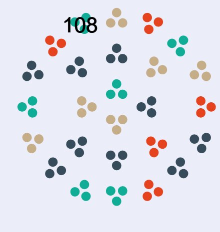


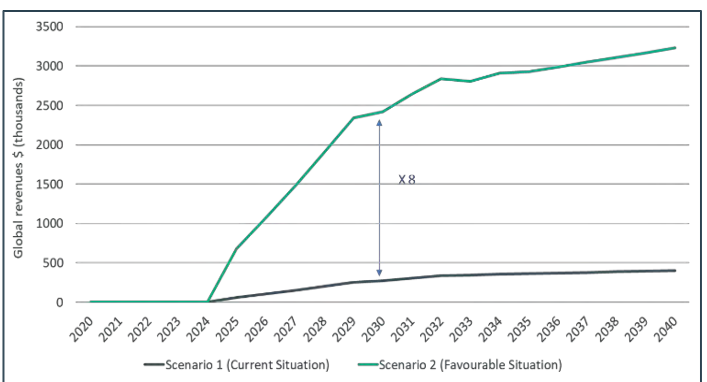


Figure A. *Dx1: Global revenue forecasts 2020-2040 (2 x scenarios)*

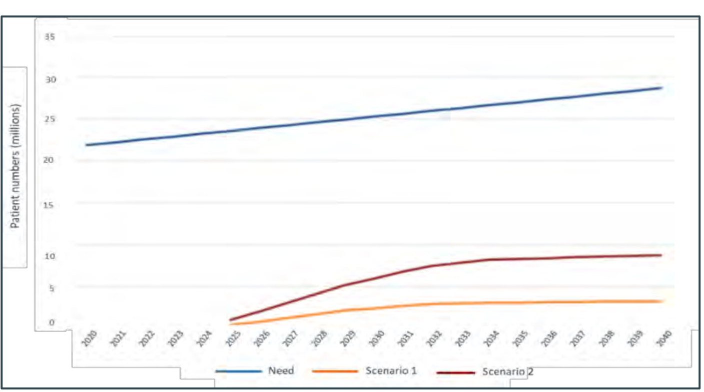


Figure B. *Dx2: Projected Need vs. Demand 2020-2040 (2 x scenarios)*

What Difference Could Policy Maker Action Make?

Figure A illustrates the substantially improved market attractiveness that could be achieved through a more favourable market context nurtured by public health actors (see Scenario box below). Dx1 revenues were forecast to increase nearly 8 times over the current situation. This represents **an additional 200 million annual diagnoses globally (i.e., an almost 14-fold increase in global patient reach)** and, by extension, a substantial decrease in unnecessary prescriptions.

❖ **Scenario 1: Base-case (current situation)**
A scenario built on the current situation. Assumes no substantive changes to the Dx context in the next 20 years.

❖ **Scenario 2: Hypothetical (favourable situation)**
Improvements in the policy/AMR context resulting in better uptake, reimbursement, clinician adoption & use (including donor-support for LMIC/LICs).

Dx2 (ID/susceptibility) is substantially less affected by the more favourable scenario, contributing only an additional $206m in revenues. Yet those increased revenues still translate into **>5 million more targeted prescriptions** – or more narrow-spectrum antibiotics being prescribed – within 20 years, were such actions (assumed by scenario 2) initiated.

The case for policy intervention to support uptake and innovation is compelling

Discrepancy in Location of Global Need & Uptake

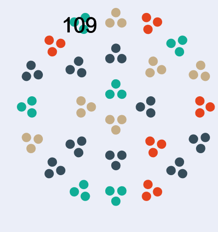
AMR is a truly global challenge yet its impact is higher and growing more rapidly outside HICs (Policy Brief #3). Forecast **global patient needs by 2040 of <800 million Dx1 (Bac. vs other) and <30 million Dx2 (ID/susceptibility)** reflect (but underestimate)[8] this and highlight that 80% of the forecast need lies outside HICs.

The global need for these diagnostics is highest where uptake will be weakest

As Dx1 (Bac. vs other) is a less sophisticated primary care device, at peak sales in around 15 years, it is forecast to reach only around **2% of its target patients or 17 million patient-diagnoses** in 80% of the world – mostly from lower-middle and low-income countries (LMIC/LICs). In contrast, despite a more favourable 'use case' (anticipated to secure up to 10% of eligible patients), Dx2 (ID/susceptibility) – the more sophisticated device for hospital use, with 20 times lower need volume – is forecast to secure **3.2 million patient-diagnoses** at its peak – mostly from upper-middle income (UMIC) countries.

Figure B shows how, under current conditions, expected global uptake and access is forecast to remain a small proportion of the overall need for such a device. With a growing number of narrow-spectrum agents in clinical development, the 'need' for a single device such as this (i.e., able to inform prescribing across multiple-agents) is only expected to grow further.

[8] The authors acknowledge that need & utility are likely both underestimated



Market Intervention Necessary – What Can be Done?

In the current market context, using a diagnostic adds cost and time to both the patient and the health care professional in the short term, while the clinical and cost benefits of their use become evident only in the long term and at a health facility or societal level. Rectifying this disconnect would have substantial public health advantages at the same time as improving the innovation system by ensuring that such urgently needed new diagnostics – to transform the AMR response – are brought to the market and patients globally.

Greater valuation, uptake & use of Dxs is possible with existing tools & would help sustain the supply of innovation

The COVID-19 response has shown how transformative change is possible. A previous study[9] by the Global AMR R&D Hub provided indications of what actions policy makers can take (see Info Boxes below) to achieve a collective progression closer towards a 'Scenario 2'.

USE OF EXISTING POLICY TOOLS

Many countries already engage in health technology assessment for medical devices, but findings could be better integrated into reimbursement policies.

BROADER DX VALUE-FRAMEWORKS

Broader value-frameworks – such as those being explored within ValueDx[10] – that capture and incorporate longer-term health and economic impacts on the whole health system would improve the flow and rate of innovation.

NOVEL POLICY OPTION

Funding of 'Dx-Tx pairs' (complementary or companion diagnostics) – as is common in other therapeutic areas – could begin to reward more targeted and precise use of novel antibiotics.

COVID-19 STEP-CHANGE IN Dx UPTAKE

The unprecedented and very rapid increase in both the availability and uptake of rapid tests – also in LMICs/LICs – for COVID-19 has catalysed important structural shifts both within the private (In vitro diagnostic manufacturers) and public sectors – that could usefully benefit and be seized upon for AMR.

On the basis of the diagnostic workstream of the study, the EAG made the **following recommendations:**

- Widespread and immediate use of existing policy levers: national-level reform of pricing and reimbursements (ideally backed by broader value-assessment frameworks) must be enhanced.
- National efforts should be coupled with additional pull support measures in order to reach a scale of return on investment attractive for developers.
- Substantially progress the dialogue on possible global access, distribution & supply chain mechanisms due to the location of the projected future AMR burden.
- Further mobilisation of donor-support / coordination options for LMICs/LICs.

"For material progress to happen healthcare systems need to leapfrog to using rapid Dx wherever possible, before using an antibiotic" *UK AMR Review 2014*[11]

With many thanks to all those who generously gave their time & insights to this study

The study is available for download from: https://globalamrhub.org/our-work/studies/market-potential-and-priority-patient-needs/

For questions or enquiries please contact Global AMR R&D Hub

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[9] National Health System Tools to Improve Valuation – Global AMR R&D Hub (globalamrhub.org)
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[11] Review on AMR, Antimicrobial resistance: Tackling a crisis for the health and wealth of nations, 2014

Review

Diagnosis of Bloodstream Infections: An Evolution of Technologies towards Accurate and Rapid Identification and Antibiotic Susceptibility Testing

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Abstract: Bloodstream infections (BSI) are a leading cause of death worldwide. The lack of timely and reliable diagnostic practices is an ongoing issue for managing BSI. The current gold standard blood culture practice for pathogen identification and antibiotic susceptibility testing is time-consuming. Delayed diagnosis warrants the use of empirical antibiotics, which could lead to poor patient outcomes, and risks the development of antibiotic resistance. Hence, novel techniques that could offer accurate and timely diagnosis and susceptibility testing are urgently needed. This review focuses on BSI and highlights both the progress and shortcomings of its current diagnosis. We surveyed clinical workflows that employ recently approved technologies and showed that, while offering improved sensitivity and selectivity, these techniques are still unable to deliver a timely result. We then discuss a number of emerging technologies that have the potential to shorten the overall turnaround time of BSI diagnosis through direct testing from whole blood—while maintaining, if not improving—the current assay's sensitivity and pathogen coverage. We concluded by providing our assessment of potential future directions for accelerating BSI pathogen identification and the antibiotic susceptibility test. While engineering solutions have enabled faster assay turnaround, further progress is still needed to supplant blood culture practice and guide appropriate antibiotic administration for BSI patients.

Keywords: infectious diseases; sepsis; pathogen diagnosis; antibiotic susceptibility; emerging technologies; sample preparation; multidrug-resistant pathogens

1. Challenges in Bloodstream Infection Diagnosis

The presence of viable microorganisms bacteria in the blood, i.e., bacteremia, when not controlled properly can lead to the development of bloodstream infection (BSI) and sepsis, a syndromic inflammatory response that contributes to a leading cause of death worldwide [1]. The survival rate of patients with sepsis drops by almost 8% per hour of delayed treatment [2]. The ability to rapidly identify invading pathogens and initiate appropriate treatment is critical yet challenging, primarily due to the typically low pathogen load (1–100 CFU/mL of blood), breadth of pathogen coverage, and the complex blood matrix [3,4]. Meanwhile, the administration of effective antibiotic treatment greatly depends on knowing the pathogens' identity and antibiotic susceptibility profile. This ongoing issue leads to poor clinical outcomes for BSI patients [5]. The lack of accurate and rapid

techniques for the timely elucidation of causative pathogens necessitates the use of broad-spectrum antibiotic agents. The 2021 report by the CDC showed that 28% of antibiotic prescriptions in the United States were unnecessary [6]. This continued misuse of antibiotics can worsen the clinical outcome and will exacerbate the emergence of antibiotic-resistant microorganisms worldwide [7].

This review highlights both the progress and shortcomings of current and emerging diagnostic tools in BSI. Key technological advancements that could pave the way for supplanting conventional blood culture practices will be discussed. Our goal is to provide an assessment of the potential future development of pathogen identification (ID) and antibiotic susceptibility tests (ASTs) to address this urgent clinical need.

2. Overview of Current Blood Culture Diagnosis Workflow

Clinical presentations of BSI are often vague and varied, making timely clinical diagnoses challenging. However, when BSI is suspected, sampling for blood culture as the laboratory diagnostic standard is usually obtained, followed by immediate, empirical broad-spectrum antibiotic treatment. Once a positive culture is detected, subsequent species ID and AST are performed.

Most of the current ID workflows still rely on a positive blood culture sample. Generally, when a blood culture flags as positive, a further overnight incubation on agar plates (subculture) is performed to obtain pure isolated colonies. Colonies are subjected to Gram stain, followed by a series of biochemical and molecular tests, and/or the matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) to confirm the bacterial species. A quantitative measure of antibiotic susceptibility using methods like broth microdilution or ETEST gradient strip (bioMérieux) usually takes place alongside or after ID to give minimum inhibitory concentration (MIC) information of antibiotics against the isolated pathogen. Depending on the growth rate of the microorganisms, it could take anywhere between 24 h and 5 days for blood culture to flag positively. Fully automated systems, such as the (bioMérieux) VITEK 2, are also commonly used for analyzing positive blood cultures, delivering faster AST turnarounds of approximately 9 to 18 h, depending on the organism [8,9]. Overall ID and AST performed after blood culture can subsequently take days or even weeks to complete (Figure 1) [10].

Since some broad-spectrum antibiotics are administered as frequently as every 6 to 8 h, clinicians could re-evaluate their choice of antibiotics during this period, based on patients' conditions. The de-escalation of antibiotics to the causative pathogen should be done as soon as possible, but antibiotic adjustment is less likely when the time to AST result is delayed [11]. In some instances, knowing the causative species could prompt an escalation in antibiotic use, such as implementing double coverage for *Pseudomonas* species or adding colistin for *Acinetobacter* species. Ideally, antibiotic adjustments should be made as early as the second dose of antibiotic administration to reduce any unnecessary use of broad-spectrum antibiotics. In particular, AST results that could be obtained within the same clinical shift are critical so that clinicians could decide on appropriate patient management [12]. Hence, the future development of ID/AST methods should take into account these clinical timepoints, while aiming to deliver results in less than 6 h.

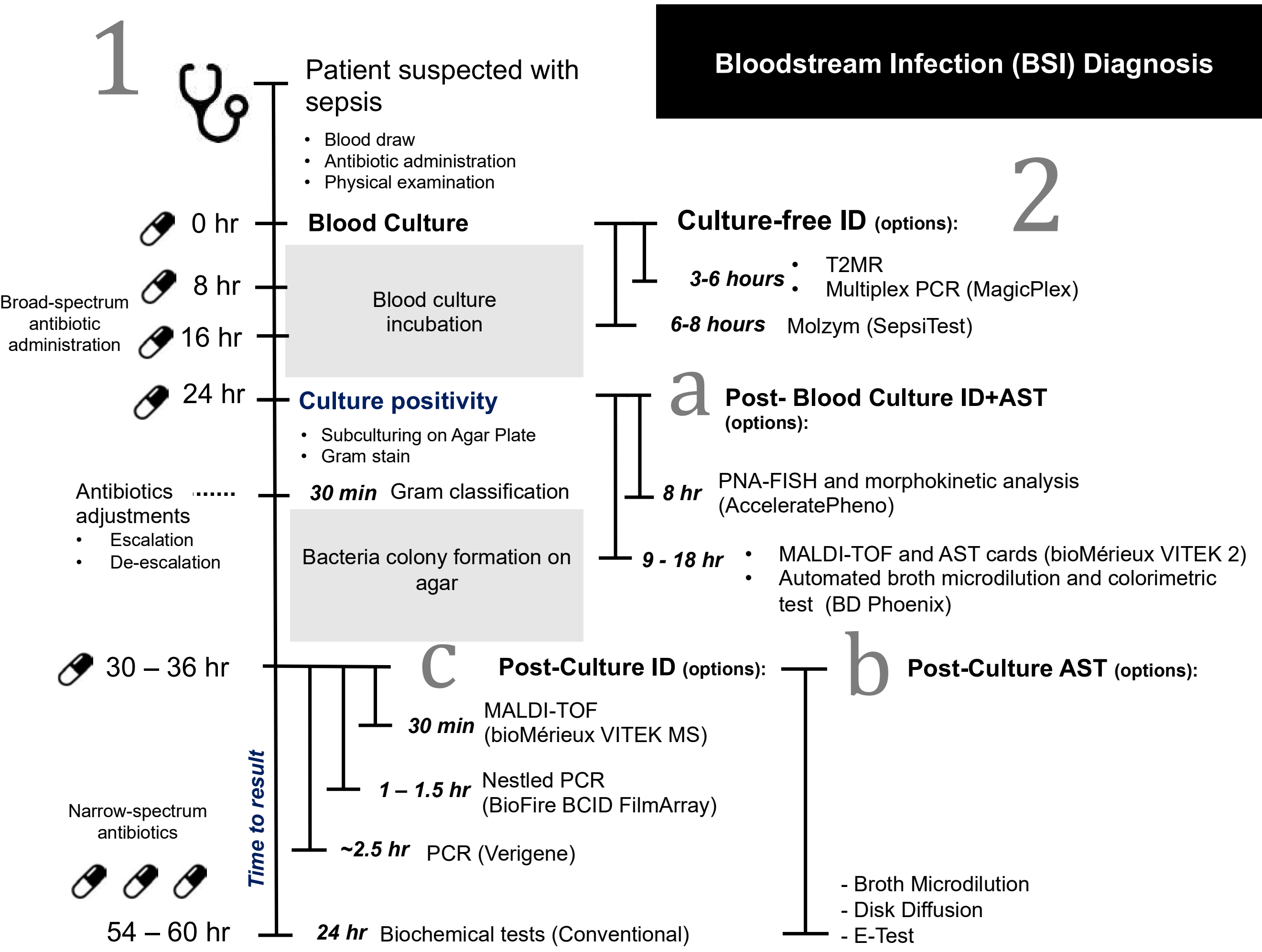

Figure 1. Workflow of current BSI diagnosis. (1) Blood collected from patients is subject to culturing followed by either an all-in-one post-culture ID/AST (a) or post-culture ID with a separate AST (b,c). (2) Culture-free ID workflow is also currently available with a separate AST from positive culture. Antibiotics administration at the point of examination. Antibiotic choices may be adjusted accordingly.

Since broad-spectrum antibiotics are administered as frequently as every 6 to 8 hours, clinicians could re-evaluate their choice of antibiotics during this period, based on patients' conditions. The de-escalation of antibiotics to the causative pathogen should be done as soon as possible, but antibiotic adjustment is less likely when the time to AST result is delayed [11]. In some instances, knowing the causative species could prompt an escalation in antibiotic use, such as implementing double coverage for *Pseudomonas* species or adding colistin for *Acinetobacter* species. Ideally, antibiotic adjustments should be made as early as the second dose of antibiotic administration to reduce any unnecessary use of broad-spectrum antibiotics. In particular, AST results that could be obtained within the same clinical shift are critical so that clinicians could decide on appropriate patient management [12]. Hence, the future development of ID/AST methods should take into account these clinical timepoints, while aiming to deliver results in less than 6 hours.

3. Challenges of BSI Diagnosis and Clinical Significance for a Novel Technique

Meeting the diagnostic needs of BSI within the acute care timescale would require direct testing from whole blood with both ID and AST capabilities. Ideally, next-generation BSI diagnostics should possess four key elements:

(1) Sample preparation directly from whole blood that requires minimal handling and can process sufficient blood volume to ensure the capture of pathogens in low abundance;
(2) Workflow that effectively separates, enriches, and concentrates pathogens or target analytes from background interferences;
(3) Sensitive, quantitative, and accurate detection and species-level identification that differentiates pathogens from contaminants or commensals;
(4) Timely and universal antibiotic susceptibility profiling independent of resistant mechanisms with MIC, reporting matches with critical clinical decision timepoints.

Previous reviews have captured a broad range of pathogen ID and AST technologies that are either in development or clinically available [13–15]. Methods that utilize techniques such as atomic force microscopy, surface plasmon resonance, electrochemical impedance, asynchronous magnetic bead, single-cell morphological imaging, and bacteriophage-based methods have also previously been described [14,16–21]. While ID

and AST do not necessarily have to be performed on a single platform, fully integrated platforms will simplify clinical workflows by requiring less sample handling. In Table 1, we summarized some of these technologies that could perform either ID or AST. These methods either target bacterial cells or their genetic contents and can be implemented either directly from whole blood or from positive blood culture samples. Those that are still in the early development stage (proof-of-concept) are not included.

Table 1. List of commercial and developing technologies for BSI diagnosis.

No.	Company	System	Approach	Status *	Sample Prep ‡	Detection/ ID	AST	TAT ^
1	Abacus Diagnostica	Genomera CDX	Rapid/ Real-Time PCR	Dev.	BC (+)	✓		50 min
2	Affinity Biosensors	LifeScale AST	Microorganism mass measurement	CE-IVD	BC (+)		✓	4 h
3	Amplex Diagnostics, GmbH, Germany	Eazyplex MRSA	LAMP ultra-rapid MRSA detection	CE-IVD	BC (+)	✓		30 min
4	Arc Bio	Galileo pathogen solution	Shotgun Sequencing	Dev.	WB	✓		48 h
5	BD	GeneOhm MRSA	Real-Time PCR	FDA, CE-IVD	BC (+)	✓		2 h
6	Becton Dickinson	BD Max StaphSR	Real-Time PCR	FDA	BC (+)	✓		~1.5 h
7	BioFire/bioMerieux Diagnostics	FilmArray DIRECT (new)	Nested PCR	FDA, CE-IVD	WB	✓		1 h
8	BioRad	Droplet dPCR	dPCR; absolute quantification using Poisson's statistics without requiring a standard curve	CE-IVD	BC (+)	✓		No report
9	BioSense Solutions (Denmark)	oCelloScope	3D optical scanning microscopy imaging	Dev.	BC (+)		✓	1 to 4 h
10	Bruker Daltonics	MALDI Biotyper + DxM MicroScan WalkAway System	Mass spectrometry	FDA, CE-IVD	BC (+)	✓		12 to 24 h
11	DNAe (electronic)	LiDia Bloodstream Infection Test	WGS/NGS/miniaturised sequencing	Dev.	WB	✓		3 to 4 h
12	FASTinov	Flow cytometry	Cell sorting fluorescence-based AST	Dev.	BC (+)		✓	<2 h
14	Roche	Smarticles	Bacteriophage-based	Dev.	BC (+)	✓		No report
15	GenMarkDx USA	ePlex BCID	Multiplex PCR	CE-IVD	BC (+)	✓		1.5 h
16	Gradientech AB	Rapid IVD; QuickMIC and CellDirector	Microfluidics Phenotypic multiplex chip	Dev.	BC (+)		✓	2 h

Table 1. *Cont.*

No.	Company	System	Approach	Status *	Sample Prep ‡	Detection/ ID	AST	TAT ˆ
17	Great Basin Corporation (Bringham Young Univ.)	OptoFluidic Platform	Single molecule fluorescence hybridization	Dev.	WB	✓		1 h
18	Hologic	AccuProbe	In situ hybridization	CE-IVD	BC (+)	✓		1 h
19	iCubate	iC GPC	Multiplex amplification assay	FDA, CE-IVD	BC (+)	✓		4 to 5 h
20	IRIDICA	BAC BSI Assay	PCR/ESI-MS	withdrawn	WB	✓		8 h
21	Karius, Inc.	Karius Test	NextGen Seq cfDNA; Genomic; Bioinformatics	Dev.	WB	✓		48 h
22	Luminex	Verigene Gram+ BC	Microarray	FDA.	BC (+)	✓		2.5 h
23	Luminex	Verigene Gram− BC	Microarray	FDA	BC (+)	✓		2.5 h
24	Master Diagnostica, Spain	Sepsis Flow Chip	Microarray	CE-IVD	BC (+)	✓		3 to 4 h
25	Molzym, Germany	SeptiTest; UMD SelectNA	Real Time PCR	CE-IVD	WB	✓		8 to 12 h
26	Momentum Biosciences (Cardiff, UK)	TBD Cognitor Minus	Enzymatic template generation and amplification	awaiting clearance	BC (+)	✓		No report
27	OpGen USA	PNA FISH	In situ hybridization	CE-IVD	BC (+)	✓		2.5 h
28	OpGen USA	Quick FISH	In situ hybridization	CE-IVD	BC (+)	✓		30 min
29	QLinea (Uppsala, Sweden)	AsTAR	High-speed time-lapse microscopy imaging of bacteria in broth	Dev.	BC (+)		✓	6 h
30	Resistell (Switzerland)	Rapid AST antibiogram	AFM, Cantilever, Nanomotion detection-based AST	unknown	BC (+)		✓	No report
31	Roche Molecular System, Switzerland	LightCycler SeptiFast	Real-Time PCR	CE-IVD	WB	✓		6 h
32	SeeGene, Korea	Magicplex Sepsis RT test	Real-Time PCR	CE-IVD	WB	✓		3 to 6 h
33	Specific Diagnostics Inc	Reveal phenotypic AST	Detection of volatile organic compounds	Dev.	BC (+)		✓	~5 h (with MIC)
34	T2Biosystem	T2 Candida Panel T2MR	Nuclear Magnetic Resonance	FDA, CE-IVD	WB	✓		3 to 5 h
35	QuantaMatrix	QMAC-dRAST	Optical Microscopy	Dev.	BC (+)		✓	4 to 6 h

* Platforms on this list are either U.S. Food and Drug Administration (FDA) and/or European CE Marking for In Vitro Diagnostic (CE-IVD) certified or under research development (Dev.); ‡ BC (+): blood culture-positive; WB: whole blood; ˆ TAT: turnaround time.

While current approaches are still short of meeting all the key elements of an ideal diagnostic, recent innovations exploring novel avenues could potentially fill the technological gaps lacking in previous developments. In this section, we discuss the challenges and

considerations of fulfilling these four elements, and highlight some emerging technologies that have the potential to address them.

3.1. Sample Preparation and Assay Workflow for Capturing, Separating, and Enriching Low-Abundance Pathogens

The first two elements of a next-generation BSI diagnosis should effectively and efficiently capture and separate pathogens from whole blood. Sample preparation is one of the most challenging aspects of the diagnostic workflow of infectious diseases [22,23]. Yet, the overall workflow of a BSI diagnosis heavily depends on this process.

Every milliliter of infected blood carries about 4 to 6 $\times$ 10^9 red blood cells, up to 1.6 $\times$ 10^7 white blood cells, and 1.3 to 4 $\times$ 10^8 platelets, with only 1 to 100 bacteria cells [24,25]. Traditionally, this low abundance of pathogens is overcome by inoculating blood in nutrient-rich broth prior to diagnosis. However, this is a process that could take days and cause severe delays in the overall assay. With the development of advanced amplification and detection techniques, bypassing the lengthy blood culture step by isolating and concentrating pathogens directly from patients' blood samples will allow dramatic time reduction in the workflow, and may enable a new generation of culture-free BSI diagnostic approaches.

Table 2 summarizes microfluidic methods for isolating bloodborne pathogens and highlights their performance in selected examples. These pathogen isolation techniques can be broadly classified into chemical and physical methods. Chemical approaches, such as affinity capture and erythrocyte lysis, rely on the biochemical properties of bacteria and blood cells for the positive and negative selection of cells in the samples. Physical approaches, such as acoustics, electrokinetics, hydrodynamics, magnetics, and filtering, isolate bacteria from blood cells based on their differences in size, density, and other physical properties. This section highlights several promising pathogen isolation methods (Figure 2). We pay specific attention to microfluidic sample preparation methods, which are amenable to automation and system integration.

Table 2. Comparison of microfluidic techniques for isolating bloodborne pathogens. Values are estimated for a single channel with a single pass.

Isolation Method	Recovery Rate #	Throughput ##	Pathogens	Sample Type	Conc. (cell/mL)	Ref
Affinity capture (magnetic; antibody)	78%	0.025 mL/h	*E. coli*	Red blood cells	5 $\times$ 10^6	[26]
Affinity capture (magnetic; antibody)	80%	20 mL/h	*C. albicans*	Whole blood	1 $\times$ 10^6	[27]
Affinity capture (magnetic; lectin)	60–90%	10 mL/h	*S. aureus, C. albicans, E. coli*	Whole blood	1 $\times$ 10^4	[28]
Filtration Affinity capture (magnetic; MBL)	68–76% 56–77%	1.2 mL/h 3 mL/h	*E. coli, P. aeruginosa, K. pneumoniae, S. saprophyticus, S. epidermidis*	Whole blood Filtered blood	10^1–10^2	[29]
Affinity capture (magnetic; Zn-DPA)	>88%	60 mL/h	*E. coli*	Whole blood	5 $\times$ 10^6	[30]
Erythrocyte depletion (detergent + water)	~100%	2.88 mL/h	*E. coli, M. luteus*	Whole blood	1 $\times$ 10^7	[31]
Erythrocyte depletion (lysis)	>90%	20 mL/h	*E. coli*	Whole blood	1 $\times$ 10^3	[32]
Erythrocyte depletion (dextran sedimentation)	50–60%	20 mL/h	*E. coli, E. faecalis, K. pneumoniae*	Whole blood	10^1–10^2	[33]

Table 2. *Cont.*

Isolation Method	Recovery Rate #	Throughput ##	Pathogens	Sample Type	Conc. (cell/mL)	Ref
Acoustophoresis	95.65%	0.03 mL/h	*E. coli*	PBMC	3×10^6	[34]
Acoustophoresis	91%	3 mL/h	*P. putida, E. coli*	Diluted blood	5×10^5	[35]
Acoustophoresis (GAF)	79.77%	0.72 mL/h	*S. aureus, S. pneumoniae, E. coli*	Blood lysates	1×10^5	[36]
Electrokinetics (DEP)	30%	0.035 mL/h	*E. coli*	Red blood cells	1×10^6	[37]
Electrokinetics (DEP)	97%	0.0009 mL/h	*E. coli, S. epidermidis, and C. albicans*	Diluted blood	1×10^4	[38]
Electrokinetics (DEP and ACEF)	30–80%	0.006–0.06 mL/h	*E. coli, A. baumannii, B. globigii*	Buffy coat	1×10^5	[39]
Inertial focusing	>60%	12 mL/h	*E. coli*	Whole blood	1×10^8	[40]
Inertial focusing (Dean flow)	>65%	0.6 mL/h	*E. coli, S. aureus, P. aeruginosa, E. faecalis*	Diluted blood	1×10^1	[41]
Elasto-inertial	76%	0.03 mL/h	*E. coli*	Whole blood	1×10^6	[42]
Elasto-inertial	>80%	0.3–1.5 mL/h	*E. coli, S. capitis*	Diluted blood	1×10^3	[43]
Elasto-inertial	60–80%	0.3 mL/h	*K. pneumoniae, S. pneumoniae*	Diluted blood	1×10^3	[44]
Margination	80–90%	1 mL/h	*E. coli and S. cerevisiae*	Whole blood	1×10^6	[45]

Removal rates are reported for blood cleansing devices. ## Throughputs for diluted blood samples are adjusted for compassion with whole blood.

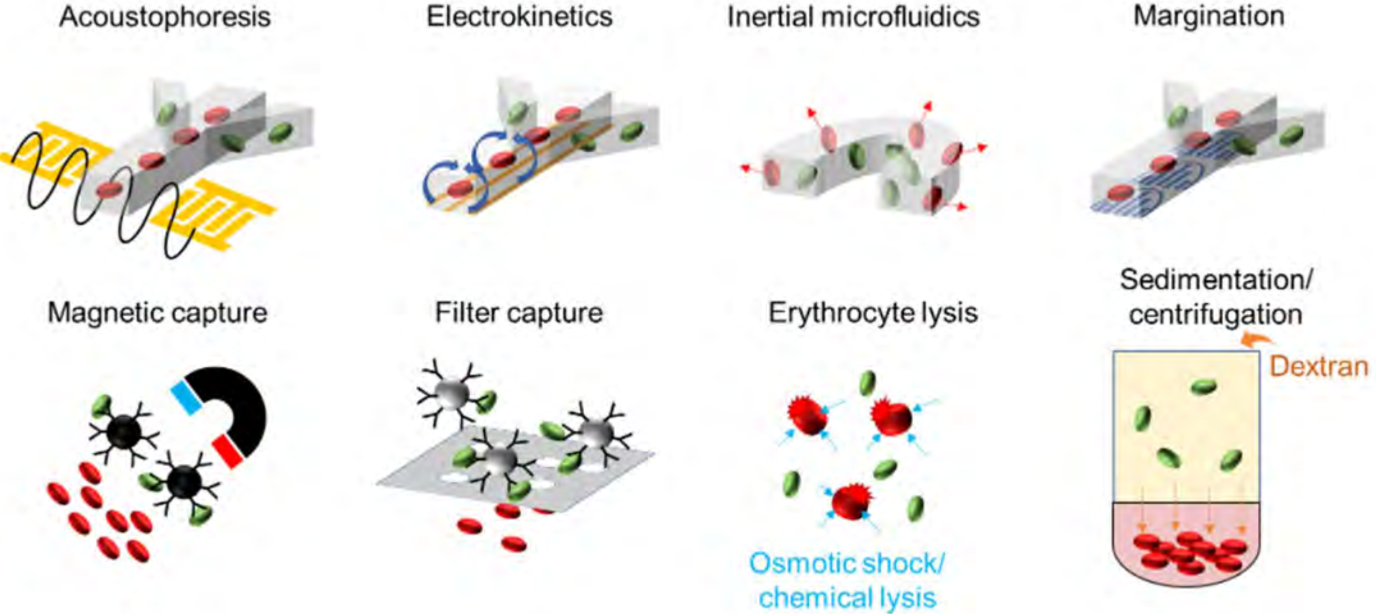

Figure 2. Sample preparation techniques for BSI diagnostics. Pathogen isolation and concentration can be achieved actively via acoustophoresis, electrokinetics, and magnetic forces, or passively via inertial focusing, margination, filtering, and erythrocyte lysis.

3.1.1. Affinity Capture

Affinity capture techniques with chemically modified particles and surfaces are popular strategies for selective cell isolation. Biochemical binding with microbeads provides a mechanism for converting chemical features to physical properties for capturing pathogens. For example, surface-modified microbeads can bind and isolate bacteria by magnetic force, gravitation/sedimentation, and filtering [46,47]. Immunoassays with antibody conjugation

bacteria is another promising candidate of broad-spectrum capture probes [30]. By using a multistage microfluidic device, Lee et al. demonstrated the removal of >95% bacteria at 60 mL/h for blood cleansing [30]. Challenges of affinity capture include false negatives associated with probe specificity and viscous drag caused by blood components. These challenges can limit the overall throughput and capture efficiency of affinity capture approaches.

Figure 2. Sample preparation techniques for BSI diagnostics. Pathogen isolation and concentration can be achieved actively via acoustophoresis, electrokinetics, and magnetic forces, or passively via inertial focusing, margination, filtering, and erythrocyte lysis.

3.1.2. Erythrocyte Depletion

Erythrocyte depletion is an important strategy for pathogen isolation. Negative selection reduces the complexity of the sample and enhances the efficiency of downstream enrichment approaches. For example, after erythrocyte depletion, the sample volume can be reduced by centrifugation or microfluidic techniques [57,58]. Osmotic shock (e.g., by adding distilled water) is a classical approach for lysing red blood cells. At the same time, most bacteria can survive the osmotic shock due to their rigid cell walls. Saponin and ammonium chloride are common chemical reagents for lysing red blood cells [31,59,60]. Sodium dodecyl sulfate (SDS) and Sepsityper are other examples of chemical lysis [61–63]. These lysis buffers and detergents are often applied in positive blood culture samples [30,64,65]. Dextran sedimentation is a physical erythrocyte depletion method, which is

on microbeads can be applied for isolating specific pathogens and have been implemented in microfluidic formats with 70–80% recovery efficiency at 10–20 mL/h [26,27,48,49]. Furthermore, antibiotics that interact with the bacterial cell wall can be applied for capturing bacteria. For instance, vancomycin and daptomycin interact and inhibit cell wall synthesis for Gram-positive bacteria [50,51]. They were modified as affinity probes on the surfaces of magnetic microbeads for isolating Gram-positive bacteria [52,53]. In addition, glycosaminoglycans, such as heparin or heparan sulfate, are widely distributed in all human tissues, and some bacteria bind specifically to them [54]. Capturing *S. aureus* from blood with over 65% efficiency has been demonstrated using surface-heparinized polyethylene microbeads [55]. These strategies are promising when there is a specific group of target pathogens. Nevertheless, finding a ligand that binds strongly and selectively to all pathogens, but not other blood components, can be challenging. In clinical scenarios where the bacterial species is unknown, multiple probes may be required to cover the large range of pathogens associated with BSI, and to avoid a false negative.

Efforts have also been devoted to the broad-spectrum capture of pathogens. In the human immune system, mannose-binding lectin (MBL) binds the exterior of pathogens and pathogen-associated molecular patterns that activate the innate immune response [41]. MBL and engineered MBL are promising approaches for the sample preparation of BSI diagnostics. Owing to their broad binding ability to pathogens, MBLs are commonly applied to bacteria capture and blood cleansing [28,33,56]. Additionally, zinc-coordinated bis(dipicolylamine) (Zn-DPA) that binds to both Gram-positive and Gram-negative bacteria is another promising candidate of broad-spectrum capture probes [30]. By using a multi-stage microfluidic device, Lee et al. demonstrated the removal of >95% bacteria at 60 mL/h for blood cleansing [30]. Challenges of affinity capture include false negatives associated with probe specificity and viscous drag caused by blood components. These challenges can limit the overall throughput and capture efficiency of affinity capture approaches.

3.1.2. Erythrocyte Depletion

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3.1.3. Acoustophoresis

Acoustophoresis refers to the migration of particles subjected to acoustic waves. In a standing acoustic wave field, a cell experiences an acoustic radiation force towards either the pressure node or the pressure antinode. The amplitude and direction of the acoustic

radiation force depend on the physical properties of the cell and the surrounding medium, including size, density, and compressibility [68,69]. Most importantly, the primary acoustic force scales with the volume, i.e., the dimension to the third power, of the cell. Therefore, acoustic separation can be achieved in heterogeneous cell mixtures, such as blood, based on the size difference between the cells. Acoustic techniques have also been applied for the manipulation of various biological entities, from circulating tumor cell clusters to exosomes in the blood [32,70].

Toward BSI diagnostics, Ai et al. showed the separation of *E. coli* from peripheral blood mononuclear cells (PBMC) in a microfluidic sheath flow device with a purity of 95.65% [71]. The flow rate in this study was 0.03 mL/h. Ohlsson et al. reported an ACUSEP system that integrates acoustic sample preparation modules (separation and enrichment) and a dry reagent PCR microchip [35]. The system achieved a detection limit of 10^3 CFU/mL at ~3 mL/h in blood samples spiked with *Pseudomonas putida* and successfully detected *E. coli* in half (2 out of 4) of the BSI patient samples. The entire process can be finished in less than 2 h and requires minimal manual processing. A challenge of acoustic separation is the domination of acoustic streaming for small objects (<2 µm) [72]. To mitigate the effect of acoustic streaming, Assche et al. report a gradient acoustic focusing (GAF) device that enables the separation of submicron particles and bacteria [36]. GAF is achieved by suppressing acoustic streaming using an acoustic impedance gradient with an inhomogeneous medium (Ficoll). The study reported a recovery rate of 79.77% with 10^5 CFU/mL *S. aureus* in blood lysates (0.72 mL/h), using a combination of cell lysing and acoustic separation.

3.1.4. Electrokinetics

Electrokinetics describes the motion of fluids and particles in external electric fields [73,74]. For example, dielectrophoresis (DEP) refers to the motion of polarizable objects (e.g., a cell) under a spatial electric field gradient. The dielectrophoretic force depends on the volume of the cell and the relative polarizability between the cell and the fluid. By tuning the frequency, it is possible to adjust the effective polarization and the cell motion toward (positive DEP) or away (negative DEP) from the electric field maxima [75]. External electric fields can also induce fluid motion, such as AC electrothermal flow (ACEF), in microfluidic systems [76]. ACEF creates a long-range (e.g., centimeter-scale) fluid circulation [77], which has been applied for improving the identification and AST of bacteria in blood samples [78,79].

Direct current electrokinetic techniques, such as capillary zone electrophoresis (CZE) and isoelectric focusing, can be applied for separating complex bacteria mixtures. Huge et al. reported a CZE device coupled with an automated fraction collection to separate bacteria from the salivary wastewater microbiome [80]. The bacteria were firstly separated based on their differences in electrophoretic mobility and then fractionated and cultured on agar plates for downstream analysis. This technique improves the sensitivity of bacteria detection with genome sequencing by eliminating the masking effect of the high-abundance bacteria over the low-abundance bacteria. For uncultivable bacteria, Jiang et al. introduced a recycling free-flow isoelectric focusing (RFFIEF) method-based electrophoresis method to separate the salivary microbiome [81]. After RFFIEF separation, the results showed that the commonly identified genera were retained, the low-abundance bacteria (e.g., Serratia) that cannot be detected by the conventional method were dramatically enriched, and the number of bacterial genera identified was increased by 225% on average. However, this technique can potentially be modified for improving the detection of bloodborne pathogens.

Kuczenski et al. reported a negative DEP device with electrodes tilted at shallow and steep angles along the flow direction for sorting *E. coli* from blood cells [37]. The device recovered 30% of viable cells at a flow rate of 0.035 mL/h [37]. Using interdigitated electrodes, Bisceglia et al. presented a positive DEP device with a 97% capture efficiency for *E. coli* spiked in diluted blood [38]. The device was also capable of simultaneously separating *E. coli*, *Staphylococcus epidermidis*, and *Candida albicans*. DEP is a short-range force field, which is strongest near the electrode edges [74]. By using a 3-parallel electrode design,

Gao et al. demonstrated a pathogen concentration device that enhances the trapping efficiency of the DEP with long-range ACEF in the conductive fluids [39]. The device concentrates *E. coli*, *Bacillus globigii*, and *A. baumannii* in urine and buffy coats for 2–3 orders of magnitude, at a flow rate between 0.006 and 0.06 mL/h. Overall, electrokinetics has a relatively low throughput; nevertheless, DEP has a high specificity, as the dielectrophoretic force depends strongly on both the size and polarizability of the cells. This specificity is important when the purity of the sample is essential for downstream procedures in the workflow.

3.1.5. Inertial Focusing

Inertial microfluidics concerns the lateral motion of particles or cells in a microchannel due to passive, inertial lift forces, which push cells away from the channel wall. The inertial lift forces are associated with fluid shear, flow disturbance near particles, and shear gradient [82–84]. In addition, the channel curvature and the rheological properties of the media can also create additional forces (e.g., Dean drag force and elastic force) on the cells [85]. These forces depend on the properties of cells and media and can be tuned by the microchannel design and flow rate. Since the equilibrium positions from the channel will depend on the cell types (e.g., bacteria and blood cells), inertial forces focus cells into different streamlines for separation. While typical microfluidic systems operate at a low flow rate, inertial focusing occurs at a relatively high flow rate (Reynolds number typically from 1–100), improving its significance in high throughput cell separation.

The high-precision inertial focusing and self-ordering of red blood cells have been demonstrated in straight and curved microchannels [83]. A cross-channel design was demonstrated for removing 80% of *E. coli* (10^8 CFU/mL) spiked in whole blood at a flow rate of 6 mL/h (240 mL/h in a 40-channel device) [40]. A spiral microfluidic device based on Dean flow fractionation reported a recovery rate of >65% for *E. coli* (10^2 cells/mL) at ~3 mL/h. The device also separated four different bacteria of various sizes and shapes (*E. coli*, *S. aureus*, *P. aeruginosa*, *Enterococcus faecalis*) at clinically relevant concentrations (~10–50 cells/mL) [41]. Furthermore, electro-inertial fluidics can enhance the separation resolution by introducing elastic force using non-Newtonian fluids [42,86]. To sort small bacteria of similar sizes (0.5 µm to 3 µm), Lu and coworkers designed an elasto-inertial microfluidic device with periodic contractions along the spiral channel and non-Newtonian fluid. The elasto-inertial microfluidic device achieved a recovery rate of 80% for *K. pneumoniae* and 60% for *Streptococcus pneumoniae* from diluted blood at 0.3 mL/h, with a bacterial load as low as 10^2 CFU/mL.

Another related phenomenon is margination, which describes the accumulation of red blood cells in the center of blood vessels and the migration of white blood cells and platelets in the near-wall region [87]. The segregation of RBCs to the low shear region and other cells to the high shear region is contributed by the high deformability of red blood cells [88]. Hou and coworkers employed the principle to design a pathogen removal microfluidic device and achieved 80% and 90% removal efficiencies for *E. coli* and *Saccharomyces cerevisiae* spiked in whole blood, respectively [45]. The device demonstrates a throughput of 1 mL/h and can be multiplexed for label-free isolation.

Overall, there are some trade-offs between the throughput, recovery rate, and purity of the sample preparation techniques. For instance, inertial microfluidics has a relatively high throughput, while electrokinetics has a high selectivity between cell types. Due to the large volume mismatch between the pathogens (femtolitre) and the blood samples (milliliter), multiple methods may be combined to achieve the required reliability in clinical diagnostics. The sample preparation modules should also be combined with microfluidic detection and characterization techniques for comprehensive BSI diagnostics. Notably, the majority of reports are based on processed or spiked blood samples. The clinical applicability and reliability of these separation technologies in direct BSI diagnostics remain to be investigated. Since these methods often depend on the properties of cells and the media, the influences of sample heterogeneity and pathogen diversity should be considered.

3.2. Sensitive and Quantitative Pathogen Detection and ID with Timely AST

Other key elements of a BSI diagnosis are the ability to provide sensitive, quantitative, and accurate detections and species-level identifications of pathogens, while also offering timely and universal antibiotic susceptibility profiling in the form of an MIC.

A sufficient volume of blood per culture (typically 40 to 60 mL) is usually needed to achieve adequate and sensitive detection of as low as 1 CFU/mL of whole blood within a 24 h timeframe [89,90]. Yet, even when ample sample volume is available, the likelihood of a false negative result is not entirely negated when using blood culture due to factors such as (1) prior antibiotic usage, (2) fastidious, slow-growing, or obligate intracellular organisms, (3) the presence of pathogens other than bacteria or yeasts, and 4) culture media bias towards the growth of certain organisms [91]. Unfortunately, ID technologies such as multiplex real-time PCR (FilmArray), fluorescence in situ hybridization with peptide nucleic acid probes (QuickFISH), and matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF/MS, VITEK MS), which are available for clinical use and have less than 60 min turnaround time, still rely on positive blood culture as their sample input [92]. This requirement limits them from supplanting blood culture.

On the other hand, the goal of AST is to guide the selection and dosage of antibiotics for effectively treating the infection. This objective can be achieved by either (1) obtaining minimum inhibitory concentration (MIC) information to predict the success of antibiotic treatment or (2) obtaining resistance information to guide antibiotic decisions. Culture-based AST alone does not always offer reliable susceptibility information. For instance, in cases where microbial biofilm is formed, a comprehensive analysis including the phenotypic measurement of biofilm production may facilitate more appropriate antibiotic choices [93–95].

The following highlights some of the recent advances in BSI diagnosis technologies that seek to address current gaps in ID/AST.

3.2.1. Digital PCR (dPCR)

PCR has been the mainstay of many disease detection methods based on the targeted amplification of nucleic acid fragments. Since its invention and application in clinical settings, a wide variety of PCR techniques have been developed. Real-time PCR or quantitative PCR (qPCR) is the most common technique used today due to its relative speed and convenience. Unlike conventional PCR, qPCR allows the real-time quantification of nucleic acid concentration via the use of fluorescent probes. Despite its wide use in various applications, qPCR suffers from inaccuracy when it comes to detecting samples with low concentrations, and is affected by PCR inhibitors typically found in the blood.

Digital PCR has been developed to overcome the shortcomings of qPCR by allowing the absolute quantification of nucleic acid [96]. By partitioning large samples of nucleic acid into individual reactions, dPCR pushes the lower limit of detection to a single-molecule level. Each partitioned reaction is amplified and analyzed to generate an absolute count of the target nucleic acid at the reaction endpoint, without the need for standards or internal controls. With an enhanced effective concentration in minute partition volume and the dilution of background human DNA or PCR inhibitors through sample partitioning, dPCR can greatly enhance detection sensitivity, particularly for BSI with a complex sample matrix [96].

Droplet digital PCR (ddPCR) is a version of dPCR where samples are partitioned into individual droplets [97]. Its application was the basis of a system being developed by Velox Biosystems, called the integrated comprehensive droplet digital detection (IC3D) (Figure 3C) [98]. Through a droplet microencapsulation technology, IC3D combines the capability of dPCR with DNAzyme-based sensors and a high-throughput 3D particle counter system to detect antibiotic-resistant genes from a whole blood sample. In short, unprocessed whole blood is mixed with dPCR reagents inside a microfluidic device that generates picoliter-sized droplets. Droplets containing the target bacterium become fluorescent after digital PCR is performed and the signal is detected and quantified using a high-throughput

3D particle counter system, allowing for a low limit of detection of 10 CFU/mL instead of the 1000 CFU/mL limit of qPCR [99].

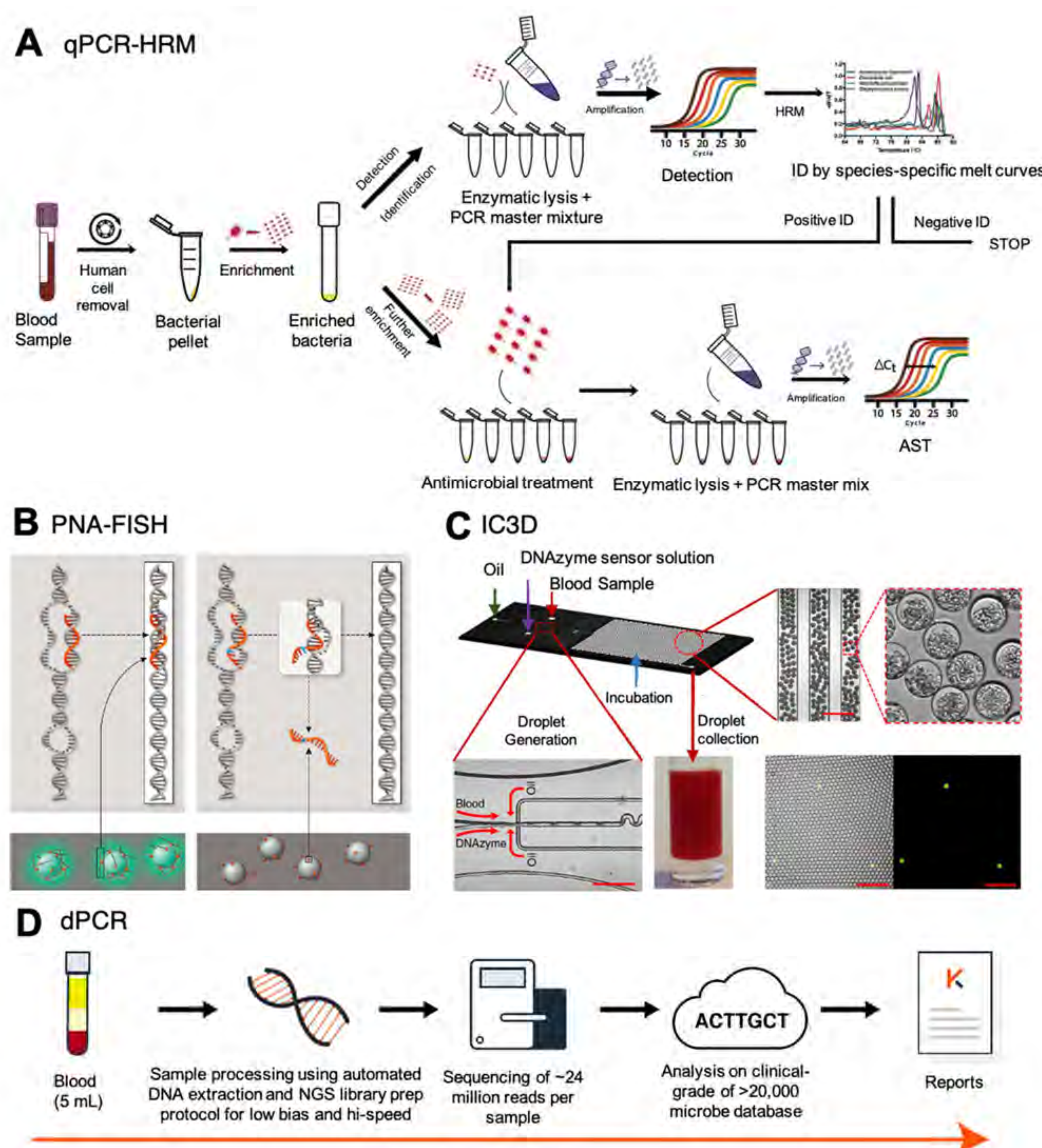

Figure 3. Recent advancements to improve BSI diagnosis. (**A**) qPCR-HRM workflow from a whole blood sample. (**B**) HelixBind PNA-FISH technology with a sequence-specific fluorescence dye, (**C**) Microfluidic-assisted whole blood compartmentalization before DNA amplification and analysis, a trademark of IC3D technology (**D**) NGS detection workflow directly from whole blood by Karius. Figures were modified and used with permission from (Andini et al., 2018; Blauwkamp et al., 2019; D. K. Kang et al., 2014; Nölling et al., 2016).

Figure 3. Recent advancements to improve BSI diagnosis. (**A**) qPCR-HRM workflow from a whole blood sample. (**B**) HelixBind PNA-FISH technology with a sequence-specific fluorescence dye, (**C**) Microfluidic-assisted whole blood compartmentalization before DNA amplification and analysis, a trademark of IC3D technology (**D**) NGS detection workflow directly from whole blood by Karius. Figures were modified and used with permission from (Andini et al., 2018; Blauwkamp et al., 2019; D. K. Kang et al., 2014; Nölling et al., 2016).

The IC3D system also has the potential to detect antibiotic-resistant bacteria from a blood sample in less than one hour through a specific primer design [98].

4. Summary and Future Outlook (Conclusions)

An accurate and timely diagnosis of BSI is important for improving the proper use of antibiotics. The lack of rapid ID/AST in current clinical approaches warranted the initiation of antibiotic treatment without a confirmed diagnosis. To date, the T2 Biosystem is the first and only FDA-approved system capable of identifying a BSI pathogen directly

inhibitor-resistant Taq polymerase mutants and PCR enhancers allows for the minimal pre-analytical processing of whole blood samples. Resistant genes found in Gram-positive (*vanA*, *nuc*, and *mecA*) and Gram-negative bacteria (extended-spectrum beta-lactamase genes; $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-2}}$, and carbapenemase genes; $bla_{\text{OXA-48}}$ and bla_{KPC}), as well as bacterial identification (*E. coli* and *Klebsiella* species) and broad bacterial detection, were tested in their proof-of-concept study [100]. Overall, the IC3D system presents itself as a potential solution for obtaining rapid pathogen ID and antibiotic resistance information.

3.2.2. Universal High-Resolution Melt (U-HRM) with Pheno-Molecular AST

High-resolution melting (HRM) analysis is a simple, yet powerful and novel solution for sequence variant scanning, genotyping, and sequence matching, which can be seamlessly integrated with PCR without any post-amplification processing steps. With the use of saturating fluorescent dye, precise reaction temperature control, and software algorithms, HRM can create sequence-dependent melting curves with single-nucleotide resolution for less than one minute [101,102]. Melting temperature and curve shape depend on sequence, % GC content, length, melt domains, and sequence complementarity. The flexibility of the technique, as well as the speed, homogenous assay format, high-throughput, and low cost, allow potential wide adoption in various research and clinical disciplines.

Over the past decade, the clinical feasibility of coupling HRM with broad-range PCR for the diagnosis of various infectious diseases has been demonstrated [103–112]. Rich melting curve profiles that enhance the breadth and analytical specificity of HRM for species-level ID were achieved when used with amplicons generated from targeting the bacterial internal transcribed spacer region (ITS).

A machine learning curve classification algorithm allowed for the automated differentiation of bacterial species based on their unique melting curve profiles when analyzed against an archived melting curve database. Coupled with this algorithm, a 90% specificity for pathogen ID was achieved from positive blood culture. Since the reference database can be incrementally updated, the assay coverage is easily expanded to include more pathogen strains [113].

To date, researchers have developed "single-organism" phenotypic, growth-based AST assays based on qPCR, digital PCR, and microfluidic digital LAMP [114–116]. Notably, Schoepp et al. were able to shorten the antibiotic incubation time in *E. coli* to 15 to 30 min by leveraging the digital-level DNA quantification [115,116]. This pheno-molecular AST was combined using qPCR with U-HRM to enable a broad bacteria ID [117,118]. In a pheno-molecular AST, bacteria are first briefly incubated with antibiotics. The amounts of bacterial DNA, as surrogates of bacterial growths between antibiotic-treated samples and controls, are quantitatively detected and compared to reveal antibiotic susceptibilities [113].

A complete molecular workflow for a sequential ID-AST can be conducted directly from whole blood for BSI [117]. Sample preparation included steps to preferentially lyse all human red and white blood cells with centrifugation to reduce the background while enriching for target cells. The turnaround time for this complete ID/AST workflow is about 8 h, which includes a 6-h pre-enrichment, 1-h rapid ID (ITS rDNA qPCR with U-HRM), and $\geq$1 h rapid AST (16S rRNA RT-qPCR) (Figure 3A). A limit of detection of 1 CFU/mL was achieved for the identification of *A. baumannii*, *E. coli*, *K. pneumoniae*, and *S. aureus* by their unique melting curves. MICs of the bug–drug pairs were subsequently determined by RT-qPCR with threshold cycle, or Ct, differences of $\geq$1 cycle [118].

One of the main limitations of traditional HRM is in resolving species in a polymicrobial infection. An ensemble, composite melting curve from all the species in the mixture is impossible to decouple into individually contributing species. This limitation can be overcome by combining digital PCR with HRM to perform the absolute quantification of target cells/DNA to resolve heterogeneous populations [110,119].

To resolve polymicrobial infections in a spiked-in polymicrobial urine specimen, U-HRM and pheno-molecular AST can be combined on a dPCR platform to yield accurate identification of multiple bacterial species and their susceptibility profiles within $\sim$4 h [112].

Despite demonstrated feasibility for measuring susceptibility based on bacterial proliferation, a prolonged doubling time of fastidious species makes this approach less than ideal. To further accelerate pheno-molecular AST independent of cell division, Yang et al. discovered RNA markers that confer ciprofloxacin susceptibility as early as 10 min after antibiotic exposure and demonstrated marker expression profiles with concordant MIC results from traditional culture-based AST for multiple isolates of *K. pneumoniae* [120].

Analysis at the single-cell level offers valuable clinical information for guiding BSI therapeutic management by (1) resolving polymicrobial infections, (2) differentiating contaminants from true pathogens, and (3) correlating pathogen quantity with disease severity and treatment efficacy.

3.2.3. Gamma Peptide Nucleic Acid (γPNA)

PNAs are polyamide-based synthetic nucleic acids that are capable of binding to complementary oligonucleotides with high specificity and thermal stability [121]. However, due to its non-ionic backbone, PNAs are only moderately soluble in water. Installation of the chiral center at the gamma-backbone renders the molecule more water-soluble [122]. The performance of PNA can be further enhanced by using a double-stranded configuration [123].

Current commercial systems, including AdvanDx QuickFISH by OpGen and AcceleratePheno, have demonstrated the capacity to deliver fast ID and AST from positive blood culture [124,125]. Leveraging on the properties of γPNA analogs with higher kinetics, sensitivity, and specificity over standard PNAs, startup company HelixBind designed a BSI diagnosis workflow that is compatible with analyzing whole blood samples [126]. Only perfect hybridization with target DNA will result in chemiluminescence that leads to optical detection (Figure 3B). Their technology incorporates a selective lysis process to almost entirely remove somatic cells without killing the microbes [126]. Comprehensive detection of more than 20 of the most common pathogens at species-level, with >95% specificity was achieved. Their entire workflow can be completed in under 2.5 h. This rapid turnaround could significantly affect the initiation of targeted BSI treatment.

3.2.4. Next-Generation Sequencing (NGS)

NGS methodologies offer an all-in-one approach to identify a broad range of BSI pathogens, as well as screening for known resistance markers in suspected BSI samples. Over the past decade, several studies have employed the NGS of cell-free DNA (cfDNA) from plasma to diagnose BSI in suspected septic patients, and shown that NGS has a 93.7% agreement with and higher sensitivity than traditional culturing methods [127,128]. Apart from these advantages of NGS over blood culture, with the advent of real-time Nanopore sequencing, pathogens can be identified within minutes of sequencing and the entire workflow can be achieved within six hours of blood draw [129].

The utility of NGS expands beyond simply identifying the BSI. Metagenomic sequencing on cfDNA facilitates screening for resistance markers as well [129]. However, identifying resistance genes does not correlate with actual resistance. Conferred resistance phenotype depends on the activation of these genes and does not suggest a MIC for the preferred antibiotic [130]. Known resistance mechanisms are limited and will continue to evolve. A secondary approach would be predicting MIC from assembled whole genomes using predictive models. Such models have been described for *Neisseria gonorrhoeae* and *Salmonella enterica* [131,132]. Comparing whole genomes of *N. gonorrhea* allows for the determination of single nucleotide polymorphisms (SNPs) in known molecular antimicrobial resistance determinants and the discovery of novel susceptibility loci using genome-wide association studies (GWAS) [131,133]. The observed range of MICs of *N. gonorrhoeae* to cephalosporins could be attributed to non-synonymous substitutions in *penA*, *porB*, *ponA*, and a disrupted mtrR promoter and the resulting model predicted MICs with an overall sensitivity and specificity of 99.9% and 97.1%, respectively [134]. Similarly, whole genomic sequencing-based AST was shown to have an 89.8% concordance between predicted and

experimental MIC in *S. enterica* for an array of 5 antimicrobials with a sensitivity and specificity of 89% and 97%, respectively [132]. Aside from a pathogenic-centric approach, NGS technologies like assay for transposase-accessible chromatin (ATAC)-seq on specific human cell types can provide sensitive pathogen detection with concurrent host response signal to the infection because only accessible host chromatin regions are sequenced. ATAC-seq on human neutrophils challenged with *S. aureus* was shown to have greater sensitivity in detecting bacterial reads (10^3 CFU/mL) in comparison to the traditional library preparation methods (10^5 CFU/mL) while simultaneously detecting host epigenomic responses to the pathogen [135].

The potential utility of NGS in ID/AST of BSI is undeniable but the application of the technology for this purpose is in its infancy. Despite the many advantages of NGS over blood culture like the breadth of information obtained from a single run or the speed of accruing this information, it has its caveats. Limitations of this technology are its dependence on extensive and well-curated databases to interpret the generated data and the choice of thresholds adopted; the inability to differentiate between DNAemia and bacteremia; and the clinical interpretation of whether the detected organisms are indeed infectious or are commensals, colonizers, or just contamination. To accurately identify the pathogen causing the BSI, reads generated on an NGS platform need to be aligned against a reference database that is comprehensive and can delineate between closely related species and potentially between strains within a species. For example, the clinically validated Karius test utilizes an advanced machine learning algorithm to analyze genomic data from cfDNA against their proprietary, constantly refined, a reference database of more than 1000 clinically relevant species of bacteria, fungi, parasites, and viruses (Figure 3D) [128]. Similar databases are required for resistance markers and whole-genome sequences of various pathogenic species to be able to predict the MIC accurately. While these databases are currently limited, the addition of more such data and the creation of analysis pipelines that combine both ID and AST will only strengthen the utility of NGS in BSI diagnosis in the near future.

3.2.5. Surface-Enhanced Raman Spectroscopy

Raman spectroscopy is a non-invasive, label-free, real-time analysis tool based on the vibrational properties of materials [136]. The spectrum obtained from the spectroscopy measurement serves as a molecular fingerprint for the analyte. The major hurdle in the use of Raman spectroscopy is in its ability to achieve highly accurate results in complex samples [137]. The relatively low probability and weak Raman scattering efficiency from bacterial cells could easily be masked by background noise, hence ruling out its potential as a sensitive diagnosis method. In addition, a long measurement time is usually required to increase the signal-to-noise ratio, making it unsuitable for high throughput analysis [137].

To overcome this hurdle, Raman signals have been improved through the use of metallic surface enhancement via surface plasmon resonance. In recent decades, metallic nanoparticles have improved the performance of Raman spectroscopy, allowing measurement with higher signal intensity, resolution, and limits of detection at a single-molecule level. Rapid pathogen detection in blood using surface-enhanced Raman spectroscopy (SERS) has received increased interest for AST measurement by observing spectral changes corresponding to the antibiotic metabolism [136,138].

Recent developments in advanced statistical data analysis and sample preparation techniques have helped to redeem the utility of SERS-based pathogen detection and ID. Utilizing electrokinetic methods, Cheng et al. were able to efficiently separate and concentrate bacteria cells from diluted blood by applying a fine-tuned AC voltage on a set of electrodes. They reported a rapid pathogen identification in less than 5 min using this SERS (Cheng et al., 2013). By using a convolutional neural network, Ho and coworkers were able to generate accurate identification of 30 common pathogens with identification accuracies of up to 99.7% from Raman spectra [139,140].

A commercial system by spectral platform (Spectral-01) uses Raman spectroscopy as its main technology for all-in-one ID and AST. The company claims to detect the pathogen in samples with as low as <1 CFU/mL pathogen load (according to their press release). Their detection method relies on the interaction between bacteria (positively charged cell wall) and human serum albumin (negatively charged surface). Free radicals produced by the bacteria metabolism oxidize the cysteine group of the albumin molecules into albumin dimer. The release of free radicals is reflected by the decrease in Raman and lycopene fluorescence signals, confirming the presence of bacteria.

The assay setup is intended for translation into ID and AST application. So far, a pathogen ID specificity of 94% for over 30 pathogens was obtained in under 20 min (according to their press release). The presence of specific bacteria can be identified by incorporating specific disulfide crosslinkers that can only be cleaved by enzymes produced by certain bacteria. Bacterial susceptibility to antibiotics is then measured by monitoring the level of Raman and fluorescence signals.

The clinical utility of SERS-based ID and AST as a universal diagnosis approach will rely on the availability of a robust spectral database. Quantification of pathogen load may be possible by measuring changes in specific net signals in the sample. While current developments are still in the early stages, the single-cell sensitivity and fast turnaround of Raman-based measurement show promise as future culture-free BSI diagnosis approach for meeting critical clinical timepoint [138,141]. Further development in this direction will determine their translational feasibility in clinical settings.

3.2.6. Flow Cytometry

Flow cytometry is a cellular analysis method widely used for studying cellular characteristics [142]. Individual cells in a liquid suspension flow through a fluidic system and pass a laser light source using laminar flow. Scattered lights are detected by photomultiplier tubes. Lights scattered at an acute angle (forward scatter), due to light diffraction upon contact with the cell surface, give information about the cell size, while wide (90°) angle light scattering (side scatter) from refracted light at the interface between the laser and the intracellular structure is indicative of the cells' roughness and granularity. Using this technique, information such as cell sizes, physiological conditions, and protein content can be obtained.

While the use of flow cytometry in microbiology is growing, its clinical application is still limited. A startup founded in 2013, FASTinov, recently patented a flow cytometry-based antibiotic susceptibility technology (FAST) that identifies and differentiates carbapenemase production in *Enterobacteriaceae*, by measuring the fluorescence intensity of a fluorochrome dye [143]. The susceptibility information correlated (98%) with the standard AST method [144]. The company also demonstrated the use of this platform to determine the MIC of bacteria challenged with colistin after only 1 h of incubation. In short, a 96-multiwell AST panel consisting of dehydrated colistin at a serial concentration of 0.125 to 65 µg/mL and a fluorescent dye is inoculated with bacterial suspension. After 1 h of incubation, bacterial cell fluorescence intensity and morphologies were analyzed using a flow cytometer. The in-house software translates the cytometer readouts into MIC values by incorporating the number of events, light scattering patterns, and fluorescence intensity of each well onto predetermined cut-off values. In a study involving 116 Gram-negative bacilli, the authors demonstrated a highly reproducible (97%), automatically generated MIC result after 1.5 h [143].

The fast <2 h turnaround, with MIC information from positive culture (equivalent to an overall <26 h turnaround) puts the FASTinov platform ahead of conventional AST methods that take at least 30 h to complete. However, the reliance on blood culture and the lack of ID capacity would set the system apart from other emerging platforms.

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4. Summary and Future Outlook (Conclusions)

An accurate and timely diagnosis of BSI is important for promoting the appropriate use of antibiotics. The lack of rapid ID/AST in current clinical approaches warranted the initiation of antibiotic treatment without a confirmed diagnosis. To date, the T2 Biosystem is the first and only FDA-approved system capable of identifying a BSI pathogen directly from whole blood. So far, no commercial systems could perform both ID and AST for diagnosing BSI directly from whole blood samples using low sample volume and in a rapid manner. Nevertheless, promising new technologies are emerging and paving the way for efficient, sensitive, selective, and comprehensive diagnoses.

Our assessment of the current ID/AST developmental progress (Figure 4, Table 3) reveals the potential for several emerging techniques to achieve highly sensitive and specific ID/AST for BSI. From our current assessment, qPCR-HRM, SERS, IC3D, and γPNA-FISH appear to be the most promising in terms of delivering highly sensitive and specific ID/AST from whole blood samples. Their relatively fast turnaround likely fits into the critical clinical timepoints concerning antibiotics selection, escalation, and de-escalation. However, only the qPCR-HRM workflow was able to deliver MIC information, and only γPNA-FISH could deliver results from whole blood in less than three hours. These features are particularly desirable for guiding clinical decisions.

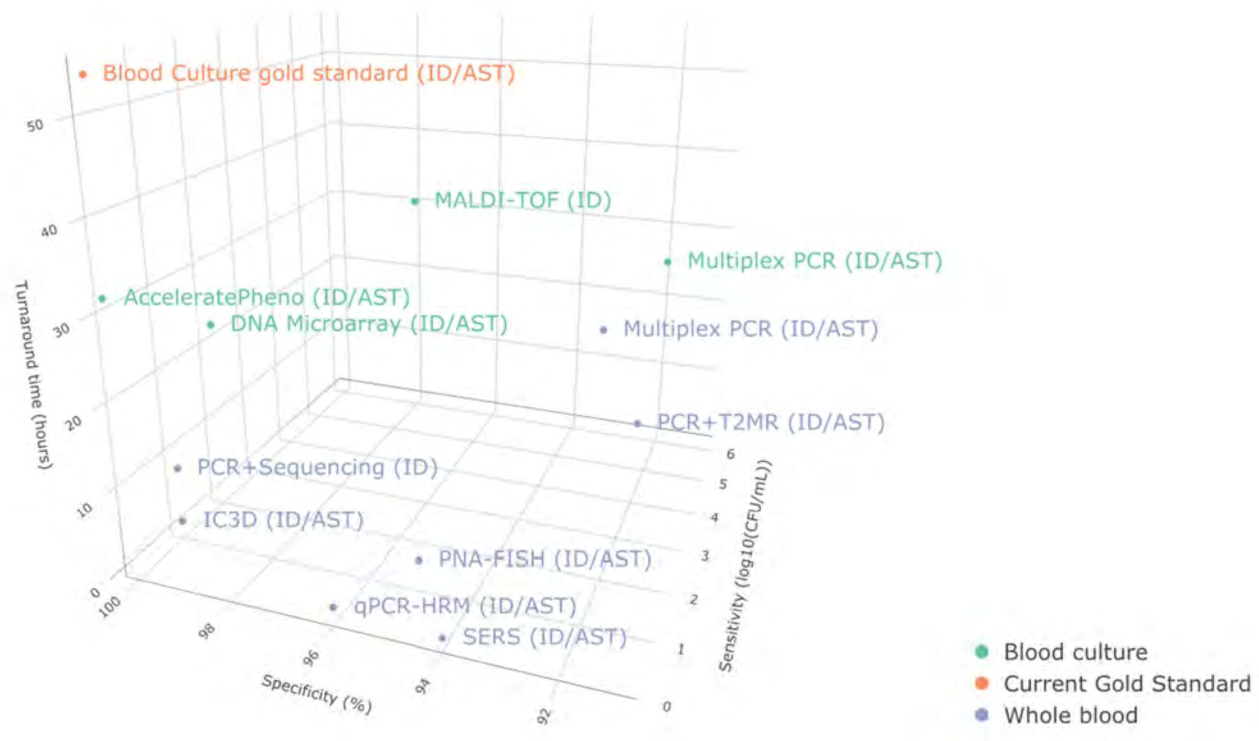

Figure 4. Performance characteristics of current and emerging technologies. Sensitivity, specificity, and turnaround time compared against the current gold standard: 1. qPCR-HRM, 2. SERS, 3. IC3D, 4. PNA-FISH 5. PCR+T2MR, 6. Multiplex PCR from whole blood, 7. PCR + sequencing, 8. Multiplex PCR from blood culture, 9. DNA Microarray, 10. MALDI-TOF, 11. AcceleratePheno, and 12. Blood culture gold standard.

Table 3. Comparison of Existing and Emerging BSI Diagnosis Technologies.

Technologies	Sample	Company	ID				AST	
			Sens. (CFU/mL)	Spec.	Breadth	TAT	Output	TAT
			EMERGING					
qPCR-HRM	WB *	Non-commercial	1	100%	37 bacteria (expandable)	8 h (with AST)	MIC	8 h (with ID)
SERS	WB	Spectral Platforms	1	94%	>30 pathogens	20 min	S/R (enzyme-based)	unspecified

Table 3. Comparison of Existing and Emerging BSI Diagnosis Technologies.

Technologies	Sample	Company	ID				AST	
			Sens. (CFU/mL)	**Spec.**	**Breadth**	**TAT**	**Output**	**TAT**
			EMERGING					
qPCR-HRM	WB *	Non-commercial	1	100%	37 bacteria (expandable)	8 h (with AST)	MIC	8 h (with ID)
SERS	WB	Spectral Platforms	1	94%	>30 pathogens	20 min	S/R (enzyme-based)	unspecified
ddPCR/ IC3D	WB	Velox Bio	10	100%	unspecified	1–4 h (with AMR)	resistance marker	1–4 h (with ID)
Flow Cytometry	BC (+)	FASTinov	N/A	N/A	N/A	N/A	MIC	<26 h
PNA-FISH	WB	HelixBind	<10	95%	21 pathogens	2.5 h (with AMR)	resistance marker	2.5 h (with ID)
			EXISTING					
PCR+T2MR	WB	T2 Biosystems	1–10	91%	5 candida species, ESKAPE organisms	27–29 h (with AMR)	resistance marker	27–29 h (with ID)
Multiplex PCR	WB	MagicPlex (SeeGene)	30	66–92%	>90 pathogens with 27 pathogens at species level	27–30 h (with AMR)	resistance marker	27–30 h (with ID)
Real-time PCR+Sequencing	WB	SepsiTest (Molzym)	10–40	86–100%	>1350 pathogens	30–31 h	N/A	N/A
Multiplex PCR	BC (+)	BioFire (FilmArray)	10^6 to 10^8	82–92%	8 Gram+/11 Gram−/5 fungi	25 h (with AMR)	resistance marker	25 h (with ID)
DNA Microarray	BC (+)	Luminex (Verigene)	10–100	84–99%	8 Gram+/5 Gram−	26.5 h (with AMR)	resistance marker	26.5 h (with ID)
MALDI-TOF + AST cards	BC (+)	Biomerieux (VITEK 2)	10^6	61–98	1316 pathogens	30–36 h (with AST)	MIC	30–36 h (with ID)
PNA FISH + morphokinetic cellular analysis	BC (+)	Accelerate Diagnostics (Accelerate Pheno)	0.8 to 1.7	86–100	7 Gram+/8 Gram−/2 fungi	32 h (with AST)	MIC	32 h (with ID)
Traditional Blood Culture	WB	BD (BACTEC)	1	100%	Broad	30 h	MIC	54 h (with ID)

* WB: direct from whole blood, BC (+): from positive blood culture; Sens.: sensitivity; Spec.: specificity; TAT: Turnaround time.

While novel approaches with rapid outcomes are a priority, discrepant results derived from new methods against imperfect conventional methods must be interpreted with caution. The clinical significance of microbial sequences identified in the blood must be interpreted along with the clinical context. For accelerated AST, the growth kinetics of the bacteria in the presence of antibiotics could be confounded by variations in inoculum size from isolated colonies, the presence of dead cells, and the growth phase of the inoculated bacteria [145]. Furthermore, isogenic bacterial populations, shown to have heteroresistance, are prone to the false categorization of susceptibility [146,147], and in some isolates, antibiotics exposure can easily enhance antibiotic resistance [148]. Therefore, understanding factors affecting antibiotic responses such as potential inoculum effect, or delayed resistance due to variation in growth phases or delayed induced resistance expression, should be

taken into account when designing a new assay that relies on a vastly different format from the current reference standard [12].

Although we have only discussed microbiological technologies, combination analysis that takes into account host responses (including immunity and hemodynamics) could be a strategy for improving future BSI diagnosis as well as the prognosis of clinical outcomes. Future development towards fulfilling the four key elements of an ideal BSI diagnosis should consider integrating suitable sample preparation techniques to enhance downstream analysis. We anticipate that future breakthroughs will likely arise from technologies that require (1) minimal handling and analysis of the low pathogen, (2) the separation, enrichment, and concentration of pathogens or target analytes from background interferences, (3) the delivery of sensitive, quantitative, and agnostic detection and broad species-level identification and (4) the provision of timely universal antibiotic susceptibility profiling with MIC reporting that matches critical clinical decision timepoints.

All in all, it remains unclear if a "one-size-fits-all" test with superior performance characteristics will ever supplant blood culture practice in BSI diagnosis. While exciting development is underway, it is easier to envision these technologies augmenting the reduction in early diagnostic uncertainty to impact clinical outcomes.

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REVIEW
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Modern Tools for Rapid Diagnostics of Antimicrobial Resistance

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Fast, robust, and affordable antimicrobial susceptibility testing (AST) is required, as roughly 50% of antibiotic treatments are started with wrong antibiotics and without a proper diagnosis of the pathogen. Validated growth-based AST according to EUCAST or CLSI (European Committee on Antimicrobial Susceptibility Testing, Clinical Laboratory Standards Institute) recommendations is currently suggested to guide the antimicrobial therapy. Any new AST should be validated against these standard methods. Many rapid diagnostic techniques can already provide pathogen identification. Some of them can additionally detect the presence of resistance genes or resistance proteins, but usually isolated pure cultures are needed for AST. We discuss the value of the technologies applying nucleic acid amplification, whole genome sequencing, and hybridization as well as immunodiagnostic and mass spectrometry-based methods and biosensor-based AST. Additionally, we evaluate the potential of integrated systems applying microfluidics to integrate cultivation, lysis, purification, and signal reading steps. We discuss technologies and commercial products with potential for Point-of-Care Testing (POCT) and their capability to analyze polymicrobial samples without pre-purification steps. The purpose of this critical review is to present the needs and drivers for AST development, to show the benefits and limitations of AST methods, to introduce promising new POCT-compatible technologies, and to discuss AST technologies that are likely to thrive in the future.

Keywords: antibiotic resistance, antimicrobial susceptibility test, antimicrobial resistance, point of care test, rapid AST

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BACKGROUND AND FOREWORD

There is an unmet need for rapid and decentralized diagnostics in outpatient clinics to reduce the misuse of antibiotics. It is important to identify the etiological pathogen and to differentiate between viral and bacterial infections, to identify the antimicrobial resistances in microbes, and to f nd out which antimicrobial agent should be used for the cure. Thereby the unnecessary use of antibiotics could be minimized and the spread of antibiotic resistance better controlled. According to the WHO, antimicrobial resistance (AMR) is the largest global health threat in the 21st century and requires urgent measures. Common infections are becoming untreatable due to the emergence of AMR. More than 700,000 people die of drug-resistant infections every year, and this f gure is expected to reach ten million by 2050 (United nations meeting on antimicrobial resistance, 2016). According to current understanding, in EU and EEA countries more than 33 000 people are killed every year due to antibiotic-resistant bacteria. They also cause close to 900 000 disability-adjusted years (Cassini et al., 2019). Bringing diagnostics closer to the general practitioners and hence to the patient would cause a paradigm shift from empirical to

evidence-based treatments of infectious diseases in outpatient clinics. Rapid diagnostics are needed for both pathogen identification and resistance testing. The prevalence of AMR may be very high for some species in certain geographic locations. According to the current recommendations on AST (antimicrobial susceptibility testing), pure culture isolates are used to test the effect of antimicrobial drugs. This is required as the sample matrix (blood, urine, mucosal) as well as the number and proportions of different microbial species may vary a lot in polymicrobial samples. It may be unclear whether the detected microbe is pathogenic or merely a commensal species. Despite significant progress in diagnostic technologies in recent years, most patients with infectious diseases are still treated empirically and thus antibiotics are heavily overused (Li et al., 2016; Mashalla et al., 2017). Even in Western countries, 30% of antibiotic prescriptions are considered to be either unnecessary or suboptimal (Centers for Disease Control Prevention, 2018). Current diagnostic tests serve hospitalized patients rather well, but they are often not available in outpatient clinics. For typical growth-based AST, several cultivation rounds are required: enrichment cultivations (e.g., blood cultures) to increase the number of bacteria, plate cultivations to obtain pure cultures, and finally AST for liquid or plate cultures using various antimicrobial loads. Microbiology laboratories apply the EUCAST-accepted breakpoint values to define whether the microbe is susceptible or resistant to the tested antibiotic. They use the disk diffusion method or other systems calibrated to EUCAST standards. Altogether, AST may require several days. Rapid molecular diagnostics has been discussed in many excellent reviews (Pulido et al., 2013; Plüddemann et al., 2015; Li et al., 2017b; Maurer et al., 2017; Syal et al., 2017; Maugeri et al., 2019). They present the progress in Nucleic Acid Amplification Technology (NAAT), electrochemical methods, microarrays, micro- and nanoparticles, as well as mass spectrometry applications, but also emphasize that very few of the molecular methods have acquired FDA approval.

The review of David Boyle, "Tuberculosis Diagnostics Technology Landscape" is worth reading, since, although not focusing on AST, it presents comprehensively new affordable molecular diagnostic technologies available in standard microscopy stations, particularly in developing countries (Boyle, 2017). The most up-to-date and concise progress compendia in the field of AST can be found in congress presentations, lectures and webinars. Prof. Mark Fisher's webinar "Rapid Antimicrobial Susceptibility Testing" (ARUP, 2020; Scientific Resource for Research and Education: Educational Resources—Rapid Antimicrobial Susceptibility Testing | University of Utah) is particularly useful.

The innovations in electronics, biosensor techniques, optics, microfluidics, hybridization technologies and DNA amplification technologies have yielded new approaches in AST. Unfortunately, the scientific papers on these technologies do not sufficiently relate these findings to the practical needs in POCT. The requirement of a microbiology laboratory and the time and resources needed for the enrichment of cultures, preparation of pure cultures. and sample treatments is often not sufficiently considered. These requirements also easily blur the total costs of AST. For these reasons, the deployment of new molecular methods for AST has been very slow (Doern, 2018). The standards of care for antibiotic prescription are quite consistent in most European countries and the USA and apply evidence-based ID and AST when available. In total, urinary and respiratory tract infections form a significant part of acute infections. Quick and accurate diagnosis for these diseases already at outpatient settings could efficiently restrict the spread of AMR bacteria and allow an early isolation of the carrier and correct treatment. Rapid diagnosis would also allow the prompt dismantling of unnecessary patient isolation, saving money and resources. However, technical improvements translate into benefits only if the structured communication and interpretation of the results are applied by the clinicians (Maurer et al., 2017) and the cost of these technologies are reasonable.

Vital emergency diagnostics for septicaemia has received a lot of resources (Marco, 2017; Hughes, 2018). New sensitive methods such as T2MR (T2Biosystems, USA) can quickly detect molecular targets directly from clinical samples, enabling rapid pathogen identification and detection of resistance factors. However, growth-based AST for blood requires a fairly high bacterial count for enrichment cultures and a well-equipped microbiology laboratory. The achievements in blood testing do not necessarily relate well to the antimicrobial stewardship in the front-line: healthcare settings.

Optimal antimicrobial therapy policy would require (1) Fast point of care analysis, (2) Identification of the etiological agent, (3) Finding of an efficient antibiotic, and (4) Determination of the functional dosage. According to Prof. Gunnar Kahlmeter (Chairman, EUCAST general committee) (Kahlmeter, 2016), the key questions for any new AST technology are:

- Is it generally applicable or suitable only for one infection (for example sepsis or one resistance type)?
- What is the capacity? How many organisms/agents per hour can be processed?
- Has the technology been validated against reference methods?
- Are there any reference installations?
- Is scientific literature available?
- When will it be on the market?

Complete answers are hard to dig up, but this review tries to address these questions. We present the needs and drivers for AST development, increase the understanding about the role of rapid AST in diagnostics of infectious diseases, show the benefits and limitations of AST methods, introduce the key

Abbreviations: AMR, Antimicrobial Resistance; AR gene, Antibiotic Resistance gene; AST, Antimicrobial susceptibility testing; CLSI, Clinical & Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; CFU, Colony forming units; FISH, Fluorescence *in situ* Hybridization; ID, Identification; LF, Lateral flow; LCR, Ligase Chain Reaction; MALDI-TOF, Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight mass spectrometry; MIC, Minimal Inhibitory Concentration; POCT, Point of Care Testing; NA= Nucleic Acid; NAAT, Nucleic Acid Amplification Technology; PIT, Plasmonic Imaging and Tracking; SERS, Surface Enhanced Raman Scattering; FEED, field effect enzymatic detection; MADM, Multiplexed Automated Digital Microscopy; PNA, Peptide-Nucleic Acid probe; WGS, Whole Genome Sequencing.

POCT-compatible technologies, and contemplate on which AST technologies are likely to thrive in the future.

CURRENT TECHNOLOGIES IN ANTIMICROBIAL SUSCEPTIBILITY TESTING AND MICROBIAL IDENTIFICATION

Bacteria can acquire resistance to antibiotics by several mechanisms. The antibiotic can be degraded or chemically modif ed (by acetylation, phosphorylation, nucleotidylation, ADP-ribosylation, mono-oxygenation, glycosylation). The drug intake can be prevented, or efflux can be enhanced. Some resistance mechanisms are based on the reprogramming of cell wall synthesis. Even slight changes in the target molecule, e.g., a point mutation in the ribosomal protein, can render the antibiotic ineÿ cient. The overwhelming variety of antimicrobials and resistance mechanisms complicates AST. Genotypic (nucleic acid-based) methods can only f nd resistances that are searched for, and the potentially found resistance genes are not necessarily from the actual pathogenic organism. According to EUCAST and CLSI guidelines, reliable antibiotic resistance diagnostics requires phenotypic testing, i.e., an experimental test whether the microorganism grows in the presence of the antibiotic. These methods work regardless of the resistance mechanism and give answers to the practical questions: which antibiotic is eÿ cient and which dose should be applied in the therapy. Classical AST techniques such as broth microdilution, disk diffusion, gradient tests, agar dilution and breakpoint tests are based on continuous exposure of a bacterial isolate to a set of antimicrobials, followed by a visual detection of growth. The use of advanced optoelectronic systems, f ber optics, microf uidics and indicator dyes sensitive to redox-state or pH can further enhance the sensitivity and performance of optical systems.

Several commercial systems have streamlined and partly automatized the follow-up of AST cultures. Systems like Vitek and Microscan perform automated turbidity measurement for multiwell liquid cultures. BD Phoenix system™ applies a redox indicator to enhance the detection of organism growth. These systems have turnaround times as short as 4 h for ID and 6–8 h for susceptibility testing (She and Bender, 2019). The CE-marked Alfed 60 AST™ system (Alifax, Italy) uses sensitive laser-light scattering technology to detect bacterial growth in a liquid culture broth and provides antimicrobial susceptibility results directly from positive blood culture bottles within 4–6 h.

Such broth dilution-based systems use ready-made AST cassettes or cards containing positive controls and wells with increasing concentrations of antibiotics. They provide continuous growth monitoring and can analyze MIC patterns for a large group of organisms through their extensive databases.

Pathogen identif cation (ID) is usually a preliminary step of AST. For blood samples, microscopy and Gram-staining are nearly always performed, as Gram-positive bacteria in general have a more limited variety of antibiotic resistances and less problems with multidrug resistance. In AST, the following sequence is typically applied: First clinical samples are cultured to obtain pure isolates. Then identif cation (with MALDI-TOF mass spectrometer, if available) is performed. Thereafter, AST and MIC determination is performed according to EUCAST or CLSI standards. This sequence, in total, requires several days. Standard healthcare settings do not have advanced microbiology laboratories with mass spectrometry instruments. Their arsenal for the diagnosis of infectious diseases may be limited to immuno-chromatographic strip tests (aka lateral f ow tests = LF or "dip-sticks") applied to the detection of viruses (e.g., inf uenza) and bacterial pathogens causing sexually transmitted diseases.

Quick identif cation can eÿ ciently restrict the search palette for certain antibiotics. Hence mass spectrometry has become a versatile workhorse in clinical laboratories. It is routinely applied for bacterial ID as soon as isolated colonies are available. Through the simultaneous measurement of several metabolites a biochemical signature of microbes can be obtained. Matrix-assisted laser desorption/ionization time of f ight (MALDI-TOF) applies laser energy to evaporate the matrix-bound sample, that is then immediately analyzed. When frequent sampling is applied, MALDI-TOF can even provide semi-quantitative growth rate data (Maxson et al., 2017). Bruker Corp. (Germany) has launched test kits such as BT STAR-Carba Assay for AST based on antibiotic degradation monitoring.

AST for blood cultures has been applied after a short-term cultivation on agar plates followed by susceptibility testing using VITEK AST cards selected on the basis of MALDI-TOF analysis (Idelevich et al., 2014; Mauri et al., 2017). By applying the MBT-ASTRA™ test with MALDI Biotyper for ID and AST, identif cation of mycobacterial strains resistant to rifampicin, isoniazid, linezolid, ethambutol, clarithromycin and rifabutin can be obtained 1 week faster than through routine cultivation-based AST (Ceyssens et al., 2017). The MS approaches combined with NAAT or microf uidics will be presented in "Future technologies" section.

CURRENT TECHNOLOGIES FOR RAPID AST

Many novel methods claim to perform AST in minutes or in few hours. Such statements usually ignore the need of time-consuming steps such as enrichment cultures and isolation of pure cultures (**Figure 1**). Methods based on NAAT, nucleic acid hybridization or immunodiagnostics in principle allow the use of non-purif ed polymicrobial clinical samples. A short cultivation with a pre-determined antibiotic load followed by NAAT (e.g., isothermal amplif cation) can reveal AR, and even provide a rough estimate of the minimal inhibitory concentration (MIC) for the tested antibiotics. Most rapid growth-based AST methods perform end-point analysis only, whilst others rely on frequent sampling from the cultivation chamber. Some sensitive immunodiagnostic systems however provide real on-line growth monitoring (**Figure 2**). Biosensor technologies detecting changes in microbial metabolism, movement or heat production have not yet provided convincing clinical demonstrations. Fast, reliable, easy-to-use and inexpensive systems applicable to AST in outpatient clinics are still elusive (van Belkum et al., 2019a).

A

Current methods for ID/AST

Steps: Sampling | Microscopy | Quick ID for pure cultures or ID by cultivation | Growth-based AST

Gram-staining

Sample preparation

MALDI/TOF
NAAT
Immunodiagnostics
FISH

Manual or automated disk diffusion test or broth dilution test

Outcome: Bacterial number increased | Isolated colonies | Pathogen identified | MIC values determined

Result 36-72 h

Identification

Enrichment | Plating | AST

1 Day | 2 Days | 3 Days

B Rapid ID/AST for pure cultures

Quant. NAAT for accelerated cultures with antibiotics, single cell monitoring methods, FISH

Result 30-40 h

C Rapid AST for clinical polymicrob. samples

NAAT for ID and AR genes, NAAT or FISH for accelerated cultures, on-line growth monitoring coupled with species-specific binders

Result 0.5-8 h

FIGURE 1 | Rapid AST or rapid result? **(A)** Current technologies. **(B)** Rapid AST applicable to pure cultures. **(C)** Rapid AST for clinical polymicrobial samples. The presented times are rough estimates and generalizations.

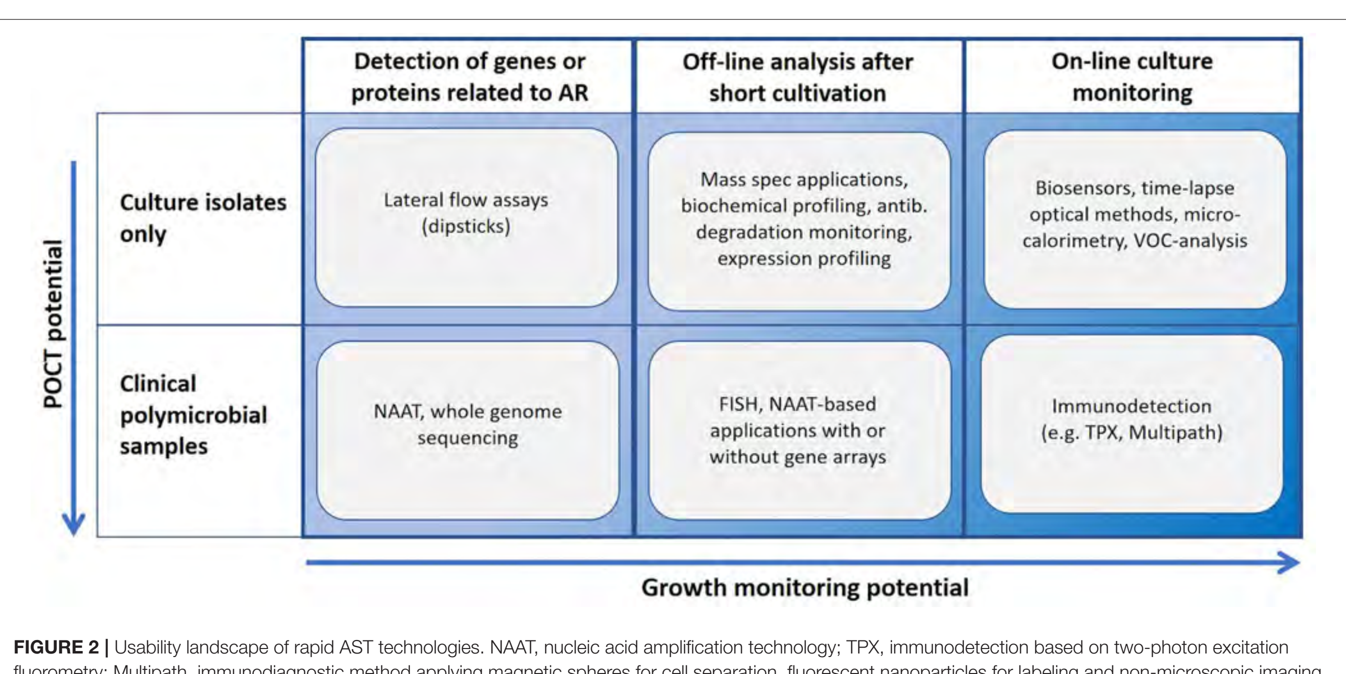

FIGURE 2 | Usability landscape of rapid AST technologies. NAAT, nucleic acid amplification technology; TPX, immunodetection based on two-photon excitation fluorometry; Multipath, immunodiagnostic method applying magnetic spheres for cell separation, fluorescent nanoparticles for labeling and non-microscopic imaging.

Microscopy

Counting of bacteria on agar plates by microscopy techniques is possible long before they have reached the number providing visible colonies. *E. coli* colonies visible by eye contain roughly 5×10^6 bacteria, but by microscopy microcolonies formed by 120 cells can already be detected (London et al., 2010). Drug susceptibility (MODS) for *Mycobacterium tuberculosis* can be assessed by observing cell aggregates (cords) microscopically in sealed microtiter plates (den Hertog et al., 2014). The Growth Direct System by Rapid Micro Biosystems Inc. detects microcolonies with digital imaging by illuminating them with blue light and directing the cellular autof uorescence directly onto a CCD chip without magnif cation. The average time for *E. coli* detection by this autof uorescence was 3.1 h compared to an average of 8.5 h for the visual plate counting method. Although the idea of applying this to AST has been declared in the patent application, clinical studies for AST have not yet been presented.

Automated microscopy systems can provide real time growth curves, and quantitative bacterial counts have been presented. Multiplexed automated digital microscopy (MADM) applying Fluorescent *in situ* Hybridization (FISH) has been commercialized by Accelerated Diagnostics (USA) for rapid on-line AST (Metzger et al., 2014; Chantell, 2015). The Accelerate Pheno® system can separate impurities from clinical samples (e.g., blood or urine) by brief electrophoresis, which runs impurities into a gel. After this a change of the electric f eld polarity repels the microbes back to the liquid. A f uorescence signal is detected every 10 min from samples taken from the bacterial culture multiplying in Mueller-Hinton media (Charnot-Katsikas et al., 2017). This system seems to be currently the only FDA-approved growth-based rapid diagnostic AST system (Doern, 2018). The performance of Accelerate Pheno® has been demonstrated with many clinical studies, e.g., with urinary tract infections (Charnot-Katsikas et al., 2017) and bloodstream infections (Charnot-Katsikas et al., 2017; Marschal et al., 2017; Descours et al., 2018). With 232 positive blood cultures tested, the overall essential agreement with routine methods was 95.1%, and the time needed for AST was decreased by 42 h in comparison to standard growth-based analysis. ID could be obtained in 1.5 h and AST in 7 h (Charnot-Katsikas et al., 2017).

Hybridization-Based Systems

FISH is a highly specif c method to visualize the presence of the target organism in a quantitative manner. The PNA-FISH technology applies peptide nucleic acid probes which allow more rapid and specif c binding than DNA or RNA probes (Perry-O'Keefe et al., 2001; Almeida et al., 2009; Cerqueira et al., 2011). It is applied in the commercial QuickFish technology (OpGen, USA) which performs ID by targeting 16S rRNA (Enroth et al., 2019). XpressFish specif cally detects the *mecA* gene in *Staphylococcus,* allowing, when used subsequent to QuickFish-based identif cation, diagnosis of methicillin resistance already 2 h after the blood culture turns positive (Salimnia et al., 2014). Since a temperature of 55°C is needed for target cell permeabilization, f xing and hybridization, PNA-FISH systems are not applicable to on-line growth monitoring. FDA-approved systems are available also from bioMérieux (Durham, NC). Clinical laboratories applying mass spectrometry are unlikely to need PNA-FISH technology; the Bruker MALDI Septityper® kit PBP2A, e.g., can detect the *mecA*-encoded PBP2A-protein in 1 h with fairly low costs.

In actively growing cells, RNA is more abundant than DNA and thus a good target for probing. Especially precursor rRNA (pre-rRNA), the intermediate stage in the formation of mature rRNA, is a good indicator of bacterial metabolism, viability, and growth rate (Halford et al., 2013). The biosensor-based AST (b-AST) system from Genef uidics Inc. (CA, USA) measures bacterial growth by quantifying 16s rRNA molecules with an electrochemical biosensor. This system uses species-specif c probes and integrates nanotechnology, plastic micro electromechanical system and microf uidics (Mach et al., 2011). This system achieved a detection limit of 10^4 cfu ml^{-1} in rapid AST of clinical urine and blood samples (Liu et al., 2014). Mohan et al. demonstrated the simultaneous detection of uropathogens and the host biomarker lactoferrin in urinary tract infection, but reached only 89% sensitivity in the pathogen identif cation (Mohan et al., 2011). In 2018 Genef uidics announced CE-IVD Marking for the UtiMax™ kit, which provides ID in 30 min and AST in 2 h from urine with an overall sensitivity of 100% and specif city of 98.2% (GeneFludics Inc., n.d.).

Nucleic Acid Amplification Technology (NAAT) in AST

The f rst generation "molecular tests" such as restriction fragment length polymorphism, pulsed-f eld electrophoresis, multiple locus tandem repeat analysis, multi-locus sequence typing and virulence genotyping were suitable rather for typing and outbreak investigation than for AST. Since these technologies require a high amount of purif ed nucleic acid, they do not allow rapid diagnostics. However, hybridization-based approaches and molecular beacon systems have persisted and are creatively combined with NAAT technologies.

NAAT is a very powerful tool for pathogen identif cation, especially when combined with a syndromic approach. Many diagnostic panels provided e.g., by BioMérieux, Elitech, Bosch, Eplex, Qiagen, or Becton Dickinson, include detection of specif c AR-genes. They can provide clinically relevant results, especially in cases where a detailed antibiogram is not needed. For example, pathogen such as *Bordetella*, *Legionella*, *Mycoplasma*, *Chlamydia trachomatis*, or *Neisseria gonorrhea* exhibit quite few antibiotic resistances. The detection of specif c AR genes, however, cannot give an undisputed proof of antibiotic resistance. The identif ed AR genes do not necessarily relate to the pathogen causing the disease, or the found resistance gene may not be functional. NAAT neither def nes the MICs nor directly indicates which antibiotics should be used. An advantage of NAAT is that the tests can be relatively quickly updated for newly emerging pathogens and resistance factors. Quantitative PCR (qPCR) allows a rough quantif cation of microbes. Quantitative reverse transcription PCR (qRT-PCR) can additionally assess the expression level of resistance genes after exposure to different antibiotic loads and thus provide

rough MIC values. The cost of devices and reagents for qRT-PCR are unfortunately currently far beyond the level acceptable for routine AST. NAAT is a powerful tool for the identif cation of both bacterial and viral pathogens. In principle it allows the use of patient samples without enrichment cultivations. However, due to the risk of losing the template during nucleic acid extraction and the sensitivity of DNA polymerases to impurities in the sample matrix, enrichment cultivations and nucleic acid purif cation are often necessary. Due to the vast choice of different f uorescent labels, several target genes can be conveniently tested in parallel from the same sample. The systems can reach further sensitivity and specif city by applying hybridization to DNA arrays. A good number of FDA-approved multiplexed diagnostic panels are already available. Such commercial systems include Xpert® (Cepheid Inc.), ePlex (GenMark Diagnostics), Unyvero (Curetis AG), BD Max (Becton-Dickinson), SeptiFast (Roche), Magicplex (SeeGene), Novodiag (Mobidiag), and GenomEra (Abacus Diagnostics). The Xpert® technology combines sample preparation, real-time PCR and nucleic acid analysis with molecular beacons. The ePlex system provides electrochemical detection of the amplif ed sequences, wherein the detection is achieved by hybridizing ferrocene-labeled probes with the sample DNA (Nijhuis et al., 2017). Hybridization techniques and microf uidics have been implemented also to Novodiag's GenomEra microarray platform. It applies time-resolved f uorescence detection of the amplif ed product on a sealable plastic chip packed with dry chemistry. Also Curetis, Becton-Dickinson, Roche and SeeGene products apply cartridge-based integrated designs (Hughes, 2018). Fast analytics of PCR products enhance the throughput of NAAT systems. T2Biosystems has recently launched a test panel able to detect 13 resistance genes from both gram-positive and gram-negative pathogens directly from blood. The amplif cation products are detected by magnetic resonance after hybridization with DNA probes conjugated with superparamagnetic particles (Hong Nguyen et al., 2019).

PCR/electrospray ionization–mass spectrometry (IRIDICA PCR/ESI-MS by Abbott Laboratories Inc., USA) allows the detection of >750 different bacterial species in a single test (Strålin et al., 2016). The High Resolution Melting system (HRM, by ThermoFisher) identif es variations in nucleic acid sequences by detecting small differences in PCR melting curves. A melting curve analysis for real-time quantitative PCR or digital PCR (wherein the sample is partitioned into a large number of individual wells each containing either 1 or 0 targets) performed for growing bacterial cultures, revealed both ID and antimicrobial susceptibility prof les for *E. coli*, *E. faecalis*, *P. mirabilis* and *S. aureus* in ∼6.5 h, when analyzed by machine learning algorithms (Athamanolap et al., 2017). For routine analytics, such systems are still too expensive, and they require separate kits for DNA extraction and PCR. However, new enzymes and technologies such as ligase chain reaction (LCR) (Barany, 1991), nucleic acid sequence-based amplif cation (NASBA) (Compton, 1991), strand displacement amplif cation (SDA) (Walker et al., 1992) and loop-mediated isothermal amplif cation (LAMP) (Notomi et al., 2000) have simplif ed nucleic acid amplif cation, made it more robust (often allowing the use of samples without NA extraction), enabled miniaturization and cut down the costs of instrumentation by allowing NAAT at a constant (isothermal) temperature. NUCLISENS® EASYQ® (bioMerieux) was the f rst automated system to combine NASBA and real-time detection using molecular beacon probes. It enabled the fast detection of *Klebsiella* carbapenemase genes (Spanu et al., 2012). LCR has been successfully used for the detection of ciprof oxacin and doxycycline resistance genes in *Bacillus anthracis*, *Francisella tularensis,* and *Yersinia pestis* (Oblath et al., 2013). LCR can be easily integrated into detection systems such as electrochemical and magnetic biosensors, quantum dots, quartz crystal and leaky surface acoustic surface biosensors, Surface Enhanced Raman Scattering (SERS), chemiluminescence and f uorescence resonance energy transfer (Oblath et al., 2013). High-throughput multiplex genotyping can be achieved also by DNAzyme (DNA oligonucleotides) technology, rolling circle amplif cation (RCA) and strand displacement amplif cation (SDA) techniques. LAMP is especially robust, since it is less sensitive to inhibitors than standard PCR. This allows analysis after a minimal processing of blood (Curtis et al., 2008), urine or stool (Francois et al., 2011). LAMP is applicable to low-resource f eld settings where DNA or RNA extraction is not possible. However multiplexing approaches are less developed for LAMP than for PCR (Sahoo et al., 2016).

Immunodetection of Pathogens

Immunodetection is a specif c and sensitive method for the identif cation of bacterial pathogens (Verma et al., 2013), toxin proteins (Zhu et al., 2014), and viruses. Since immunodetection does not necessarily require disruption of the target microbes, it can potentially provide pathogen identif cation and growth monitoring in a single step. It is applicable as simple lateral f ow (LF) tests, but can also be integrated to biosensor technology, microf uidics and even to DNA/RNA-based analysis. Companies like Mizuho Medy, Alere, and Beckton Dickinson have launched several easy-to-use stick tests for clinical diagnostics of (inf uenza) viruses and bacteria causing sexually transmitted diseases. The binders are typically antibodies that are immobilized onto strips, micro/nanoparticle beads or biosensor surfaces providing an eÿ cient and specif c target binding. The detection antibody can be labeled with f uorescent dyes or redox enzymes to provide a quantitative signal.

Only few products are available for the direct detection of antibiotic resistance proteins. The LF-test developed by Kitao et al. detects chloramphenicol resistance in *P. aeruginosa* samples (Kitao et al., 2010). Alere Inc. has launched an immunochromatography test for the detection of MRSA, based on a PBP2a-specif c chicken IgY antibody (Yamada et al., 2013). The PBP2a SA Culture Colony Test can identify MRSA in 6 min (Trienski et al., 2013; Delport et al., 2016). Coris Bioconcept (Belgium) has launched tests for the detection of carbapenemases (OXA-48-like, KPC, and NDM type) from enterobacterial isolates (Bogaerts et al., 2013; Glupczynski et al., 2017). Boutal et al. have presented LF tests for the carbapenemases TX-M-15, NDM, OXA-48-like, KPC, IMP, and VIM (Boutal et al., 2017). LF tests work well with isolated clinical isolates. They are,

however, generally not applicable for direct analysis of clinical samples. The commercial MultiPath™ platform (First Light Diagnostics Inc., USA) applies non-magnif ed digital imaging for the detection of biomolecules tagged with antibody-coated f uorescent nanoparticles. The mariPOC® system by ArcDia Ltd, based on Two-Photon eXcitation f uoroscopy technology (TPX), is already in diagnostic use for immunogenic detection of pathogens. These two technologies will be discussed closer in the following sections.

FUTURE TECHNOLOGIES UNDER COMMERCIALIZATION

Mass spectrometry is likely to become tightly integrated into other AST technologies, especially in the diagnostics of septicaemia. In the MALDI-TOF Direct-On-Target Microdroplet Growth Assay (DOT-MGA), sample droplets (culture plus antibiotics in 6 μL volume) are spotted directly onto disposable MS-target plates, incubated for 3–4 h and then analyzed with MS (Idelevich et al., 2018). Screening panels for ESBL and AmpC β-lactamases of enterobacteria are already available (Correa-Martínez et al., 2019). The fast progress in microf uidics, biosensor technologies, isothermal amplif cation-based NAAT, and immunodetection has recently provided several potent systems which may eventually change the paradigms of AST.

Gradientech's QuickMIC system combines microf uidics with automated time-lapse photomicrography to follow growth inhibition along a linear drug gradient. It measures the greyscale intensity changes in the images caused by the formation of microcolonies and provides AST in 2–5 h (Malmberg et al., 2016). The system is currently seeking CE-IVD marking and FDA approval for AST in blood samples.

Q-Linea ASTar® applies time-lapse microscopy to fully automated monitoring of blood cultures as well as preparation and monitoring of bacterial isolates. It processes 12 samples at a time and 50 samples a day, delivering true MIC values for up to 48 antibiotics within 6 h. The system does not perform ID, but it can be connected to any ID system. Clinical trials will start in 2020.

The BacterioScan 216Dx system (St. Louis, MO, USA) measures both a sample's optical density (OD) and the scattered intensity by forward laser light scattering, allowing 10–100-fold higher sensitivity compared to normal OD measurements. This system can process 16 samples simultaneously and perform real-time continuous growth measurement. It can detect bacterial growth in 3 h from clinical urine samples containing $>10^4$ cfu/ml of *E. coli*, *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus antracis, Yersinia pestis,* or *Burkholderia pseudomallei* (Bugrysheva et al., 2016; Hayden et al., 2016; Montgomery et al., 2017). The BacterioScan 216R Rapid AST System is currently under testing.

The oCelloscope™ system (BioSense Solutions, Denmark) applies digital time-lapse angled f eld microscopy and image analysis for standard 96-well plates. The time-to-result for positive blood cultures ranged from 1 to 4.2 h (Fredborg et al., 2015). However, the overall performance for AST has not yet been suÿ ciently tested.

Changes in individual cell morphology or size indicate growth long before bacteria have multiplied. Single-cell morphological analysis (SCMA) uses bright-f eld microscopy to determine antibiotic-induced changes in cells immobilized on an agarose channel chip (Choi et al., 2014). The commercial MultiPath™ platform applies non-magnif ed digital imaging for the detection of biomolecules tagged with antibody-coated f uorescent nanoparticles. Antibody-coated magnetic particles are used to bind to target cells and to pull them down to the camera surface, thus eliminating background signal and enabling a wash-free assay for clinical samples. The system counts individual targets in large areas and performs growth monitoring for multiple targets cells and enables the determination of MIC values. The MultiPath™ system is currently seeking FDA clearance.

The Two-Photon eXcitation f uoroscopy technology (TPX), commercialized by ArcDia Ltd. (Finland), allows separation-free detection of biological molecules in small reaction volumes by immunodetection (Vakkila et al., 2015). The mariPOC® test system was developed for rapid pathogen identif cation. It applies polystyrene microparticles as solid carriers for immunocomplex formation, that leads to three-component immunocomplexes (monoclonal antibody—antigen—labeled monoclonal antibody) on the microspheres in proportion to the analyte concentration. The detection of the immunoassay f uorescence signal is achieved by two-photon excitation from the surface of individual microspheres. This mechanism allows the use of unpurif ed clinical samples and on-line monitoring of viable cells in AST cultures. At present this system is under clinical testing for AST.

Nanostring Technologies Inc. combine genotypic and phenotypic AST through RNA detection. The GoPhAST-R platform detects mRNA expression signatures in bacteria after antibiotic exposure. The system can be used directly for positive blood culture bottles. It couples machine learning analysis of transcriptional changes with the detection of resistance genes (Bhattacharyya et al., 2019).

Colorimetric sensor arrays provide an inexpensive method to detect volatile organic compounds (VOCs) associated with microbial metabolism (Lonsdale et al., 2013; Lim et al., 2014). Specif c Technologies Inc (USA) has commercialized the small molecule sensor (SMS) array technology which reacts with the metabolic products of bacteria produced during their growth. Their Reveal-AST printed sensor array system responds to the volatiles emitted during growth producing a colorimetric pattern. It reveals also species ID with 94% accuracy (Sharp, 2020).

The SlipChip/dLAMP technology by Caltech integrates seamlessly cultivation and semiquantitative smartphone-based visual analysis of NA products (Schoepp et al., 2017). This system, currently being commercialized by Talis Inc. (USA), will be described closer in the chapter "POCT-compatible technologies."

Whole Genome Sequencing

Progress in Whole Genome Sequencing (WGS) technologies has made them a feasible system for pathogen ID and AST (van Belkum and Rochas, 2018). 3rd generation systems such as Illumina MiniSeq, Pacif c Biosciences PacBio Sequel system, or Oxford Nanopore MiniON and PromethION can provide fairly long reads at high speed. In principle, WGS can simultaneously

provide fast pathogen ID, epidemiological typing, and detection of drug susceptibility genes. Since WGS provides a massive amount of data in fragmented form, sophisticated software is needed to interpret the results (Quainoo et al., 2017). The European Committee on Antimicrobial Susceptibility Testing reviewed in 2017 the development status of WGS for AST (Ellington et al., 2017). They concluded that, for most bacteria, the available evidence for WGS as an AST tool is still either poor or non-existent and thus inadequate for clinical decision making. They pointed out the urgent need for a single database of all known resistance genes/mutations to facilitate comparison between different systems and bioinformatics tools.

PROOF-OF-CONCEPT TECHNOLOGIES

Smartphone-Based Readers

Kadlec et al. combined smartphone technology to a microphotometric system applying microwell plates coated with antibiotics and the yellow redox indicator dye tetrazolium salt WST-8, which turns orange by the metabolic activity of growing cells. The system could correctly monitor the growth of several pathogens associated with urine tract infection. Samples with concentrations of 10^1 to 10^6 cfu/mL could be tested directly without preliminary enrichment cultivations (Kadlec et al., 2014). Feng et al. presented an automated smartphone-based device with a 3D-printed attachment holding a microwell plate. A light-emitting diode array and f ber-based optics enabled detection of turbidity changes in wells already after 1 min (Feng et al., 2016). This system, tested for 17 antibiotics targeting Gram-negative bacteria on clinical isolates of *K. pneumoniae*, provided drug susceptibility interpretation with accuracy of 99.23%. Cui et al. have presented a smartphone-based system for the monitoring of viable bacteria in droplet-based single-cell microdroplet cultures (Cui et al., 2018). In this system, single bacteria were encapsulated in monodisperse microdroplets. This dSPC (Digital Standard Plate Count) platform could quantify *E. coli* and *B. subtilis* in 6 h, compared to 24 h needed for traditional plate counting. These smartphone demonstrations discussed above were performed using pure culture isolates.

Smartphone-based systems applying immunodetection have been demonstrated for ID, but so far not for growth-based AST. Wang et al. introduced a microwell plate-based microphotometric system, which applied a f eld-of-view adapter and a microprism array between the mobile phone camera and the 96-well plates. In a serological analysis (771 patient samples in 12 serology assays for bacterial/viral infections) the system exhibited 97.59∼99.90% analytical accuracy in pathogen identif cation with costs of ∼50 USD per 96-well plate and analytical quality suÿ cient for POCT (Wang et al., 2018).

An inexpensive (under 100 USD) smartphone-based monitoring system for nucleic acid amplif cation, smaRT-LAMP, was introduced by Barnes et al. The system contains a hot plate for isothermal amplif cation, two f exible cables and 96 LED lights f tted into a cardboard box. The Bacticount software detects the emitted green light as a result of a successful amplif cation and automatically determines the genome copy number in real time. smaRT-LAMP was shown to work well with diverse Gram-negative and Gram-positive pathogens in biological specimens, giving in ∼1 h results with matched standard cultivation-based tests. Reliable pathogen ID was obtained for spiked urea and blood samples as well as for urea samples of sepsis patients (10^5 -10^8 CFUs). The small sample size (2 µl per reaction), however, limits its use for very diluted samples (Barnes et al., 2018).

Priya et al. have successfully coupled loop-mediated isothermal amplif cation (RT-LAMP) and sensitive quenching of unincorporated amplif cation signal reporters (QUASR) detection technologies to visual detection with a smartphone. The portable "LAMP box" was successfully used for the sensitive and specif c detection of Zika, chikungunya, and dengue viruses (Priye et al., 2017). This system has not yet been applied to growth-based AST.

Optical or Microscopic Methods

Choi et al. have developed a rapid antimicrobial susceptibility testing system, dRAST. It can determine the AR from a positive blood culture bottle in 6 h (Choi et al., 2017). The sample is mixed with agarose and inoculated into a well of a plastic microchip. Addition of cultivation medium forms a liquid bridge between the growth chamber and the satellite well, which contains the antibiotic agent. Using microscopic detection of bacterial colony formation in agarose, the total time-to-result was only 6 h with a wide range of bacterial concentrations. The tested clinical isolates (n=206) included 16 Gram-negative species and seven Gram-positive species, and the dRAST system agreed with a standard microdilution test with an accuracy rate of 91.11% (Choi et al., 2017).

Matsumoto et al. described a microf uidic channel method for rapid (3 h) AST for *P. aeruginosa* by automated microscopic detection of cell number and cell morphology (Matsumoto et al., 2016). Their Drug Susceptibility Testing Microf uidic device (DSTM) consisted of f ve sets of four microf uidic channels and allowed simultaneous microscopic observation. Susceptibilities to the antibiotics (pre-dried into each channel) were evaluated by the differences in cell number and shape between drug-treated and control cells. Hundred and one clinically isolated strains of *P. aeruginosa* tested with DSTM correlated strongly with the results obtained using the conventional microbroth dilution method (Matsumoto et al., 2016). This system waits for applicability testing with other organisms.

In nanowell AST, morphotyping with a phase contrast microscopy and optical signal analysis is performed for 0.5 µl cultures. Antibiotic susceptibility data can be obtained for uropathogens in <4 h. The system showed a total categorical agreement of 97.9% with standard disk diffusion assays, but a careful standardization of cell densities prior to cultivation was found necessary (Veses-Garcia et al., 2018).

Hybridization Methods

Mezger et al. presented a generic method for rapid species identif cation and AST after 0.5-2 h cultivation (Mezger et al., 2015). Cultured bacteria from urine samples were lysed by sodium hydroxide and heat, and DNA was captured onto magnetic beads. Padlock probes targeting the 16S rRNA gene

were hybridized, ligated and amplif ed by the circle-to-circle amplif cation method. Optical imaging system then performed digital quantif cation. Antibiotic susceptibility prof les of *E. coli* for ciprof oxacin and trimethoprim could be determined with 100% accuracy in 3.5 h (Mezger et al., 2015).

Detection of Growth-Related Molecules or Antibiotic Degradation Products

Devices detecting volatile compounds, so-called electronic noses (eNose), can recognize a smell-print characteristic for bacterial species and their metabolic prof le. Since 1982, electronic noses have been applied in diagnostics (Persaud and Dodd, 1982), but mainly for pathogen ID. The Cyranose system (Smiths Detection) was able to distinguish between controls and samples positive for *S. aureus*, *S. pneumoniae*, *Haemophilus inf uenzae*, and *P. aeruginosa* in upper respiratory tract infections (Lai et al., 2002). Gas chromatography connected to ion mobility spectrometry (GC-IMS E-nose) could reliably distinguish bacterial infections from viral respiratory tract infections (Lewis et al., 2017). Saviauk et al. performed an applicability test for the ChemPro 100i Ion Mobility Spectrometry sensor (Environics Inc.). They could discriminate MRSA from MSSA with 83% sensitivity and 100% specif city (Saviauk et al., 2018) and were able to identify also other pathogens (*P. aeruginosa*, *Enterococcus*, *E. coli,* and *Clostridium perfringens*) from culture plates with 78% accuracy. In order to access the AST market, the eNose systems should outperform the simple and inexpensive colorimetric sensor array system "Reveal-AST" (Specif c Technologies, USA) which is applicable to growth-based AST.

NAAT in Growth Monitoring

Real-time qPCR can detect quantitative differences between cultures exposed to various antibiotics and different concentrations. Already a 15 min cultivation can provide a detectable increase of nucleic acids (Schoepp et al., 2017). Although qPCR devices are expensive and require experienced personnel for their operation, low-cost devices based on isothermal amplif cation might change the game thoroughly. Applying chip electronics and microf uidics, CalTech (the Technical University of California) has developed a device applicable to AST. This system is currently under commercialization by SlipChip Corp and will be described closer later in this review in the section "POC-compatible technology."

Biosensor Systems

Biosensors are devices that measure biological or chemical reactions by generating signals proportional to the concentration of an analyte in the reaction. Exposure to antibiotics causes detectable changes in bacterial membranes, morphology, metabolism, movements, mass, heat production and nucleic acid content. In microcalorimetry approaches, heat production correlates with the number of cells arising over time (von Ah et al., 2009). This approach is applicable to both solid and liquid cultures (Howell et al., 2012). Dynamic heat f ow patterns have served species identif cation from urine samples (Bonkat et al., 2012). Isothermal microcalorimetry revealed vancomycin-resistant *Staphylococcus aureus* in <8 h (Entenza et al., 2014). Butini et al. applied isothermal microcalorimetry to real-time monitoring of microbial viability in biof lms in the presence or absence of antimicrobial compounds (Butini et al., 2018). Microcalorimetric methods, although fast and sensitive, require pure cultures and a fairly high number of bacterial cells. In 2017, the Swedish company SymCel announced an extensive 28-months clinical testing of their microcalorimeter calScreener™ for AST. However, currently no clinically validated microcalorimetry systems are available.

For some antibiotics (beta-lactams, chloramphenicol) AMR can be assayed by the follow-up of antibiotic degradation. The BYG Carba test detects conductivity changes caused by the enzymatic hydrolysis reaction of imipenem antibiotics on an electrode coated with polyaniline, which is highly sensitive to changes in pH or redox potential. With a loop-full of bacteria (10 µl) from a fresh plate as a sample, this home-made inexpensive instrument could detect carbapenem resistance in <35 min displaying 95% sensitivity and 100% specif city in comparison to PCR-based analysis (Bogaerts et al., 2016). Mecklenburg et al. developed an assay that directly detects the thermal signal generated from the enzymatic breakdown of antibiotics. The system was able to distinguish between penicillinase and metallo-β-lactamase (Mecklenburg et al., 2017). Its value for clinical work needs to be evaluated, as it requires pure cultures and does not provide pathogen ID.

A variety of electrochemical reporters for cell viability have been applied to viability analysis and drug susceptibility measurements. The system by Besant et al. uses resazurin dye (an oxidation-reduction indicator) to monitor cells trapped in nanoliter wells (Besant et al., 2015). Within 1 h the microfabricated device could detect the response of *E. coli* and *K. pneumoniae* exposed to ampicillin and ciprof oxacin in urine samples spiked with bacteria in concentrations as low as 1 cfu/µL. The level of commercialization of this technology is not known.

In microelectromechanical systems (MEMS) the def ections associated with the micromotions of bacteria attached to a microcantilever provide a signature of bacterial metabolism. Such changes can indicate growth long before the bacteria replicate. With bacteria captured in bi-material microchannel cantilevers, quantitative antibiograms have been obtained for *E. coli* and *S. aureus* within 2 h (Etayash et al., 2016). The bacteria absorb infrared photons and release heat to the support matrix by a process of vibrational energy relaxation, inducing bending of the bimetallic cantilever proportional to the quantity of the released energy. High sensitivity, corresponding to a single cell per µl, was obtained with *Listeria*-containing samples. The researchers plan to further integrate sample separation techniques into this BioMaterial Cantilever platform. LifeScale Analytics (NC, USA) has already launched a commercial product which correlates cantilever vibration to biomass for MIC determination (Burg et al., 2007). This system performs automated cell counting, mass measurement, and visual observation of liquid samples for AST in <3 h, but requires cell concentrations above 10^4 cells/ml. The company has not yet presented peer-reviewed clinical AST studies for this instrument. Micromotions are affected by f owing liquids, and ineÿ cient transfer of antibiotics to immobilized bacteria can distort the results. Therefore pre-enrichment and pre-purif cation of bacteria may be necessary (Li et al., 2017b; Syal et al., 2017).

A low-cost and rapid biosensor has been developed employing photoluminescence emission of photo-corroded GaAs/AlGaAs biochips. Growing bacteria protect the biosensor surface against photo-corrosion, while non-growing or dead cells give a higher signal. These biochips exposed to a *E. coli* and *Legionella pneumophila* cultures (w/o antibiotics) were capable of quantifying electrically charged bacteria in 4.5 h (Nazemi et al., 2017).

The Field Effect Enzymatic Detection (FEED) biosensor platform can detect extremely low bacterial concentrations (below 10 c.f.u./ml). An electrical field between the working electrode and the immune complex multiplies the biocatalytic output current, enabling a direct detection of bacteria without sample processing (Shi et al., 2018). These biosensors apply horse radish peroxidase (HRP) as a redox source in the sandwich hybridization complex. Commercial devices applying this technology for AST may potentially emerge in coming years.

Other Proof-of-Concept Technologies

Flow cytometry (FC) can provide excitation/emission spectra of cells and give information about cell-counts, morphology and viability, enabling AST in 2–3 h. Due to the large amount of raw data, mathematical methods such as adaptive multidimensional statistics must be applied for analysis (Huang et al., 2015). Successful demonstrations with clinical polymicrobial samples are currently missing. Flow cytometric assays struggle with complex patient samples, inefficient staining, the presence of autofluorescence, the inability to differentiate cellular damage for the influence of antibiotics, and lack of clinical databases for validation. Flow cytometry instruments are very costly, making this technology an unlikely candidate for POCT. Atomic force microscope (AFM) allows real time monitoring of bacterial activity. This principle is applicable to both cultivable and non-cultivable cells (Longo et al., 2013), but is an expensive and tedious system for clinical work. Plasmonic imaging and tracking (PIT) can be used for the monitoring of nanometer-scale motions of single bacterial cells before and after antibiotic addition. The observed image contrast fluctuations were found to indicate changes in bacterial metabolism long before cell replication (Syal et al., 2016). Asynchronous magnetic bead rotation (AMBR) is a non-microscopy-based approach capable of monitoring individual cells for elongation, generation time, lag time, division, as well as sensitivity to antibiotics. It has been successfully tested with *E. coli* attached to anti-*E. coli* functionalized beads (Kinnunen et al., 2011). This system is still at the research stage and a complex technology regarding handling, setup and the need for expertise (Schumacher et al., 2018).

MINIATURIZED AND CHIP-BASED GROWTH MONITORING SYSTEMS

Lab-on-a-chip systems combine one or several laboratory functions into a single integrated circuit. The microfluidic AST platforms typically apply channels containing pre-loaded dried antibiotics. Following on-chip cultivation, the channels can be monitored with a detection device such as a phase contrast microscope. Some systems use agar to immobilize the bacteria and to form growth chambers. Also gradient-forming microfluidic platforms have been applied (Luka et al., 2015; Campbell et al., 2016). He et al. introduced a chip applying antibody-coated glass beads to capture *E. coli* O157 and to provide fluorescence detection for ID and AST. Their device had an integrated antibiotic release system, which could identify this strain at a range of 10^4-10^8 cfu/ml within 30 min (He et al., 2014). Li et al. integrated antibody-coated carbon nanotubes with isothermal amplification. The carbon nanotube multilayer could perform selective capture, cultivation and release of the bacteria. After cultivation the antibody-entrapped bacteria were lysed, and their DNA was accurately quantified by LAMP. This system could detect *E. coli* O157:H7 and its toxin at concentrations as low as 1 cfu/ml without any complicated instrumentation (Li et al., 2017a). Its use for growth-based AST has not yet been presented. Researchers at Hong Kong Baptist University have developed a multidimensional AST system for growth-based AST, providing detection through automated microscopy in 4 h. They established a hydrogel microfluidic chip which simulates drug diffusion and pathogen killing processes inside the human body. Due to the chip's multidimensionality, several antibiotics, nutrients or immunologic substances could be tested simultaneously. This system is currently under commercialization (Sun et al., 2016; Liu et al., 2017a,b). Weibull et al. have presented a nanowell AST device capable of real-time optical reading and growth data analysis. This "stationary nanoliter droplet array" (SNDA) system can provide ID and AST for urine samples within one working shift. A filtering process first isolates bacteria from the clinical urine samples into a growth medium, supplemented with 10% resazurin (oxidation-reduction indicator). Applying 12 bacteria–antibiotic combinations precise MIC determinations could be obtained in 3 h, which is 6-fold faster than traditional broth microdilution assays in 96-well plates. However, follow-up studies with clinical isolates of both Gram-negative and Gram-positive bacteria are needed (Weibull et al., 2014). Veses-Garcia et al. applied 0.5 µl cultures for uropathogens on a 672-nanowell slide equipped with optical signal analysis. They were able to define a precise minimum inhibitory concentration for 70 clinical *E. coli* isolates. Algorithm-assisted optical analysis determined antibiotic susceptibility in 3 h 40 min, showing a total categorical agreement of 97.9% (Veses-Garcia et al., 2018).

The "Integrated Comprehensive Droplet Digital Detection" (IC 3D) system is capable of detecting bacteria directly from diluted blood within 1.5–4 h. It consists of bacteria-specific DNAzyme-based sensors, a droplet microencapsulation system, lysozyme and a 3D particle counter system. The ongoing work aims to develop an automated, portable device for multiplexed and rapid detection of antibiotic-resistant strains (Kang et al., 2014). The Ultrafast Parallelized Microfluidic Platform consists of four arrays (each holding 110 pL droplets with 1–4 bacteria) which are screened by dynamic imaging over 2 h. This imaging-based AST was successfully tested with four types of pathogens causing urinary tract infection (UTI) (Kang et al., 2019).

POCT-COMPATIBLE DEVICES FOR ID AND AST

Systems with a low price and high speed are needed in all healthcare sectors, but especially in outpatient clinics and in developing countries. Point-of-Care Tests (POCT) could bring the diagnostics into outpatient clinics and thus facilitate evidence-based medication in places where extensive prescription of antibiotics happens. Very few rapid AST systems are currently available, but the ongoing development is promising. David Boyle has made an excellent review of affordable technologies and devices applicable to "standard microscopy stations" (Boyle, 2017). The devices presented below can provide a fast pathogen identif cation. Some of these are already now applicable to AST, and some devices can be even applied to diagnostics of non-infectious diseases as well.

Scanogen Inc. (USA) develops DNA-based (non-amplif cation-based) "single-molecule biosensors" bound to microparticles, which can convert the hybridization signal to an optical signal. The device contains an inexpensive and low-power light-emitting diode ring and uses disposable sample cartridges, abolishing the need for manual sample preparation. The company is currently developing diagnostic assays for infectious diseases and drug resistance. The MicrobeDx technology (MicrobeDx Inc., CA, USA) applies a transformational ribosomal RNA-based assay on a microf uidic disc platform. The disc holds 150 nucleic acid capture probes spotted onto a glass slide. This system can discriminate four clinically relevant *Staphylococcus* species that differ by a single nucleotide polymorphism (SNP) in diagnostic probe sequences. The protocol includes hybridization, washing, rinsing, and drying steps and does not require purif cation of the target nucleic acids (Peytavi, 2005). In a clinical testing phase f nanced by NIH during the years 2018-2019 (so far unpublished), MicrobeDx has tested UroLogic, a highly automated instrument and cartridge system that performs rapid pathogen ID and AST in 30 and 150 min, respectively. The Alveo platform (Alveo Technologies Inc, CA) applies hybridization-based electrochemical nucleic acid detection in single-use cartridges. The cloud-connected device is capable of analyzing 100 infectious diseases. Sample preparation, nucleic acid amplif cation, and real-time hybridization-based detection are performed in a single isothermal microf uidic channel. This concept has not yet been applied to growth-based AST. The Loopamp™ technology by Eiken Chemical Corp. (China) has already received WHO endorsement for tuberculosis diagnostics (Noncommercial culture drug-susceptibility testing methods for screening patients at risk for multidrug-resistant tuberculosis: policy statement, 2011). The Loop-mediated isothermal amplif cation (strand displacement reaction) employs four different primers designed for six distinct regions on the target gene. It has been used successfully for the detection of *Neisseria meningitidis* in clinical cerebrospinal f uid samples (Lee et al., 2015) and for the detection of the OXA-23 carbapenemase (Yang et al., 2018) gene of *Acinetobacter baumannii*.

Schoepp et al. (2017) demonstrated with 51 clinical samples that the antibiotic exposure time in phenotypic AST can be shortened to 15 min when dLAMP (digital real-time loop-mediated isothermal amplif cation) or dPCR (droplet-PCR) is applied. The changes in DNA concentrations (control vs. antibiotic-treated samples) were determined by a "digital single-molecule counting" system after incubation. In dLAMP, the target molecules or lysed cells are partitioned into thousands of nanodroplets so that each compartment contains approximately a single molecule (Schoepp et al., 2017). The 1 nl droplets with DNA concentrations relevant to clinical urine tract infection samples showed 1.23-fold difference in amplif cation products between resistant and susceptible strains, and 98.1% of the tested samples matched the standard AST results. The LAMP chemistry was optimized and applied to a SlipChip microf uidic device equipped with an electrophoretic system concentrating the bacteria in the sample. This concept was tested for AST of positive blood cultures. The results were consistent with standard microdilution tests or the BD Phoenix System when several broad-spectrum antibiotics and clinical *E. coli* samples as well as the *S. aureus* ATCC 6538 strain were tested (Yi et al., 2019). The Talis Biomedical Corporation (CA, USA) is currently commercializing dAST/SlipChip technology to a product which combines a single-use cartridge integrating f uid partitioning for parallel treatment with different antimicrobials, reagent additions, target lysis, extraction and amplif cation. The NAAT-based system presented by Priye et al. (2017) applies a reverse-transcription loop-mediated isothermal amplif cation (RT-LAMP) coupled with the QUASR technique (quenching of unincorporated amplif cation signal reporters). The device was found to be f ve times more sensitive than traditional POCT for the detection of the dengue, chikungunya, or Zika viruses. Hassibi et al. have presented a fully integrated, miniaturized semiconductor biochip with closed-tube detection chemistry for multiplex NA amplif cation and sequence analysis, which they claimed to have a high dynamic quantif cation range for the microbial load, while at the same time performing comprehensive mutation analysis on up to 1,000 sequences or strands simultaneously in <2 h. This chip was able to correctly detect and quantify multiple DNA and RNA respiratory viruses in clinical samples, while at the same time detecting 54 drug-resistance-associated mutations in six genes of *Mycobacterium tuberculosis* (Hassibi et al., 2018). QuantuMDx Ltd (UK) aims to launch a miniaturized portable diagnostic platform that runs on battery power. This Q-POC™ device would be a portable molecular diagnostic instrument with built-in NA amplif cation for multiplexed diagnostics and drug susceptibility testing within 20 min. It applies nanowires coated with specif c probes for the detection of genetic variants and has cloud-based connectivity to share and utilize epidemiologic data. This technology is currently under clinical evaluation for diagnostics of malaria (protozoan *Plasmodium falciparum*) and tuberculosis (*Mycobacterium tuberculosis*). The device, although not designed for growth-based AST, might have a very wide applicability range including non-infectious diseases such as cancer or genetic disorders. The outcome of the clinical tests has not yet been published. The Multipath instrument (First Light Diagnostics Inc., USA) applies f uorescence-labeled nanoparticles attaching to the target cells by immunobinding. The nanoparticle/cell complex can then be pulled toward the detection system

by magnetic beads conjugated with another microbe-specif c antibody. Thereby the system is capable of monitoring viable cells using non-magnif ed digital imaging. A POCT-compatible device is currently under testing. Immunodiagnostic TPX-technology (two-photon excitation f uorometry), commercialized by ArcDia Ltd (Finland) to mariPOC and mariAST platforms, can analyze 40–100 samples a day. It has a cloud-based connectivity allowing eÿ cient sharing of epidemiologic data. TPX allows the use of untreated polymicrobial clinical samples on-line, providing bacterial ID in 20 min and antibiotic resistance in a few hours. Unlike most other AST systems, it can also provide sensitive immunodetection of viruses Sanbonmatsu-Gámez et al., 2015; Bruning et al., 2018.

POCT devices using smartphone technology have long been used for personalized medication applications such as the colorimetric analysis of urine strips (Barnes et al., 2018). The use of smartphones in growth-based AST for miniaturized cultures has been presented in several studies (Kadlec et al., 2014; Schoepp et al., 2017; Cui et al., 2018; Hernández-Neuta et al., 2019), although instrument manufacturers seem to be reluctant to incorporate standard commercial smartphones to their systems. Photolithography, plotting, plasma etching, inkjet printing, cutting, and wax printing can be used to pattern paper for inexpensive diagnostic tests (López-Marzo and Merkoçi, 2016). Paper-based systems have been applied for the immunodetection of *Helicobacter pylori*, *Chlamydia* infections and Zika virus infections, but platforms integrating LF-testing into growth-based AST have not been presented.

PROBLEMS WITH MINIATURIZATION AND RAPID AST

Due to the small sample size (at lowest a single bacterial cell), rapid AST may not give results representing the whole bacterial population in the diagnostic sample. This problem can be mitigated by analyzing a large number of individual cells. An example of such a strategy is the stochastic conf nement of bacteria in nanoliter droplets, so that a high number of individual droplets can be analyzed quickly and eÿ ciently (Boedicker et al., 2008). The direct analysis of clinical samples also demands the use of valid internal standards. Additionally any growth-based analysis may utterly fail if the growth rate is very low or the microbes cease to grow due to the lack of specif c growth factors, a non-optimal atmosphere, or the accumulation of growth-inhibiting substances. For intracellular pathogens the use of NAAT may be necessary. Compared to standard microbial cultivations, favorable growth conditions may be diÿ cult to establish for miniaturized set-ups. These problems can be mitigated by analyzing cell viability, morphology or movements. Miniaturization can improve the performance and capacity of cultivation-based AST only if the need for manual steps is minimized. As reagent dilutions are often done before loading onto the chip device, set-up complexity may stay a similar level as in broth dilution methods. Due to the relatively low number of pathogens in most clinical samples and the impurities of the sample matrices, miniaturized cultivations benef t from methods concentrating the cells and removing the impurities. The FISH-based Accelerate Pheno system applies electrokinetic/electrophoretic system for these purposes. Coarse cell sorting for blood samples can be achieved through simple inertial microf uidic systems, where centrifugal (inertial) force drives bacteria to the outer side of a spiral-formed microcapillary tube, while blood cells stay on the inner side (Bhattacharyya et al., 2017). Some systems bypass the problem of impurities by bringing the target close to the biosensor surface with magnetic and antibody-coupled beads or nanoparticles. Miniaturization sets high demands to the standardization of the conditions, as the samples should represent similar growth states and culture densities. Robots may be needed for accurate pipetting.

Serious doubts concerning the ability of accelerated cultures to fully substitute standard growth-based tests have been presented. Accelerated cultivations may struggle to differentiate between wild type and resistant strains (Leclercq et al., 2013; Kahlmeter, 2014; Maurer et al., 2017). This is especially a concern in the case of induced expression of the resistance factors, e.g., in the detection of AmpC in enterobacteria or macrolide-resistance in streptococci (Leclercq and Courvalin, 2002; Jacoby, 2009; Harris and Ferguson, 2012).

ECONOMIC DRIVERS FOR RAPID AST

Substantial overall savings can be obtained with rapid AST through the reduction of hospital days, disability days and the saving of lives (Cassini et al., 2019). Despite the potential total cost savings, the high price of molecular testing forms an eÿ cient barrier for the installation of new technologies. A price tag ranging from $100 to $250 per test is common for molecular tests (Li et al., 2017b). For a typical 500-bed community hospital, detailed multiplex testing of positive blood cultures by NAAT could cost more than $500,000/year in reagents alone (She and Bender, 2019). Also the instrument prices may be signif cant. The total costs of testing are quite diÿ cult to estimate. The prices of test kits and diagnostic instruments are based on the distributors' offers. Testing costs include clinical sampling and usually also cultivations in microbiology labs (enrichment, preparation of bacterial isolates) before AST can be performed. As an example, Patel et al. estimated the total costs for mass spectrometry-based AST to be roughly 79 € per patient, when the cost of the MALDI-TOF device, reagents, pharmacist time and the antimicrobial stewardship program are pooled together (Patel et al., 2017). Yet the reagent costs for one MS-sample can be pressed close to 1 €. A typical MALDI-TOF MS system with all the accessories, software databases and maintenance is costly (up to €200,000 per year) (Wieser et al., 2012). This implies that the use of the instrument must be high for an acceptable cost eÿ ciency. This unfortunately rules out their use in outpatient clinics. MS-instruments are, however, very versatile and can be applied also to other routine diagnostics in central laboratories (Vrioni et al., 2018). Direct inoculation from plates to automated identif cation systems such as Vitek, Microscan and others has been validated and used in many clinical laboratories. These systems apply multiwell liquid cultures and have a turnaround

time as short as 4 h for ID and 6–8 h for AST (She and Bender, 2019), but the prices of these instruments are high. The cost of any rapid AST should not signif cantly exceed the cost level currently considered acceptable for routine testing: this might be roughly 30 to 50 €, including sampling, culturing and AST (EU, 2013). Five phenotypic tests for a targeted detection of enterobacterial carbapenemases have been recently evaluated for their performance and costs. All these tests required the use of culture isolates. Per sample, the costs of multiplexed PCR-based analysis was 30 €, immunochromatographic methods ∼15 €, a colorimetric assay 5 € and carbapenem hydrolysis test 1 € per sample (Baeza et al., 2019). Apart from turnover time, also the capacity of the test system is important. Among automated microscopy systems, Accelerate Pheno handles one sample per unit module (max. four modules per device, cost per sample ∼250 €), whilst the Q-Linea ASTar system can handle 50 samples per day. The capacities of NAAT-based systems can be even higher. Automated MIC-determination systems such as VITEK-2 can manage tens of samples per time. For POCT usage, however, a throughput capacity of a few tens of samples per time would be suÿ cient.

SUMMARY: HOW DO THE AST TECHNOLOGIES MEET THE REQUIREMENTS?

Several identif cation systems based on NAAT, NA hybridization and immunodiagnostics are already available for rapid diagnostics, but only few are applicable to POCT. This is due to high costs, lack of appropriate facilities or expert labor, or insuÿ cient performance with clinical samples. Multiplexing and high-throughput capacities are important for central laboratories, but most healthcare settings process only few samples per time. POCT use rules out expensive devices, systems requiring demanding sample preparation, and systems requiring standard microbiology laboratory facilities. NAAT serves well for the identif cation of viral and bacterial pathogens, and in some cases provides also the detection of AR genes. However, the commercial multiplexed diagnostic NAAT panels are far too expensive for routine diagnostics. Immunochromatographic tests, especially dip-sticks, are a low-cost and handy option for virus diagnostics and detection of inf ammatory factors. Unfortunately systems capable of detection of antibiotic resistance proteins directly from clinical samples without enrichment cultures, culture isolation or sample purif cation do not yet exist. The routine diagnostic tools for infectious diseases should serve a wide f eld of requirements: both the identif cation of the pathogen (viral and bacterial), the detection of antibiotic resistance and the determination of the correct antibiotic dosing. Systems based on microscopy are not compatible with the detection of viruses or inf ammatory factors. Diagnostic systems differ very much in their potential to provide all relevant data for medication (**Table 1**). Multiplexing NAAT-based systems can seamlessly incorporate the detection of emerging resistance-related genes or mutations. However, every update for a diagnostic panel requires new validations. While central laboratories have resources to validate new tests against the standardized tests, smaller healthcare units are bound to use tests validated by authorities such as the FDA. The few systems currently capable of providing simultaneous ID and growth-based AST directly from clinical samples are based either on FISH (Accelerate Pheno), immunodiagnostics (e.g., mariAST, MultiPath, immunobiosensors), or digital AST (dAST) (**Table 1**). As their properties and application areas differ, all these systems may have a good future. Rapid growth-based diagnostic systems rely on accelerated cultivations. For this reason, it is necessary to validate every new technology carefully against the standard tests accepted by EUCAST and CLSI. The MIC breakpoints (sensitive, resistant, or in-between) must be re-def ned for each system and each tested organism. This requirement def nitely retards the deployment of new technologies. While most novel AST systems only perform an endpoint analysis, the systems based on microscopy, heat production, movements, immunodetection or detection of mass changes in principle facilitate on-line monitoring. Biosensor-based AST systems, however, yet miss convincing clinical demonstrations.

In practice clinical diagnosis should be obtained during oÿ ce hours, meaning <8 h from sampling to results. This does not leave time for enrichment cultivations or preparation of culture isolates. The strategy "take a sample, store and analyze later" works f ne for diagnosis of slowly advancing infections, but also for NAAT-based systems which do not require viable microbes. Growth-based AST, on the other hand, need fresh samples to ensure pathogen survival until the point of analysis.

Regarding the speed and the need to handle clinical polybacterial samples, the immunodiagnostic TPX-technology (ArcDia Ltd), growth-based FISH (Accelerate Pheno), the Multipath digital imaging technology based on nanoparticles for labeling and magnetic beads for capturing (First Light Diagnostics Inc.), and the NAAT-based dAST (Talis Inc.) seem promising options for rapid point-of-care testing of antimicrobial susceptibility. The Talis system is, however, not yet on the market. Immunobiosensors are still in their infancy, but may in the future become important in testing non-culturable microbes. Lateral Flow (immuno-chromatographic "dip-sticks") systems would be an ideal product format for clinical work, but their applicability has been so far proven only with isolated cultures (i.e., colonies on plate).

Due to the plethora of different resistance mechanisms, NAAT struggles with the detection of antibiotic resistances in Gram-negative bacteria (Maurer et al., 2017) and with the analysis of samples containing commensal f ora. NAAT may fail to identify several ESBL genes and genes providing f uoroquinolone or aminoglycoside resistance. NAAT is, however, a powerful and necessary technology for the detection of fastidious, slow-growing or intracellular pathogens, toxin-producing bacteria such as *E. coli* O157:H7, and viruses (Miller et al., 2018). Still, the increased use of NAAT has raised a concern about the fate of bacterial samples required for further studies (Marder et al., 2017; McAdam, 2017). Since NAAT does not require viable samples, subsequent epidemiologic studies or cultivation-based conf rmatory AST may be impossible with the stored samples. Whole genome sequencing (WGS) is still in its infancy regarding

TABLE 1 | Properties of technologies applicable to rapid identification and AST.

Technology	Company or product (examples)	Time for AST (h)	Simultaneous ID and AST	Clinical polymicrob. samples	Online AST	Provides MICs	Detects new resistances	AST for non-culturable microbes	Enables virus ID	Level of commercialization	References
Standard cultivation tests											
Broth dilution test	Several	18–36	–	–	✓	✓	✓	–	–	Gold standard	
Disk diffusion test and E-test	Several	18–24	–	–	✓	✓	✓	–	–	Gold standard	
Automated readers for cards or microtiter plates											
Broth microdilution-based instruments	bioMerieux, BD, Siemens	5–16 h	–	–	✓	✓	✓	–	–	Commercial	
Disk diffusion-based instruments	Giles Scientific, Oriana, BioRad, BD	5–16 h	–	–	✓	✓	✓	–	–	Commercial	
Mass spectrometry (biochemical profiling, follow-up of antibiotic degradation, detection of anti-microbial protein)											
MALDI-TOF (Bruker MBT)	Bruker, Shimadzu, Sciex, Waters	2-4 h	–	–	–	–	✓	–	–	Commercial	
MBT-ASTRA (biochem. profiling after antibiotic exposure)	Bruker Daltonik GmbH	2-4 h	–	–	–	✓	✓	–	–	Commercial	Sparbier et al., 2016
Direct-On-Target Microbial Growth Assay (DOT-MGA)	All instrument providers	4 h	–	–	–	✓	✓	–	–	Experimental	Idelevich et al., 2018; Correa-Martínez et al., 2019
Fluorescence & hybridization											
FISH (fluorescent probes, microscope)	XpressFISH	2–4 h	✓	✓	–	–	✓	–	–	Commercial	Salimnia et al., 2014
Multiplexed automated microscopy/FISH	Accelerate Diagnostics	6.5 h	✓	✓	✓ (fs)	✓	✓	–	–	Commercial	Hill et al., 2017
Automated fluorescence detection for expression profiling	NanoString Technologies	24 h	✓	✓	✓ (fs)	✓	✓	–	✓	Commercial, under testing	Barczak et al., 2012; Bhattacharyya et al., 2017, 2019; Kelley, 2017; Koehler et al., 2018
Non-microscopic imaging, fluorescent antibody-bound nanoparticles, magnetic beads for concentrating	First Light Diagnostics	4 h	✓	✓	✓	✓	✓	–	✓	Commercial, under testing	https://www.firstlightdx.com/publications/

(Continued)

TABLE 1 | Continued

Technology	Company or product (examples)	Time for AST (h)	Simultaneous ID and AST	Clinical polymicrob. samples	Online AST	Provides MICs	Detects new resistances	AST for non-culturable microbes	Enables virus ID	Level of commercialization	References
Other imaging or spectroscopy-based systems											
Surface plasmon resonance (SPR)	Biacore 3000 and exp. devices	0.5–4h	–	–	✓	✓	✓	✓	–	Experimental	Chen et al., 2011; Tao and Syal, 2016
Raman spectroscopy (SERS)	Several	2h	✓	–	✓	✓	✓	–	–	Experimental	Liu et al., 2016; Wang et al., 2018
Smartphone-based growth monitoring of microplates, capillaries or chips	Experimental	2–4 h	–	–	✓	✓	✓	–	–	Experimental	Kadlec et al., 2014; Feng et al., 2016; Cui et al., 2018
Cell sorting systems, flow cytometry											
Flow cytometry	FASTinov	2 h	–	✓	✓	✓	✓	(✓)	–	Commercial	Costa-de-Oliveira et al., 2017
Sensor-based detection of micromotions or mass changes											
Plasmonic imaging and tracking for nanomotions	Experimental	<1 h	–	–	✓	✓	✓	✓	–	Experimental	Syal et al., 2016
Atomic force microscopy cantilever	Experimental	0.25-4h	–	–	✓	(✓)	✓	(✓)	–	Inverted microscope	Longo et al., 2013
SAW and other mass sensitive biosensors	Experimental	0.5-6h	–	–	✓	(✓)	✓	(✓)	✓	Experimental	Chang et al., 2007; Hoß and Bendas, 2017
Heat production											
Microcalorimetry	SymCel AB, TA Instruments	Few hours	–	–	✓	(✓)	✓	(✓)	–	Commercial	https://www.laboratoryequipment.com/article/2017/11/how-use-calorimetry-tackle-antibiotic-resistance
Immunochromatography (lateral flow tests, "dip-sticks")											
Resistance factor specific binders	Coris Bioconcept	0.25–4	–	–	–	–	–	–	–	Commercial	ECCMID 2015 Booth #243
Immunodetection, fluorescence-based											
Two-photon fluorescence microscopy TPX	ArcDia	2–4 h	✓	✓	✓	✓	✓	–	✓	Commercial, under testing	Koskinen, 2008

(Continued)

TABLE 1 | Continued

Technology	Company or product (examples)	Time for AST (h)	Simultaneous ID and AST	Clinical polymicrob. samples	Online AST	Provides MICs	Detects new resistances	AST for non-culturable microbes	Enables virus ID	Level of commercialization	References
Multipath (magnetic beads, non-microscopy imaging)	First Light Diagnostics	2–4 h	✓	✓	✓	✓	✓	–	✓	Commercial, under testing	
Electrochemical biosensors or detection of volatile organic compounds											
rRNA-hybridization, peroxidase signaling	GeneFluidics	2–5 h	✓	✓	–	✓	✓	(✓)	–	Commercial	Mach et al., 2011; Liu et al., 2014
Colorimetric sensor array for VOC detection	Specific Diagnostics	3–4 h	✓	–	✓	✓	✓	–	–	Commercial	https://www.specific-dx.com/reveal-ast
Redox-indicator resazurin	Experimental	1 h	–	–	✓	✓	✓	(✓)	–	Experimental	Besant et al., 2015; Avesar et al., 2017
Field effect enzymatic immunosensor	Experimental	1–2 h	✓	✓	✓	✓	✓	(✓)	–	Experimental	Shi et al., 2018
Electronic nose: ion mobility spectrometry sensor	Environics, Olfactomics	Few minutes	(✓)	–	(✓)	–	–	–	–	Commercial	Lewis et al., 2017; Saviauk et al., 2018
NAAT											
PCR, qPCR	Several	2–4 h	✓	✓	–	–	–	✓	✓	Commercial	
Integrated cassette-based NAAT solutions	Several	4 h	✓	✓	–	–	–	✓	✓	Commercial	
Isothermal amplification	Several	0.5–4	✓	✓	–	–	–	✓	✓	Commercial	
Whole Genome Sequencing	Several	1–24 h	✓	✓	–	–	✓	✓	✓	Commercial	
NAAT combined to cultivation											
Isothermal amplification, digital AST	Talis Biomedical	0.5 h	✓	✓	✓(fs)	✓	✓	(✓)	✓	Under commercialization	Schoepp et al., 2017, 2020; https://talis.bio

The marking ✓ is in brackets, if the possible feature lacks experimental demonstrations. – indicates a missing property. fr indicates the need for frequent sampling.

TABLE 2 | Contemplation on Prof. Kahlmeter's criteria for new technologies (Kahlmeter, 2016).

Criteria	Contemplation
Generally applicable or restricted to certain infections?	In principle all growth-based rapid AST systems are generic and work with culture isolates. However, for polymicrobial clinical samples they must be coupled with specific probes or antibodies which provide ID. Therefore, specific test panels have been developed e.g., for respiratory, urinary, and blood samples. The pathogen load may not be high enough for direct analysis, and especially blood samples may require culturing prior to analysis. AST for fastidious, non-culturable, or intracellular pathogens call for NAAT. The complexity of the sample matrix affects the choice of the diagnostic system and the methods for sample preparations.
Capacity: how many organisms/agents per hour can be processed	DNA-arrays and PCR systems (including multiplexed cassette designs) have a high throughput capacity. Mass-spectrometry performed on PCR products can handle hundreds of samples per hour in central laboratories. In outpatient clinics speed is more essential than the capacity. High multiplexing (parameters per sample) and high-throughput capacity (number of samples) may be challenging to combine. Progress in NAAT, immunodiagnostics, biosensor technologies and microfluidics has yielded several systems capable of analyzing tens of samples per day or even during a single work shift.
Has the technology been validated against reference methods?	So far quite few quick technologies have received FDA-approval. Currently they include PCR-tests, cartridge-based NAAT-systems and Accelerate Pheno (automated microscopy). Several clinical trials are in progress to achieve CE-marking or FDA-clearance.
Are there any reference installations?	Commercial analysis systems in general do have, and manufacturers tend to publish successful clinical trials. However, finding a lab which lines up to the specific needs may be challenging.
Is scientific literature available?	For mature commercial systems scientific references can be fairly easily found. For near-market products this is much more challenging. Companies often only declare on-going tests, but provide only limited info about the progress. Scientific articles typically present proof-of-concept level data obtained with isolated cultures spiked into sample matrices.
When on market?	Many systems are already available, but they may have a limited scope for ID/AST. Due to lack of clinical data, some systems have a "research use only" status. Some have been accepted only for veterinary use. Commercially mature products include several NAAT systems, FISH-systems and immunodiagnostic system.

its use for rapid AST. The required bioinformatics is challenging, and universal open databases are needed to interpret the results.

In the near future, the progress in chip, microfluidics and biosensor technologies may provide new inexpensive AST systems. Integration of many sophisticated technologies will be needed to resolve problems with a low initial pathogen number and the presence of contaminating sample matrices. Several scientific publications have already demonstrated the successful use of smartphone optics and telecommunication capacity for monitoring of microwell or microcapillary cultivations, pH and redox changes and for delivering the read-outs of biosensor data (Berg et al., 2015; Feng et al., 2016; Cui et al., 2018; Hernández-Neuta et al., 2019). The obvious lack of IPR protection for smartphone-based analytic devices and the requirement to validate analytic devices as an entity unfortunately discourages commercialization of these technologies.

CONCLUSIONS

Standard growth-based technologies based on disc diffusion and broth dilution still dominate in AST. They are slow and require pure cultures, but in other aspects serve the purpose well. Only few rapid growth-based AST methods work directly with polymicrobial clinical samples, which is required in POCT. Sensitive growth monitoring can be achieved either by frequent sampling (applicable to disruptive methods like FISH or NAAT) or by on-line immunodiagnostic methods. dAST with chip-based microfluidics devices and isothermal amplification can potentially revolutionize phenotypic AST. With this approach, thousands of individual single bacterium droplet samples can be categorized according whether the amount of amplified DNA reaches the limit defined for growing cells. The FISH-based Accelerate Pheno system has already reached FDA approval. The mariPOC device based on immunodetection with two-photon excitation fluoroscopy allows non-disruptive microbial identification. Its clinical validation for AST should be followed with interest, as this technology enables the use of non-purified clinical samples and also allows follow-up studies to confirm the results. Additionally it already provides a rapid and sensitive identification of both bacterial and viral pathogens. The Multipath technology (First Light Diagnostics Inc.) may provide a functional platform for automated on-line detection by applying fluorescent nanoparticles for signaling, magnetic beads for binding, and non-microscopic imaging for detection. Multiplexing cartridge-based NAAT solutions are likely to reach a significant customer base in central laboratories, since they offer high speed, work well with non-culturable bacteria and viruses and possess an excellent high-throughput power for pathogen

ID. However, all new emerging technologies struggle to meet all of the criteria Prof. Kahlmeter set for AST technologies (**Table 2**). As none of the presented technologies is optimal in all aspects, it is probable that many of them will reach a large customer base. Therefore the consensus statement of the PIAMR AMR- RDT Working Group on Antimicrobial Resistance and Rapid Diagnostic Testing is still valid: "*There is no single major, or broadly accepted, technological breakthrough that leads the f eld of rapid AST platform development*" (van Belkum et al., 2019b).

AUTHOR'S NOTE

The described technologies have been taken into consideration without any pre-selection and have been judged only by their applicability to clinical diagnostics.

AUTHOR CONTRIBUTIONS

AV was the main responsible for the acquisition and structuring of the scientif c literature. The text and conclusions were processed by all the authors. VH and OL mediated valuable contacts to the expert biotech scientists and healthcare experts consulted for this review. All authors contributed to the writing and accepted the f nal version.

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Conf ict of Interest: VH was employed by Fimlab Laboratories.

The remaining authors declare that the research was conducted in the absence of any commercial or f nancial relationships that could be construed as a potential conf ict of interest.

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MAJOR ARTICLE

Randomized Trial of Rapid Multiplex Polymerase Chain Reaction–Based Blood Culture Identification and Susceptibility Testing

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(See the Editorial Commentary by Caliendo on pages 1081–3.)

Background. The value of rapid, panel-based molecular diagnostics for positive blood culture bottles (BCBs) has not been rigorously assessed. We performed a prospective randomized controlled trial evaluating outcomes associated with rapid multiplex PCR (rmPCR) detection of bacteria, fungi, and resistance genes directly from positive BCBs.

Methods. A total of 617 patients with positive BCBs underwent stratified randomization into 3 arms: standard BCB processing (control, n = 207), rmPCR reported with templated comments (rmPCR, n = 198), or rmPCR reported with templated comments and real-time audit and feedback of antimicrobial orders by an antimicrobial stewardship team (rmPCR/AS, n = 212). The primary outcome was antimicrobial therapy duration. Secondary outcomes were time to antimicrobial de-escalation or escalation, length of stay (LOS), mortality, and cost.

Results. Time from BCB Gram stain to microorganism identification was shorter in the intervention group (1.3 hours) vs control (22.3 hours) ($P < .001$). Compared to the control group, both intervention groups had decreased broad-spectrum piperacillin-tazobactam (control 56 hours, rmPCR 44 hours, rmPCR/AS 45 hours; $P = .01$) and increased narrow-spectrum β-lactam (control 42 hours, rmPCR 71 hours, rmPCR/AS 85 hours; $P = .04$) use, and less treatment of contaminants (control 25%, rmPCR 11%, rmPCR/AS 8%; $P = .015$). Time from Gram stain to appropriate antimicrobial de-escalation or escalation was shortest in the rmPCR/AS group (de-escalation: rmPCR/AS 21 hours, control 34 hours, rmPCR 38 hours, $P < .001$; escalation: rmPCR/AS 5 hours, control 24 hours, rmPCR 6 hours, $P = .04$). Groups did not differ in mortality, LOS, or cost.

Conclusions. rmPCR reported with templated comments reduced treatment of contaminants and use of broad-spectrum antimicrobials. Addition of antimicrobial stewardship enhanced antimicrobial de-escalation.

Clinical Trials Registration. NCT01898208.

Keywords. antimicrobial stewardship; blood culture; diagnostic; PCR.

Conventional methods for identification and susceptibility testing of microorganisms from blood cultures takes ≥2 days, during which time patients may be receiving ineffective or unnecessarily broad-spectrum antibiotics [1, 2]. Panel-based molecular diagnostic assays are now available for direct testing of positive blood culture bottles (BCBs), providing timelier results than conventional subculture and phenotypic susceptibility testing. Faster identification and resistance characterization of pathogens may lead to earlier administration of directed antimicrobial therapy, promote earlier de-escalation of broad-spectrum agents, and potentially result in better outcomes, fewer antibiotic-associated adverse effects (eg, *Clostridium difficile* infection), and less emergence of antimicrobial-resistant

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organisms [3]. However, rapid blood culture diagnostics are "add-on" tests performed in addition to conventional testing, and therefore increase complexity of laboratory testing and cost of patient care. The Infectious Diseases Society of America has recently called for research to evaluate how novel diagnostics impact patients and healthcare systems and to identify methods to integrate novel diagnostic testing into clinical practice [4].

Studies evaluating the clinical impact of rapid blood culture diagnostics have been limited by observational study designs and use of historical controls [5–11]. Whereas studies suggest that antimicrobial use, length of stay (LOS), mortality, and/or cost may be reduced through use of rapid blood culture diagnostic tests [5, 6, 9, 11–14], practice changes other than implementation of the novel tests may have affected outcomes. Additionally, prior studies have evaluated bundled interventions in which rapid tests were implemented with antimicrobial stewardship initiatives, making it impossible to determine whether the rapid test or the stewardship intervention alone might have been effective [5, 6, 8, 11, 15, 16]. Furthermore, real-time antimicrobial stewardship is not feasible in hospitals without stewardship programs. Ideally, a randomized controlled trial (RCT) is needed to evaluate outcomes associated with use of rapid diagnostics for bacterial identification and susceptibility testing directly from positive BCBs, but to date, none has been performed.

We conducted a prospective RCT comparing antimicrobial utilization and outcomes among patients with positive blood cultures who received standard culture and antimicrobial susceptibility testing alone, or with a rapid multiplex polymerase chain reaction (PCR) panel that identifies bacteria and *Candida* species and select antimicrobial resistance genes in approximately 1 hour [17, 18]. To determine the optimal method of communicating results, PCR test results were delivered in 2 ways, with templated comments to guide antimicrobial prescribing, or with templated comments in conjunction with real-time antimicrobial stewardship team recommendations to prescribers.

METHODS

Study Design, Randomization, and Masking

The study was a prospective RCT conducted at the Mayo Clinic, Rochester, Minnesota. Eligible patients were adults and children who had positive blood cultures processed in the clinical microbiology laboratory using the Becton Dickinson BACTEC FX system between August 2013 and March 2014. Patients were randomly assigned to either standard BCB processing (control), rapid multiplex PCR with templated comments (rmPCR), or rmPCR with templated comments and real-time antimicrobial stewardship (rmPCR/AS). Patients were excluded if they had a positive blood culture in the prior week, had not provided the Minnesota state research authorization (Minnesota Statute 144.335), were previously enrolled in the study, died or were transitioned to comfort care within 24 hours of enrollment, or had a negative BCB Gram stain. This study was approved by the Mayo Clinic Institutional Review Board with a waiver of informed consent.

Stratified randomization (based on age <65 or ≥65 years, intensive care unit admission, and admission to solid organ or bone marrow transplant services) was done once a BCB signaled positive. Laboratory technologists and investigators were not blinded to study arm assignment. For all groups, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for pathogen identification of colonies isolated from positive BCBs, and rapid testing for methicillin resistance on *Staphylococcus aureus* colonies with the Alere PBP2a test (Alere, Waltham, Massachusetts) were used. Baseline institutional antimicrobial stewardship interventions were in place for all study groups including requiring infectious diseases approval for restricted antimicrobials, and Monday–Friday daytime prospective audit and feedback of select inpatient antimicrobial orders. A computer-based monitoring system that integrates pharmacy, laboratory, and microbiology databases was used to identify opportunities for audit and feedback [19] (see Supplementary Methods for details).

Intervention

The rmPCR panel used in both intervention arms was the FilmArray Blood Culture ID Panel (BioFire Diagnostics/bioMérieux, Salt Lake City, Utah), which was performed as soon as a BCB signaled positive, 24 hours a day, 7 days a week. This assay detects *Staphylococcus* species, *S. aureus*, *Streptococcus* species, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Enterococcus* species, *Listeria monocytogenes*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Serratia* species, *Proteus* species, *Acinetobacter baumannii*, *Haemophilus influenzae*, *Neisseria meningitidis*, *Pseudomonas aeruginosa*, Enterobacteriaceae, *Escherichia coli*, *Enterobacter cloacae* complex, *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis*, and 3 antimicrobial resistance genes, *mecA*, *vanA/B*, and bla_{KPC}. Results of the rmPCR test and templated comments regarding optimal antimicrobial therapy (Supplementary Table 1) were communicated to the service by telephone by a laboratory technologist and entered into the electronic medical record in real time. Gram stain–positive blood cultures that had negative rmPCR results were reported with Gram stain result only.

In the rmPCR/AS group, the rmPCR test was performed and reported as above, and an infectious diseases clinician or pharmacist (R. B. or C. B. T.) was paged with the result, 24 hours a day, 7 days a week. The subject's rmPCR result and medical record were reviewed and the primary service (or consulting infectious diseases physician, if applicable) contacted immediately over the 3 days following enrollment if a modification to antimicrobial therapy was deemed appropriate. Discordant results between rmPCR and conventional identification and

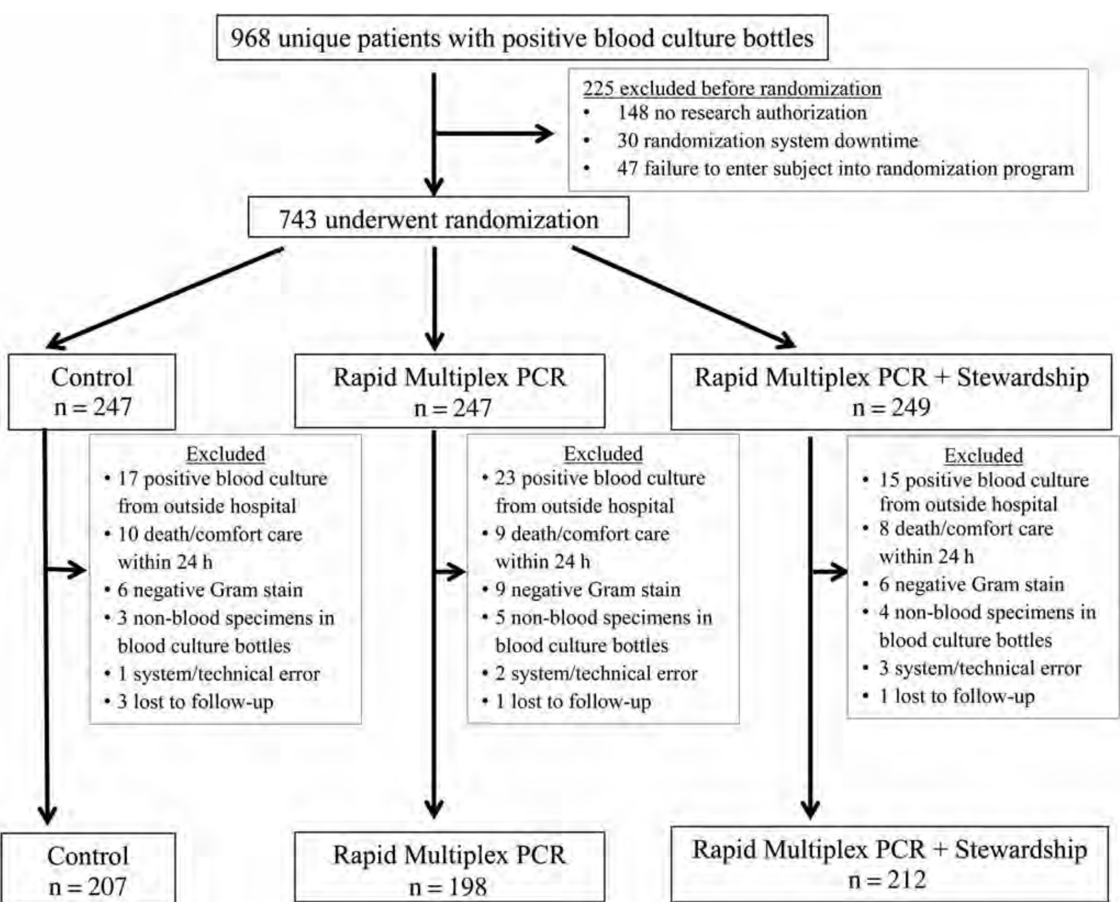

Figure 1. Participant enrollment. System/technical errors included randomization software downtime, typographical errors causing an ineligible subject to be erroneously randomized, and failure to enter an eligible subject into the randomization program. Abbreviation: PCR, polymerase chain reaction.

susceptibility testing were reviewed in real time by the bacteriology laboratory director (R. P.).

Outcome Measures

Subjects were followed for 30 days after enrollment. The primary outcome was duration of antimicrobial therapy (in hours) in the 4 days after enrollment. Duration of antimicrobial therapy was calculated as the difference between the date and time of the antibiotic start order (or Gram stain–positive blood culture, if antibiotics were started prior to the positive culture result) and the date and time of the antibiotic stop order. Secondary outcomes included time from positive Gram stain result to first active antibiotic; time to first appropriate antibiotic escalation (initiation of 1 or more antibiotics or switch from a narrow- to a broad-spectrum antibiotic) or de-escalation (discontinuation of 1 or more antibiotics and/or switch from a broad- to a narrow-spectrum antibiotic); proportion of contaminants not treated; time to pathogen identification and blood culture clearance; LOS; mortality; antibiotic-associated toxicities; infectious disease consultation; and costs per patient [20, 21] (see Supplementary Methods for details).

Statistical Analysis

We anticipated that two-thirds of patients (130/200 per arm) would receive vancomycin or an antipseudomonal antibiotic and that the standard deviation (SD) for antibiotic duration would be about 24 hours. Thus, we would be able to detect a difference in either vancomycin or antipseudomonal antibiotic duration of at least 0.403 SD (approximately 10 hours) with 80% power at an α level of .017 (to account for 3 comparisons) using a 2-sample *t* test. Comparisons among 3 groups were performed using the Kruskal–Wallis test for continuous variables and Fisher exact test or χ^2 test for categorical variables. When the overall test was significant, pairwise comparisons were made using Wilcoxon rank-sum, Fisher exact, or χ^2 test, as appropriate. Analysis was performed using SAS software, version 9.3 (SAS Inc, Cary, North Carolina).

RESULTS

Patients

A total of 2646 positive blood cultures from 968 unique patients were identified, 743 patients underwent randomization, and 126

Table 1. Baseline Demographic and Clinical Characteristics According to Study Group

Characteristic	Control (n = 207)	Rapid Multiplex PCR (n = 198)	Rapid Multiplex PCR + Stewardship (n = 212)
Demographics			
Male sex	142 (69)	116 (59)	127 (60)
Age, y, mean ± SD	61.5 ± 19.32	61.4 ± 21.22	61.2 ± 20.08
Race, white	176 (85)	186 (93.9)	179 (84.4)
Location before admission			
Nursing home	22 (10.6)	13 (6.6)	15 (7.1)
Outside hospital	27 (13)	34 (17.2)	30 (14.2)
Patient home	114 (55.1)	103 (52)	104 (49.1)
Outpatient clinic	22 (10.6)	13 (6.6)	15 (7.1)
Location at enrollment			
Outpatient	14 (6.8)	20 (10.1)	28 (13.2)
General ward	125 (60.4)	120 (60.6)	125 (59)
Intensive care unit	68 (32.9)	58 (29.3)	59 (27.8)
Comorbidities			
Charlson comorbidity score, mean ± SD	5.3 ± 3.2	5.2 ± 4.7	4.9 ± 3.0
Myocardial infarction	17 (8.2)	16 (8.1)	9 (4.2)
Chronic heart failure	31 (15)	32 (16.2)	22 (10.4)
Cerebrovascular accident	24 (11.6)	21 (10.6)	18 (8.5)
Chronic obstructive pulmonary disease	25 (12.1)	22 (11.1)	27 (12.7)
Peptic ulcer disease	27 (13)	15 (7.6)	21 (9.9)
Lymphoma	21 (10.1)	21 (10.6)	25 (11.8)
Leukemia	15 (7.2)	21 (10.6)	21 (9.9)
Solid tumor	41 (19.8)	34 (17.2)	31 (14.6)
Diabetes mellitus	68 (32.9)	50 (25.3)	63 (29.7)
Immunosuppressant use[a]	79 (38.2)	77 (38.9)	82 (38.7)
Surgery in prior 30 d	30 (14.5)	27 (13.6)	45 (21.2)
Renal replacement therapy	16 (7.7)	13 (6.6)	14 (6.6)
Any malignancy	76 (36.7)	72 (36.4)	73 (34.4)
Chronic heart disease	81 (39.1)	87 (43.9)	73 (34.4)
Chronic renal disease	62 (30)	49 (24.7)	52 (24.5)
Chronic liver disease	35 (16.9)	24 (12.1)	33 (15.6)
Chronic lung disease	43 (20.8)	44 (22.2)	50 (23.6)
Central venous catheter	95 (45.9)	91 (46)	94 (44.3)
Acute kidney injury	53 (25.6)	35 (17.7)	37 (17.5)
Concurrent infectious syndromes[b]	97 (46.9)	87 (43.9)	108 (50.9)
Neutropenic fever	24 (11.6)	24 (12.1)	27 (12.7)
Respiratory infection	31 (15)	21 (10.6)	29 (13.7)
Urinary tract infection	15 (7.3)	17 (8.6)	19 (9)
Intra-abdominal infection	22 (10.6)	21 (10.6)	26 (12.3)
Source of bacteremia			
Central venous catheter	32 (15.5)	38 (19.2)	37 (17.5)
Urinary	31 (15)	34 (17.2)	33 (15.6)
Intra-abdominal	38 (18.4)	22 (11.1)	37 (17.5)
Skin/soft tissue	11 (5.3)	13 (6.6)	8 (3.8)
Respiratory	9 (4.3)	11 (5.6)	8 (3.8)
Bone/joint	2 (1)	4 (2)	12 (5.7)
Eyes, ears, nose, throat	1 (0.5)	2 (1)	1 (0.5)
Cardiovascular	2 (1)	3 (1.5)	2 (0.9)
Surgical site infection	0 (0)	0 (0)	2 (0.9)
Unidentified	18 (8.7)	16 (8.1)	9 (4.2)

Table 1 continued.

Characteristic	Control (n = 207)	Rapid Multiplex PCR (n = 198)	Rapid Multiplex PCR + Stewardship (n = 212)
Complicated bloodstream infection[c]	14 (6.8)	21 (10.6)	13 (6.1)
Source control within 5 d[d]	44 (21.3)	46 (23.2)	57 (26.9)
Contact isolation for MRSA, VRE, or *Clostridium difficile*	51 (24.6)	40 (20.2)	43 (20.3)
Possible contaminant[e]	63 (30.4)	55 (27.8)	62 (29.3)
Severity of illness			
APACHE II score, mean ± SD[f]	18.3 ± 8.2	17.4 ± 7.8	16.4 ± 7.3
Requiring mechanical ventilation	21 (10.1)	23 (11.6)	16 (7.5)
Hypotension[g]	80 (38.6)	76 (38.4)	63 (29.7)
Pitt bacteremia score, mean ± SD[f]	2.0 ± 2.5	2.0 ± 2.3	1.6 ± 2.0
Infectious diseases consultation within 72 h of enrollment	103 (49.8)	97 (49)	96 (45.3)
On active antibiotic at the time of enrollment[h]	99 (69)	102 (71)	113 (75)

Data are presented as No. (%) unless otherwise specified. There were no significant differences in baseline characteristics between the study groups with the exception of race ($P = .005$).

Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; MRSA, methicillin-resistant *Staphylococcus aureus*; PCR, polymerase chain reaction; SD, standard deviation; VRE, vancomycin-resistant *Enterococcus* species.

[a] Received cytotoxic agents within prior 6 weeks, >15 mg prednisone for >1 week in prior 4 weeks, or any other immunosuppressant within 2 weeks of blood culture.

[b] A patient was classified with concurrent infection other than neutropenic fever when a site of infection was documented in the medical record (eg, pneumonia, intraabdominal infection), and culture from the concurrent infection site grew at least 1 organism that was not isolated from blood.

[c] Positive blood culture after 3 days of effective antimicrobial therapy, metastatic infection, or infective endocarditis.

[d] Catheter removal or surgical drainage procedure.

[e] Growth of common contaminant (eg, coagulase-negative *Staphylococcus* species) from a single blood culture set when ≥2 blood culture sets were collected, except among subjects suspected to have true bacteremia associated with central venous catheters or devices.

[f] Excludes outpatients.

[g] Requiring vasopressors or systolic blood pressure decrease by >20 mm Hg.

[h] Active antibiotic defined as an agent to which the blood culture organism was susceptible by conventional antimicrobial susceptibility testing.

(17%) were excluded, leaving 617 patients included in the study (Figure 1). Compared to nonrandomized subjects, randomized patients were slightly older (61.3 vs 56.7 years, $P = .01$), but not different by sex. Clinical and demographic characteristics were similar between the groups (Table 1).

Microbiology

Blood cultures grew 54.8% gram-positive bacteria, 32.6% gram-negative bacteria, 2% *Candida* species, and 10.5% multiple organisms. One-third of organisms isolated (29.2%) were considered contaminants. Among subjects with rmPCR testing, 81% of organisms isolated were detectable by the rmPCR panel. Study groups did not differ in terms of the distribution of microorganisms or the proportion that were contaminants or detectable by rmPCR (Supplementary Table 2).

Rapid Multiplex PCR Performance and Stewardship Interventions

Among subjects with pathogens represented on the rmPCR panel, median time from Gram stain result to organism identification was shorter in both intervention groups (both 1.3 hours) vs the control group (22 hours) ($P < .0001$; Figure 2). Most discrepancies between rmPCR and standard culture and susceptibility results occurred because microorganisms were not represented on the rmPCR panel (78/410 [19%]). In 13 of 410 (3.2%) cases, there were discrepancies in organism identification or susceptibility result (Table 2).

In the rmPCR/AS group, investigators made 159 recommendations for the following: antibiotic de-escalation (58%), antibiotic escalation (18%), optimization of antibiotic dose or duration (15%), and infectious diseases consultation (9%); 78% of recommendations were accepted within 24 hours. In contrast, the baseline stewardship program in place for all groups identified fewer audit and feedback opportunities (50 in control, 26 in rmPCR, and 34 in rmPCR/AS) and made fewer recommendations to modify therapy (7 in control, 0 in rmPCR, and 6 in rmPCR/AS).

Antimicrobial Utilization

Within the first 4 days after enrollment, duration of vancomycin was not different between groups. However, among subjects with bloodstream infections caused by organisms not requiring vancomycin therapy (eg, monomicrobial cultures of methicillin-susceptible *S. aureus*, *S. pyogenes*, *S. agalactiae*, or gram-negative or fungal organisms), the median duration

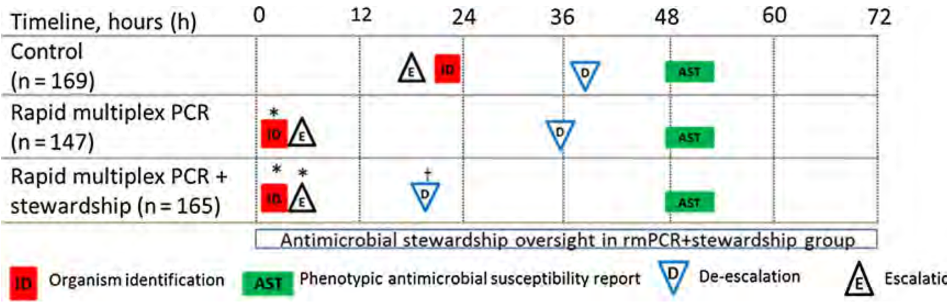

Figure 2. Comparison of time to organism identification, availability of phenotypic antimicrobial susceptibility results, and first appropriate modification of antimicrobial therapy for the subset of study subjects with organisms represented on the rapid multiplex polymerase chain reaction (rmPCR) panel (n = 481). Time 0 is when the positive Gram stain result was reported. Median time in hours (interquartile range [IQR]) to organism identification: control 22.3 (17–28), both rmPCR and rmPCR + stewardship 1.3 (0.9–1.6); de-escalation: control 39 (19–56), rmPCR 36 (22–61), rmPCR + stewardship 20 (6–36); escalation: control 18 (2–63), rmPCR 4 (1.5–24), rmPCR + stewardship 4 (1.8–9). *$P < .05$ vs control; †$P < .05$ vs control and rmPCR groups.

of vancomycin use was shorter in the rmPCR (0 hours) and rmPCR/AS (0 hours) groups compared with the control group (8.2 hours, $P = .03$) (Table 3). Conversely, for subjects with bloodstream infections caused by vancomycin-susceptible enterococci, vancomycin use was greater in the rmPCR (70 hours) and rmPCR/AS (82 hours) groups than the control group (20 hours, $P = .037$). Duration of narrow-spectrum β-lactam use (cefazolin, nafcillin, oxacillin) was greater in both the rmPCR (71 hours) and rmPCR/AS (85 hours) groups than in the control group (42 hours, $P = .04$). Among all

Table 2. Discrepancies Between Rapid Multiplex Polymerase Chain Reaction Assay and Standard Culture/Phenotypic Antimicrobial Susceptibility Testing

Rapid Multiplex PCR Panel Result	Standard Culture and Phenotypic Susceptibility Testing	No.
Single/few organisms detected	Multiple organisms detected	6
1. *Klebsiella pneumoniae*	1. *K. pneumoniae*, *Staphylococcus epidermidis*	
2. *Enterococcus* species	2. *Enterococcus faecium*, *Staphylococcus hemolyticus*, *Micrococcus luteus*	
3. *Enterobacter cloacae*, *Enterococcus* species (*vanA/B* positive)	3. *Enterococcus faecalis* (vancomycin susceptible), *E. faecium* (vancomycin resistant), *E. cloacae*, *Streptococcus mitis*, *S. epidermidis*, *Acinetobacter* species	
4. (bottle a) *Streptococcus agalactiae* (bottle b) *Escherichia coli*[a]	4. (bottle a) *S. agalactiae*, *E. coli* (bottle b) *E. coli*, *Proteus* species	
5. *Streptococcus* species, *Haemophilus influenzae*	5. *Haemophilus sputorum*, *Neisseria subflava*, *Streptococcus salivarius*, *Streptococcus viridans* group, *Streptococcus mitis*	
6. *Enterococcus* species (*vanA/B* negative)	6. *E. faecium* (vancomycin susceptible), *S. anginosus*	
Discrepancy in susceptibility result		4
1. *Enterococcus* species (*vanA/B* negative)	1. *Enterococcus casseliflavus* (vancomycin MIC, 8 µg/mL)	
2. *Enterococcus* species (*vanA/B* positive)	2. *E. faecium*, vancomycin MIC 2 µg/mL, isolate *vanA* positive by PCR	
3. *Staphylococcus* species (*mecA* positive)	3. *Staphylococcus capitis*, oxacillin MIC 0.25 µg/mL, isolate *mecA* negative by PCR	
4. *Staphylococcus* species (*mecA* negative)	4. Coagulase-negative *Staphylococcus* species, oxacillin 1 µg/mL, isolate *mecA* negative by PCR	
Discrepancy in organism identification		3
1. *Enterococcus* species and *Staphylococcus* species (*mecA* positive)[b]	1. *S. epidermidis*	
2. Negative	2. *Enterobacter* species	
3. *K. pneumoniae*	3. *Enterobacter aerogenes*	

Abbreviations: MIC, minimum inhibitory concentration; PCR, polymerase chain reaction.

[a] Gram stain results differed between 2 blood culture bottles collected on the same day for this subject. Both bottles were evaluated according to the study arm assignment of bottle a, which signaled positive first.

[b] Heavy growth of coagulase-negative staphylococci may lead to cross-reactivity with *Enterococcus* species; thus, the rapid multiplex PCR result was not reported.

Table 3. Antibiotic Utilization Among All Study Subjects in the First 96 Hours Following Enrollment

Outcome	Control	Rapid Multiplex PCR	Rapid Multiplex PCR + Stewardship	*P* Value Comparing 3 Groups
Duration of therapy[a], h				
Vancomycin				
All patients (n = 357)	44 (22–72)	42 (21–93)	42 (19–90)	.92
Organisms not requiring vancomycin[b] (n = 169)	8.2 (0–26)	0 (0–16)	0 (0–3)[c]	.032
Vancomycin-susceptible enterococci (n = 32)	20 (1–59)	70 (48–88)[c]	82 (40–96)[c]	.037
Methicillin-susceptible *Staphylococcus aureus* (n = 42)	23 (20–53)	11 (0–26)	8 (0–44)	.2
Nafcillin, oxacillin, or cefazolin (n = 50)	42 (24–57)	71 (51–79)[c]	85 (42–92)[c]	.035
Piperacillin-tazobactam (n = 214)	56 (39–82)	44 (27–74)[c]	45 (19–78)[c]	.012
Cefepime (n = 181)	55 (28–96)	71 (43–96)	58 (32–96)	.56
Antibiotic modifications				
Time to first appropriate de-escalation[d] (n = 344)	34 (21–55)	38 (22–66)	21 (7–37)[c,e]	<.0001
Time to first appropriate escalation[f] (n = 122)	24 (3–67)	6 (2–36)	5 (2–22)[c]	.04
Time to administration of active antibiotics (n = 123)[g]	11 (2–51)	6 (2–31)	4 (2–20)	.55
Contaminated blood cultures not treated or treated for <24 h, No. (%)[h]	47 (75)	49 (89)[c]	57 (92)[c]	.015

Data are presented as median (IQR) unless otherwise specified.

Abbreviations: IQR, interquartile range; PCR, polymerase chain reaction.

[a] Duration of therapy (hours) was calculated as the difference between the date and time of the antibiotic start order (or Gram stain–positive blood culture, if antibiotics were started prior to the positive culture result) and the date and time of the antibiotic stop order, for subjects who received the specified antibiotics, according to the organisms identified and study group. Shorter duration of broad-spectrum antibiotics, longer duration of narrow-spectrum antibiotics, faster antibiotic escalation or de-escalation, and less treatment of contaminants were considered favorable outcomes.

[b] Organisms not requiring vancomycin included monomicrobial cultures with methicillin-susceptible *Staphylococcus aureus*; groups A, B, C, or G streptococci; *Streptococcus anginosus* species group; or gram-negative or fungal organisms.

[c] Statistically significant compared to control group.

[d] From positive Gram stain to 96 hours after enrollment. De-escalation included discontinuation of 1 or more antibiotics and/or switching from a broad- to a narrow-spectrum antibiotic.

[e] Statistically significant comparison between the 2 intervention groups.

[f] From positive Gram stain to 96 hours after enrollment. Escalation included initiation of 1 or more antibiotics and/or switching from a narrow- to a broad-spectrum antibiotic.

[g] From positive Gram stain to start of active antibiotic among patients not on active therapy at enrollment; excludes patients with contaminated blood cultures.

[h] Contaminated blood cultures were defined as growth of organisms such as coagulase-negative staphylococci from a single blood culture set when ≥2 blood culture sets were collected, except among subjects suspected to have true bacteremia associated with central venous catheters or devices.

subjects, the median duration of piperacillin-tazobactam was shorter in the rmPCR (44 hours) and rmPCR/AS (45 hours) groups compared with control (56 hours, $P = .012$). Utilization of other antibiotics was not different between groups.

The proportion of subjects with any de-escalation of antimicrobial therapy after Gram stain or rmPCR result was higher in the rmPCR/AS group (24.1%) compared with the rmPCR (14.7%) and control (12.1%) groups ($P = .003$). Time from Gram stain result to first appropriate antimicrobial de-escalation was shorter in the rmPCR/AS group (21 hours) compared with the control (34 hours) and the rmPCR (38 hours) groups ($P < .001$) (Table 3). Time from Gram stain result to first appropriate antimicrobial escalation was shorter in the rmPCR/AS group (5 hours) than in the control group (24 hours, $P = .04$; Table 3). Results were similar among the subset of subjects with organisms on the rmPCR panel (Figure 2). In the groups with rmPCR testing, time to antimicrobial de-escalation or escalation did not change significantly over the course of the study. The proportion of contaminant blood cultures that was not treated or treated for <24 hours was higher in the rmPCR (89%) and rmPCR/AS (92%) groups vs control (75%, $P = .015$; Table 3). Antimicrobial utilization was not different among patients with and without infectious diseases consultation.

Other Outcomes

There were no differences in clinical or microbiologic outcomes among the groups (Table 4). Among the few patients with discrepant rmPCR and conventional culture results, no adverse consequences were observed. Both intervention groups had increased test costs but similar hospitalization costs compared with the control group. Antimicrobial costs tended to be lower for both intervention groups than for the control group,

Table 4. Comparison of Clinical, Microbiologic, and Cost Outcomes According to Study Group

Outcome	Control (n = 207)	Rapid Multiplex PCR (n = 198)	Rapid Multiplex PCR + Stewardship (n = 212)	*P* Value Comparing 3 Groups
Clinical outcome				
Disposition				.12
Home	68 (32.9)	62 (31.3)	78 (36.8)	
Home with outpatient antimicrobial therapy	39 (18.8)	52 (26.3)	38 (17.9)	
Nursing home/skilled nursing facility	63 (30.4)	42 (21.2)	54 (25.5)	
Hospice/comfort care	12 (5.8)	8 (4)	7 (3.3)	
Death	11 (5.3)	11 (5.6)	8 (3.8)	
Length of stay (entire hospitalization), d, median (IQR)	8 (5–15)	8 (5–15)	8 (5–16)	.60
Length of stay (after enrollment), d, median (IQR)	7 (4–12)	6 (4–12)	7 (4–12)	.61
Intensive care unit admission within 14 d after enrollment	16 (7.7)	5 (2.5)	10 (4.7)	.06
Length of stay in intensive care unit (after enrollment), d, median (IQR)	3 (2–4)	2 (1–5)	3 (2–4)	.90
30-day mortality	22 (10.6)	20 (10.1)	18 (8.5)	.74
30-day attributable mortality	7 (3.4)	7 (3.5)	2 (0.9)	.42
30-day readmission for infection with same organism	6 (2.9)	6 (3)	8 (3.8)	.88
Toxicity/adverse drug reaction[a]	3 (1.4)	3 (1.5)	2 (0.9)	.82
Microbiologic outcomes				
Blood culture clearance within 3 d after enrollment	147 (71)	131 (66.2)	146 (68.9)	.79
Acquisition of *Clostridium difficile* or multidrug-resistant organisms[b] within 30 days after enrollment	15 (7.2)	16 (8.1)	21 (9.9)	.62
Cost per hospitalized patient, mean (median)				
Overall hospitalization costs	$65 450 ($27 192)	$66 887 ($23 935)	$68 729 ($29 064)	.78
Test costs	$5377 ($2082)	$5680 ($2585)[c]	$5743 ($2774)[c]	<.001
Antimicrobial costs	$2194 ($990)	$1932 ($866)	$1741 ($890)	.65

Data are presented as No. (%) unless otherwise specified.

Abbreviations: IQR, interquartile range; PCR, polymerase chain reaction.

[a] Toxicities include seizures, *Clostridium difficile* infection, hepatitis, myelosuppression, renal insufficiency, prolonged QTc interval, and rash that occurred within 2 weeks following enrollment and were documented in the medical record.

[b] Multidrug-resistant organisms including vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus*, extended-spectrum cephalosporin-resistant Enterobacteriaceae, and *Pseudomonas aeruginosa* and *Acinetobacter* species resistant to ≥3 antibiotic classes.

[c] Statistically significant compared to control group.

but this difference was not statistically significant. Total costs were not significantly different between intervention and control groups in sensitivity analysis of rmPCR test cost.

DISCUSSION

We report the first prospective RCT to demonstrate benefit of an rmPCR-based blood culture diagnostic test. We found that use of the rmPCR test led to more judicious antibiotic use. Additionally, we delivered rapid test results along with templated comments guiding interpretation and antimicrobial prescribing, and separately evaluated this approach with and without real-time antimicrobial stewardship. Both rapid test reporting strategies reduced unnecessary antibiotic use, although rmPCR testing combined with antimicrobial stewardship resulted in the most rapid antibiotic de-escalation.

In earlier observational studies, rapid pathogen identification methods have been associated with decreased mortality, LOS, and cost [5, 6, 9, 11–14]. However, these studies were limited by their retrospective designs, use of historical controls [5, 6, 10, 22], lack of randomization, and failure to match subjects by severity of illness [7]. In contrast, in this trial we did not observe differences in clinical or cost outcomes between patients in intervention and control arms. This may be due to the use of different study designs, the fact that this trial was not powered to detect differences in LOS, mortality, or cost, and/or the fact that 70% of our study subjects were receiving at least 1 active agent at time of enrollment and were generally being overtreated, rather than undertreated. In addition, our institution's baseline antimicrobial stewardship program, low resistance rates, high rate of infectious diseases consultation (48%), and baseline use of advanced technologies including MALDI-TOF MS and

rapid PBP2a testing, even in the control arm, may have reduced differences between the control and intervention arms.

The rmPCR test enabled clinicians to quickly initiate "pathogen-directed" therapy and appropriately scale up or scale down antibiotic therapy, as needed. Specifically, in both intervention groups, we observed increased narrow-spectrum antibiotic use, less unnecessary vancomycin use, decreased treatment of blood culture contaminants, and more timely antibiotic escalation compared with the control group. Because timely initiation of effective therapy is a critical step in the management of patients with sepsis [1, 23–25], reducing time to appropriate antibiotic escalation by 14 hours, as observed in both intervention arms (Figure 2), is clinically significant. Antibiotic de-escalation occurred nearly 1 day (19 hours) faster in the rmPCR/AS group compared with control (Figure 2), which is almost a 25% reduction in broad-spectrum antibiotic days of therapy, as median duration of piperacillin-tazobactam or meropenem therapy in the control group was 4 days. This is likely to be significant at a population level; models estimate that a 5% reduction in broad-spectrum antibiotic use among hospitalized patients would result in a 26% decrease in *C. difficile* infection rates [26]. Among subjects with vancomycin-susceptible enterococci, vancomycin use increased in both intervention groups because of prompt de-escalation from daptomycin to vancomycin. In contrast, cefepime use did not decrease in the intervention groups, likely because this drug is commonly used for management of suspected infection in neutropenic hosts, in whom antibiotic de-escalation is often not indicated, despite negative blood cultures.

We observed that provider response differed according to how rapid test results were delivered. Others have noted that despite the availability of a rapid test result (eg, presence or absence of *mecA*), providers may fail to modify antibiotic therapy without pharmacist intervention [8, 27]. Observational studies have found favorable outcomes when rapid testing was implemented together with antimicrobial stewardship interventions [5, 6, 11–16]. To our knowledge, no prior studies have implemented a rapid blood culture diagnostic test using electronic decision support in the form of templated comments to guide prescribing, as done in this study. In the group that received the rmPCR test results delivered with templated comments communicated verbally and in the medical record, we observed more rapid antibiotic escalation, more narrow-spectrum antibiotic use, and less treatment of contaminants compared with the control group. Templated comments are an inexpensive and effective means of communicating straightforward results to providers, such as when coagulase-negative staphylococci or *Micrococcus* species represent contaminants, or when a *Staphylococcus* species is methicillin susceptible or resistant.

When the rmPCR test result was delivered with guidance from the antimicrobial stewardship team, there was also more frequent and timelier antibiotic de-escalation. We speculate that this is in part because notification from a stewardship team member might prompt a busy clinician to act on a test result more so than a telephone call from a laboratory technologist. Additionally, in complex clinical scenarios when critically ill or immunocompromised patients or patients with polymicrobial infections are on multiple broad-spectrum antibiotics, providers may prefer discussion with infectious disease specialists prior to modifying antimicrobial management. Although in this study the stewardship team provided feedback to providers 24 hours a day, most antibiotic de-escalation occurred during the day because housestaff preferred not to contact supervising providers at night regarding nonurgent de-escalation questions. Thus, around-the-clock antimicrobial stewardship team oversight, which is costly, may not be necessary. Rather, we estimate that at our institution, an additional 1–2 hours of stewardship effort (during the daytime) would be sufficient for daily review of positive blood cultures.

Unlike other rapid PCR-based platforms that target a limited number of organisms, the rmPCR test detected multiple targets, characterizing >80% of positive blood cultures and providing accurate results nearly a day faster than standard techniques. Additional advantages of the rmPCR test studied are that it is a closed system, can be used whether blood cultures contain gram-positive or gram-negative bacteria or yeast, and does not require significant technologist training or time. Known limitations of the test include its lack of sensitivity in detecting all organisms in polymicrobial cultures (Table 2), and the limited susceptibility information provided for gram-negative bacteria. The rmPCR test studied is "add-on" testing that does not replace conventional BCB subculture workup methods.

This study has limitations, including that it was performed at a single center and may not be generalizable to other institutions with different patient populations, prescribing and stewardship practices, and antimicrobial resistance rates. The study was not powered for subgroup analyses or to detect differences in secondary outcomes including LOS and mortality. Investigators were not blinded to study arm, and infectious diseases "curbside" consultations were not captured. We did not include a control group without rmPCR testing but with stewardship of all positive blood cultures. We were unable to account for contamination between study arms, although rarely were multiple patients in the intervention arms cared for by the same clinical service at the same time. Despite these limitations, this is the first prospective RCT to evaluate the value of a rapid diagnostic test for blood culture pathogen identification and to compare strategies to communicate rapid test results to clinicians.

In conclusion, rapid pathogen and susceptibility detection directly from blood cultures implemented with templated comments or antimicrobial stewardship oversight can optimize

antibiotic prescribing for bloodstream infections. To influence clinical decision making, rapid results should be delivered with real-time decision support (using automated systems or antimicrobial stewardship programs) that assists clinicians to interpret and act on results.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Clinical Infectious Diseases

VIEWPOINTS

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Diagnostic Stewardship: Opportunity for a Laboratory–Infectious Diseases Partnership

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Recent advances in microbial diagnostics are providing clinicians with information about microbes causing infections and their resistance to antimicrobial agents more rapidly than ever before. Diagnostic stewardship refers to the appropriate use of laboratory testing to guide patient management, including treatment, in order to optimize clinical outcomes and limit the spread of antimicrobial resistance. Fulfilling the promise of diagnostic stewardship requires a seamless partnership between clinical laboratories, pharmacists, and infectious diseases clinicians, so that appropriate tests are ordered and diagnostic information is translated into appropriate management in real time.

Keywords. diagnostics; stewardship; clinical microbiology; infectious diseases; culture-independent.

Rapid precision diagnostics are revolutionizing clinical microbiology and promise to improve patient outcomes and curb the antimicrobial resistance (AMR) crisis by improving the use of antibiotics. For this potential to be fully realized, infectious diseases (ID) clinicians will play an essential role in a collaborative effort referred to as *diagnostic stewardship* (not to be confused with the cost-effective use of laboratory tests which, though part of diagnostic stewardship, is more limited in scope) [1–4]. Diagnostic stewardship requires a serious reconsideration of current practices, as empiricism gives way to diagnostics-guided therapy.

The goal of new diagnostic methods is to improve human health, but technological advances alone cannot achieve this goal. Decisions must be made about which new diagnostics are needed, how they will be used, and whether they are worth paying for. Laboratory tests currently account for only 4% of healthcare costs [5] but represent the most rapidly growing segment of the healthcare budget, mainly as a result of new molecular assays [6]. Although conventional clinical microbiology diagnostics are relatively inexpensive, some newer and technologically advanced tests can be costly, elevating the need to address value. It is estimated that approximately one-fi h of available tests are overused, with even more being underused [7]. Overuse of tests adds unnecessary costs, and both overuse and underuse can lead to incorrect diagnoses and inappropriate treatment. Moreover, many microbiology tests have become outdated, and optimal testing methods are unavailable in many settings. Appropriate use of testing is becoming more challenging as the number of available diagnostic tests increases. ID specialists can help to determine the appropriate tests for specific patients and situations.

ID clinicians currently partner with laboratory scientists to determine which antimicrobial susceptibility results are routinely reported for specific microorganisms and when additional testing should be performed. Th s facilitates antimicrobial stewardship by encouraging appropriate antibiotic use. With recent diagnostic advances that allow the identifi ation of microorganisms virtually as soon as they are grown on plates or in blood culture bottles, and sometimes even earlier [8], ID expertise plays an essential role in translating this information into appropriate treatment.

Many studies have shown that rapid diagnostics only improve clinical outcomes if they are coupled with stewardship teams that properly interpret results and apply them to treatment decisions [9–20]. Th s approach may require expanding the hours of laboratory operation and providing real-time ID consultative support. ID physicians and pharmacists may be asked to work alongside their laboratorians on diagnostic management teams [21], or clinical microbial sequencing boards (modeled after tumor boards) (https://www.genomeweb.com/sequencing/ucsf-lab-readies-launch-metagenomic-ngs-test-infectious-disease) that assist clinicians with the interpretation of complex test results in a specific linical fi ld.

ID clinicians can assist laboratories in devising appropriate comments to accompany test results in the electronic medical record (EMR), such as "possible contaminant which may not require antibiotic treatment" when coagulase-negative staphylococci are reported from a single blood culture; such comments can be tailored to the needs and unique epidemiology of

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individual institutions [22]. In this way, the EMR can provide a solution to AMR. Integration of laboratories with the EMR, antimicrobial stewardship teams, clinical pharmacists, and clinicians can ensure that treatment decisions are appropriately modifi d in response to test results in real-time [13]. Likewise, detection of certain drug-resistant organisms can automatically trigger inpatient isolation, preventing the spread of these organisms.

Sophisticated diagnostics can augment antimicrobial stewardship efforts by allowing the replacement of broad-spectrum antimicrobial agents with narrow-spectrum agents that target the microbes specifi ally responsible for individual patients' infections, facilitating early discontinuation of antimicrobial agents, or abrogating their use in the fi st place. To support the safety and efficacy of this approach, more studies of the application of precision diagnostics to optimize patient outcomes and reduce AMR (ie, implementation science) will be needed. In the context of AMR, improvements at the patient level promise to provide benefits at the population level.

Test menu selection is another activity in which ID clinicians can partner with their laboratories. An ever-increasing number of powerful but expensive technologies, ranging from point-of-care molecular diagnostics [23, 24] to multiplex panels [25, 26] and next-generation sequencing methods [27], require careful and discerning application. Laboratories can benefit from clinical input to select cost-effective diagnostics that best address patient needs. Similarly, if diagnostics manufacturers market tests directly to ID clinicians, then ID clinicians should work with their clinical microbiologists to ensure that the needs of their patients are optimally served.

As new technologies become available, local laboratory methods and a catalog of send-out tests should continually be reassessed to ensure that patients benefit from the latest diagnostic advances; ID clinicians should help ensure that testing is both available and appropriately ordered (eg, by helping to build smart ordering systems in the EMR). For example, ID clinicians can provide guidance with regard to the appropriate clinical criteria for testing of patients with clinical syndromes, such as acute gastroenteritis [28–30] or suspected *Clostridioides diffi le* infections [31, 32].

Finally, by defini g important unmet diagnostic needs [33], ID clinicians will play an increasingly important role in defi -ing the future tests that should be developed by industry. For example, the development of syndromic molecular diagnostic panels can benefit from clinical guidance [25, 26]. Molecular diagnostics allow the rapid and sensitive detection of pathogens that were not previously detectable with conventional methods [30], and ID expertise will be required to determine the implications of these diagnoses for specific anagement.

For example, it is not unusual for multiplex molecular platforms to detect multiple potential pathogens in a single clinical sample [25]. ID physicians can help interpret apparent coinfections with multiple potential pathogens and determine when a pathogen is likely to be responsible for a patient's symptoms, as well as establish appropriate criteria for the use of multiplex tests and assist in the design of such assays so that appropriate target organisms are included [26]. Newer technologies, such as whole-genome sequencing, shotgun metagenomic methods to diagnose infection, and methods to characterize the host microbiome [34, 35] likewise pose both an opportunity and a challenge, and ID clinicians can help establish interpretive criteria and applications.

Achieving a clinician-laboratorian collaboration will not necessarily be simple. The consolidation of laboratory services [36] has created obstacles for direct clinician-laboratory interactions at a time when such interactions are needed more than ever. In addition, new clinical guidelines and testing algorithms will need to keep pace with the development of novel diagnostic methods, which will require the input of both laboratory scientists and ID clinicians (eg, on guidelines panels).

In a recent commentary in *Clinical Infectious Diseases* [37], Arturo Casadevall pointed out that the role of ID specialists has historically been to provide "intellectual input in the form of consultation." Nowadays he suggests that ID specialists should "use (their) expertise to command an important position in the information and decision fl ws in medicine" but worries that empiricism in the use of antimicrobial agents has "fostered a neglect of new diagnostics." The current diagnostics revolution promises to transform clinical practice to more closely conform with Casadevall's vision of diagnostics-driven therapy for ID.

Developers of new diagnostic technologies will be best served by a team-based approach. ID specialists are ideal partners to develop and implement systems to ensure appropriate diagnostic testing and the seamless translation of laboratory results into personalized treatment. Improved diagnostics may increase the costs of diagnostics, so assessing value will become increasingly important, with regard to both specific tests and approaches to patient care. Diagnostic stewardship means selecting the right test for the right patient at the right time, to optimize clinical care and antimicrobial use [4]. Th s is a mission for ID specialists and clinical microbiologists to take on together.

Notes

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parapertussis PCR and an anti-biofilm substance and a patent on a device/method for sonication with royalties paid by Samsung to Mayo Clinic; and has served on an Actelion data monitoring board. F. C. F. has participated in research studies supported by BioFire, Cepheid, ELITech, and Luminex (formerly Nanosphere). R. P. and F. C. F. both receive editors' stipends from the Infectious Diseases Society of America. Both authors have submitted the ICMJE Form for Disclosure of Potential Confli ts of Interest. Confli ts that the editors consider relevant to the content of the manuscript have been disclosed.

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Review

Rapid Methods for Antimicrobial Resistance Diagnostics

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Abstract: Antimicrobial resistance (AMR) is one of the most challenging threats in public health; thus, there is a growing demand for methods and technologies that enable rapid antimicrobial susceptibility testing (AST). The conventional methods and technologies addressing AMR diagnostics and AST employed in clinical microbiology are tedious, with high turnaround times (TAT), and are usually expensive. As a result, empirical antimicrobial therapies are prescribed leading to AMR spread, which in turn causes higher mortality rates and increased healthcare costs. This review describes the developments in current cutting-edge methods and technologies, organized by key enabling research domains, towards fighting the looming AMR menace by employing recent advances in AMR diagnostic tools. First, we summarize the conventional methods addressing AMR detection, surveillance, and AST. Thereafter, we examine more recent non-conventional methods and the advancements in each field, including whole genome sequencing (WGS), matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) spectrometry, Fourier transform infrared (FTIR) spectroscopy, and microfluidics technology. Following, we provide examples of commercially available diagnostic platforms for AST. Finally, perspectives on the implementation of emerging concepts towards developing paradigm-changing technologies and methodologies for AMR diagnostics are discussed.

Keywords: molecular diagnostics; antimicrobial resistance; antibiotic susceptibility testing; microfluidics; point-of-care; lab-on-a-chip; MALDI-TOF; FTIR; sequencing

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1. Introduction

Antimicrobial Resistance (AMR) has become one of the dominant health challenges of our times. Antibiotic resistance occurs as a natural evolutionary process in bacteria, but can be accelerated by a number of factors [1,2]. More specifically, the excessive and inadequate use of antibiotics in both humans and animals leads to the wide spread of resistant bacteria and their antimicrobial resistant genes (ARGs) [3–5]. AMR has severe adverse effects on humans, healthcare systems, farm animals, agriculture, environmental health, and, consequently, on national economies [6]. AMR is a challenging threat undermining key features of current medical care at enormous costs in terms of patient mortality and morbidity, but also in terms of patient treatment expenses [7,8]. Modern, mainstream antibiotic therapeutic strategies are responsible for their own regression by actively selecting for resistant strains, compelling the need for supporting the continuous discovery of new antibiotics in order to remain ahead of the AMR challenge [9]. Therefore, it is urgent to prolong the lifespan of current antibiotics while research and development of new-generation antibiotics takes its course. In addition, it is important to implement efficient control measures for antibiotic use in order to slow down the need for continuous discovery of new antibiotics [1].

The costs related to the soaring AMR rates are forecasted to grow dramatically if no measures are taken [10]. The lack of effective antimicrobials is leading to common infections becoming life-threatening, hence, rendering treatments, such as chemotherapy and surgical procedures being more prone to becoming life-threatening due to common infections. Thus, constraining the misuse and the overuse of antimicrobials is crucial for impeding the dispersion of AMR. According to recent studies [10–13] more than 33,000 people die every year in the European Union (EU) as a result of infections stemming from antibiotic-resistant bacteria. The annual economic burden related to AMR in the EU is considerable, accounting for an estimated 1.5 billion euros, including healthcare costs and productivity losses [10]. Globally, antibiotic resistance is responsible for more than 500,000 deaths every year, from which more than 40% involve infant deaths [14].

Early detection of pathogens is required for the optimal treatment of infectious diseases. Although great leaps have been made in medical technology, the turnaround time (TAT), both for the detection and the characterization of microbial pathogens, often takes up to several days [15–17]. As a result, clinicians are pushed to start empiric antibiotic therapies, typically broad-spectrum, before a diagnosis can be reached. This practice may lead to detrimental consequences not only for the health of the patient (i.e. microbiome dysbiosis), but also for the exacerbation of the ongoing AMR challenge. Thus, the need for rapid, highly sensitive, affordable, and cost-effective detection platforms for AMR diagnostics has become urgent. The utilization of such platforms will significantly reduce the TAT for antibiotic susceptibility determination, thus enabling the selection of enhanced, target-specific therapies [15]. Diagnostic tests are considered an essential weapon in any strategy against AMR. Rapid diagnostic tests (RDTs) related to infectious diseases are considered an indispensable tool for antimicrobial stewardship programs. RDTs have shown to reduce mortality, lessen hospital stay, and shrink healthcare costs. Indeed, such diagnostic tests have proven to be more cost-effective, not only by providing a significant cost reduction, but also by decreasing antibiotic use [17–20].

The present review aims to give an outline of the current and emerging methods and technologies being implemented, or under development, targeting fast detection of antimicrobial resistance. Moreover, the main advantages and limitations of these methods and technologies are summarized. Already established methods, such as phenotypic and molecular-based techniques, as well as the more recently developed sequencing (whole genome sequencing (WGS) and whole genome metasequencing (WGM), MALDI-TOF MS, and IR spectroscopy, are also subjected to a critical overview. A special focus is placed on those state-of-the-art approaches, such as microfluidics and lab-on-a-chip technologies, which have a promising potential in AMR detection. Finally, we provide a short summary of the commercially available platforms designed for AST. Figure 1 depicts a summarizing chart of the methods and technologies analyzed in the present review.

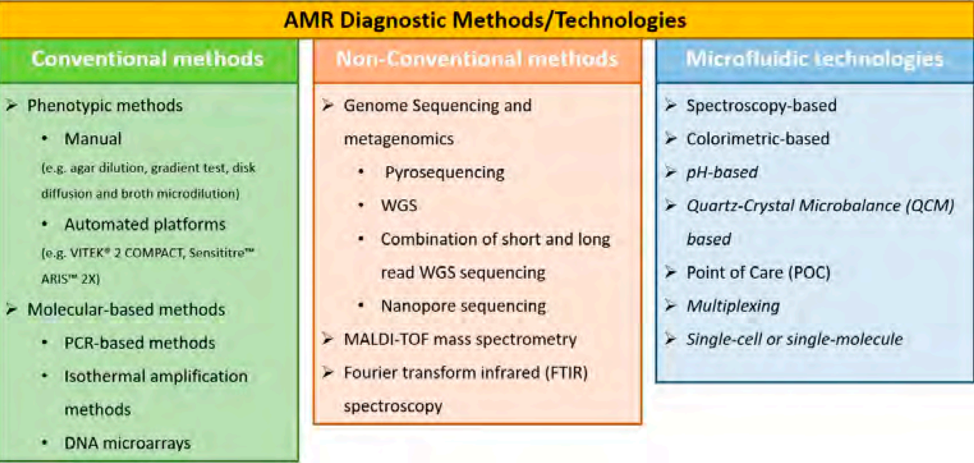

Figure 1. Summarizing chart of the methods and technologies analyzed in the present review.

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2. Conventional AMR Diagnostic Methods

Although new time-saving technologies have been introduced to obtain antimicrobial resistance data, the classic, conventional technologies are still being used. These mainly include culture-based and molecular-based approaches. More recently, microscopy-based and spectrometry-based approaches have also been incorporated in the tools for developing diagnostics.

2. Conventional AMR Diagnostic Methods

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2.1. Phenotypic Methods

Culture-based methods rely on the phenotypic resistance detection by evaluating the bacterial growth in the presence of antibiotics, and can be classified in two categories, manual and automated. Manual tests include agar dilution, gradient test, disk diffusion, and broth microdilution antimicrobial susceptibility testing methods. The automated commercial platforms (VITEK®2 COMPACT, Sensititre™ ARIS™ 2X, and Alfred 60AST system) use some of the aforementioned methods. Broth dilution-based platforms typically use ready-made cartridges or plates including positive controls and gradient concentrations of antibiotics. Sensititre panels belong to the category of microdilution methods. Typically, such panels are plastic multi-well micro-titer plates precision-dosed with dried antimicrobial agents. For instance, the Sensititre panel method was used for the determination of the susceptibility of carbapenem-resistant *Klebsiella pneumoniae* to polymyxins [21]. Such platforms usually offer real-time growth monitoring and minimum inhibitory concentration (MIC) analysis through their comprehensive databases which include a broad spectrum of organisms. The above-mentioned technologies offer qualitative and quantitative data for the strain under investigation. For example, dilution methods and Epsilometer tests (E-tests) provide quantitative values [22] for the minimum inhibitory concentration (MIC), as the lowest concentration of a given antimicrobial which prevents the visible overnight growth of a culture [23]. Disk diffusion provides a zone of inhibition. E-Test belongs to the gradient test methods [24] and is especially useful for fastidious microorganisms [25], such as *Campylobacter* spp. [26]. Various methods have been traditionally employed regarding the phenotypic analysis for susceptibility of bacteria to antibiotics, and different standards, criteria, and guidelines have been proposed by several international organizations for the interpretation of Alfred 60 antimicrobial susceptibility testing (AST) results. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) in the USA are two of the main organizations responsible for the annual revision and update of the AST standards. However, several discrepancies have been observed in the interpretation of the criteria regarding different bacterial species. For example, in the case of amikacin resistant *Escherichia coli*, a more stringent susceptibility breakpoint is provided by EUCAST ($\leq$8 mg/L) compared to CLSI ($\leq$16 mg/L) [22].

2.2. Molecular-Based Methods

Molecular-based assays addressing the detection of ARG can offer advantages over phenotypic assays, such as multiplex targeting and more precise characterization and detection of AMR genes. For some taxonomic units, susceptibility breakpoints have not been established, and molecular-based methods represent an acceptable alternative. Another advantage is the elimination of isolate purification since non-purified polymicrobial samples can be used. Moreover, they allow for relatively quick adaptation to newly introduced resistance factors [27]. Nevertheless, molecular-based assays for AMR detection have some limitations. Molecular-based methods are not capable of defining MIC. Besides, some ARGs could be missed in terms of both sensitivity and coverage since they can only detect resistances that are searched for and not newly evolved ones. Moreover, the wide diversity of different genes related to AMR poses a challenge in assay development due to the cost involved, thus competing with phenotypic assays is sometimes difficult. However, advancements in the field of molecular based techniques are gaining a place in routine diagnostics [28]. Molecular-based methods for detecting ARGs as well as their expression take advantage of the developments in amplification and nucleic acid hybridiza-

tion techniques [29]. Molecular-based techniques can offer ARGs detection in a fast and sensitive manner. ARGs encode the ability of bacteria to survive and grow in the presence of antibiotics. In the past, scientists were solely targeting a small fraction of ARGs, but with the decrease in the cost of next-generation sequencing (NGS) technologies and the subsequent expansion in bacterial whole genome sequencing (WGS), the availability of ARG targets in various databases has enormously been expanded [30]. In the following sections, nucleic acid amplification-based techniques, such as polymerase chain reaction (PCR) and isothermal techniques, as well as DNA microarrays, will be discussed.

2.2.1. PCR-Based Methods

PCR is the most commonly used nucleic acid amplification technique for the detection of ARGs [31,32]. More recently, real-time [33], quantitative [34], digital [35,36], and multiplex [37] PCR assays have further boosted clinical acceptance of genetic testing.

The changes in NGS and WGS have impacted the availability of ARG targets, paving the way for high throughput quantitative PCR (HT-qPCR), which is comparatively fast, convenient, and allows for simultaneous investigation of a large number of ARGs [30]. HT-qPCR is cost effective and it has already been employed in many studies for the analysis of ARGs stemming from various sample types [38]. For example, Wang et al. used HT-qPCR to provide a comprehensive profiling of ARGs in bacteria isolated from park soils [39], whereas a novel high-throughput screening method (simultaneous screening 48 isolates against three antibiotics) employing HT-qPCR, tested the antimicrobial susceptibility of *Orientia tsutsugamushi* clinical isolates [40]. Xu et al. demonstrated the versatility of chemically synthesized double-stranded (ds) DNA, which can be employed as a qPCR standard for ARGs offering comparable performance, in terms of sensitivity and reliability, to natural DNA. This qPCR method has been successfully used with various sample types, such as animal feces, soil, and surface water [41]. A multiplex real-time PCR was used for AMR characterization in *Neisseria gonorrhoeae* including resistance to ciprofloxacin, ceftriaxone, cefixime, azithromycin, and spectinomycin. Although this methodology accurately detected mutations generating resistance to antibiotics employed for gonorrhea treatment, the low assay sensitivity prohibits the direct application for diagnostic testing in clinical specimens. Nevertheless, it can be used as a screening method for AMR in gonococcal isolates since it is faster than current conventional culture-based AMR testing [42]. Wang et al. developed a singleplex and a multiplex real-time PCR assays for methicillin resistant *S. aureus* (MRSA) in pediatric samples. The assay proved fast, reliable, and capable of detecting and differentiating MRSA and methicillin susceptible *S. aureus* MSSA [43]. Two decades ago, ligation mediated PCR (LM PCR) coupled with low denaturation temperature method has been proposed leading to specific melting-profile DNA patterns, both fungal and bacterial isolates. This method is suitable for strain characterization and differentiation [44]. This method has been used for epidemiological typing of various pathogens, such as extended-spectrum-beta-lactamase-producing *Escherichia coli* [45,46] as well as *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* [47].

2.2.2. Isothermal Amplification Methods

A more recent development, in molecular biology, is the use of isothermal DNA amplification eliminating the need for thermocycling, which is indispensable in the case of traditional PCR methods. Several methods of isothermal nucleic acid amplification have been developed, such as strand displacement amplification (SDA), transcription mediated amplification (TMA), nucleic acid sequence-based amplification (NASBA), rolling circle amplification (RCA), recombinase polymerase amplification (RPA), loop-mediated isothermal amplification (LAMP), and helicase-dependent amplification (HDA) [48]. These methods have paved the way for the implementation of rapid, next-generation molecular diagnostics [49].

The main advantages of the isothermal over the conventional PCR-based methods are the circumvention of thermocycling, which in turns lead to low power consumption and reduced analysis time. Thermocyclers are no longer needed, since a water bath or a hotplate can regulate the temperature [50]. Moreover, unlike PCR, isothermal amplification is faster and more sensitive [51] since it does not depend on discrete thermal cycles, but rather relies on continuous amplification, which can yield traceable amplicons in less than 10 min. Another advantage offered by some isothermal methods, such as LAMP, RCA, and HDA is the elimination of template denaturation and the tolerance to biological components for LAMP and HDA [52]. Moreover, although some isothermal methods have complex primer design (e.g., LAMP) they offer greater specificity compared to PCR. A recent evaluation of several isothermal methods in terms of simplicity, sensitivity, cost, and reproducibility showed that LAMP and RPA hold great potential for point-of-need (PON) diagnostics employed in low resource settings. Both of them are single step (incubation at a single temperature) and require minimum amount of DNA template [50]. Isothermal methods are also preferable for microfluidic-based approaches due to all of the aforementioned reasons [53]. In addition, LAMP amplicons can be detected even with naked-eye through turbidity or color change [54]. On the other hand, isothermal methods also have some limitations. Multiplexing approaches of isothermal methods are less successful, since the difficulty of the experimental design is increased [55]. Furthermore, some isothermal amplification methods have complex reaction mechanisms and need several primers, for example LAMP needs 4–6 primers, or several enzymatic steps are involved, such as in NASBA [52].

During the last two decades, significant investments in engineering, reagent formulations, and software have resulted in the commercialization of in vitro diagnostic (IVD) products based on PCR and isothermal nucleic acid amplification technology (NAAT) [56]. The integration and automation of processes, such as nucleic acid extraction, purification, amplification, and detection, coupled with sophisticated data analysis software have led to integrated and automated platforms (discussed in subsequent sections of this review article) providing accurate results [57].

2.2.3. DNA Microarrays

A DNA microarray is a tool, which allows for the assessment of the bacterial genomic diversity. This approach relies on the detection of the presence or absence of genes in a target organism when compared to a reference strain or genome. Initially, DNA microarrays were based on glass slides [58], which were spotted with numerous specific DNA probes relying on reference genes present in a characterized strain for which the whole-genome sequence was available. Comparative genomic hybridizations were performed followed by the analysis of the hybridization results. However, the use of glass slides as well as fluorescent dyes made the process costly and time-consuming. Nonetheless, there have been numerous advancements in the DNA microarray technology during the past two decades [59]. A fast and simple DNA labeling system based on biotinylated primers specific for the linkers has been developed for disposable microarrays [60]. A DNA microarray for the simultaneous (multiplex asymmetric PCR amplification) detection of ARGs among *Staphylococcus* clinical isolates based on fluorescently labeled PCR products has been developed [61]. More recently, Havlicek et al. proposed a rapid cartridge based, melting curve assay for the detection of pyrazinamide resistant *Mycobacterium tuberculosis* [62]. The assay can be automatically implemented using a closed cartridge coupled with a battery powered Alere™ q analyzer, as a point-of-care test in resource-limited settings [62].

3. Non-Conventional AST Methods

In this section, some of the most promising non-conventional methods for AST will be described. Those method include: sequencing, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), and Fourier transform infrared (FTIR) spectroscopy.

3.1. Genome Sequencing and Metagenomics in AMR Diagnostics

The first DNA sequencing methods were developed in the mid-1970s and were able to decode hundreds of nucleotide bases of DNA per day. At that time, the two most widely accepted methods were the chain terminator [63] and the chemical cleavage procedures [64]. Single-base resolution was enabled by polyacrylamide gel electrophoresis for each base-specific reaction. In 1995, the first complete bacterial genome (*Haemophilus influenzae*, 1,830,137 bp) was obtained with the first automated sequencers employing fluorescence chemistry based on the Sanger method [65]. Until 2005, the Sanger sequencing prevailed as the primary sequencing technology. Although these first-generation sequencing methods had low throughput, they could produce high-quality, relatively long DNA sequences. Multiple sample sequencing was feasible by integrating numerous capillaries on the same instrument, thus enabling the sequencing of each individual sample. The major technical advancement of next-generation sequencing (NGS) was multiplexing, allowing for the simultaneous analysis of thousands of samples. Typically, a NGS workflow comprises DNA extraction and fragmentation, adaptors ligation, DNA amplification, and sequencing.

In second generation sequencing, or short-read sequencing, the template amplification encompasses intrinsic drawbacks, such as copying errors, sequence-dependent biases, and information loss. In 2005, the 454 pyrosequencing platform was introduced [66]. Pyrosequencing is based on the detection of pyrophosphate release along with the light generation on nucleotide incorporation, unlike the chain termination with dideoxynucleotides used in Sanger sequencing. The Illumina platforms, which use synthesis technology where reversible terminator nucleotides labeled with fluorescence are incorporated into DNA strands and visualized via their fluorophore excitation, were subsequently incorporated with the same aim [67].

On the other hand, the third-generation sequencing, first developed in 2011 by Pacific Biosciences, is a real-time and single molecule based long-read sequencing relying on an optical approach coupled with a zero-mode waveguide on a nanostructured device [68]. Oxford Nanopore Technologies developed another approach relying on DNA molecules movement through a nanopore and measuring an electrical signal changing analogously to the base presently passing the pore [69]. These newly introduced second and third generation sequencing approaches have paved the way for single genome sequencing, as well as for the characterization of complex microbial communities and the identification of antibiotic resistance determinants [70]. Whole metagenome sequencing (WMS) and analysis of genetic material in patient samples allows for the identification of ARG directly from clinical specimens without the need for prior isolation or identification of specific bacteria.

Bacterial sequence data availability has increased due to the advancements in sequencing technologies. Improved computational methods coupled with the continual cost decrease (due to the intense competition among different companies) made sequencing an affordable and viable tool for ARG identification, characterization, and surveillance [71]. Numerous methods, tools, and databases (Table 1) have been reported in recent years for the detection of genetic determinants related to AMR from WGS [72] and WMS data [73]. These evolving methods and technologies act as complementary tools to traditional culture-based methods, providing opportunities for rapid and sensitive resistance determination in uncultivable and cultivable bacteria. More information on the use of databases for AMR detection can be found in two recent reviews [74,75]. The organization of sequencing data is considered a crucial pre-processing step prior to ARG analysis. Short reads, produced by technologies like Illumina, could be processed employing assembly-based methods (sequencing reads are initially assembled into contiguous fragments (contigs) followed by annotation where comparison takes place with public or custom reference databases), or directly analyzed utilizing read-based methods where resistance determinants are forecasted by mapping reads to a reference database [75].

Table 1. Bioinformatic tools and databases for antimicrobial resistant gene (ARG) detection from whole genome sequencing (WGS) or whole metagenome sequencing (WMS) data.

Name	Type of Tool	Link
RGI	Assembly-based	https://card.mcmaster.ca/analyze/rgi (accessed on 15 January 2021)
CARD	Assembly-based	https://card.mcmaster.ca/ (accessed on 15 January 2021)
ARGs-OAP (v2	Assembly-based	https://galaxyproject.org/use/args-oap/ (accessed on 15 January 2021)
ARIBA	Assembly-based	https://github.com/sanger-pathogens/ariba (accessed on 15 January 2021)
NCBI–AMRFinder	Assembly-based	https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/AMRFinder/ (accessed on 15 January 2021)
PointFinder	Assembly-based	https://cge.cbs.dtu.dk/services/ResFinder/ (accessed on 15 January 2021)
ShortBRED	Read-based	http://huttenhower.sph.harvard.edu/shortbred (accessed on 15 January 2021)
SEAR	Read-based	https://github.com/will-rowe/SEAR (accessed on 15 January 2021)
KmerResistance	Read-based	https://cge.cbs.dtu.dk/services/KmerResistance/ (accessed on 15 January 2021)
PATRIC	Read-based	www.patricbrc.org (accessed on 15 January 2021)
SSTAR	Read-based	https://github.com/tomdemanbio/Sequence-Search-Toolfor-Antimicrobial-Resistance-SSTAR (accessed on 15 January 2021)
DeepArgs	Read-based	https://bench.cs.vt.edu/deeparg (accessed on 15 January 2021)
GROOT	Read-based	https://github.com/will-rowe/groot (accessed on 15 January 2021)

A main advancement facilitating resistome surveillance is the established power for AMR prediction from solely genomic data. Various studies along with those focused on foodborne pathogens have demonstrated a high (>96%) concordance between the presence of known mutations or ARGs and MIC of various antimicrobials [76–79]. In addition, a growing body of evidence shows that it is feasible to predict AMR and sometimes also the MIC of an antimicrobial, by employing machine learning techniques to genome sequencing data [80,81].

Although long-read sequencing platforms can provide comprehensive entire genome capturing, they require substantial investment not only in equipment, but also in laboratory expertise. In addition, such systems typically require substantial quantities of DNA (i.e., more than 5 µg) and longer preparatory time, and they have higher error rates as compared to short-read sequencing platforms. Alternative sequencing platforms relying on nanopore technology are considered capable of providing libraries of high quality from long reads, as well as producing closed bacterial genomes. In addition, advantages, such as portability and affordability, less laboratory space, and on-site sequencing, have been highlighted [82]. The MinION nanopore system (Oxford Nanopore, Oxford, UK), is a portable (palm-sized, 100 g), real-time device for DNA and RNA sequencing, able to detect changes in ionic current upon DNA or RNA passing through the nanopores.

3.1.1. Pyrosequencing

In 2012, pyrosequencing was suggested as an innovative, rapid tool for the detection of *Yersinia pestis* strains towards fighting bioterrorism. The detection and identification relied on virulence genes, which led to an assay based on pyrosequencing for characterizing ARG profiles was developed by Amoako et al. [83]. Pyrosequencing was also evaluated as a tool for the detection of clinical drug-resistant *Mycobacterium tuberculosis*. The pyrosequencing assay was capable of reliably and robustly detecting resistance-associated mutations in *M. tuberculosis* isolates with great specificity (96–100%) [84]. The efficiency of the pyrosequencing was evaluated based on the rapid detection of resistance to fluoroquinolones (FQs), rifampicin (RIF), kanamycin (KAN), and capreomycin (CAP) in *M. tuberculosis* clinical isolates [85]. The sensitivity of the assay for detecting the resistance to RIF, FQs, CAP, and KAN was 100%, 100%, 40%, and 50%, respectively, with 100% specificity. This assay was considered as a fast and effective method for the detection of mutations associated with drug resistance in *M. tuberculosis* clinical isolates [85]; however, it has been superseded by other sequencing technologies (see below).

3.1.2. WGS

WGS for predicting AMR in non-typhoidal *Salmonella* was evaluated in human and food isolates employing v2 or v3 chemistry with paired-end 2- by 25- or 2- by 300-bp reads on the MiSeq platform (Illumina, San Diego, CA, USA) [86]. The data suggested that acquired resistance is highly correlated with the presence of known resistance determinants, useful for risk assessment linked to drug use in food animal production [86]. Velez et al. proposed the use of WGS for the determination of the occurrence of ARGs in *Streptococcus uberis* and *Streptococcus dysgalactiae* isolates, stemming from dairy cows [87]. A paired-end 125 bp sequencing was implemented using the Illumina HiSeq 2500 platform with v4 chemistry. In addition, they investigated the relation between genomic and epidemiological characteristics and phenotypic AMR profile. The outcome showed the association between a number of unique ARG sequences and phenotypic resistance (MIC data) [87]. Zhao et al. tried to identify AMR genotypes for *Campylobacter* investigating the correlation between resistance genotypes and phenotypes employing in vitro AST and WGS [88]. A strong correlation (99.2%) was observed between resistance phenotypes and genotypes. These outcomes suggested that WGS is a reliable resistance indicator (for tetracycline, ciprofloxacin, nalidixic acid, erythromycin, gentamicin, azithromycin, clindamycin, telithromycin, and florfenicol). From these initial screenings, several studies [74,89,90] also highlighted that WGS is a powerful tool for AMR surveillance programs [88]. More recently, an ongoing epidemiological change was studied using WGS revealing the co-existence of antibiotic resistance and virulence factors in carbapenem-resistant *Klebsiella pneumoniae* isolates, suggesting that this finding should be taken into account for future genomic surveillance studies [91].

3.1.3. Combination of Short and Long Read WGS Sequencing

Plasmids are capable of transferring ARGs among bacterial isolates. Nonetheless, plasmids are difficult to assemble from short-read WGS data. Berbers et al. used short and long read WGS sequencing to characterize ARGs on plasmids as well as establishing their localization [92]. Due to the rising concern of the spread of ARGs, it is of crucial importance to establish their location, especially when they are in mobile elements. Risk assessment of AMR spread was feasible by overcoming the challenges of plasmid reconstruction when employing the combination of long and short read sequencing [92].

3.1.4. Nanopore Sequencing

Nanopore sequencing has been widely used on viruses [93], yeasts [94] and for performing de novo bacterial assembly [95]. It has also been used for identifying viral pathogens [96], undertaking metagenomics studies [97] and detecting ARGs [98]. The MinION nanopore sequencer was implemented to resolve the structure as well as the chromosomal insertion site of a composite antibiotic resistance island in *Salmonella* Typhi [99]. It was also employed for the identification of the position as well as the structure of bacterial AMR determinants in a multidrug-resistant (MDR) strain of Enteroaggregative *E. coli* [100]. Long-read analysis of WGS data facilitated the identification of mobile genetic elements where AMR determinants were positioned and revealed the combination of various AMR determinants co-located on the same mobile element. These findings provided a deeper understanding regarding the transmission of co-located AMR determinants in MDR *E. coli* [100]. Schmidt et al. showed that MinION could successfully identify bacterial pathogens as well as acquired resistance genes without culturing directly from urine samples within 4 h [101]. This study highlights the importance of WMS-based diagnosis towards adjusting antimicrobial therapy [101]. The Oxford Nanopore MinION long read DNA sequencing device was exploited for the detection of ARGs, the assessment of ARGs' taxonomic origin as well as to decoding their genetic organization and possible correlation with mobilization markers. Based on the findings, targeted intervention measures could be implemented in order to mitigate the risks of ARGs transferring among sites and, thus, improve biosecurity practices in hospitals and other environments [102]. Nanopore se-

quencing was also used for the fast determination of plasmids, virulence markers, phages and ARG in Shiga toxin-producing *E. coli* [82]. More recently, MinION nanopore sequencing was employed for rapid pathogen, plasmids and ARG identification in bacterial DNA extracted from positive blood cultures [103]. After only 10 min of sequencing, pathogen identification was possible. The detection of predefined ARGs and plasmids stemming from monoculture experiments was achieved within 1 h employing raw nanopore sequencing data. This is one crucial difference between Illumina and nanopore sequencing. Nanopore sequencing offers real-time data availability whereas when Illumina is used, the data become accessible once the sequencing run is finished [103]. The use of the MinION sequencer was also examined both for whole genome generation and characterization of *Streptococcus suis*. The genomes from the MinION sequencer were capable of accurately predicting the multilocus sequence type (8 out of 10 samples) and identifying AMR profiles (100% of the samples) [104]. The ultra-long read Nanopore sequencing technology was used for AMR detection in *Mannheimia haemolytica* [105]. De novo assembly generated a complete genome for a non-resistant and an almost complete assembly for a drug resistant strain. Successful ARG detection was achieved with only 5437 MinION reads [105].

Contrary to phenotypic tests, providing information solely related to AST, NGS, can reveal the molecular basis of the AMR resistance. The acquired information can be fed in monitoring schemes, aiding the understanding of the events leading to resistance acquisition. Furthermore, NGS is capable of characterizing novel mechanisms of resistance when they are detected. This can be achieved by sequencing isolates previously proven to be phenotypically resistant, thus providing an exquisite added value when compared to various nucleic-acid based techniques (e.g., PCR) [106].

3.2. MALDI-TOF Mass Spectrometry in AMR Diagnostics

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) can be used for the detection of AMR, alternatively to traditional genotypic or phenotypic bacterial characterization [107–111]. MALDI-TOF MS relies on the cellular proteome and is capable of profiling proteins (mainly ribosomal, 2–20 kD) from whole bacterial cell extracts creating a bacterial spectral fingerprint or profiles that discriminates microorganisms at a genus, species, and subspecies level [112,113].

In the assay preparation, the sample is mixed with a matrix, an energy absorbent solution. The entrapped sample within the matrix crystalizes upon drying. Once the sample is hit by a laser beam it gets ionized, producing protonated ions which are accelerated by using a constant potential leading to their separation based on their mass to charge (m/z) ratio. This ratio is determined by measuring the time needed for each protonated ion to move along the length of the tube. A "Peptide Mass Fingerprint" (PMF), which is a distinctive mass spectrum, is generated according to the TOF information. The peaks obtained from the PMF are compared to a database with reference peaks specific to genera and species of known, well characterized microorganisms, thus, allowing for the identification of the sample [114,115].

MALDI-TOF MS has also allowed the detection of antibiotic resistance mechanisms (e.g., carbapenemases) [116]. The standardization of the procedure is necessary to obtain reproducible results [117]. MALDI-TOF MS is considered a reliable, rapid (within minutes), accurate, easy to use, cost-effective, and environmentally friendly methodology [114]. Although MALDI-TOF MS has enormously decreased the TAT for bacterial identification and progress have been made towards the determination of AMR, the high cost (purchase and maintenance) as well as the large size of such systems pose significant restrictions for its implementation in low-resource settings or as a point-of-care (POC) AMR or AST testing platform [113]. Moreover, MALDI-TOF MS is not suitable for the characterization of mixed samples, since purification, cultivation as well as sample preparation procedures are required beforehand. Furthermore, additional chemicals, such as the matrix, are required for the execution of the tests [118]. Databases with spectra able to differentiate susceptible and resistant strains should be available. Table 2 includes recent works where MALDI-

TOF MS has been applied to AMR detection. MALDI Biotyper (Bruker Daltonik Bremen, Germany), and VITEK MS (bioMérieux, Marcy l'Étoile, France) are the two commercially available MALDI-TOF MS systems. Comparison studies regarding the performance of both platforms can be found in the literature [119–121].

Table 2. Applications of MALDI-TOF MS for specific antimicrobial resistance (AMR) detection.

Organism	Antibiotic	Year (Reference)
E. coli	Polymyxins	2018 [122]
E. coli	Colistin	2019 [123]
E. coli *Klebsiella pneumoniae*	Beta-lactams (ESBL-producing isolates)	2019 [124]
Staphylococcus aureus *S. intermedius* *S. pseudintermedius*	Novobiocin Polymyxin B Acriflavine	2019 [125]
S. aureus	Methicillin	2019 [126]
Candida auris	Echinocandins	2019 [127]
K. pneumoniae *Bacteroides fragilis* *S. aureus*	Carbapenems (carbapenemase-producing isolates) Methicillin	2019 [128]
Enterobacteriaceae	Carbapenems (carbapenemase-producing isolates)	2019 [129]
Enterobacteriaceae	Carbapenem	2019 [130]
Pseudomonas aeruginosa	Beta-lactams (MBL)	2019 [131]
Enterococcus faecium	Vancomycin	2019 [132]
K. pneumoniae	Carbapenems (carbapenemase-producing isolates)	2019 [133]
Acinetobacter baumannii	Colistin	2020 [134]
E. coli *K. pneumoniae*	Cefotaxime Meropenem, Ciprofloxacin	2020 [135]
S. aureus *Enterococcus* species *E. coli* *K. pneumoniae*	Oxacillin (methicillin) Vancomycin Ceftriaxone Meropenem	2020 [136]
Enterobacterales	Imipenem/Relebactam	2020 [137]
S. aureus	Methicillin	2020 [138]

3.3. Fourier Transform Infrared (FTIR) Spectroscopy in AMR Diagnostics

Recently, great progress has been achieved in optical technologies and their applications in the biomedical and microbiology fields. Infrared (IR) spectroscopy and microscopy allows for enhanced spectral and spatial resolution facilitating the acquisition of biochemical information at molecular level for microorganisms. With respect to clinical microbiology applications, Fourier transform infrared (FTIR) spectroscopy is a phenotypic method that has emerged as an attractive and dynamic weapon enriching the tools employed for biochemical analysis, owing to the detailed information it can provide the chemical composition at molecular level. FTIR spectroscopy allows for the quantification of the IR light absorption by molecules such as lipids, lipopolysaccharides, carbohydrates, proteins, and nucleic acids, resulting in a characteristic FTIR spectrum that represents the complete composition of the sample [139]. These characteristic spectra of the cell biomolecules offer ample functional and structural information. IR spectroscopy has been applied to differentiate the molecular changes associated with the development of AMR in prokaryotes [140–142].

The coupling of IR spectroscopy of bacterial samples with data analysis employing artificial neural networks (ANNs) was able to detect uropathogenic *E. coli* strains susceptible to cephalothin, achieving a success rate of 95% [143]. In 2017, Sharaha et al. used FTIR to identify bacterial susceptibility to certain antibiotics based on the obtained IR bacterial spectra. An IR microscope was utilized, and a computational classification method was developed to analyze the IR spectra by novel pattern-recognition tools, to determine *E. coli* susceptibility to ceftazidime, gentamicin, nitrofurantoin, nalidixic acid, and ofloxacin. The results showed an 85% success rate in the classification into sensitive and resistant strains [144]. In 2017, Salman et al. demonstrated the detection of structural molecular changes linked AMR by employing FTIR microscopy coupled with a novel statistical classification approach developed in-house for spectral analysis [140]. Kochan et al. recently reported the identification of changes in the chemical composition of *S. aureus* associated with vancomycin and daptomycin antibiotic resistance. An innovative, single cell, nanoscale technique, namely atomic force microscopy-infrared spectroscopy (AFM-IR), coupled with chemometric analysis was employed [145]. AFM-IR combines IR and scanning probe microscopy to improve resolution and capacity to map cell structures at the atomic scale.

FTIR shows many advantages, such as reliability, speed, cost-effectiveness, and environmentally friendly methodology in AMR study. Similar to other instrumental systems (i.e., MALDI-TOF MS), the purchase and maintenance costs and equipment size make its implementation very difficult in low-resource settings, or as a POC AMR, or AST testing platform. Purification, cultivation, as well as sample preparation procedures are required previously, and databases with spectra able to differentiate susceptible and resistant strains should be available.

4. Microfluidics and Lab-on-a-Chip Technologies towards Rapid Diagnostics

Lab-on-a-chip (LoC) devices using microfluidics represent a promising tool in numerous fields, such as clinical diagnostics [146], food safety [147] and environmental monitoring [148]. Recently, LoC technology has also been applied in the detection of antibiotic-resistant bacteria [3]. Some of the advantages offered by the LoC technology compared to macro-scale methods are: fast and high throughput analysis, accurate fluid manipulation, low cost, low reagent, and power consumption, smaller sample volume, automation, integration, compactness, and portability [149–152]. Genotypic and phenotypic assays are the two main categories of microfluidic-based detection methods. Genotypic microfluidic assays (e.g. PCR, LAMP) target genetic markers (e.g., ARG), thus circumventing bacterial growth and allowing for shorter TAT (several hours) [153]. The implementation of microfluidics combined with isothermal DNA amplification protocols offer enhanced features due to the elimination of thermal cycling [50]. This approach is highly promising for the development of cheap, convenient, and efficient diagnostic tools for food safety, clinical, and environmental applications [154]. On the other hand, phenotypic microfluidic assays monitor bacterial growth of bacteria in the presence of antibiotics, thus offering accurate AST results. In them, in general, bacteria are confined in small volumes (e.g., chambers, channels, or droplets) [155], captured with the aid of antibodies on magnetic beads or membranes [156], or encapsulated in chambers containing agarose [157] and hydrodynamic trapping [158]. For example, hydrodynamic trapping is a method used for the immobilization of the bacteria and is compatible with microfluidics offering highly dense trap arrays, easy integration, high scalability, and easy biosensing though, the trapping efficiency is quite low. A drawback regarding the use of antibodies is the high cost as well as the restricted availability to specific strains. As for the droplet-based method, typically they require expensive and sophisticated readout. The agarose-based method, although it can be applied to conventional multi-well plates, the arraying is not straightforward, which hinders both the automated detection and the data analysis. Due to these limitations, more research and improvements are needed in order for these systems to become commercially available. In the following sections, various approaches will be dis-

cussed; namely spectroscopy-based, colorimetric-based, pH-based, and, last but not least, quartz-crystal microbalance (QCM) based, point-of-care (POC), multiplexing, single-cell, or single-molecule.

4.1. Spectroscopy-Based Approaches

Surface enhanced Raman spectroscopy (SERS) is considered a main biochemical fingerprinting approach since it can precisely reflect the macromolecular profiles as well as the changes occurring within the bacterial cells as a result of antibiotic action [118,159]. SERS has been applied for the investigation of the resistance or susceptibility to antibiotics of bacteria, as well as for studying the working mechanism of antibiotics relying on the whole cells' spectral fingerprint. SERS is capable of providing rapid, accurate, and ultra-sensitive detection of resistant bacteria with minimum requirement for sample preparation and handling [118,160]. SERS has also been used in LoC platforms. Lu et al. reported the development of a microfluidic chip combined with SERS providing rapid detection and differentiation of MSSA and MRSA [161]. Chang et al. presented the development of an integrated multimodal microfluidic system capable of performing on-chip enrichment of bacteria, collection of metabolites, and in situ SERS measurements for AST, with a limit of detection (LoD) of 10^3 CFU/mL [162]. Liao et al. reported the development of a microfluidic platform integrating SERS with microwells allowing for low concentration (10^3 CFU/mL) encapsulation of bacteria followed by label-free detection and in situ AST [163].

However, while current advancements in SERS methodology have substantially improved the selectivity and sensitivity in bacterial biosensing, it still has some limitations. It usually requires a drying step of the sample prior to analysis that can lead to reproducibility issues. Although liquid phase detection of bacteria is favored when interrogation of cells is performed under their natural environment, this is frequently challenging because of scattering of the Raman laser source. Another limitation associated with SERS is the sample and the experimental conditions, i.e., typically, samples containing a single bacterial species are employed under regulated laboratory conditions. Furthermore, despite the progress made in the identification of the molecular spectral fingerprints (e.g. nucleobases), comprehensive databases of SERS spectra of biomolecules are still needed, as well as mathematical interpretation and processing of spectra (e.g., multivariate data analysis) [164]. Ideally, bacterial SERS biosensors should facilitate the simultaneous detection of multiple strains from complex samples. Further information on the SERS method can be found in the review of Galvan et al. [159].

4.2. Colorimetric-Based Approaches

Several studies also report the development of colorimetric-based microfluidic platforms addressing pathogen identification and AST. Lee et al. proposed an integrated, automated, microfluidic platform capable of performing AST for 1–2 antibiotic combinations against bacterial pathogens [165]. On-chip determination of MIC is also provided via a colorimetric assay using a pH-dependent colorimetric broth. The total TAT of the on-chip microfluidic assay is 16–24 h, approximately. Automated fluidic control (e.g., transportation, mixing) is achieved using a pneumatically controlled custom-made module connected to the microfluidic chip. The initial loading of all samples [250 μL of bacterial suspension (10^6 CFU/mL) /chamber] and reagents is performed manually [165]. Recently, Ma et al. proposed a polymer-based microfluidic device addressing the identification and AST of *Campylobacter* spp. The microdevice consisted of an array of incubation micro-chambers loaded with chromogenic medium and antibiotics. Bacterial growth was visualized through a color change (chromogenic reaction). Rapid and reliable on-chip identification and AST was performed within 24 h with a LoD of 10^2 CFU/mL. Some variations in terms of the TAT and the LoD were observed according to the food matrix used [166].

4.3. pH-Based Approaches

Tang et al. proposed a microfluidic device integrating polymer-based microfluidic channels with a pH-sensitive chitosan hydrogel capable of detecting small pH changes for rapid AST [167]. Fourier transform reflectometric interference spectroscopy (FT-RIFS) was used for the real-time observation of the changes in the pH. The TAT for detection of whole bacterial growth was less than 2 h [167]. Hu et al. developed a real-time, ultra-fast electronic detection microdevice for ARG detection (resistance genes from *E. coli* and *Klebsiella pneumoniae*) using the RPA method for isothermal amplification coupled with a thin film transistor sensor for measuring changes in the pH. The TAT was less than 3 min for a LoD of 100 copies [168]. Xu et al. presented a polymer/paper hybrid microfluidic chip for a one-step identification and AST of multiple uropathogens. The multiplexed colorimetric assay was facilitated via the use of paper substrates within the cell culture microchambers, allowing for a versatile combination of the antimicrobial agents and the chromogenic media. The assay was completed within 15 h and the outcome of the chromogenic reaction was monitored via a camera. Snapshots were taken every 30 min and analyzed with an image analysis software [169]. Recently, He et al. reported a laser-pattern paper-based microfluidic device capable of performing *E. coli* identification and susceptibility testing via visual observation of a simple color change (colorimetric readout). Such a micro-device is suitable for low resource settings and can be used by minimally trained personnel [170].

4.4. Quartz-Crystal Microbalance (QCM)-Based Approaches

Quartz-crystal microbalance (QCM) is a physical nanogram-sensitive device with a piezoelectric sensor. QCM facilitates the real-time, rapid, on-site detection of AMR bacteria [171]. Reyes et al. have demonstrated a highly sensitive, accurate, and dynamic (real-time) system with a dual purpose, allowing both for monitoring of antimicrobial effects on *E. coli* and *Saccharomyces cerevisiae*, as well as ARG detection employing a magnesium zinc oxide (MZO) nanostructure-modified quartz crystal microbalance (MZOnano-QCM) biosensor [172]. Low cost, low demand in clinical samples volume, and rapidity (within 10 min) are the main advantages of the proposed method [173].

4.5. POC Approaches

Toosky et al. developed a POC system for AMR diagnostics and phenotypic AST addressing bacteriuria and urinary tract infection (UTI) [174]. The TAT is 2h with the ability of detection and quantification of bacterial concentrations ranging from 50 to 10^5 CFU/mL. The detection is based on a portable particle-counting instrument comprising a miniature confocal microscope coupled with a software for real-time data analysis. The detection system allows for growth curve measurements of fluorescently stained bacterial cells in control and antibiotic-treated samples. The main advantages of the proposed POC lie in the elimination of pre-processing steps (e.g., pre-culture, enrichment, centrifugation) of urine samples as well as in the sensitivity of the instrument [174]. Only preliminary data are available for this method; thus, further studies are needed. One limitation of this method, which is common in all AST methods, is the negative effect of mixed cultures both on the specificity and the sensitivity of the results. Recently, Abram et al. reported a RDT platform integrating a novel single step blood droplet digital PCR assay with a high throughput three-dimensional (3D) particle counter system capable of performing bacterial identification and AST directly from whole blood samples, eliminating the need of culture and sample processing steps [175]. The demonstrated technology could simultaneously achieve high sensitivity of 10 CFU/mL and fast TAT of one hour [175].

4.6. Multiplex Approaches

A multiplex (eight samples) microfluidic chip for high throughput rapid phenotypic AST was proposed [176]. A mix of bacterial isolates and agarose was loaded in an array of microchambers within the chip. The growth rate of bacterial colonies under antibiotic gradients is monitored with the aid of a custom-built dark-field microscope coupled to a

motorized camera (taking snapshots every 10 min) followed by automated image analysis. The TAT is 5 h and the method achieves stable MIC values showing 100% agreement with reference (broth microdilution) MIC values. The key advantage of the proposed system is the ability of simultaneously and rapidly analyzing eight samples on a single chip, which can also allow for parallel testing of several antibiotics [176].

4.7. Single-Cell or Single-Molecule Approaches

A rapid AST system based on a microfluidic agarose channel with immobilized bacteria allows for single cell growth and monitoring by microscopy [177]. MIC values were determined by analyzing the time lapse images of the single cell bacteria cultured under various antibiotic concentrations. The TAT for the aforementioned system was less than 4 h [177]. Baltekin et al. presented their rapid AST system, also based on single-cell imaging (phase contrast microscope), using a microfluidic chip (made of a micromolded silicon elastomer and a cover glass) with cell traps. The rapid AST system was used for the determination of urinary tract infections (UTIs) caused by resistant bacteria with a TAT of 30 min even when urine samples with low CFUs were used [178]. Li et al. reported a versatile microfluidic system for fast bacterial classification (3 min) and phenotypic AST at the single-cell level. The incorporation of tunable microfluidic valves coupled with real-time visual detection (microscopy) facilitated the cell entrapment and classification based on their size and shape. The TAT for determining susceptibility, by monitoring the growth of the bacteria (single-cell level) in the presence of antibiotics, was 30 min. Moreover, the proposed system can be extensively applied for bacteria detection and complex (blood cultures, urine, whole blood) polymicrobial samples analysis [179]. Table 3 summarizes the microfluidic platforms that have been described in the literature, together with their main characteristics.

Table 3. Microfluidic platforms described in the literature.

Category	Method	LoD [1]	TAT [2]	Reference
Microfluidic	Optical (laser)	20-25 cells	3-5 h	[15]
Microfluidic	Colorimetric	100 CFU/mL	24 h	[166]
Microfluidic	Colorimetric	N/A [3]	24 h	[165]
Microfluidic	Colorimetric	N/A	15 h	[169]
Microfluidic	Colorimetric	N/A	Overnight	[170]
Microfluidic	Microscopy	N/A	4 h	[177]
Microfluidic	Microscopy	N/A	30 min	[178]
Microfluidic	Microscopy	N/A	33 min	[179]
Microfluidic	Microscopy	N/A	5 h	[176]
Cuvette	Microscopy	50 CFU/mL	2 h	[174]
Microfluidic	Electrochemical (pH)	100 cells	3 min	[168]
Microfluidic	FT-RIFS	N/A	2 h	[167]
Microfluidic	QCM	N/A	10 min	[172]
Microfluidic	Digital PCR	10 CFU/mL	1 h	[175]
Microfluidic	SERS	10^3 CFU/mL	N/A	[162]
Microfluidic	SERS	10^3 CFU/mL	N/A	[163]

[1] LoD: limit of detection, [2] TAT: turnaround time, [3] N/A: not applicable (this piece of information was not mentioned in the article).

5. Overview of Commercially Available AST Platforms

In this section, a description of some common, commercially available systems for AST follows.

Adagio™ Antimicrobial Susceptibility Testing System (Bio-Rad Laboratories) [180] is an automated system built around an imaging device. It measures the size of the inhibition zone around antibiotic discs. It is coupled with a sophisticated data management software allowing for rapid and accurate result generation and automated AST interpretation [181]. The Adagio system was evaluated for the automated reading and interpretation of disk

diffusion AST results in bacteria. Good categorical agreement was observed after visual validation of the automated results [182].

VITEK®2 COMPACT (bioMérieux, Marcy l'Étoile, France) is a compact, automated instrument addressing microbial identification and AST by reducing hands-on time for enhanced workflow and rapid reporting. The TAT is 2 to 18 h, although primary organism isolation is required. VITEK®2 COMPACT is considered a cost-effective, space-saving system. The technology used by VITEK®2 COMPACT relies on a fluorogenic methodology for organism identification and a turbidimetric method for AST.

Accelerate Pheno™ (Accelerated Diagnostics, USA) comprises a fully automated system capable of performing identification in approximately 2 h and AST within approximately 7 h directly from the sample without requiring culturing for isolates [183]. The clean-up process of the samples relies on gel electrofiltration. Pathogen detection, species identification, and quantitation are performed in a fast and fully automated manner using fluorescence in situ hybridization. It also incorporates an automated digital microscope for the morphokinetic cellular analysis (MCA), thus allowing tracking phenotypic features, such as size, shape, division rate of individual live cells, while being challenged by antibiotics, as well as extrapolating MIC values. The main advantage of this system is the user-friendliness, whereas the main disadvantages are the lack of freedom for any intervention and the necessity of processing solely fresh blood cultures.

Alfred 60AST system (Alifax, S.r.l., Italy) is a fully automated system capable of performing bacterial culture, residual antimicrobial activity (RAA) and susceptibility testing including the processes of sample inoculation, reading, and result transmission. This system, which relies on light scattering, is capable of detecting not only the presence of live bacteria, providing real-time information on growth curves as well as bacterial counts, but also their drug resistance in a few hours (4–6 h) with high sensitivity and specificity. The Alfred 60AST system coupled with MALDI-TOF MS for direct identification is considered a rapid AST. The main advantage of this system is its plasticity, since it allows for interventions by the user, which could also be considered as its main drawback, since such interventions dictate the need of skilled personnel able to interpret the results (i.e., growth curves).

MicroScan WalkAway plus System (Beckman Coulter, Inc.) (40 or 96-panel capacity models) provides identification of microorganisms and AST results efficiently with minimal labor in an automated manner from an isolate inoculum within 4 h (or overnight for some samples).

BD Phoenix™ (Becton, Dickinson, and Company) is an AST system providing rapid, reliable and accurate results from colony inoculums. It employs an oxidation/reduction indicator and a turbidimetric growth detector. Moreover, 200 identifications (ID)/AST sets could be processed in less than 4.5 h.

Sensititre™ ARIS™ 2X (Thermo Fisher) provides bacterial pathogen identification and emerging antibiotic resistance detection relying on the gold-standard of broth microdilution coupled with the time-saving advantages of automation, thus improving patient care and enhancing lab efficiency. Growth measurements and endpoint MIC determinations are based on the hydrolysis of a fluorogenic substrate by the bacterial isolates.

GeneFluidics (GeneFluidics, Inc.) offers automated platforms for research use, addressing both identification and AST. More specifically, ProMax®, UtiMax®, and BsiMax®platforms are capable of providing identification (TAT: n/a, 1 h, and 6 h, respectively) and AST (TAT: 3 h, 2 h, and 3.5 h, respectively) results from isolates, urine, and whole blood samples, respectively. GeneFluidics' products rely on molecular-based, PCR-less identification of species-specific phenotypic markers of resistance and susceptibility (resistance profiling determined by the change in 16S rRNA content of each target pathogen under various antibiotics conditions). The detection technology relies on an electrochemical sensor array.

Comparison of Platforms

Alfred 60AST coupled with MALDI-TOF MS has a faster TAT for identification and AST and it is more cost-effective compared to Accelerate Pheno™. However, Accelerate Pheno™ can provide identification and MIC determination using a single cartridge. Thus, it is considered an excellent candidate for small and medium laboratories, where MALDI-TOF MS equipment is not available [17]. From the above-mentioned platforms, VITEK2, BD Phoenix, MicroScan WalkAway and Sensititre ARIS 2X are cleared by the Food and Drug Administration (FDA) as IVD diagnostics. Although these platforms generate fast (2–18 h) results, it must be highlighted that a standardized microbial inoculum is required, which entails a culturing step of the specimen for 1–2 days prior introducing the inoculum into the AST platform [184]. Table 4 summarizes some of the most commonly used commercially available platforms for AST.

Table 4. Commercially available AST platforms.

Name	Link	Detection Method	TAT [1]	ID [2]	AST [3]	MIC [4]	Reference
VITEK®2 Compact (bioMérieux SA)	https://www.biomerieux-diagnostics.com/vitekr-2-compact-0 (accessed on 15 January 2021)	Turbidity	2–18 h	•	•		[185]
Adagio™ Antimicrobial Susceptibility Testing System (Bio-Rad Laboratories)	https://www.diagnostics-bio-rad.com/wp-content/uploads/2016/11/2015-Adagio-Brochure-EN.pdf (accessed on 15 January 2021)	Imaging device measuring the size of the inhibition zone around antibiotic discs			•	•	[180]
Accelerate Pheno™ (Accelerate Diagnostics, Inc.)	https://acceleratediagnostics.com/products/accelerate-pheno-system/ (accessed on 15 January 2021)	Fluorescence in-situ hybridization (FISH)	≈ 7	•	•	•	[186]
Alfred 60AST system (Alifax S.r.l.)	https://www.alifax.com/products/bacteriology-line/show/alfred-60 (accessed on 15 January 2021)	Light Scattering Technology/ Turbidity	4–6 h		•		[187]
ProMax ® (GeneFluidics, Inc.)	http://genefluidics.com/20151123/wp-content/uploads/2018/08/ProMax.pdf (accessed on 15 January 2021)	Electrochemical-based sensors based on sandwich hybridization of capture and detector probes with target 16S rRNA	3 h		•		
UtiMax® (GeneFluidics, Inc.)	http://genefluidics.com/20151123/wp-content/uploads/2018/08/UtiMax.pdf (accessed on 15 January 2021)	Electrochemical-based sensors based on sandwich hybridization of capture and detector probes with target 16S rRNA	3 h	•	•		[27]
BsiMax® (GeneFluidics, Inc.)	http://genefluidics.com/20151123/wp-content/uploads/2018/08/BsiMax.pdf (accessed on 15 January 2021)	Electrochemical-based sensors based on sandwich hybridization of capture and detector probes with target 16S rRNA	9.5 h	•	•		[188]
MicroScan WalkAway plus System (Beckman Coulter, Inc.)	https://www.beckmancoulter.com/es/products/microbiology/microscan-walkaway-plus-system (accessed on 15 January 2021)	Turbidity	4 h–overnight	•	•	•	[189]
BD Phoenix™ (Becton, Dickinson and Company)	https://www.bd.com/en-us/offerings/capabilities/microbiology-solutions/identification-and-susceptibility-testing/bd-phoenix-automated-identification-and-susceptibility-testing-system (accessed on 15 January 2021)	Turbidity and colorimetric change	4.5 h	•	•		[190]
Sensititre™ ARIS™ 2X (Thermo Scientific™)	https://www.fishersci.com/shop/products/sensititre-aris-2x-id-ast-inst/stv3090 (accessed on 15 January 2021)	Fluorescence measurement	Overnight (18 h–24 h)		•	•	[191]

[1] TAT: turnaround time (TAT refers to both ID and AST when applicable), [2] ID: identification, [3] AST: antimicrobial Scheme, [4] MIC: minimal inhibitory concentration.

6. Conclusions and Future Perspectives

The AMR crisis is imposing a joint response from academia, risk managers, risk assessors, government, and industry to enhance the current methodologies, both for diagnosis and treatment, by developing novel tools circumventing the drawbacks and limitations of the golden standards and existing AST methods. The main limitations of the currently available tools are: (i) the need for sample pre-treatment steps; (ii) their low sensitivity; (iii) the incapacity of microorganism identification in some occasions; and (iv) the lack of integration, automation, and portability. In relation to the three first points, lengthy biological protocols (culturing, isolation, identification) are required in order to detect a number of pathogens. It is highly important to focus on and strive for substantial advancement towards the development of new testing platforms with superior performance characteristics in this regard, in order to allow for their approval and marketing as soon as possible. Spending time and effort on improvements on existing methods, technologies, and platforms is also plausible.

According to MarketsandMarkets™, by 2025, the AST market is projected to reach USD $4.2 billion. In this market report, it is highlighted that, despite the use of automated AST platforms reducing both the incubation and detection times, the high prices of these platforms are considerable constraining factors for the widespread adoption of such platforms by end-users, principally for small-budget institutions [192]. In terms of product type, manual AST products held the largest share of the overall AST market in 2019. This is mainly attributed to the lower cost of such products. Based on the method, in 2019, disk diffusion accounted for the biggest share of the AST market mainly attributed to the relatively low cost and the diversity of the commercially available disks. Regarding the end users, hospitals and diagnostic laboratories commanded the largest share of the AST market in 2019 [193]. The cost estimation of the methods and technologies reviewed in this paper is out of scope. Albeit some rough estimations on the cost related to AST methods is described in the following publications of El-Bouri et al, Vrioni et al. and Vasala et al. [26,114,194].

All of the methods and technologies described above have shown great potential towards the AMR challenge, though various issues remain unanswered. For example, how many of these methods are generally applicable? Have these methodologies been validated against reference methods? For those not commercialized yet, when is it anticipated to become commercially available on the market for broad use? Although many methods presented in the literature claim to be capable of performing AMR detection in a short amount of time (minutes–few hours), in reality, they do not consider tedious pre-treatment steps, such as culture enrichment and culture isolation.

To sum up, standard cultivation tests for AST typically have a TAT of 18–36 h and can provide MIC, though they are not suitable for non-culturable pathogens. The commercially available automated platforms have a TAT of 2–24 h, some of them provide MIC, but they are not compatible with non-culturable pathogens. MALDI-TOF MS has a lower TAT of 2–4 h and, in some cases, MIC determination is also possible, though it shares the same limitation as the two previous technologies. In addition, it is not yet endowed with standardized AST protocols as well as companion software for data analysis. NAAT-based approaches have a TAT of 0.5–4 h, though MIC determination is not possible. On the other hand, NAAT is suitable for AST for non-culturable pathogens. In addition, NAAT-based systems are capable of easily integrating the detection of emerging ARGs or mutations. Nevertheless, new validations and standardization are needed for the diagnostics for each update. As for the WGS, it is still relatively newly introduced in the field of rapid AST. The biggest challenge related to WGS is the bioinformatics, since universal databases are required for the result interpretation. Microfluidics is an ever-growing field with great potential and versatility. Various microfluidic technologies, coupled with miniaturized biosensing schemes, hold a great promise for the future. Such microfluidic devices offer many advantages over conventional platforms, such as minimal resource (sample, reagents, power) use, low cost, user-friendly handling, rapid TAT, integration (multimodal), automa-

tion, and portability. Regarding the microfluidic approaches, apart from the upscaling of the fabrication processes to allow for mass production at a low price [195], a high degree of integration is needed for the pretreatment steps (e.g., sample preparation) and user-friendly interfacing, so as to become more appealing to users.

Although all of these technologies struggle to meet the requirements for rapid AST, none of them is optimal. It is highly probable that some of them will claim a large share of rapid AST diagnostics market in the future. This market can be divided into two categories, the central lab-based and the PON-based. The first refers to organized and well-equipped labs (e.g., hospitals, research, and diagnostic centers) where WGS, WGM, PCR, MALDI-TOF MS, FTIR, and automated AST platforms can also be integrated, and the latter would be useful for small laboratories, practitioners, and pharmacists where microfluidic-based, portable AST platforms would be more appropriate, as they are superior in terms of portability and affordability, needing less laboratory space, and providing fast TAT at the same time. Table 5 summarizes the main advantages and disadvantages of the methods and technologies described.

In conclusion, the development of reliable, sensitive and affordable diagnostics will facilitate combating the threat of AMR. Rapid diagnostic technologies employed mainly in primary care locations (i.e., rapid diagnostic tests), could enhance and facilitate the effective and targeted treatment. Moreover, advanced monitoring systems, such as mobile applications, coupled with surveillance programs, are essential to track antimicrobial consumption. Emerging approaches, such as machine learning and data mining in combination with automation, will play a key role for the next generation diagnostics. Epidemiological surveillance is of upmost importance for AMR since it provides the necessary input for developing and monitoring therapy guidelines, antibiotic stewardship programs, public health interventions, and novel antimicrobials and vaccines [196]. The developments on the cutting-edge methods and technologies addressing AMR and AST, coupled with the surveillance programs allowing for increased and simplified data transmission, would hugely contribute towards minimizing the detrimental effects of the AMR threat.

Table 5. Advantages and disadvantages of AMR diagnostic methods and technologies.

Method	Advantages	Disadvantages
Conventional methods		
Phenotypic methods	• Reference, validated methods • Simple methodology • MIC [1] values can be estimated • Usually, pathogen identification is also achieved	• Testing of individual, purified strains • Previous cultivation is needed (difficult for fastidious microorganisms, not possible for non-culturable ones) • Some disagreements between standards • For some taxonomic units, susceptibility cut-off values have not been yet established
Molecular-based assays	• Elimination of sample purification • Polymicrobial samples analyzed • Multiplex targeting of AMR [2] determinants • More precise detection and characterization of ARG [3] • Relatively quick adaptation to newly introduced resistance factors	• Need trained personal • Expensive lab equipment • Not capable of defining MIC • Some ARGs could be missed (sensitivity and coverage) • Diversity of ARG poses a difficulty in generating assays due to the cost involved • Not total correlation with phenotype

Table 5. *Cont.*

Method	Advantages	Disadvantages
Non-conventional methods		
WGS [4], WMS [5]	• Adequate for fastidious, non-culturable microorganisms • For long-read sequencing platforms, portability and affordability, less laboratory space, and on-site sequencing • Genetic basis of AMR established. Novel mechanisms of resistance can be characterized • Simultaneous study of multiple AMR determinants (for WMS, from different hosts)	• Large equipment costs • Complex, laborious methodology • Trained personnel needed • Sometimes discrepancies with phenotypic tests (false positive, false negative results) • For WMS, host of the AMR determinant is not known sometimes • Not capable of defining MIC • Not total correlation with phenotype
MALDI-TOF MS [6]	• Fast analysis • High throughput • Automated procedure • Simple sample manipulation • Low running costs • Small sample volume • Molecular basis of AMR established	• Large equipment costs • Testing of individual, purified strains. Previous cultivation is needed • Databases (including spectra from resistant and susceptible strains) should be developed • Need to find AMR biomarker (peak pattern). Not applicable to all microorganisms • Mathematical discrimination procedure needed • No portability • Not capable of defining MIC
FT-IR [7] spectroscopy	• Fast analysis • High throughput • Automated procedure • Simple sample manipulation • Low running costs • Small sample volume	• Large equipment costs • Testing of individual, purified strains. Previous cultivation is needed • Databases (including spectra from resistant and susceptible strains) should be developed • Need to find AMR biomarker (spectral pattern). Not applicable to all microorganisms • Mathematical discrimination procedure needed • No portability • IR [8] spectra influenced by culture conditions • Not capable of defining MIC
Technology		
Microfluidics and Lab-on-a-chip (LoC [9])	• Fast and high throughput analysis • Accurate fluid manipulation • Low cost, low reagent and power consumption • Small sample volume • Automated procedure • Integration, compactness, portability • Easy sample manipulation	• Not capable of defining MIC • Scalability issues • Reproducibility issues in terms of fabrication • Large surface to volume ratio • Surface treatment (minimize adsorption) • Commercialization

[1] MIC: minimal inhibition concentration, [2] AMR: antimicrobial resistance, [3] ARG: antimicrobial resistance gene, [4] WGS: whole genome sequencing, [5] WMS: whole metagenome sequencing, [6] MALDI-TOF MS: matrix-assisted laser desorption/ionization- time of flight mass spectrometry, [7] FT-IR: Fourier-transform infrared spectroscopy, [8] IR: infrared, [9] LoC: lab-on-a-chip.

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Abbreviations

AFM-IR	Atomic Force Microscopy-Infrared Spectroscopy
AMR	Antimicrobial Resistance
ANN	Artificial Neural Networks
ARG	Antimicrobial Resistance Gene
AST	Antimicrobial Susceptibility Testing
CLSI	Clinical and Laboratory Standards Institute
EFSA	European Food Safety Authority
ESBL	Extended-spectrum β-lactamase
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FDA	Food and Drug Administration
FTIR	Fourier Transform Infrared
FT-RIFS	Fourier Transform Reflectometric Interference Spectroscopy
GMO	Genetically Modified Organism
HAD	Helicase-Dependent Amplification
HT-qPCR	High Throughput Quantitative PCR
IR	Infrared
IVD	in vitro Diagnostic
KPC	*Klebsiella pneumoniae* Carbapenemase
LAMP	Loop-Mediated Isothermal Amplification
LoC	Lab-on-a-Chip
LoD	limit of detection
MALDI-TOF MS	Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry
MCA	Morphokinetic Cellular Analysis
MDR	Multidrug-Resistant
MIC	Minimum Inhibitory Concentration
MLST	multi-locus sequence typing
MRSA	methicillin resistant *S. aureus*
MSSA	methicillin susceptible *S. aureus*
MZO	Magnesium Zinc Oxide
NAAT	Nucleic Acid Amplification Technology
NASBA	Nucleic Acid Sequence-Based Amplification
NGS	Next-Generation Sequencing
PCR	Polymerase Chain Reaction
PMF	Peptide Mass Fingerprint
POC	Point-of-Care
QCM	Quartz-Crystal Microbalance
RAA	Residual Antimicrobial Activity
RCA	Rolling Circle Amplification
RDT	Rapid Diagnostic Test
RPA	Recombinase Polymerase Amplification
SDA	Strand Displacement Amplification
SERS	Surface Enhanced Raman Spectroscopy
TAT	Turnaround Time
TMA	Transcription Mediated Amplification
UTI	Urinary Tract Infection
WGS	Whole Genomic Sequencing
WHO	World Health Organization
WMS	Whole Metagenome Sequencing

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LANDSCAPE OF DIAGNOSTICS AGAINST ANTIBACTERIAL RESISTANCE, GAPS AND PRIORITIES

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Landscape of diagnostics against antibacterial resistance, gaps and priorities
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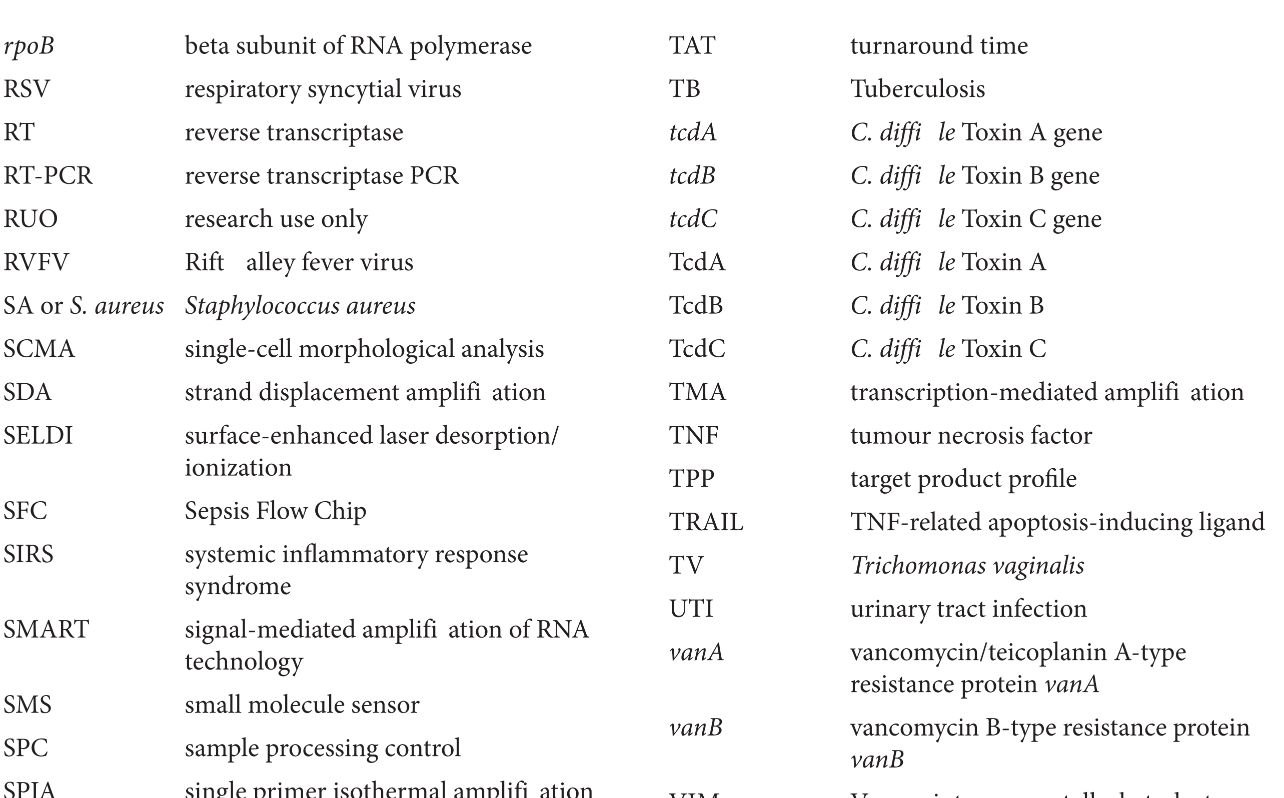
Glossary

ABR	antibacterial resistance
AFB	acid-fast bacilli
AMR	antimicrobial resistance
ARI	acute respiratory infection
ASO	antistreptolysin O
AST	antimicrobial susceptibility testing
BC	blood culture
BCID	blood culture identification
BSI	bloodstream infection
CAI	community-acquired infection
CAP	community-acquired pneumonia
C_2CA	circle-to-circle amplification
CDAD	*Clostridium difficile*-associated disease
CDC	US Centers for Disease Control and Prevention
CDI	*Clostridium difficile* (or *C. diff*) infection
C. difficile	*Clostridium difficile*
cDNA	complementary DNA molecule
CE	Conformité Européenne
CFU	culture-forming unit
cHDA	circulator helicase-dependent amplification
CLIA	Clinical Laboratory Improvement Amendments
CLSI	Clinical and Laboratory Standards Institute
CMV	cytomegalovirus
CNS	coagulase-negative staphylococci
CO_2	carbon dioxide
CPE	carbapenemase-producing *Enterobacteriaceae*
CPO	carbapenemase-producing organism
CRE	carbapenem-resistant *Enterobacteriaceae*
CRP	C-reactive protein
CSF	cerebrospinal fluid
CT	*Chlamydia trachomatis*
CTX-M	CTX-M beta-lactamases
DNA	deoxyribonucleic acid
dPCR	digital PCR
DST	drug susceptibility testing
DTR	discrete test region
DTS	direct tube sampling
ECDC	European Centre for Disease Prevention and Control
E. coli	*Escherichia coli*
EDL	WHO Model List of Essential In Vitro Diagnostics
E. faecalis	*Enterococcus faecalis*
EIEC	enteroinvasive *Escherichia coli*
ELISA	enzyme-linked immunosorbent assay
ESBL	extended spectrum beta-lactamase
ESI	electron spray ionization
ET	fluorescent energy transfer
ETEC	enterotoxigenic *Escherichia coli*
ETGA	Enzymatic Template Generation and Amplifi ation
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FDA	US Food and Drug Administration
FFPE	formalin-fi ed, paraffin- bedded
FISH	fluorescence in situ hybridization
FLLS	forward laser light scattering
GARDP	Global Antibiotic Research and Development Partnership
GBS	Group B *Streptococcus*
GC	*Neisseria gonorrhoeae*, also gas chromatography
gDNA	genomic DNA
GEL	gel electrofiltration
GES	GES-type beta-lactamase
GI	gastrointestinal
GLASS	Global Antimicrobial Surveillance System
HAI	hospital- and/or health-centre-acquired infections
HbA1c	glycated haemoglobin
HBV	hepatitis B virus
HCV	hepatitis C virus
HDA	helicase-dependent amplifi ation
HIC	high-income country
HIV-1	human immunodeficie cy virus-1

HPV	human papilloma virus
HSV 1	herpes simplex virus 1 (oral herpes)
HSV 2	herpes simplex virus 2 (genital herpes)
ICR	inducible clindamycin resistance
ICU	intensive care unit
ID	identifi ation
IgG	immunoglobulin G
IgM	immunoglobulin M
IL-6	interleukin-6
IMBI	Inhibition Magnetic Binding Immunoassay
IMDA	isothermal multiple displacement amplifi ation
IMP	IMP-type metallo-beta-lactamase
iNAAT	isothermal nucleic acid amplifi ation test
IP-10	interferon gamma-induced protein-10
IVDs	in vitro diagnostics
KPC	*Klebsiella pneumoniae* carbapenemase
K. pneumoniae	*Klebsiella pneumoniae*
LAMP	loop-mediated isothermal amplifi ation
LATE	late-linear-after-the-exponential
LDT	laboratory-developed test
LED	light-emitting diode
LFIA	lateral fl w immunoassay
LIMS	laboratory information and management system
LIS	laboratory Information system
LMICs	low- and middle-income countries
LOD	limit of detection
LPA	line probe assay
LPS	lipopolysaccharide
LRTI	lower respiratory tract infection
MAC	microfluidic agarose channel
MALDI-TOF MS	matrix-assisted laser desorption ionization-time of fli ht mass spectrometry
McF	McFarland unit
mcr	mobilized colistin resistance gene
mecA	methicillin resistance *mecA* gene
mecC	methicillin resistance *mecC* gene
MEMS	microelectromechanical systems
MG	*Mycoplasma genitalium*
MIC	minimum inhibitory concentration
mL	millilitre
MLSb	macrolide-lincosamide-streptogramin B resistance
MREJ	*mec* right extremity junction
mRNA	messenger RNA
MRSA	methicillin-resistant *Staphylococcus aureus*
MS	mass spectrometry
MSSA	methicillin-susceptible *Staphylococcus aureus*
MTB	*Mycobacterium tuberculosis*
MTB complex	*Mycobacterium tuberculosis* complex
MxA	myxovirus resistance A
m/z	mass-to-charge
NAAT	nucleic acid amplifi ation test
NASBA	nucleic acid sequence-based amplifi ation
NDM	New Delhi metallo-beta-lactamase
NEAR	nicking enzyme amplifi ation reaction
NG	*Neisseria gonorrhoeae* or *N. gonorrhoeae*
NGS	next-generation sequencing
nm	nanometre
nvCT	new variant of CT
NTM	nontuberculous mycobacteria
OXA	OXA-type carbapenem
P. aeruginosa	*Pseudomonas aeruginosa*
PaLoc	pathogenicity locus
PBP	penicillin binding protein
PCR	polymerase chain reaction
PCT	procalcitonin
PNA	peptide nucleic acid
POC	point of care
PT-INR	prothrombin time-international normalized ratio
qPCR	quantitative PCR
RAA	residual antimicrobial activity
R&D	research and development
RALF	resuspension and lysis fluid
RCA	rolling circle amplifi ation
RDT	rapid diagnostic test
RIF	resistance to rifampicin
RNA	ribonucleic acid
RP	respiratory panel
RPA	recombinase polymerase amplifi ation
rRNA	ribosomal RNA



rpoB beta subunit of RNA polymerase
RSV respiratory syncytial virus
RT reverse transcriptase
RT-PCR reverse transcriptase PCR
RUO research use only
RVFV Rift alley fever virus
SA or *S. aureus* *Staphylococcus aureus*
SCMA single-cell morphological analysis
SDA strand displacement amplifi ation
SELDI surface-enhanced laser desorption/ ionization
SFC Sepsis Flow Chip
SIRS systemic inflammatory response syndrome
SMART signal-mediated amplifi ation of RNA technology
SMS small molecule sensor
SPC sample processing control
SPIA single primer isothermal amplifi ation
STAT short turnaround time
STEC Shiga toxin-producing *Escherichia coli*
STI sexually transmitted infection
stx1 Shiga toxin 1 gene
stx2 Shiga toxin 2 gene
S. typhi *Salmonella typhi*
TAT turnaround time
TB Tuberculosis
tcdA *C. diffi le* Toxin A gene
tcdB *C. diffi le* Toxin B gene
tcdC *C. diffi le* Toxin C gene
TcdA *C. diffi le* Toxin A
TcdB *C. diffi le* Toxin B
TcdC *C. diffi le* Toxin C
TMA transcription-mediated amplifi ation
TNF tumour necrosis factor
TPP target product profile
TRAIL TNF-related apoptosis-inducing ligand
TV *Trichomonas vaginalis*
UTI urinary tract infection
vanA vancomycin/teicoplanin A-type resistance protein *vanA*
vanB vancomycin B-type resistance protein *vanB*
VIM Verona integron metallo-beta-lactamase
VOC volatile organic compound
VRE vancomycin-resistant enterococci
VZV varicella-zoster virus
WHO World Health Organization

Executive Summary

Background

The increasing prevalence of antimicrobial resistance (AMR) is a serious threat to global public health and is especially burdensome in low- and middle-income countries (LMICs). The need for new diagnostics to combat AMR has been recognized (*1*, *2*). Among the diagnostics needed are: (i) rapid tests that distinguish between bacterial and viral infections; (ii) tests for pathogen identification (ID); and (iii) tests for antimicrobial susceptibility patterns (*3*).

In order to address these needs, the World Health Organization (WHO) has undertaken in this report to map available and pipeline diagnostics against AMR, identify gaps in the availability of such diagnostics in LMICs, and establish a research and development (R&D) priority list of diagnostics against AMR for the next 3–5 years. In the second phase of the project, WHO will develop consensus target product profiles (TPPs) for the highest-priority diagnostics on the R&D priority list.

Scope

While the increasing prevalence of AMR is a growing concern for viral, parasitic and fungal infections, antibacterial resistance (ABR) has now become a major global health issue that requires urgent solutions, including new diagnostics. Therefore, this report focuses on commercially available diagnostics to combat ABR and prioritizes the following key parameters: (i) diagnostics to improve clinical/syndromic management of patients to reduce the overprescription of antibiotics; (ii) antibiotics exhibiting the highest proportion of resistance as set forth in Annex I hereto; (iii) diagnostics that can be performed at primary and secondary care facilities in LMICs; (iv) diagnostics targeted at pathogens primarily related to community-acquired infections (CAIs) and secondarily to bacterial infections that are most frequently acquired in hospitals (HAIs); and (v) diagnostics to help distinguish bacterial from nonbacterial infections.

It should be noted that tuberculosis (TB) is a leading infectious disease that causes mortality worldwide, and drug-resistant *Mycobacterium tuberculosis* (MTB), the cause of TB in humans, is in the top 10 list of WHO priorities, as shown in Annex I. Extensive landscaping of diagnostics and drug-resistance testing for MTB has already been performed, and a number of TPPs have been developed for priority diagnostic needs. Links to these materials are provided later in this report. Given the extensive work that has already been done with respect to diagnostics for MTB, these diagnostics are not a focus of this report. Nonetheless, because of the importance of TB, the report includes priorities for TB diagnostics R&D in addition to priorities for other targeted bacterial pathogens.

In addition, although the report does not cover issues related to uptake and implementation of new diagnostics in LMICs, to provide context for the diagnostic mapping, the report describes in general terms public health laboratory systems in LMICs. It maps typical facilities and capabilities from the lowest level of the system to the highest level – primary care facilities (Level I), secondary care/district hospital facilities (Level II), regional and provincial laboratories (Level III), and national and multicountry reference laboratories (Level IV). The laboratory structure and testing capabilities at each level of the public healthcare system have important implications for improving access to diagnostics to combat ABR. The needs for human capacity, infrastructure and quality systems in clinical microbiology laboratories mean that bacterial cultivation, antimicrobial susceptibility testing (AST) and even molecular testing are generally found only at Level III and Level IV facilities, limiting testing access to most patients.

The question and the findings

The question that frames this diagnostics landscape report is, What are the gaps in diagnostics to combat ABR for prioritized, drug-resistant bacterial pathogens, with an emphasis on CAIs, at Level I and Level II of the public healthcare system in LMICs? In order to answer that question, the report maps phenotypic and nonphenotypic methods of specific bacterial ID as well as antimicrobial susceptibility and resistance testing methods available at all levels of the healthcare system in all settings. It also considers tests that detect the host response to bacterial pathogens, but do not specifically identify them.

The report details phenotypic methods of bacterial ID, including culture, both manual and automated, and biochemical testing. Although these methods are

still the backbone of diagnostic bacteriology, the test methods are slow and, when done manually, cumbersome. Given the lack of well trained, highly skilled microscopists in LMICs and the need for sophisticated infrastructure for automated methods of bacterial cultivation, such testing is generally limited to Level III and Level IV laboratories in LMICs. In general, simpler, faster methods of bacterial pathogen ID are needed, especially for low-resource settings.

The report details nonphenotypic methods of bacterial ID. These include rapid immunoassays, which have the advantage of being fast and often require no equipment other than a test cartridge. This makes them suitable for use at Level I in LMICs. Disadvantages include that the assays can generally only identify one bacterial pathogen at a time and the performance of some assays has been questioned.

There are also numerous commercial genotypic test systems, including molecular-based platforms (using hybridization or amplification methods), DNA microarrays and sequencing, as well as mass spectrometry (MS) methods for bacterial pathogen ID. All of these methods are more rapid than traditional phenotypic testing; however, like bacterial cultivation, these methods require sophisticated laboratories and well trained laboratory technicians. This means that many of these tests and test platforms are best placed in Level III and Level IV settings, again limiting access to testing in LMICs.

Following pathogen ID, it is also important to conduct AST or to detect resistance in individual bacterial isolates in order to guide treatment decisions. The report maps commercially available phenotypic and genotypic methods of combined bacterial ID and AST/resistance testing, as well as AST/resistance assays only.

Classical phenotypic AST methods can be done manually using various media, both solid and liquid, in which the growth of bacteria along with the organism's resistance or susceptibility to a select antimicrobial agent are measured. In addition to manual methods, automated instruments that combine bacterial pathogen ID and AST offer improved turnaround time (TAT). While widely used in high-income countries (HICs), these systems are not generally available in resource-limited settings, except at the highest levels of the system (*4*, *5*).

Nonphenotypic methods, in particular molecular-based platforms and MS, are now being used routinely in clinical microbiology laboratories for both bacterial pathogen ID and resistance testing. Although all of the platforms offer faster results than phenotypic methods, most platforms, especially those for bloodstream infections (BSIs), which require culture samples, are systems best used in sophisticated laboratory settings with strong infrastructure and well trained laboratory staff. Again, these systems are not widely available in LMICs.

There are also a number of phenotypic and nonphenotypic diagnostic systems in the development pipeline designed for use in LMICs. The systems are smaller and simpler to use than conventional systems designed for use in large laboratories. Some of these pipeline diagnostics provide pathogen ID as well as AST or resistance testing capabilities; some do not. Some perform monoplex testing only and some will only process swabs and urine, which limits the pathogens they are able to detect. Some systems will process complex matrices, including whole blood, which would offer the possibility of avoiding culture. However, detecting and identifying bacteria direct from whole blood with performance at least equivalent to blood culture has proven to be very difficult. It is a challenge that has not yet been met.

Finally, there are a number of assays that incorporate biomarkers that are host derived, including C-reactive protein (CRP) and procalcitonin (PCT), and that are capable of indicating host response to a pathogen. For CRP there are rapid diagnostic tests (RDTs) available for use in Level I settings in LMICs, while for PCT testing there are one or two instrument-based platforms available for use in Level II settings. Although not a complete solution to detecting bacterial pathogens, these tests could be used in primary healthcare settings for triage as indications of severity of infection and to determine whether an infection is more likely to be bacterial than nonbacterial, which could aid in antimicrobial stewardship.

Diagnostic gaps

The answer to the question that frames this report is that although there are many commercially available diagnostic systems to identify and/or perform AST/resistance testing for prioritized bacterial pathogens, the tests are not well suited to primary and secondary healthcare facilities. Most systems are predicated on sophisticated, well equipped laboratories with well trained laboratory staff. In LMICs, this effectively limits access to these tests to Level III and Level IV.

In other words, what emerges from this report, as well as from additional work by WHO with respect to TB, is a series of significant gaps in tests and testing platforms for Level I and Level II facilities in LMICs, where most patients initially present when they are ill.

Gaps include:

- inadequate near-patient testing for (i) biomarker-based, non-sputum-based detection of TB; (ii) patient triage evaluation for TB; (iii) sputum-based replacement for acid-fast bacilli (AFB) smear microscopy; and (iv) TB drug susceptibility testing (DST) (see https://www.who.int/tb/publications/tpp_report/en/);

- little or no ability to perform simplified phenotypic bacterial ID and AST to enable definitive therapeutic decision-making at Level III, and potentially Level II, in LMICs, particularly in the context of BSIs, in particular sepsis;
- inadequate near-patient testing options for ID and susceptibility testing for multidrug-resistant *Neisseria gonorrhoeae* (NG);
- few RDTs or easy-to-use, robust diagnostic platforms for use in primary (or secondary) healthcare settings that can reliably distinguish between bacterial and nonbacterial infections from accessible, minimally invasive clinical specimens (e.g., whole blood, urine, stool and nasal swabs);
- no multiplex platform suitable for Level II and/or Level I settings to detect bacterial pathogens, including BSIs, from whole blood (no culture required) with AST/resistance testing done on a separate platform or combined with AST/resistance testing on the same platform; and
- no simple, easy-to-use test/platform suitable for use at Level II and/or Level I settings for AST from whole blood or other sample matrices (urine, stool, respiratory specimens) for which culture is not required.

R&D priorities

These findings suggest the following R&D priority diagnostics against AMR for primary and secondary healthcare facilities over the next 3–5 years, for which consensus TPPs to stimulate product development are proposed:

- Improved near-patient testing for TB: to enable point-of-care (POC) assays capable of: (i) detecting all forms of TB by identifying characteristic biomarkers or biosignatures in specimen(s) other than sputum; (ii) low-cost patient triage by first-contact healthcare providers to identify those patients who need further testing; (iii) replacing AFB smear microscopy for detecting pulmonary TB; and (iv) determining first-line regimen-based therapy via DST that can be used at the microscopy-centre level of the healthcare system. These proposed TPPs have been developed. For details, see https://www.who.int/tb/publications/tpp_report/en/.
- Simplified phenotypic ID and AST: to enable the performance of culture and AST in key resistance categories, in particular sepsis, at Level II and higher facilities. Review published TPP and build on it as needed.
- Improved diagnostics and AST for NG: to provide a (i) rapid test to detect and distinguish NG and *Chlamydia trachomatis* (CT) for use in primary care settings, and (ii) a comprehensive test to both confirm NG infection and enable genotypic resistance testing of NG infection at primary/secondary care settings. WHO, FIND, and the Global Antibiotic Research and Development Partnership (GARDP) are already developing a TPP for each of these tests. Assuming alignment with this initiative, support it as needed.
- Host response tests: to provide additional tests to help distinguish between bacterial and nonbacterial infections at primary healthcare facilities. A consensus TPP for such tests has already been developed, but should be revisited to consider whether it should be refined/revised.
- Multiplex diagnostic platform to identify bacterial pathogens and perform AST/resistance testing without culture: to provide a platform suitable for Level II facilities and higher that could identify a broad range of bacterial pathogens from whole blood as well as from other sample matrices – e.g., urine, stool, nasal swabs – and that optimally could perform AST/resistance testing on the same platform. A consensus TPP should be developed.
- Simple, easy-to-use test/platform for AST only: to perform susceptibility testing at Level II or Level I settings from sample matrices such as urine, stool and nasal swabs, minimally, and optimally from whole blood, to be used following bacterial ID on a separate platform. A consensus TPP should be developed.

Introduction

The increasing prevalence of AMR, which WHO defines as the "ability of a microorganism (like bacteria, viruses and some parasites) to stop an antimicrobial (such as antibiotics, antivirals and antimalarials) from working against it" (*6*), is a serious threat to global public health and disproportionately burdens low-resource countries (*1*, *2*). The growing resistance to antibiotics of bacterial pathogens is recognized as the largest of these threats (*7*).

The urgency of the threat was highlighted when, in May 2015, the Sixty-eighth World Health Assembly endorsed a global action plan to combat AMR, including ABR (*8*). An extensive review of AMR published the previous year had concluded that by 2050 the lives of 10 million people per year would be at risk due to the increase in drug-resistant infections if solutions were not found (*3*). Among the recommendations made in that report was to promote new, rapid diagnostics to reduce the unnecessary use of antibiotics (*3*).

In recognition of the need for new diagnostics against AMR, in 2014 WHO had convened a consultation to facilitate dialogue among stakeholders with respect to creating a roadmap to stimulate the development of, and access to, appropriate rapid diagnostic tools for AST at all levels of the healthcare system in LMICs (*9*). The consultation also considered the need for diagnostics for pathogen ID and for discriminating between bacterial and viral infections in those settings and concluded that:

- the priority need is for rapid tests to distinguish between bacterial and viral infections;
- the next need is tests for pathogen ID; and then
- tests for susceptibility patterns (*9*).

In order to address the needs articulated in the 2014 consultation, including the finding that there was no authoritative list of commercially available diagnostics to combat AMR, WHO, with funding from Wellcome Trust, is undertaking the following initiative with respect to in vitro diagnostics (IVDs) against AMR, which is being carried out in two phases:

- In Phase I, WHO is:
  - mapping available and pipeline diagnostics against AMR;
  - identifying gaps in the availability of such diagnostics; and
  - establishing an R&D priority list of diagnostics against AMR for LMICs for the next 3–5 years.
- In Phase II, WHO will:
  - develop consensus target product profiles (TPPs) for the highest-priority diagnostics against AMR on the R&D policy priority list.

As required by Phase I of the work, this report maps available and pipeline in vitro diagnostics against ABR and identifies gaps in the availability of such diagnostics. The gaps on which this report focuses are those that are the result of the nonexistence or lack of technologies fit for purpose in LMICs as opposed to barriers to access to such diagnostics that exist for other reasons, including in-country policies or system failures. Detailed information on in vitro diagnostics and laboratories can be found at: https://www.who.int/in-vitro-diagnostic/en/.

Because resistance to antibiotics is the largest public health threat globally, the report focuses on IVDs that can play a role in limiting ABR. With respect to bacterial pathogens, such diagnostic tools comprise a spectrum of tests, including phenotypic, genotypic and immunologic test methods for identifying bacterial pathogens as well as various methods of AST, which measure bacterial growth in the presence of antimicrobial agents, and testing methods that identify bacterial resistance but do not measure susceptibility.

Within this framework, the following are the key parameters that define the mapping and scope of this report:

- Clinical/syndromic patient management. With respect to IVDs to address ABR, the primary focus is diagnostics to improve clinical/syndromic management of patients to reduce overprescribing of antibiotics, that is, the focus is on IVDs for antibiotic stewardship. A secondary focus is on IVDs for surveillance. The focus on stewardship implies an emphasis on faster/more accurate diagnostic testing, reducing the time to appropriate antibiotics, reducing their unnecessary

use and informing decisions regarding antibiotic de-escalation or discontinuation.[1] With appropriate tools, including diagnostic connectivity, rapid ID and susceptibility or resistance determination of bacterial pathogens will inform surveillance as well.

With respect to IVDs for national surveillance, the Global Antimicrobial Surveillance System (GLASS) has undertaken a landscape – *Molecular methods for antimicrobial resistance (AMR) diagnostics to enhance the Global Antimicrobial Resistance Surveillance System* – which is available at: https://www.who.int/glass/resources/publications/molecular-methods-for-amr-diagnostics/en/. It is a resource that is complementary to this report.

- High-priority bacterial pathogens. The mapping focuses on IVDs to detect and categorize priority bacterial pathogens identified by WHO (*11*), the US Centers for Disease Control and Prevention (CDC) (*12*), and the European Centre for Disease Prevention and Control (ECDC) (*13*) that exhibit the highest proportion of resistance (i.e., those categorized as Critical/Serious or High/Serious, all of which are resistant to two or more classes of antibiotics, e.g., carbapenem-resistant, extended-spectrum beta-lactamase [ESBL]-producing) (*14*, *15*), as well as bacterial pathogens that most affect LMICs (*3*). These are set out in Annex I.

  TB is a leading infectious disease causing mortality worldwide, and drug-resistant MTB, the cause of TB in humans, is in the top 10 list of WHO priorities. Extensive landscaping of diagnostics and drug-resistance testing for MTB has already been performed, and a number of TPPs have been developed for priority diagnostic needs, which are highlighted in this report. In 2018, the WHO Global TB Programme issued a series of documents to improve drug susceptibility testing for TB in laboratories worldwide. The technical documents put together the latest knowledge on molecular mechanisms of drug resistance in MTB and describe state-of-the art testing methods for determining drug resistance in the laboratory in order to design the most appropriate regimens for patients requiring treatment for drug-resistant TB. These documents include a technical guide for detecting mutations associated with drug resistance in MTB; a technical report that includes internationally agreed critical concentrations for drug-susceptibility testing for detecting drug-resistant TB; and a technical manual of medicines used in treating TB. Given the extensive work that has been done with respect to diagnostics for MTB, such diagnostics are not a focus of this report. However, diagnostic platforms that include ID and/or resistance testing for MTB as well as other assays of relevance to this report are highlighted; the report also highlights priorities for TB diagnostics R&D. For a comprehensive landscape of diagnostics for MTB, see https://unitaid.org/assets/TB-Dx-Landscape-5Ed_May2017_V2.pdf.
- Primary care. Given the WHO emphasis on universal healthcare (*16*) and essential diagnostics for healthcare systems articulated in the WHO Model List of Essential In Vitro Diagnostics (EDL) (*17*), the landscape focuses on IVDs that can be performed at primary and secondary care facilities in LMICs, which are generally referred to as Level I and Level II settings and are described more fully below.
- Community-acquired infections. Since Levels I and II are primarily outpatient facilities, the landscape focuses on bacterial pathogens that are most often community-acquired (CAIs), but also considers diagnostics for bacterial infections that are most frequently acquired in hospitals or in other in-patient healthcare facilities (HAIs) (also referred to as nosocomial infections), in the context of testing at higher-level facilities in LMICs. It should be noted that some pathogens are found commonly in both settings, e.g., MTB and *Staphylococcus aureus*). See Annex I. CAI pathogens considered are *Escherichia coli*, NG, Helicobacter *pylori*, *Campylobacter* spp., *Salmonellae*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Shigella* spp. and MTB. HAI pathogens include *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterococcus faecium* and *Staphylococcus aureus* (*18–20*).
- Host response tests. The landscape also considers and maps other potential IVDs that may be useful in combating ABR. These include host response tests that incorporate host-derived biomarkers, including PCT and CRP, and novel biomarkers, which may be able to classify infections as bacterial or nonbacterial (*21–23*).

[1] WHO defines diagnostic stewardship as “coordinated guidance and interventions to improve appropriate use of microbiological diagnostics to guide therapeutic decisions. It should promote appropriate, timely diagnostic testing, including specimen collection, and pathogen ID and accurate, timely reporting of results to guide patient treatment” (*10*).

Methods

The material in this diagnostics landscape report has been gathered from the following sources:

- An extensive review of publicly available information, published and unpublished reports, and prospectuses. Databases may include Embase, the Cochrane Database of Systematic Reviews and PubMed, among others.
- With respect to individual commercial diagnostic platforms, company websites as well as the website of the US Food and Drug Administration (www.fda.gov).
- Relevant, publicly available diagnostic landscapes published by Unitaid, FIND, WHO and others, as well as additional sources, including, but not limited to, the Wellcome Trust, the Joint Programming Initiative on Antimicrobial Resistance, RAPP-ID, VALUE-Dx and the Longitude Prize.

Organization of the landscape report

This diagnostics landscape report provides a description of the basic public healthcare delivery and laboratory system in LMICs, which sets the stage for understanding the types of IVDs that can be utilized at each level of the system.

The landscape then maps current diagnostic methods of identifying bacterial pathogens, including manual and automated methods primarily done at higher levels of the healthcare system in LMICs, with a primary focus on commercialized platforms. This includes light-touch mapping of current phenotypic methods of identifying bacterial pathogens, including classical phenotypic methods: morphologic characterization (microscopy and staining); growth in culture; and phenotypic and metabolic characterization by biochemical tests. The landscape also maps current molecular and other nonphenotypic methods of identifying bacterial pathogens (e.g., immunoassays, microarrays, sequencing, MS), describing, where available, commercial systems that have the potential to be used at primary and secondary healthcare facilities.

The landscape provides a mapping of current and emerging phenotypic methods combining bacterial pathogen ID and AST, including both manual and automated methods, most of which are best suited to higher levels of the healthcare system in LMICs. The landscape then maps commercially available nonphenotypic methods for simultaneous pathogen ID and detection of ABR or methods of identifying genes that directly confer antibiotic resistance only.

Throughout the mapping of diagnostics for ABR, emerging and pipeline diagnostics for bacterial pathogen ID are also considered. These include phenotypic and nonphenotypic methods of bacterial detection, AST and resistance testing. The focus is on commercial platforms that have the potential to be used at primary and secondary levels of the healthcare system in LMICs.

Finally, the landscape maps current and pipeline diagnostics that differentiate between bacterial and nonbacterial causes of infection. These are host response assays, including tests that incorporate host-derived biomarkers like CRP and PCT, as well as novel biomarkers, a combination of host biomarkers or combinations of protein biomarkers. The landscape considers the role such tests could play in combating ABR, particularly in primary and secondary healthcare settings in LMICs.

All of the commercial diagnostic platforms landscaped in detail in this report are summarized in Annex II.

For purposes of this report, of particular interest is the applicability of available and pipeline tests to the rapid ID and AST/resistance testing of multidrug-resistant bacteria found in community settings in primary and secondary care facilities in LMICs. The landscape also identifies primary gaps in the current diagnostics landscape and what IVDs are needed to improve clinical/syndromic management of patients in order to reduce overprescribing of antibiotics. The hypothesis is that the diagnostic needs going forward are faster, easier and less expensive testing at all levels of the healthcare system, and basic accessibility of appropriate testing at Level I and Level II, including syndromic testing.

Laboratory systems in LMICs

In HICs, there are generally a large number of laboratories. For example, in the United States, there are approximately 5700 hospital-associated laboratories, 2000 independent laboratories and 10 000 physician-office labs/clinics (*24*). They offer a wide array of diagnostic testing from routine to the most targeted. Hospitals in HICs have access to nearly all requested testing with TAT of 24 hours or less. Some testing is performed in-house and some is sent out to large laboratory service companies. Tests for which results are needed immediately in order to manage medical emergencies will generally be performed in-house with TAT of an hour or less. In general, therefore, testing availability and access are not particularly problematic in HICs.

The same cannot be said for testing in LMICs. Over the last 10 years, access to treatment for people living with such priority diseases as HIV/AIDS, TB and malaria has substantially increased. However, the lack of laboratory and diagnostic capacity in resource-poor settings continues to be a barrier to achieving treatment targets outlined by countries and by international organizations. Simpler technology that is low cost and adapted to the needs of public laboratories in LMICs, in particular, is required in order to expand testing services to the communities that need them.

In order to understand laboratory testing in resource-limited settings, one needs to consider the typical public healthcare facilities and testing services available, which are usually characterized as a tiered system as follows (*25*).[2]

Level I – Primary:[3] Health post and health centre laboratories that primarily serve outpatients. Often, health posts have no laboratory capability, but are able to perform some POC testing. Generally, no clinicians are on-site at a health post. Health centres, however, usually have a simple laboratory where basic testing can be performed – e.g., POC assays and some microscopy (AFB smear by light microscopy), if a microscopist is available – and clinicians are generally on-site.

Level II – District: Laboratories in intermediate referral facilities, e.g., a district hospital. These facilities can perform all services provided at Level I and additionally provide a broader menu of tests, including Gram staining. They usually have automated equipment for tests such as CD4 count and blood chemistries. Physicians and other clinicians (e.g., nurses) are commonly available on-site.

Level III – Regional and provincial: Laboratories in a regional and provincial referral hospital that may be part of a regional or provincial health bureau. These facilities will have still more expansive test menus than those found at Level II facilities. In addition to performing all of the tests and services provided at Levels I/II, regional and provincial facilities can usually provide additional testing capabilities such as blood cultures, full chemistry testing and AFB smear (by fluorescent technique), as well as AFB culture, ID and susceptibility testing for first-line drugs. In addition, qualitative and quantitative nucleic acid amplification tests (NAATs) may also be available.

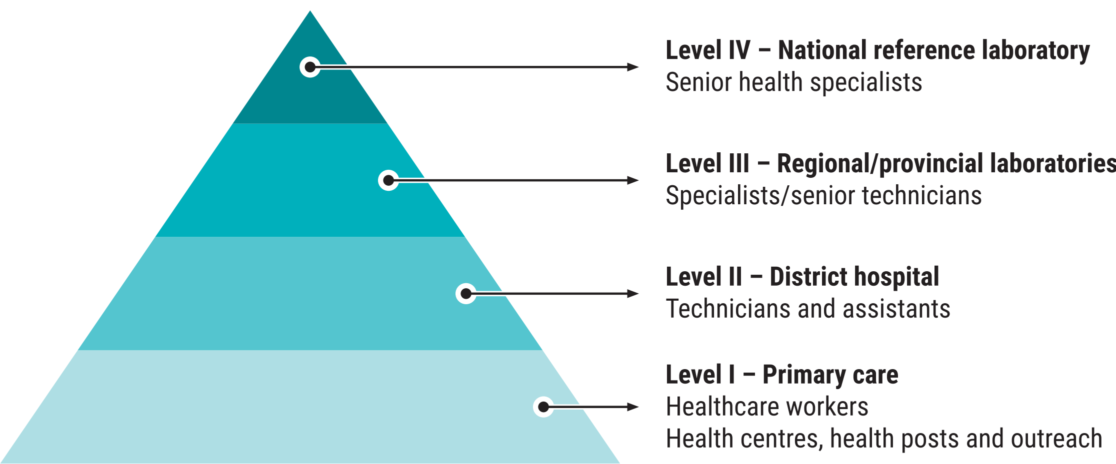
Level IV – National and multicountry reference laboratory: The national reference laboratories are specialized facilities charged with strengthening laboratory capacity for diseases of public health concern. They often provide linkages with research laboratories, academic institutions and other public health laboratories, forming integrated laboratory networks that can provide assistance in clinical trials, evaluation of new technologies and surveillance. In addition, national reference laboratories perform molecular and other sophisticated testing beyond the capabilities of Level III facilities – e.g., NAATs, HIV drug-resistance studies and AFB susceptibility testing for both first- and second-line drugs.

The laboratory system is often depicted as a pyramid (Fig. 1), which shows that there are generally a large number of Level I facilities and they serve the most patients directly. As one goes up the levels of the system, there are a smaller number of centralized facilities. In the case of national reference laboratories

[2] The Maputo meeting report notes that the tiered levels of a laboratory system and the testing performed at each level may vary depending on the population served (e.g., infants, adults), level of service available, physical infrastructure, electricity, water, road conditions and the availability of trained technical personnel in-country (*25*).

[3] The Maputo meeting report does not specifically place outreach services at Level I of the tiered laboratory system. Although some experts place outreach efforts at Level I, some consider patient outreach to be at a level below Level I and add a fifth tier to the system, referred to as sub-primary care or Level 0 (*25*).

Fig. 1. Diagram of tiered laboratory system in LMICs



and some provincial laboratories, they may not serve patients with a broad set of consultative services, but rather are referral centres for quality assurance and training or for conducting complex tests (either using samples drawn at facilities lower in the system and transported or by receiving patients referred directly from other facilities).

This laboratory structure and the testing capabilities provided at each level of the system in LMICs have important implications for improving access to diagnostics to combat ABR. Bacterial pathogen ID, quantification and AST typically take place in clinical microbiology laboratories using culture-based techniques dating from the late 1800s (*4*). The majority of tests available today in LMICs were created for developed-country settings, where laboratory-based diagnostics are operated by highly trained technicians on sophisticated instrumentation that is costly, often run in standard 96-well formats (high patient loads), and require dedicated laboratory infrastructure and equipment, as well as strong quality assurance programmes (*2*). Also, these instruments rely on a complex medical infrastructure that uses extensive sample transport networks to collect samples from multiple hospitals and clinics and uses sophisticated patient-tracking mechanisms that enable doctors and hospitals to return results to patients over weeks. These systems are not easily adapted for use in most regions of LMICs, where access, cost, infrastructure and patient loss are significant barriers to increasing case detection rates.

These needs for human capacity, infrastructure and quality systems in clinical microbiology laboratories mean that cultures, susceptibility testing and even molecular testing are generally limited to Level III and Level IV laboratories in LMICs, which in turn means that such testing is often not available to most patients. Indeed, the lack of equipment and assays for such testing has been called the Achilles heel of the containment of ABR in low-resource settings (*26*, *27*). In order to improve access to diagnostics to combat ABR in LMICs, easier, faster and simpler methods of testing are needed.

IVDs for identifying bacterial pathogens

Phenotypic ID methods

Phenotypic methods of identifying bacterial pathogens are often referred to as traditional or classical methods. They rely on features of the organism beyond its genetic make-up, including cell and colony morphology and biochemical reactions. The basic methods of phenotypic ID of bacterial pathogens are discussed below.

Bacterial cultivation

The lynchpin of phenotypic ID of bacteria is culture. The cause of infection is determined by isolating and culturing bacteria in artificial media. Cultivation of bacteria involves growing it in a culture medium that is suited to its metabolic needs; a pure culture (i.e., containing one strain of a single species) is required. Cultivation necessitates the use of optimal artificial media (liquid or solid) and incubation conditions (carbon dioxide [CO_2] concentration and temperature) to isolate and identify the bacterial etiologies of an infection (*28*).

The cultivation of bacteria from various patient specimens is done by inoculating processed specimens directly onto the artificial media. Incubation conditions are selected for their ability to support the growth of the bacteria most likely to be involved in the infectious process. To enhance the growth, isolation and selection of etiologic agents, a small, measured amount of specimen inocula (less than 2–5 µL) is usually streaked over the surface of plates containing culture medium in a standard pattern, so that individual bacterial colonies are obtained and semi-quantitative analysis can be performed.

Culture media can be broth (liquid) or agar (solid). In the laboratory, nutrients are incorporated into culture media on or in which bacteria are grown, and because different bacterial pathogens require different nutrients, various types of culture media have been developed for use in diagnostic microbiology; these are widely commercially available (*28*). Some bacteria (fastidious) have complex nutrient needs, while most (nonfastidious) do not.

Culture media are generally one of four types: enrichment, nutritive, selective or differential.[4] As an example, most bacteriology specimens are inoculated onto plates containing sheep blood agar (a selective medium), because this medium supports growth for all but the most fastidious clinically significant bacteria (*28*). On the other hand, MacConkey agar is both a selective and differential medium and is used to isolate and differentiate lactose-fermenting and non-lactose-fermenting enteric bacilli. The suspected pathogen as well as tissue type informs laboratories about the type of media on which to culture the sample.

In addition to the importance of nutrients required for growth, environmental factors that influence bacterial growth are also important to consider. One of the primary distinguishing characteristics of bacteria is whether it grows aerobically (in the presence of oxygen), anaerobically (in the absence of oxygen), facultatively (in the presence or absence of oxygen) or microaerobically (in the presence of a less than atmospheric partial pressure of oxygen) (*29*). Therefore, the oxygen/CO_2 levels of culture media are critical. Temperature, pH and moisture conditions are also important. Temperature is controlled by incubating the medium, usually at 35–37 °C. Moisture levels may be controlled by using humidified incubators, while the appropriate pH is generally provided in commercially available plates/tubes.

If appropriate conditions are met, after approximately 12–24 hours of incubation bacterial colonies will develop sufficiently to be seen by the naked eye; standard incubation times, however, are up to 5 days, which is sufficient to recover the majority of bacteria, including fastidious bacteria (*30*).

Microscopic morphology

Microscopy confirms the presence of bacteria, allows detection of different organisms present in the same specimen and determines the organism's clinical significance (*28*). One of the initial steps in identifying bacteria is to determine their size and shape, which can be done quickly using direct microscopic examination of a specimen (from sterile and nonsterile body fluids, biopsies and positive cultures) on a wet mount slide; this will also determine whether the organism is a prokaryote, i.e., single celled.

[4] For a detailed discussion of culture media, see Tille (*28*).

Microscopy, enhanced by staining techniques, like Gram staining, is commonly used to differentiate among various types of bacteria based on the biochemical properties of their cell walls. It can detect nearly all clinically important bacteria and will indicate the Gram reaction of the organism, including its shape, whether it is acid fast, its motility and its cell arrangement. It will also indicate the bacteria's staining patterns, another important characteristic. Various types of microscopy, e.g., bright-field/light microscopy and fluorescence microscopy, may be utilized, and the method chosen will depend on the microorganisms to be detected (*28*).

Gram staining can also distinguish between gram-positive and gram-negative bacteria, the two major groups of bacteria, although some bacteria are morphologically indistinct. More specifically, Gram staining will generally allow the categorization of bacteria into one of four groups: gram-negative cocci, gram-positive cocci, gram-negative bacilli and gram-positive bacilli (*28*).

Because specimens often contain a low quantity of microbes, cultured specimens are generally used for bacterial ID and can be performed as soon as the culture is positive. For example, in suspected BSIs, blood specimens are collected and used to inoculate two standard bottles containing rich media (one aerobic bottle and one anaerobic bottle). As soon as the culture is positive, blood sample aliquots may be examined microscopically using a Gram stain.

Sometimes the Gram stain provides enough information to begin appropriate antimicrobial therapy while awaiting additional testing. For example, the Gram stain has been used effectively to diagnose urinary tract infections (UTIs), including those caused by *Escherichia coli* (*31,32*). But, in most cases, the Gram stain alone is not sufficient to definitively identify bacteria.

Macroscopic morphology

The macroscopic (colony) morphology of bacteria can also be examined via culture. The properties of an individual bacterial colony, including its form, size, elevation, margin/border, surface, opacity (e.g., glistening, opaque, dry), colour (pigment) and in some cases odour, can be examined. These characteristics provide clues to the identity of the bacteria; for example, colonies of streptococci are generally fairly small relative to many other bacteria, such as staphylococci, and may be identified in this way (*31*).

Biochemical tests

Testing of the nutritional and metabolic capabilities of a bacterial isolate is typically used to determine its genus and species (*31*). In other words, testing is done to determine the enzymatic capabilities of a given bacteria and to determine its ability to grow or survive in the presence of inhibitors, e.g., salts, surfactants, toxins and antibiotics. Biochemical test-based bacterial ID systems generally consist of single-enzyme-based tests to measure the presence of a specific enzyme and/or tests to determine a complete metabolic pathway that may contain several different enzymes.

Single enzyme tests are easy to perform and can be used to determine what subsequent bacterial ID steps should be performed. Certain of these tests, including the catalase test and the oxidase test, are very commonly used. The catalase test is important in the ID scheme of many gram-positive bacteria, whereas the oxidase test plays a similar role for gram-negative bacteria. Other commonly used single-enzyme-based tests include the following: indole, urease, PYR and Hippurate hydrolysis.

Tests to determine the metabolic pathways of bacteria fall into three general categories: carbohydrate oxidation and fermentation (to determine whether bacteria substrate utilization is an oxidative or fermentative process), amino acid degradation (to assess the ability of bacteria to produce enzymes that deaminate, dehydrolyse or decarboxylate certain amino acids) and single substrate utilizations (to determine whether bacteria can grow in the presence of a single nutrient or carbon source). The detection methods used for determining the end products of different metabolic pathways use colorimetry, fluorescence or turbidity.

In general, a battery of tests will be run. The number and types of tests chosen will depend on the type of bacteria to be identified, the clinical significance of isolates and the availability of reliable methods. In the end, a metabolic profile of the bacteria will be determined based on the results of the test battery, and this profile will be compared with an extensive ID database to establish the identity of the specific isolate.

Automated or semi-automated phenotypic testing methods

Essentially all of the steps involved in the phenotypic ID of bacteria described above can be done manually. There are, however, automated systems available for most elements of the process. These include commercially available systems for Gram staining specimens for microscopic examination, inoculation and specimen processing, bacterial culture and biochemical testing/metabolic profiling. Most of these systems are best suited to sophisticated, high-throughput laboratories. They are discussed below.

Automated Gram staining

Several automated Gram staining systems are commercially available. These include, but are not limited to, the PREVI® COLOR GRAM (bioMérieux, France), MULTISTAINER® (ALL.DIAG - BIOSYNEX, S.A.,

France), Aerospray® Gram series 2 (ELITechGroup Solutions, France) and QuickSlide™ GramPRO 1™ automated Gram stain instrument (Hardy Diagnostics, USA), each of which is described briefly below.

PREVI® COLOR GRAM

The PREVI® COLOR GRAM, pictured in Fig. 2, is a benchtop automated Gram staining system that uses a patented spray technology to provide rapid, standardized results for all specimen types; the system is closed and avoids cross-contamination. Standardized slides can be read in about 5 minutes. The system is high throughput, and at a minimum can process 12–120 slides per hour.

Fig. 2. PREVI® COLOR GRAM Gram staining platform

MULTISTAINER®

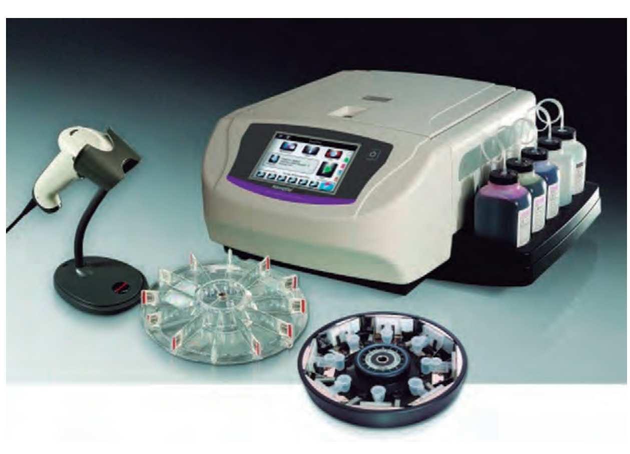
The MULTISTAINER®, pictured in Fig. 3, is a benchtop system that can perform most cold staining – e.g., Gram stain and acid-fast staining – with a capacity of up to 20 slides simultaneously. A Gram staining cycle takes approximately 5 minutes.

Fig. 3. MULTISTAINER®

Aerospray® Gram series 2

The Aerospray® Gram series 2, pictured in Fig. 4, is a benchtop staining system that can process more than 144 slides per hour. There is no cross-contamination with the system as specimens contact only fresh stain, precisely metered from separate spray nozzles. The cycle time is as short as 5 minutes.

Fig. 4. Aerospray® Gram series 2



QuickSlide™ GramPRO1™ automated Gram stain instrument

The QuickSlide™ GramPRO 1™ automated Gram stain instrument is one of several entirely automated instruments offered by Hardy Diagnostics. The GramPRO 1™ stains, decolourizes and counterstains each slide in 3.5–4.5 minutes. The system, pictured in Fig. 5, fits on a countertop. It has an easy user interface and operates on a push-button system with little hands-on time.

Fig. 5. QuickSlide™ GramPRO 1™ automated Gram stain instrument

Each of the automated gram staining instruments described above is easy to use, compact and could likely be used in Level II (and possibly Level I) laboratories in LMICs, but they are high-throughput instruments intended for use in laboratories where such capacity is required. In LMICs, this would most likely be in Level III settings and above.

Automated specimen processing and inoculation of media

Although specimen preparation and processing may be done manually, there are also commercially available, semi-automated or automated instruments that provide standardized specimen processing and inoculation of media. The systems automate four main steps: (i) selecting the appropriate petri dish; (ii) inoculating the sample; (iii) spreading the inoculum on agar plates to obtain, upon incubation, well separated bacterial colonies; and (iv) accurate labelling and sorting of each inoculated medium.

These are large systems most appropriate for use in high-throughput, sophisticated laboratories at Level III and Level IV in LMICs. Systems include the BD™ Innova automated microbiology specimen processor (Becton Dickinson [BD], USA), BD Kiestra™ InoqulA+™ (BD, USA), PREVI® Isola (bioMérieux, France) and Copan WASP® DT: Walk-Away Specimen Processor (Beckman Coulter, a Danaher Corporation, USA).

BD™ Innova automated microbiology specimen processor

The BD™ Innova automated microbiology specimen processor provides for complete automation of "front-end" processing of a variety of liquid samples. It can also streak various types of specimens without manual handling or changing components. The Innova, pictured in Fig. 6, is especially well suited to a high-volume laboratory due to its high capacity and extended walk-away time, although it can be used in smaller laboratories as well.

Fig. 6. BD™ Innova automated microbiology specimen processor

BD Kiestra™ InoqulA+™

The BD Kiestra™ InoqulA+™ is a specimen processor that can handle both liquid and nonliquid bacteriology specimens – swabs, urine and other nonfluid samples. The system's rolling bead technology ensures discrete bacterial colonies and standardized streaking patterns. The system, pictured in Fig. 7, is high throughput, processing up to 250–400 inoculations per hour.

Fig.7. BD Kiestra™ InoqulA+™

PREVI® Isola

The PREVI® Isola is an automated plate streaker that has five different-sized racks, one for each diameter of specimen tube. There are five input cassettes with a capacity of 30 plates in each stack; different agar plates can be loaded into each stack or each stack can hold the same type of agar. The PREVI® Isola, pictured in Fig. 8, processes a variety of specimens, including liquid specimens and swab systems with transport media such as liquid Amies medium to improve the diagnosis of aerobes, anaerobes and fastidious bacteria. The system offers consistent, automated and standardized inoculation/streaking, which optimizes bacterial colony isolation and eliminates risk of cross-contamination. The instrument is high throughput, processing 180 plates per hour, and as such is best suited to large laboratories.

Fig. 8. PREVI® Isola

Copan WASP® DT: Walk-Away Specimen Processor

The Copan WASP® DT is an open-platform, modular instrument for specimen processing, including streaking as well as Gram slide preparation and enrichment broth inoculation. Pictured in Fig. 9, the system employs specimen load and unload conveyers with different-sized pallets for different-diameter tubes; it uses a universal decapper that decaps and recaps different-sized containers without any manual interface. The WASP® DT uses metal loops with 1, 10 and 30 µL sizes to inoculate plates rather than pipette tips. Samples can be loaded continually onto the instrument without batching, and the system accommodates most specimen types, including swabs, urine, faeces, sputum, body fluids and pre-enrichment broths. Like the other specimen systems described above, the WASP® DT is most appropriate for large, sophisticated labs that require high throughput.

Fig. 9. Copan WASP® DT

For a laboratory considering the selection of any microbiology specimen processing instrument, the following factors should be included in the selection: accuracy, capacity, manufacturer's technical support, modularity, flexibility (e.g., specimen types, loops, inoculation protocols, medium options, interface, if any, with laboratory information systems and cost) (*33*).

Automated culture systems

Bacterial cultivation can be done manually, but a number of automated culture systems, which generally use blood or other sterile body fluids, are also available. The primary commercial culture incubation systems currently available are: BD BACTEC™ FX (BD, USA), BACT/ALERT® 3D (bioMérieux, France), BACT/ALERT® VIRTUO® (bioMérieux, France) and VersaTREK™ (Thermo Fisher Scientific, USA). These systems are discussed in more detail below.

Because in LMICs most bacterial culture is done in Level III and Level IV laboratories, the systems would be most appropriate for use in those facilities. Further, most of the systems described below are high throughput, and their selection and implementation should be driven by testing needs.

BD BACTEC™ FX

The BD BACTEC™ FX is a fully automated microbiology system designed to detect microbial growth from blood specimens. The system has a very sensitive fluorescent sensor of CO_2 production and a vial-activated workflow that allows for hands-off processing. It uses standard aerobic and anaerobic broth media specifically designed for small blood volume inoculation. The system has specific algorithms for fastidious organisms.

The most common configuration of the FX, pictured in Fig. 10, is a two-module system designed as a stack. The stack contains four drawers, each with a 100-vial capacity. Smaller-volume laboratories can choose a single, top-unit system. For high-volume capacity, multiple (up to 20) stack/top units can be seamlessly integrated into a single system using BD EpiCenter™.

Fig. 10. BD BACTEC™ FX

BACT/ALERT® 3D

The BACT/ALERT® 3D platform is used for detecting the presence or absence of microorganisms in blood and sterile body fluids. It is a culture system that uses the colorimetric sensing of CO_2 production, which is designed to detect bacteria (as well as fungi and yeasts) early, even with delayed entry of 24 hours or more depending on culture bottle type.

The BACT/ALERT® 3D is modular, as illustrated in Fig. 11, which enables flexible, ergonomic configuration to meet space limitations. The control module manages all bottle inventory and data, while the incubator module performs the testing. Modules can be configured to accommodate volumes of 7000–84 000 bottles yearly. The system can be configured with or without laboratory information system (LIS) connectivity.

Fig. 11. BACT/ALERT® 3D

BACT/ALERT® VIRTUO®

The BACT/ALERT® VIRTUO®, pictured in Fig. 12, is a more advanced and automated version of the BACT/ALERT® 3D blood culture system and uses the same detection principle – colorimetric detection by pH sensors of CO_2 produced by growing microorganisms (bacteria, fungi and yeasts). Improvements over the BACT/ALERT® 3D include incorporation of new instrument design to improve temperature stability, workflow improvement via automation of processes that are currently performed manually, an improved user interface and an enhanced proprietary algorithm to shorten time to detection of positive cultures.

Fig. 12. BACT/ALERT® VIRTUO® system

The VIRTUO® is a very high throughput system that holds 428 bottles; four instruments can be linked with a common loading area. The loading bay is motion activated with optical sensors to scan and automatically load BACT/ALERT® bottles via a conveyor belt and internal robotic arm. The scanning station rotates and images the entire BACT/ALERT® bottle for definitive ID from barcoded labels. Positive bottles are unloaded into an external chute automatically or on demand, and negative bottles are unloaded into a removable waste container at the end of the culture period. The instrument's software has automated detector cell quality control and calibration and may be connected to an LIS or to Myla middleware (bioMérieux) (*34*).

VersaTREK™

Thermo Fisher Scientific offers both manual and automated blood culture systems, including the only instrument that has four FDA-cleared tests on one platform for blood culture, sterile body fluids, mycobacteria detection and MTB susceptibility testing. VersaTREK™ is the only culture system capable of detecting any gas produced or consumed by organisms, not just CO_2, which means that it is able to detect a wider range of both common and fastidious bacteria.

The VersaTREK™ system, pictured in Fig. 13, uses only two bottles and uses VersaTREK™ REDOX™ media, which have distinct characteristics, including specimen draws as low as 0.1 mL and true direct-draw bottles, both of which are FDA cleared. The VersaTREK™ system features scan functions, simple bottle removal and is generally designed for ease of use.

Fig. 13. VersaTREK™

Biochemical testing

In addition to the commercial, automated or semi-automated culture systems described above, there are also commercially available manual and automated systems for biochemical testing to identify bacterial pathogens. In general, these systems simultaneously inoculate and incubate a series of miniaturized biochemical reactions based either on detecting bacterial enzymes or on cellular products that do not require bacterial growth. To identify bacteria, the enzymatic or biochemical results of the tests are combined and compared to the characteristics of bacterial organisms contained in a computer database. Some of these methods have reasonably rapid TAT, e.g., 2–4 hours,

compared to methods that require microbial growth, which require overnight incubation. But some of these assays still require 18–24 hours. Commercially available manual and automated systems for bacterial ID are described below. Note that diagnostic systems that both identify bacterial pathogens and perform AST are discussed later in this report.

Manual biochemical ID systems are typically easy to use and low cost relative to automated phenotypic and genotypic methods of pathogen ID. They do, however, require access to a computer database, usually via the Internet, for comparison of the characteristics of bacterial organisms found. In general, manual tests rely importantly on the skills of a well trained microscopist. Automated pathogen ID systems provide higher throughput as well as automatic analysis and comparison of pathogens, reducing the role of the microscopist. Results from either manual or automated systems are influenced by culture conditions. It is likely that automated biochemical ID systems, in particular, should not be implemented below Level III in the laboratory structure in LMICs for reasons of throughput, infrastructure requirements and human resource requirements.

Manual bacterial ID systems

API® (bioMérieux, France)

BioMérieux offers a family of test strips (Fig. 14) for manually identifying bacteria to the species level from microorganisms isolated in an appropriate culture medium. The test kits, which consist of microvials on a plastic strip that contain dehydrated substrates to demonstrate enzymatic activity or carbohydrate fermentation, can identify gram-positive and gram-negative bacteria as well as yeast. The system offers a large database which can be accessed through the Internet via the company's APIWEB™ service.

Fig. 14. API 20E test strips

The API® product line includes the following bacterial ID strips:

API gram-negative ID

- API 20E – 18–24-hour ID of *Enterobacteriaceae* and other nonfastidious gram-negative bacteria
- API Rapid 20E – 4-hour ID of *Enterobacteriaceae*
- API 20NE – 24–48-hour ID of gram-negative non-*Enterobacteriaceae*
- API NH – 2-hour ID of *Neisseria/Haemophilus*

API gram-positive ID

- API Staph – overnight ID of clinical staphylococci and micrococci
- RAPIDEC® Staph – 2-hour ID of commonly occurring staphylococci
- API 20 Strep – 4- or 24-hour ID of streptococci and enterococci

API anaerobe ID

- API 20A – 24-hour ID of anaerobes
- Rapid ID 32A – 4-hour ID of anaerobes.

BBL™ Crystal™ identification system (BD, USA)

The BD BBL™ Crystal™ identification system is a manual method of biochemical testing which is considered high complexity by the FDA. It utilizes miniaturized fluorogen- and/or chromogen-linked substrates to detect enzymes that bacteria use to metabolize a variety of substrates. The system, pictured in Fig. 15, consists of BBL Crystal panel lids, bases and inoculum fluid tubes. Only one step is required for inoculation, and the tubes provide a closed system when the bases and lids are snapped into place. Following the recommended incubation time, the vials are examined manually for colour changes or the presence of fluorescence. The resulting pattern of positive and negative test scores is used for bacterial ID with the BBL Crystal RGP (rapid gram-positive) database. Identification is derived from a comparative analysis of the reaction pattern of the test isolate to those contained in the database.

Fig. 15. BBL™ Crystal™ identification system

The BD™ BBL Crystal™ identification system is not intended for use directly with clinical specimens, but rather from culture isolates. It includes the following assays:

- Enteric/nonfermenter ID kit: an overnight ID method for identifying clinically significant aerobic gram-negative *Enterobacteriaceae* isolates and non-fermenting gram-negative rods;
- *Neisseria/Haemophilus* ID kit: a 4-hour ID method for identifying *Neisseria*, *Haemophilus* and other fastidious bacteria;
- Gram-positive ID kit: an 18-hour ID method for identifying both gram-positive cocci and bacilli;
- Rapid Gram-positive ID kit: a 4-hour ID method using conventional, fluorogenic and chromogenic substrates to identify gram-positive bacteria isolated from clinical specimens; and
- Anaerobe ID kit: a 4-hour ID method for identifying clinically significant anaerobic organisms.

RapID™ systems (Thermo Fisher Scientific, USA)

Thermo Fisher Scientific's RapID™ systems (Fig. 16) comprise microbial ID systems based on enzyme technology. These are manual systems that simplify identifying bacteria and other microorganisms from cultured specimens with TAT of 4 hours.

Fig. 16. RapID™ system

Although the systems are manual, RapID™ requires no oil and no pipetting; the systems produce visible colour reactions. Time to result is 4 hours. The product line includes, but is not limited to:

- RapID™ ONE system: identifies over 70 medically important oxidase-negative, gram-negative bacilli;
- RapID™ ANA II system: identifies over 90 clinically important anaerobes;
- RapID™ NH system: identifies 30 taxa, including *Neisseria*, *Moraxella*, *Haemophilus* and related microorganisms;
- RapID™ NF PLUS system: identifies over 70 clinically important oxidase-positive, gram-negative bacilli, including *Vibrio* spp.;
- RapID™ STAPH PLUS system: identifies 40 different staphylococci and related genera;
- RapID™ STR system: identifies streptococci and related genera; and
- RapID™ SS/u system: identifies commonly isolated urinary tract pathogens in 2 hours.

RapID™ systems are paired with ERIC™ software and databases for faster ID of bacteria. The databases are updated regularly to provide better reporting coverage for thorough analysis.

Oxoid™ Microbact™ biochemical systems (Thermo Fisher Scientific, USA)

Oxoid™ Microbact™ biochemical systems (Fig. 17) are manual systems currently available for identifying gram-negative organisms, *Staphylococcus aureus* and *Listeria* from pure culture isolates. ID is based on pH changes in various substrates and substrate utilization tests. The systems are designed for use with Microbact™ software, which features an expanded and regularly updated database compatible with 64-bit computer systems. Kits include:

- Microbact™ GNB kits: identify *Enterobacteriaceae* and common miscellaneous gram-negative bacilli. TAT is between 24 and 48 hours; and

Fig. 17. Microbact™ biochemical identification kit 24E for gram-negative bacteria

- Oxoid™ Microbact™ Staphylococcal 12S kit: identifies staphylococci, including *Staphylococcus aureus* and coagulase-negative staphylococci (CNS). TAT is 24–36 hours.

Automated bacterial ID systems

Biolog microbial identification systems (Biolog, Inc., USA)

Biolog offers three microbial ID systems: the OmniLog ID, a fully automated system (pictured in Fig. 18); the MicroStation ID, a semi-automated system; and the MicroLog M system, a manual ID system. Each system is capable of identifying bacteria, yeast and fungi, and all use the GENIII MicroPlate test panel, which is capable of identifying both gram-negative and gram-positive bacteria at the same time on a single panel. Gram stain and other pretests are not required on the systems.

Fig. 18. OmniLog ID system

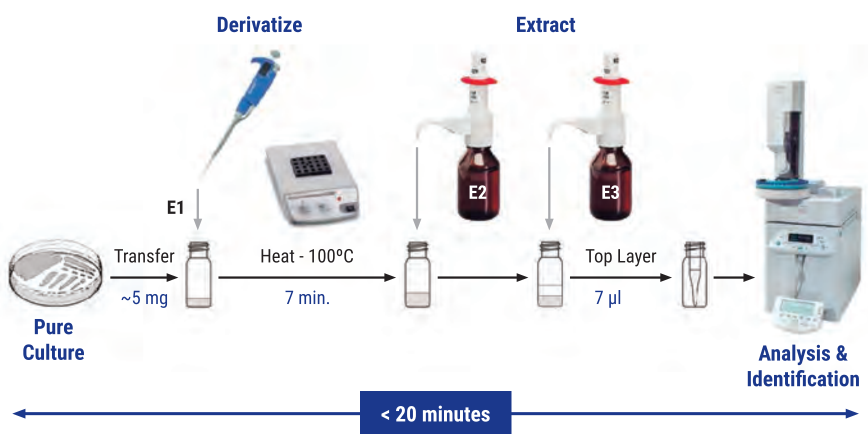
Biolog systems use oxidation-reduction chemistry by which GENIII dissects and analyses the ability of a cell to metabolize all major classes of biochemicals, in addition to determining other important physiological properties such as pH, salt and lactic acid tolerance. Biolog systems produce a characteristic pattern or "metabolic fingerprint" from discrete test reactions performed in a 96-well microplate. Culture suspensions are tested with a panel of preselected assays, then incubated, read and compared to databases of human pathogens using the company's RetroSpect 2.0 software tool. Depending on the panel run, TAT is 4–6 hours or 16–24 hours (*35*).

Sherlock™ microbial identification system (MIDI, Inc., USA)

The Sherlock™ microbial identification system (MIS) analyses and identifies microorganisms isolated in pure culture or artificial media. The MIS uses a sample preparation procedure and gas chromatography (GC) analysis of extracted microbial fatty acid methyl esters (FAMEs) to yield qualitatively and quantitatively reproducible fatty acid composition profiles. Fatty acids extracted from unknown bacteria are automatically quantified and identified by the Sherlock software to determine the fatty acid composition. The fatty acid profile is then compared to a library of profiles of reference strains stored in the computer to determine the identity of the bacteria.

The recently introduced Sherlock™ E-FAME™ assay, an acid-catalysed GC-FAME extraction and analysis method, is used to identify clinically important aerobic bacteria in less than 20 minutes. As illustrated in Fig. 19, the system is labour intensive and is designed for use in reference laboratories.

Fig. 19. E-FAME™ bacterial identification system workflow



216Dx UTI system (BacterioScan, USA)

BacterioScan has developed the 216Dx UTI system, which is a compact, semi-automated in vitro diagnostic system that uses forward laser light scattering (FLLS), analysing the angular variation in the intensity of the scattered light to measure bacterial growth directly from urine specimens incubated in trypticase soy broth. The BacterioScan 216Dx is for the qualitative determination (presumptive positive or presumptive negative) of bacteriuria at a defined cut-off, and is intended for use in conjunction with other clinical and laboratory findings to aid in diagnosing UTIs. The BacterioScan 216Dx has a limited scope as it is not intended to provide bacteriuria levels, bacterial ID or differentiation; presumptive positive urine samples must be cultured (as must presumptive negative urine samples if a low level of bacteriuria is suspected and is clinically relevant).

The BacterioScan 216Dx system, pictured in Fig. 20, consists of an instrument and handheld barcode scanner, a network appliance computer with preloaded software, required power and interconnecting Ethernet cable(s). BacterioScan provides single-use, disposable cartridges and multicuvettes for use in the system. One multicuvette contains four cartridge wells and can be used to test up to four samples; up to four multicuvettes can be processed by the 216Dx in a single run – or 16 samples total.

Fig. 20. BacterioScan 216Dx and multicuvettes

The 216Dx, which is FDA cleared, examines changes in optical signals which represent bacterial growth in the urine samples over a 3-hour period. Once the testing is complete in the multicuvette, the analysis is available.

It should be noted that despite advances with respect to automated specimen processing, plating and biochemical testing, nonautomated methods are often still needed for unusual pathogens or fastidious microorganisms that fail to grow. In addition, some clinical isolates of microorganisms may produce a biofilm or be too viscous for automated instruments, which will result in the inability to make an ID (*28*).

Conclusion

Although the phenotypic methods of bacterial ID described above are still the backbone of diagnostic bacteriology, there are drawbacks to their use that affect LMICs significantly. The test methods are slow and, when done manually, cumbersome. The growth of bacteria in culture, for example, can take days to obtain results, especially for fastidious or slow-growing bacteria. In addition, there are operational challenges to performing bacterial culture in LMICs. These include, but are not limited to, inconsistent electricity, dust, lack of climate control and human resource constraints (*36*, *37*).

As indicated above, bacterial cultivation for pathogen ID is generally limited to Level III and Level IV laboratories in LMICs. Even the use of manual phenotypic methods of bacterial ID requires well trained and highly skilled microscopists, who are often not available at Level II settings and below. Automated systems are often high throughput and require suitable infrastructure to accommodate the instrumentation, including appropriate space, consistent power supply, climate control, running water and access to transport. These capabilities and requirements also suggest the use of these phenotypic testing methods at Level III and above.

Recently, Ombelet and colleagues proposed a framework for implementing clinical bacteriology that would be suitable for use in basic diagnostic laboratories operated by technicians without expertise in microbiology in LMICs (*37*). This approach, together with using existing diagnostics (e.g., manual bacterial ID and biochemical test methods) and improved algorithms, could make clinical bacteriology possible in Level II facilities. In addition, however, simpler, faster methods of bacterial pathogen ID are needed. Immunoassay and molecular testing to identify bacterial pathogens are alternatives and are discussed next (*3*).

Immunoassay methods of identifying bacterial pathogens

Rapid immunoassays, which use the binding of antibodies to antigens to identify and measure certain substances, are also available for detecting bacterial pathogens. The tests below, all of which are rapid diagnostic lateral flow assays which, unless otherwise indicated, require little or no ancillary equipment, are a selection of commercially available immunoassays relevant to ABR. While these tests can and are used in Level I settings in LMICs, it is important to consider the performance of each assay to determine whether its sensitivity and specificity are adequate for the intended use of the test and whether its ease of use is appropriate for the intended setting. Also, given

the frequent need to perform syndromic testing on patients presenting at Level I and Level II settings – e.g., for acute febrile illness, respiratory and enteric infections, among others – it is important to note that most lateral flow assays can only perform limited multiplexing. In addition to the physical limitations of such tests, there are also technical challenges, the most important of which is potential cross-reactivity (*38*, *39*).

- Oxoid™ PBP2′ latex agglutination test (Thermo Fisher Scientific, USA): a rapid latex slide agglutination test for detecting PBP2' from culture as an aid in identifying methicillin-resistant *Staphylococcus aureus* (MRSA) and in CNS. TAT is approximately 3 minutes after positive culture. The assay is FDA cleared.
- Clearview® Exact PBP2a test (Abbott, USA): a rapid immunochromatographic qualitative assay for detecting PBP2a direct from *Staphylococcus aureus* culture isolates as an aid in detecting MRSA. TAT is approximately 5 minutes after positive culture. The assay is FDA cleared.
- *RAPID*™ Hp StAR™ (Thermo Fisher Scientific, USA): an immunochromatographic assay for detecting *Helicobacter pylori* antigen in human stool samples. TAT is 15–20 minutes. The assay is CE marked.
- RAPIRUN® *H. pylori* antibody detection kit (Otsuka Pharmaceutical Co., Ltd., Japan): a rapid immunochromatographic assay intended for qualitatively detecting antibodies against *Helicobacter pylori* in urine to aid in diagnosing the infection. TAT is about 20 minutes. The assay is FDA cleared.
- Immuno*Card* STAT!® CAMPY (Meridian Bioscience, Inc., USA): an immunochromatographic rapid test for qualitatively detecting specific *Campylobacter* antigens (*C. jejuni* and *C. coli*) in human stool, where stool may be either unpreserved or preserved in Cary-Blair-based transport media. TAT is 20 minutes. The assay is CE marked.
- *C. DIFF QUIK CHEK COMPLETE*® (Abbott, USA): a rapid membrane enzyme immunoassay for simultaneously detecting *Clostridium difficile* glutamate dehydrogenase antigen and *C. difficile* Toxin A (TcdA) and *C. difficile* Toxin B (TcdB) in a single reaction well. The test is to be used as an aid in diagnosing *C. difficile* infection (CDI). TAT is less than 30 minutes. The assay is FDA cleared.
- Xpect™ C. difficile Toxin A/B test (Thermo Fisher Scientific, USA): a rapid in vitro immunochromatographic assay for the direct, qualitative detection of TcdA and/or TcdB in human faecal specimens from patients suspected of having CDI. The test is intended as an aid in diagnosis. TAT is 20 minutes. The assay is FDA cleared.
- Immuno*Card*® Toxins A&B (Meridian Bioscience, Inc., USA): a rapid, qualitative, horizontal-flow enzyme immunoassay for detecting TcdA and TcdB in human stool. The test is to be used as an aid in diagnosing CDI. TAT is about 15 minutes. The assay is CE marked.
- BioStar® OIA GC (Thermo Fisher Scientific/BioStar, USA): a rapid optical immunoassay test for qualitatively detecting gonococcal antigen in female endocervical swab and male urine specimens. The test is intended for use as an aid in identifying the presence of NG antigen. TAT is 25–40 minutes.
- BinaxNOW® *S. pneumoniae* Antigen Card (Abbott, USA): a rapid, qualitative in vitro immunochromatographic assay that detects the C-polysaccharide antigen of *Streptococcus pneumoniae* in 15 minutes from human urine. It is intended as an aid in diagnosing community-acquired pneumonia (CAP). The test can be read visually or with the use of the DIGIVAL reader. The assay is CE-IVD marked.
- BIOSYNEX *S. pneumoniae* (BIOSYNEX, S.A., France): a rapid immunochromatographic test for detecting *Streptococcus pneumoniae*-specific antigen in urine and cerebrospinal fluid (CSF). It is intended as an aid in diagnosing CAP. TAT is 15 minutes. The test can be read visually or with the use of the BIOSYNEX Flexireader®. The assay is CE-IVD marked.
- Typhidot® (Malaysian Biodiagnostic Research, Malaysia): a qualitative enzyme-linked immunosorbent assay (ELISA) in vitro diagnostic assay for detecting *Salmonella typhi* in serum. It detects either immunoglobulin M (IgM) or immunoglobulin G (IgG) antibodies against a specific antigen on the outer membrane protein of *S. typhi*. For specimens that are indeterminate (IgM negative and IgG positive), a confirmatory test, Typhidot-M, is recommended by the manufacturer. TAT is 60 minutes.
- TUBEX® (IDL Biotech, Sweden): a semi-quantitative in vitro diagnostic Inhibition Magnetic Binding Immunoassay (IMBI) assay that tests for antibodies against *Salmonella typhi* lipopolysaccharide (LPS) antigen in serum by quantifying the inhibition of binding between O9 monoclonal antibodies and LPS-coupled magnetic particles. A visible decolourization of the serum in the test reagent solution through magnetic particle separation indicates a positive result.

Samples are graded 0 to 10 according to the colour reaction; those with a grade greater than 2 are considered positive. TAT is 3 minutes. The assay is CE-IVD marked.

- Wellcogen™ Haemophilus influenzae b Rapid latex agglutination test (Thermo Fisher Scientific, USA): a rapid latex diagnostic test for the qualitative detection of antigen from *Haemophilus influenzae* type b from CSF, serum, urine or blood cultures. TAT is 3 minutes. The assay is CE-IVD marked.
- O157 Coli-Strip (Coris BioConcept, Belgium): a qualitative in vitro immunochromatographic assay for detecting *Escherichia coli* O157 bacteria in stool specimens (after broth enrichment). TAT is 15 minutes. The assay is CE-IVD marked.
- Immuno*Card* STAT!® *E. coli* O157 Plus (Meridian Bioscience, Inc., USA): a rapid, qualitative, horizontal-flow enzyme immunoassay for detecting *Escherichia coli* O157:H7 in stool specimens or culture (broth enrichment or plate culture). TAT is less than 20 minutes. The assay is CE-IVD marked.

Molecular methods of identifying bacterial pathogens

Molecular testing methods, which detect specific sequences of nucleic acids in DNA and RNA, have substantially changed the microbiological diagnosis of pathogens over the last decade. In particular, nucleic-acid-based test methods are widely used in clinical laboratory diagnostics. Molecular methods generally fall into one of three categories: (i) hybridization, (ii) amplification or (iii) sequencing (*40*).

Hybridization methods

Hybridization assays use labelled oligonucleotide probes, which can confirm ID by culture or can directly detect microorganisms. For example, probe hybridization can be useful for identifying slow-growing bacteria after isolation in either liquid or solid media. Although this method displays high specificity, it requires a large number of target cells to achieve high sensitivity.

Fluorescence in situ hybridization (FISH) is a method that allows rapid detection and ID of the genus and species of bacteria (as well as yeasts and protozoa), combining the speed and ease of use of conventional bacterial staining methods with the specificity of molecular methods (*41*). The technique involves fluorescent-labelled probes that hybridize with target sequences of ribosomal RNA (rRNA); the probes fluoresce upon binding to the target and are detected by fluorescent microscopy (*41*).

In general, FISH methods have a short time to result (60–90 minutes) and hands-on time of about 15–20 minutes. FISH can be used to detect bacteria and other microorganisms in primary specimens (i.e., tissue samples), which also shortens the time to result, and it can be used to identify certain antimicrobial drug resistance, primarily in enterococci (*41*). FISH requires little equipment, but it does require experienced and well trained technicians, including a knowledgeable microscopist. In addition, detecting bacteria using FISH requires a targeted approach – meaning that the nature of the bacterial infection must be anticipated before probes are chosen.

There have been a number of innovations and improvements on the basic FISH method since its introduction in 1980, one of which replaces standard DNA and RNA nucleic acids with a synthetic peptide nucleic acid (PNA) probe (*41*). So-called PNA-FISH reduces the number of steps in the test procedure and improves its specificity, although it is still laborious because individual probes have to be created for each bacterial species. PNA-FISH has also been standardized, and FDA-approved assays are available.

PNA-FISH requires only a microscope equipped with a fluorescent lamp and dual band filters for interpreting results. Therefore, the capital equipment cost of implementing the technology is low, and the technique is relatively easy to perform in a clinical laboratory. PNA-FISH does, however, require an accurate choice of PNA-FISH probe, which is contingent upon the correct interpretation of a Gram stain. As mentioned above, good performance of FISH methods generally requires an experienced and highly trained microscopist. In addition, the commercialized PNA-FISH methods described below require culture. Collectively, these factors suggest that PNA-FISH could be performed in LMICs, but likely only at Level III and above facilities.

AdvanDx (OpGen, USA)

OpGen offers two AdvanDx FISH product lines, AdvanDx PNA FISH® and *Quick*FISH®, both of which are for the early pathogen ID of BSIs (bacteria and yeast) from positive blood cultures. The tests utilize FISH with PNA probes. Results are available about 48–72 hours earlier than using conventional phenotypic methods. AdvanDx PNA FISH® offers the following tests: *S. aureus*/CNS, *E. faecalis*/OE (other enterococci), gram-negative organisms and *Candida*. *Quick*FISH® offers tests for *Staphylococcus*, *Enterococcus*, gram-negative organisms and *Candida*. The *Quick*FISH® procedure is illustrated in Fig. 21. A number of the available tests are FDA cleared and CE-IVD marked.

Fig. 21. *Quick*FISH® procedure

Quick and Easy Procedure

3 Easy Steps: Fix Sample, Hybridize Probes and View Results.
5 Min. Hands-on Time. 20 Min. Turn-around Time.

Fix

5 Min.
Fix 10 µl of Blood Culture Sample to *Quick*FISH Slide.

Hybridize

15 Min.
Add PNA Reagents. Hybridize for 15 Min. at 55°C.

Examine

View Results
Examine on Fluorescence Microscope (60x or 100x Oil Objective).

Amplification methods

This section of the landscape sets out the various molecular amplification methods to detect bacteria and other pathogens. Unless otherwise noted, there are commercially available platforms relevant to bacterial pathogen ID using each of the methods described. In this report, all of the test methods are described first; applicable commercial platforms are then described in some detail.

Although molecular hybridization methods are highly specific for bacterial detection and ID, their sensitivity is limited, particularly for fastidious organisms. Also, fluorescent imaging techniques require sufficient quantities of target sequences to generate adequate signal-to-noise ratios and reduce the probability of false positive results. In contrast, molecular methods that amplify target nucleic acid reaction in an organism can enhance bacterial pathogen detection and ID without sacrificing specificity (*28*). NAATs work on this principle.

Amplification methods are either designed to increase the number of target molecules (selected sections of DNA or RNA) to a level that permits detection (target amplification methods) or are aimed at increasing the signal generated by the method (signal amplification methods). The majority of commercially available NAAT platforms today are based on target amplification.

Regardless of whether a NAAT assay is based on target amplification or signal amplification, it will consist of the following three steps: (i) pre-amplification sample preparation and/or nucleic acid extraction; (ii) amplification of either the nucleic acid target or detection signal; and (iii) post-amplification detection and/or quantification of the amplified nucleic acids.

Pre-amplification. Pre-amplification methods (sample preparation and/or nucleic acid extraction) are critical to the NAAT process. For each sample to be analysed correctly and to produce an accurate result, the nucleic acid must be both available for the reaction and purified. Protocols for pre-amplification steps include purifying the sample for specific organisms or cells, extracting nucleic acids and capturing target sequences in specimens. Molecular methods are sensitive to the extraction and processing steps prior to amplification; prompt processing of samples, rapid extraction methods and appropriate storage of specimens lead to improved sensitivity of the assays.

Amplification. There are several amplification methods used to detect RNA or DNA after preparing samples. In target amplification, the target sequence is identified and millions of copies of a portion of the nucleic acid are synthesized via an amplification reaction; in effect, this method enhances the ability to detect very low levels of nucleic acids that occur naturally in the specimen. In signal amplification methods, the reporter molecule generates a signal that is amplified. Some benefits to signal amplification include higher specificity and the ability to conduct isothermal reactions that do not require thermocycling.

Post-amplification. Finally, post-amplification methods require the detection and/or quantification of either the amplification products (in target amplification methods), called amplicons, or the signals

that have been generated (in signal amplification methods). Detection can be achieved using any one of a number of reagents, e.g., colorimetric, radioactive or fluorescence. Detection can either be done at the endpoint of the process (after a fixed number of amplification cycles) or in "real time" (after each amplification cycle).

Specific molecular methods of amplification and detection commonly used in commercial NAAT platforms are described below.

Polymerase chain reaction (PCR)

Of the NAAT-based molecular methods, PCR, which was first introduced by Kary Mullis as a research tool in 1983, is the most common method used and "forms the backbone of molecular diagnostics in clinical microbiology" (*42*). PCR is an enzymatic process that exponentially amplifies a single copy of a nucleic acid target (selected sections of DNA), which may be undetectable by standard hybridization methods, to 10^7 or more copies in a relatively short time. There are three primary steps in conventional PCR: (i) denaturing double-stranded DNA at 95 °C; (ii) binding (annealing) PCR primers to the target sequence at 50–60 °C; and (iii) extending and polymerizing nucleic acids to the primer at 72 °C to generate amplicons of the target sequence (*43*). These steps are followed sequentially over multiple cycles ("thermocycling"); each cycle exponentially increases the amount of amplicon, as each amplicon serves as a template for additional amplification. Following amplification (i.e., a predetermined number of cycles), amplicons are available in sufficient quantities to be detected/visualized by fluorescence, hybridization or other methods. Detection requires using a labelled probe specific for the target sequence in the amplicon; this allows the amplicon to be visualized and ensures that the amplicon is the target sequence of interest (*28*).

Over the years, there have been variations and improvements on basic PCR-based NAAT. These methods include nested PCR, which can achieve greater specificity; quantitative PCR (qPCR), which quantitates the number of targets in a specimen; and digital PCR (dPCR), used to directly quantify and clonally amplify nucleic acid strands (DNA or RNA). Also of note, these methods include real-time PCR and multiplex PCR, which are discussed in more detail below.

Real-time PCR

Real-time PCR arguably has had the greatest impact on detecting and identifying human pathogens in clinical microbiology laboratories (*44*). This method monitors the quantity of amplicons over time (after each cycle) rather than at the end-point of the reaction; this real-time monitoring of the amplicon enables mathematical extraction of the starting concentration of target sequence (quantification). The method combines thermal cycling (repeated heating and cooling cycles) with fluorescent probes which bind to the amplicons as they are generated in the same reaction vessel; as such it is a closed system, which minimizes the risk of contamination. Since signal detection is monitored in real time, real-time PCR often leads to faster results than end-point PCR.

Multiplex PCR

Also of note is the development of multiplex PCR testing, which combines a number of primer pairs into a single PCR for simultaneously detecting several targets. This allows for the inclusion of control primers as well as test primers that can be directed to a sequence specific to the particular organism or gene of interest. This approach is particularly useful in testing patients presenting with symptoms that could be attributable to a number of different pathogens – e.g., for use in patients with symptoms of an upper respiratory infection or enteritis.

A number of different PCR-based instruments and detection probe formats are available commercially (*40*). Many of these are real-time systems and some are capable of multiplexing.

Non-PCR-based molecular methods – isothermal amplification

Non-PCR-based molecular methods include signal amplification (e.g., branched DNA) and some methods that use both target and signal amplification. But, in recent years, amplification techniques have turned to isothermal methods of nucleic acid amplification, or iNAATs, which eliminate the need for the rapid thermal cycling required by PCR-based techniques and can be more specific due to the non-temperature dependence of the reactions (*45*). They can also be combined with other detection technologies, e.g., fluorescent-probe-independent methods that eliminate the requirement of sophisticated optics. The most common iNAAT methods for which commercial platforms are available are described briefly below.

Transcription-mediated amplification (TMA) and nucleic acid sequence-based amplification (NASBA)

Unlike PCR-based testing, TMA and NASBA amplify RNA rather than DNA. They use reverse transcriptase (RT) replication mechanisms to produce a modified complementary DNA molecule (cDNA) from an RNA template, which is then rapidly amplified into RNA amplicons; in other words, they effectively imitate in vivo retroviral replication

mechanisms to produce RNA amplicons from the RNA template (*46*). To date, most assays using NASBA or TMA target a single or a few analytes, using one or only a few oligonucleotide primer sets (*46*). Both methods can be used for identifying microorganisms.

Loop-mediated isothermal amplification (LAMP) and helicase-dependent amplification (HDA)

LAMP amplifies DNA under isothermal conditions. It generally uses four specifically designed primers to recognize six different areas of the DNA target combined with strand displacement activity. In brief, unlike PCR, which uses heat to denature and anneal primers to the target sequence, LAMP relies on complex binding kinetics and physical proximity of the target sequences and primers in a loop in order to generate a single-strand template without the need for heat denaturation (*42*, *46*). Although high levels of amplicons can be generated at 60–65 °C and are generally achieved in less than an hour, sensitivity and specificity are reduced with LAMP.

HDA uses thermostable helicase enzymes to effect DNA strand separation. Once separated, single-stranded DNA binding proteins stabilize the single strands to allow binding of the PCR primers. DNA polymerase extends the primers, and the newly synthesized DNA duplexes serve as templates that are then hybridized by sequence-specific primers for further amplification cycles (*28*, *41*, *42*). Exponential amplification can be achieved at a single amplification temperature (60–65 °C), generally in 60–90 minutes (*40*).

Strand displacement amplification (SDA)

SDA uses bifunctional primers that incorporate both target recognition and endonuclease target regions. First, endonucleases make cuts or nicks at a specific site in the target sequence; then strand-displacing DNA polymerase, typically *Bst* DNA polymerase, Large Fragment (the original polymerase for LAMP), is used to initiate replication (*41*). The nicking site is then regenerated with each polymerase displacement step, and DNA is exponentially amplified. Amplification takes place at a temperature range of 55–59 °C.

In addition to the isothermal methods of nucleic acid amplification described above, there are additional methods described in the literature. These include the nicking enzyme amplification reaction (NEAR), recombinase polymerase amplification (RPA), signal-mediated amplification of RNA technology (SMART), rolling circle amplification (RCA), isothermal multiple displacement amplification (IMDA), single primer isothermal amplification (SPIA) and circular helicase-dependent amplification (cHDA).[5] These methods are not described in detail in this report because currently no commercialized platforms utilize them.

Commercially available platforms using molecular amplification technologies for detecting bacterial pathogens

Below are some of the major commercial IVD platforms that use molecular amplification techniques to identify bacterial and other pathogens. Generally, in the early years NAATs were focused on detecting viruses; real-time PCR has paved the way for their use in detecting bacterial pathogens (*40*). Most tests are qualitative, and until recently, they were monoparametric – one analyte per assay (*40*). Only in recent years have multiparametric NAATs been developed, in particular for detecting BSIs direct from whole blood specimens, as opposed to blood cultures.

The commercial NAAT platforms described below are automated and have single and/or multiparametric assays for detecting bacterial pathogens; many of them have separate automated instruments to perform sample preparation and nucleic acid extraction. Except where indicated that the platform is available only for laboratory-developed tests (LDTs), the assays are standardized and available in quality-assured kits. For the most part, these platforms are designed for use in sophisticated laboratories with highly trained laboratorians where high throughput is needed. As such, the platforms are not well suited for use at or near POC; where platforms are potentially appropriate for near-patient testing, it is indicated. This is a nonexclusive list of NAAT platforms, which is limited to commercial platforms that can identify at least one bacterial pathogen on the prioritized list in Annex I.

Abbott *m*2000 system (Abbott, USA)

Abbott manufactures the Abbott RealTi*m*e CT/NG assay, which is a real-time PCR assay for direct, qualitative detection of the genomic DNA (gDNA) of NG and the plasma DNA of CT on its automated *m*2000 and *m*24 systems. The CT/NG assay is currently the only one on the *m*2000 system targeted at detecting a WHO priority bacterial pathogen.

The assay may be used to test the following swabs from symptomatic patients: female endocervical

[5] For details of these methods, see Gill and Ghaemi (*45*).

swabs, clinician- or patient-collected vaginal swabs; male urethral swab specimens; and male and female urine swabs. The assay may also be used to test the following swabs from asymptomatic patients: clinician- or patient-collected vaginal swabs; and male and female urine swabs.

Additional RealTi*me* assays offered by Abbott for the *m*2000 system include cytomegalovirus (CMV), human immunodeficiency virus-1 (HIV-1 Quantitative), hepatitis B virus (HBV), hepatitis C virus (HCV), HCV Genotyping II and Zika virus (Emergency Use Authorization test).

The Abbott RealTi*me* CT/NG assay (and the other assays listed above) can be automated using the Abbott *m*2000*rt* for amplification and detection and one of three methods for sample preparation: (i) manual (for laboratories with low-throughput requirements); (ii) the *m*24*sp* instrument (for laboratories with low- to medium-throughput requirements); or (iii) the *m*2000*sp* instrument (for laboratories with medium- to high-throughput requirements).

The *m*24*sp* pictured in Fig. 22 is a benchtop sample preparation and extraction device with a small footprint that is generally appropriate for facilities with medium-throughput requirements. It provides a variable extraction system (extraction output can be stored either in deepwell trays or 1.5 mL tubes) with ready-to-use and reusable reagents as well as flexible batch size capabilities.

Fig. 22. Abbott *m*24*sp* instrument

The *m*2000*sp* by Abbott (pictured on the left in Fig. 23) is a larger and more automated sample preparation device than the *m*24*sp*. With complete automation comes increased walk-away time for the operator. It is a high-throughput system with a maximum batch size of 96 samples per run; reagents and tips required for extraction are loaded manually by the operator. When combined with the Abbott *m*2000*rt*, the amplification and detection instrument, the system can provide automation from barcoded laboratory tube through to patient result.

Fig. 23. Abbott *m*2000 system: *m*2000*sp* (left) and *m*2000*rt* (centre)

The Abbott *m*2000*rt* is the amplification and detection platform for use with manual extraction, the *m*24*sp* and the *m*2000*sp* instruments, as described above. It is a high-performance system, but is relatively compact, weighing just over 75 lbs. The *m*2000*rt* (pictured in Fig. 23, centre) can run 96 samples at a time in about 3 hours of cycling time (not including time for sample preparation). The system will run both quantitative and qualitative analyses.

cobas® 6800/cobas® 8800 systems (Roche Molecular Diagnostics [Roche], USA)

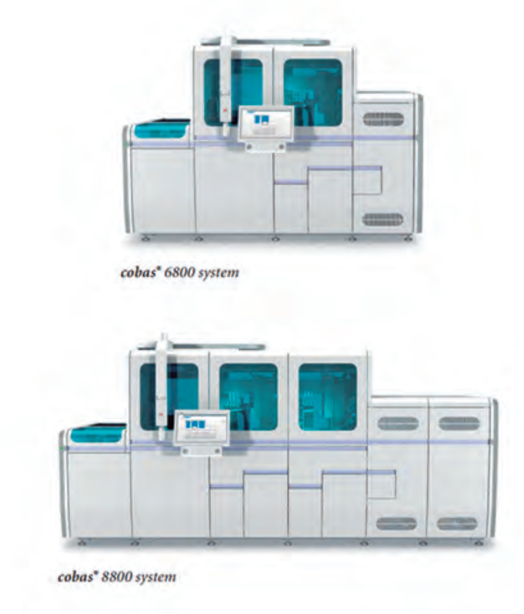
Roche offers the cobas® CT/NG assay for use on cobas® 6800/8800 systems. The assay is an automated, qualitative in vitro NAAT that utilizes real-time PCR for direct detection of CT and/or NG DNA in male and female urine, clinician-instructed self-collected vaginal swab specimens (collected in a clinical setting), clinician-collected vaginal swab specimens and endocervical swab specimens, all collected in cobas® PCR Media, and cervical specimens collected in PreservCyt® solution. The test is intended as an aid in diagnosing chlamydial and gonococcal disease in both symptomatic and asymptomatic individuals.

With respect to the cobas® CT/NG assay, target-specific primers and two probes are used to detect, but not discriminate between, the CT cryptic plasmid and the *ompA* gene. Additionally, target-specific primers and two probes are used to detect, but not discriminate between, two conserved sequences in the NG DR-9 region. A DNA internal control, used to monitor the entire sample preparation and PCR amplification process, is introduced into each specimen during sample processing.

The cobas® 6800/8800 systems, pictured in Fig. 24, are CE-IVD marked, but not FDA cleared; they are therefore not currently available in the United States.

The systems offer the fastest time to results with the highest throughput available. The cobas® 6800/8800 systems are fully automated solutions designed for donor screening, viral load monitoring, women's health and microbiology testing.

Fig. 24. cobas® 6800 and 8800 systems



The 6800/8800 systems are available in medium- and high-throughput models. Each system provides results for the first 96 tests in less than 3.5 hours, with the 6800 system delivering up to 384 results in an 8-hour shift, and the 8800 system generating up to 960 results in the same amount of time. Both systems also allow for simultaneous processing of multiple assays and are designed to enable up to 8 hours (cobas 6800®) and 4 hours (cobas 8800®) of walk-away time with minimal user interaction.

In addition to the CT/NG assay, additional assays available for the 6800/8800 platforms include the quantitative cobas® HIV-1, HBV and HCV assays.

cobas® 4800 system (Roche, USA)

Roche manufactures a qualitative multiplex NAAT, the cobas® 4800 CT/NG, which detects DR-9, a direct repeat region and target of the NG assay. It also simultaneously detects two CT independent DNA targets – one in the cryptic plasmid and the other on the CT genome. This design can detect infections caused by wild-type CT, the Swedish variant (nvCT) and other *Chlamydia* strains that may harbour deletions in the cryptic plasmid, or those that do not carry the cryptic plasmid. Approved samples include DNA in endocervical swab specimens, clinician-collected vaginal swab specimens, clinician-instructed self-collected vaginal swab specimens, and male and female urine in cobas® PCR Media.

The cobas® 4800 CT/NG test utilizes amplification of target DNA by PCR and nucleic acid hybridization to detect these pathogens, and is intended to be used as a diagnostic as well as a screening tool in both symptomatic and asymptomatic individuals. These assays can only be run on the automated cobas® 4800 system.

Also available for the cobas® 4800 system is a diagnostic test for CDI. The cobas® Cdiff test selectively detects a specific *Clostridium difficile tcdB* gene directly from unformed (liquid or soft) stool specimens using real-time PCR technology. The test, which is not FDA cleared and therefore is not available in the United States, is intended for use as an aid in diagnosing CDI in humans in conjunction with clinical and epidemiological risk factors.

Additional assays from Roche available for use on the cobas® 4800 system include HPV, HSV 1 and 2, and MRSA/SA (*Staphylococcus aureus*) (which is not intended to diagnose, guide or monitor treatment for MRSA or *S. aureus* infections, or provide results of susceptibility to methicillin; it is for surveillance purposes).

The cobas® 4800 system, pictured in Fig. 25, integrates fully automated total nucleic acid isolation directly from primary and secondary tubes, automated PCR setup and real-time PCR. It is intended for laboratories with a medium workflow. The system comprises the cobas® x 480 instrument and the cobas® z 480 analyser and, per Roche, features minimal hands-on time (30 minutes for a run of 24–96 samples). It can take multiple sample types, can detect multiple test targets and has what the company describes as an intuitive workflow.

Fig. 25. Roche cobas® 4800 system

cobas® Liat® system (Roche, USA)

Roche offers the cobas® Cdiff nucleic acid test for use on the cobas® Liat® system. The Cdiff test is an automated qualitative in vitro diagnostic test that utilizes real-time PCR to detect the *tcdB* gene of toxigenic *Clostridium difficile* in unformed (liquid or soft) stool specimens obtained from patients suspected of having CDI. The cobas® Cdiff nucleic acid test for use on the cobas® Liat® system is intended as an aid in diagnosing CDI in humans in conjunction with clinical and epidemiological risk factors. The assay is CE-IVD marked and FDA cleared.

Additional, clinically validated assays for use on the cobas® Liat® system include Influenza A/B, Strep A and Influenza A/B & RSV (respiratory syncytial virus), all of which are CE-IVD marked and FDA cleared. These assays, together with the Cdiff assay, have received a Clinical Laboratory Improvement Amendments (CLIA) Waiver from the FDA. A CLIA Waiver determines that there is little risk of error due to the simple use of the test and that no special training is required.

All of the assays listed above are designed to be run on the cobas® Liat® system, pictured in Fig. 26, which is a compact, real-time PCR platform designed for on-demand short turnaround time (STAT) testing at POC or in the laboratory to support time-sensitive diagnoses and treatment decisions. Although the system is very easy to use, it does require a cold chain, which could impede its use in some settings in LMICs. All NAAT processes are fully automated, including sample preparation, amplification and real-time detection for qualitative and quantitative results, as well as results interpretation. Each cobas® Liat® assay tube contains all assay reagents for a single test.

Fig. 26. cobas® Liat® system

To aid the operator and provide reliable results, the cobas® Liat® system incorporates a variety of intelligent and advanced features. The system self-checks at power on and has an error diagnostic system with comprehensive real-time monitoring, continuous self-calibrations and error message display. The graphical user interface provides on-screen prompts for easy-to-follow directions to guide the operator through sample loading and tube insertion. An on-board scanner supports a variety of barcode types for ease of use. Volume sensing ensures the appropriate amount of sample is used for the test, or delivers a warning if the sample volume is insufficient. A comprehensive set of sensors further monitors system operations in real time. Internal controls are pre-packed and process through every step, and quality-control reagents are used with each new assay tube lot.

As illustrated in Fig. 27, the cobas® Liat® test procedure is straightforward, with no sample manipulation or reagent loading steps, other than inputting

Fig. 27. cobas® Liat® test procedure

Sample

Add your patient sample to the **cobas**® Liat® assay tube with provided transfer pipette.

Scan

Scan assay tube using built-in barcode reader.

Start

Insert assay tube into the **cobas**® Liat® Analyzer.

the sample directly into the cobas® Liat® assay tube. The cobas® Liat® system is a closed system, thus minimizing cross-contamination and biohazard risks, and allowing testing to be performed in nonlaboratory or near-patient facilities. The cobas® Liat® system is small and portable, weighing 8.3 lbs. It executes all required assay steps and reports a test result in about 20 minutes after loading a specimen on the system. The system runs one test at a time, and therefore can run a maximum of three tests per hour or approximately 24 tests in an 8-hour day.

The cobas® Liat® system has an internal optical system that provides independent optical detection channels, allowing for the detection of multiple targets in each test and providing future expandability for detection of multiple diseases. It is powered by AC mains.

Hologic Panther® system (Hologic, USA)

Hologic manufactures the Aptima Combo 2® assay, which is a target amplification nucleic acid probe test that utilizes target capture for in vitro qualitative detection and differentiation of rRNA from CT and/or NG. The test is intended for use in the diagnosis of chlamydial and/or gonococcal urogenital diseases using the Panther® system or the Tigris® DTS® (direct tube sampling) automated analyser or semi-automated instrumentation, described below. The following specimens from both symptomatic and asymptomatic individuals may be used for the test: clinician-collected endocervical, vaginal and male urethral swab specimens, clinician-collected gynaecological specimens collected in PreservCyt® solution, patient-collected vaginal swab specimens, and female and male urine specimens.

Additional assays that can be run on the Panther® system include Aptima *Trichomonas vaginalis* (TV), HPV, HPV 16 18/45 genotyping assay and HIV-1 viral load. Assays in development for the system include HBV viral load, HCV viral load, *Mycoplasma genitalium*, HSV 1 and 2, bacterial vaginosis and *Candida*.

The Panther® system, pictured in Fig. 28, is a molecular diagnostic platform with random access testing capability on a fully integrated and automated NAAT system.

The Aptima Combo 2® assay and other assays run on the Panther® system involve three main steps, all of which take place in a single tube: target capture, target amplification by TMA, and detection of amplicons by the fluorescent-labelled probes (torches). Within the Panther®, all nucleic acid testing steps, from primary sample tube to results, are fully automated in one system with first reportable results within 3 hours after loading samples, and five results every 5 minutes thereafter. Samples can be continuously loaded with up to 120 samples at a time. Reagent controls and calibration are valid for 24 hours. At least 275 samples can be run within an 8-hour shift, or 500 in a 12-hour period (an additional 225 samples can be run without operator attendance). Four reagent lanes allow up to four Aptima® test kits to be onboard and randomly accessed at any time: this could be four kits of the Combo 2® assay or any combination of the other molecular diagnostic assays available on the Panther®, including the HIV-1 Quant Dx, TV, HPV and HPV genotyping, HCV Quant Dx, HBV Quant and HSV 1 & 2 assays.

Fig. 28. The Panther® system

The Aptima Combo 2® assay as well as the Aptima® assay for *Neisseria gonorrhoeae* (GC assay) and the Aptima® assay for TV can also be run on the Tigris® DTS® (direct tube sampling) system (pictured in Fig. 29), which automates all phases of molecular diagnostics testing, from sample preparation, through amplification and detection, to results reporting. The Tigris® system uses target capture, which isolates the target nucleic acid from the sample and purifies it. TMA, using two enzymes (RT and RNA polymerase), amplifies the purified target RNA. The system hybridizes the amplicon to single-stranded nucleic acid probes and labels them.

Fig. 29. Tigris® DTS® system

The Tigris® system accommodates multiple assays on one system and has the ability to process approximately 450 samples in an 8-hour shift, and up to 1000 samples in approximately 13.5 hours. Because of the degree of automation of the system, it maximizes walk-away time during test processing.

Hologic Panther Fusion® system (Hologic, USA)

In addition to the assays for its Panther® system previously described, Hologic offers a series of assays for its Panther Fusion® system. These currently include an assay for MRSA (CE-IVD marked) and the following additional assays: Flu A/B/RSV (CE marked and FDA cleared), Paraflu (CE marked and FDA cleared), AdV/hMPV/RV (adenovirus, human metapneumovirus and rhinovirus) (CE marked and FDA cleared), Group B streptococcal disease (GBS) (CE marked and FDA cleared) and *Bordetella* (CE marked). The company is also developing a gastrointestinal assay for the platform.

The Panther Fusion® system is a fully automated, high-throughput platform that combines the TMA capabilities of the Panther® with PCR-based testing capabilities on the Panther Fusion® instrument; the two instruments can be linked with one another as illustrated in Fig. 30. Together the instruments provide test menu consolidation, random access testing (any combination of sample types and assays may be performed at the same time) and continuous loading.

Fig. 30. Hologic Panther Fusion® system

BD ProbeTec™ ET system (BD, USA)

BD Diagnostics offers the BD ProbeTec ET amplified DNA assays, which allow direct, qualitative detection of CT and GC DNA in endocervical swabs, male urethral swabs, and male and female urine specimens (with and without preservative) that are collected from either symptomatic or asymptomatic patients. The assays may be run on the BD ProbeTec™ ET system, pictured in Fig. 31, which uses SDA technology as the amplification method and fluorescent energy transfer (ET) as the detection method to test for the presence of CT and GC in the clinical specimens indicated.

Fig. 31. BD ProbeTec™ ET system

While the BD ProbeTec™ is relatively compact and requires no special room, in addition to the ET instrument, pictured above centre, the system requires separate priming and amplification microwells, a pipettor, and a lysing rack and heater. Nonetheless, the system can generate CT/GC results for up to 46 patient samples in 3 hours – or 276 CT/GC results in one 8-hour shift. Total hands-on time is less than 2 minutes per sample.

BD Viper™ LT system (BD, USA)

BD offers the GC Q^x and CT Q^x amplified DNA assays, which use SDA technology for the direct, qualitative detection of NG DNA or CT DNA, respectively, in clinician-collected female endocervical and male urethral swab specimens, patient-collected vaginal swab specimens (in a clinical setting), and male and female urine specimens. The assays are indicated for use with asymptomatic and symptomatic female individuals and symptomatic male individuals to aid in diagnosing gonococcal urogenital disease. The assays are based on the simultaneous amplification and detection of target DNA using amplification primers and a fluorescently labelled detector probe.

The GC and CT Q^x amplified DNA assays can be performed using the BD Viper™ LT system, pictured in Fig. 32, which uses homogeneous SDA as the amplification method and ET as the detection method to test for the presence of pathogens by their genetic content in clinical specimens. The system uses four fluorescent channels with advanced optics and LED light sources for detection, and a thermocycler with 24 control zones designed to ensure uniform temperature control. The BD Viper™ LT system provides up to 30 CT/GC sample results in 3.5 hours, and 120 results per day. As such, the system is designed for a medium-throughput laboratory.

Fig. 32. BD Viper™ LT system

All of the assays for use on the BD Viper™ LT system are for sexually transmitted infections (STIs). In addition to the GC and CT assays, assays for detecting HSV 1 and 2, TV and HPV are also available.

BD MAX™ system (BD, USA)

BD offers several bacterial detection assays to be run on its BD MAX™ system. One of these is a CT/GC/TV assay. The BD MAX™ system incorporates automated DNA extraction and PCR for the direct, qualitative detection of DNA from CT, GC and/or TV. The assay may be used to detect CT and/or GC DNA in male urine specimens, and CT, GC and/or TV DNA in female urine specimens, clinician-collected female endocervical swab specimens and patient-collected vaginal swab specimens (in clinical settings). The assay is indicated for use to aid in diagnosing chlamydial urogenital disease, gonococcal urogenital disease and/or trichomoniasis in asymptomatic and symptomatic individuals.

In addition to the CT/GC/TV assay, there is also the BD MAX™ Cdiff assay. As performed on the BD MAX™ system, the assay is an automated in vitro diagnostic test for the direct qualitative detection of *Clostridium difficile tcdB* in liquid or soft stool specimens from patients suspected of having CDI. The test is performed directly on the specimen and utilizes real-time PCR to amplify *tcdB* DNA and fluorogenic target-specific hybridization probes to detect the amplified DNA. The Cdiff assay is intended to aid in diagnosing CDI.

A third assay, the BD MAX enteric bacterial panel, also performed on the BD MAX™ system, is an automated in vitro diagnostic test for the direct qualitative detection and differentiation of enteric bacterial pathogens. The panel detects nucleic acids from *Salmonella* spp.; *Campylobacter* spp. (*C. jejuni* and *C. coli*); *Shigella* spp./enteroinvasive *Escherichia coli* (EIEC); and Shiga toxin 1 (*stx1*)/Shiga toxin 2 (*stx2*) genes (found in Shiga toxin-producing *E. coli* [STEC]).

The BD MAX™ enteric bacterial panel is performed on unpreserved soft to liquid stool specimens or Cary-Blair preserved stool specimens from symptomatic patients with suspected acute gastroenteritis, enteritis or colitis. The test is performed directly on the specimen, utilizing real-time PCR to amplify *SpaO*, a *Campylobacter*-specific *tuf* gene sequence, *ipaH* and *stx1*/*stx2*. The test utilizes fluorogenic sequence-specific hybridization probes to detect the amplified DNA.

BD indicates that the test is intended for use, in conjunction with clinical presentation, laboratory findings and epidemiological information, as an aid in the differential diagnosis of *Salmonella*, *Shigella*/EIEC, *Campylobacter* and STEC infections. Results of this test should not be used as the sole basis for diagnosis, treatment or other patient management decisions.

BD also offers the BD MAX™ extended enteric bacterial panel. Performed on the BD MAX™ system, it is an automated in vitro diagnostic test for the direct qualitative detection and differentiation of enteric bacterial pathogens. It is used in conjunction with the BD MAX™ enteric bacterial panel as an optional master mix. The BD MAX™ extended enteric bacterial panel detects nucleic acids from *Plesiomonas shigelloides*; *Vibrio* (*V. vulnificus*, *V. parahaemolyticus* and *V. cholerae*); enterotoxigenic *Escherichia coli* (ETEC) heat-labile enterotoxin (LT)/heat-stable enterotoxin (ST) genes; and *Yersinia enterocolitica*.

Additional assays available for use on the BD MAX™ system that are not discussed in detail in this report include the BD MAX™ vaginal panel, BD Max™ GBS, BD MAX™ enteric parasite panel and the BD MAX™ enteric viral panel. BD also offers two additional assays to combat transmission prevention and infection control for HAIs: BD MAX™ MRSA XT (as an aid in preventing and controlling MRSA infections in healthcare settings and not for in vitro use) and BD MAX™ StaphSR (for surveillance only).[6]

All of these assays are run on the BD MAX™ platform, pictured in Fig. 33, which automates sample preparation, including target lysis, DNA extraction and concentration, reagent rehydration and target nucleic acid amplification using real-time PCR. The amplified DNA targets are detected using hydrolysis (TaqMan®) probes, labelled at one end with a fluorescent reporter dye (fluorophore) and at the other end with a quencher moiety. The system software automatically interprets test results. The BD MAX™ platform is capable of batch processing and analysing up to 24 specimens simultaneously. Test results

[6] For more detail on BD MAX™ MRSA XT and StaphSR, which are not covered in detail in this report as they are not assays for in vitro use, see WHO Global AMR Surveillance System (GLASS) (*10*).

generally take about 2.5–3 hours with an additional 15–20 minutes of hands-on time for completing 24 specimens.

Fig. 33. BD MAX™ instrument

Great Basin analyser system (Vela Diagnostics, Singapore)

Vela Diagnostics recently acquired Great Basin Scientific, Inc., and now offers several FDA-cleared assays for detecting bacterial pathogens. These are the Great Basin Stool Bacterial Pathogens panel, Great Basin Staph ID/R Blood Culture panel and Great Basin Toxigenic *C. difficile* test. The assays are configured for use on the Great Basin analyser, the PA 500 Portrait analyser.

The Great Basin Stool Bacterial Pathogens panel is a multiplexed, qualitative in vitro diagnostic for detecting and identifying DNA targets of enteric bacterial pathogens directly from Cary-Blair or C&S medium preserved stool specimens from symptomatic patients with suspected acute gastroenteritis, enteritis or colitis. The panel detects *Campylobacter* (*C. coli* and *C. jejuni*), *Salmonella*, *stx1*, *stx2*, *Escherichia coli* serotype 0157 and *Shigella*. The test is intended for use as an aid in diagnosing specific agents of gastrointestinal illness in conjunction with clinical and epidemiological information, but is not intended for use in monitoring these infections. TAT is less than 2 hours.

The Great Basin Staph ID/R Blood Culture panel is a qualitative, multiplex, in vitro diagnostic assay intended for simultaneously identifying nucleic acid from *Staphylococcus aureus*, *Staphylococcus lugdunensis* and various *Staphylococcus* spp. to the genus level, and detection of the *mecA* gene directly from patient positive blood culture specimens. The assay is intended for use in conjunction with other clinical or laboratory findings to aid in diagnosing BSIs, but is not intended for monitoring these infections. TAT is less than 2 hours.

The Great Basin Toxigenic *C. difficile* test is a qualitative in vitro diagnostic test that detects the *tcdB* gene in human stool samples collected from patients suspected of having CDI. It is intended as an aid in diagnosing CDI. The test is not intended for use in near-patient care settings. TAT is approximately 90 minutes.

The Great Basin analyser system, pictured in Fig. 34, is a fully automated system that comprises two major components: the control platform (touchscreen) and the PA500 Portrait analyser, below it, which is a molecular in vitro diagnostic device. Resident software in the device is used to control, analyse and determine test results.

Fig. 34. Great Basin analyser system

The Great Basin analyser system utilizes automated, hot-start PCR amplification technology to amplify specific nucleic acid sequences that are then detected using hybridization probes immobilized on a modified silicon chip surface, in a single-use, self-contained disposable test cartridge. The system contains linear actuators that open small reagent containers, linear actuators that open and close valves, linear motors that depress reagent blisters to move fluid through the cartridge, pictured in Fig. 35, a motor to facilitate mixing processes and analog sensors that detect the presence of fluid.

Fig. 35. Great Basin test cartridge

The system takes unprocessed samples, and the cartridge contains, either in blister packs or lyophilized, all of the reagents required to run the test. The analytical steps of the assay, including sample prep, amplification and detection, are performed in chambers present on the cartridge.

Additional assays available for the platform and not described here in detail are the Great Basin Shiga Toxin Direct test, the Great Basin *Bordetella* Direct test and the Great Basin Group B *Streptococcus* test.

illumigene™ molecular diagnostic system (Meridian Bioscience, Inc., USA)

Meridian Bioscience offers an FDA-cleared *Clostridium difficile* assay, the illumigene™ *C. difficile* DNA amplification assay, for use on its Alethia™ platform which, it should be noted, the company indicates is only for use in hospital, reference or state laboratory settings and not for use at POC. The assay utilizes LAMP technology to detect the pathogenicity locus (PaLoc) of toxigenic *Clostridium difficile* strains from stool specimens. The illumigene™ *Clostridium difficile* assay detects the PaLoc by targeting a partial DNA fragment on the *C. difficile* Toxin A gene (*tcdA*). The *tcdA* target region was selected as an intact region remaining in all known A^+B^+ and A^-B^+ toxinotypes.

Meridian Bioscience also offers assays for respiratory infections – Group A *Streptococcus*, *Mycoplasma* Direct and Pertussis – as well as two assays for sexual health – Group B *Streptococcus*, and HSV 1&2.

These assays are intended to be performed on the Alethia™ platform, pictured in Fig. 36, which is a compact, automated isothermal amplification and detection system. There is a separate sample preparation device. The Alethia™ is a menu-driven laboratory instrument with two independent sample-processing blocks, identified as Block A and Block B. Sample heating and optical detection is carried out for up to five two-chambered illumigene™ devices per block. Each two-chambered Alethia™ device contains a sample chamber and a control chamber. Amplification of target DNA occurs during the heat cycle and results in the formation of precipitate detected by the Alethia™ optics system. The precipitate generated by the presence of amplified target DNA leads to a turbid sample/control reaction solution which is then measured by absorbance.

One to 10 qualitative results are available in less than an hour on the Alethia™ instrument.

Fig. 36. Alethia™ instrument

Solana® platform (Quidel Corporation, USA)

Quidel corporation offers the Solana® *C. difficile* assay, which is an in vitro diagnostic test for the direct, qualitative detection of *tcdA* in unformed stool specimens of patients suspected of having CDI. The assay is intended for use as an aid in diagnosing CDI. The assay utilizes HDA to amplify a highly conserved fragment of the *tcdA* sequence. The Solana® *C. difficile* assay is intended for use only with the Solana® instrument platform.

The Solana® platform, pictured in Fig. 37, uses a helicase enzyme to unwind double-stranded DNA into single strands, eliminating the need for a thermocycler. The company emphasizes that unlike other isothermal amplification methods, HDA uses a probe-based detection method, thereby resulting in greater specificity. In addition, because HDA only detects amplicons, rather than turbidity caused by amplification as with LAMP, it provides assurance that amplification of only the intended target will be identified as positive. HDA can also multiplex in a single tube.

Fig. 37. Solana® platform

Solana® is a compact benchtop instrument measuring 9.4 × 9.4 × 5.9 inches that allows rapid detection of targets. TAT for the tests is as short as 35 minutes. In addition, the platform permits operators to batch up to 12 samples in a single run, allowing for testing scale-up as needed.

The company believes the platform is ideal for small- to medium-sized microbiology labs where the low total cost of the instrument and disposables enables molecular testing at the volumes seen in these settings. In resource-limited settings, this would likely translate into use at Level II settings and above.

Additional assays that can be run on the Solana® platform include Influenza A+B, Group A *Streptococcus*, Group B *Streptococcus*, HSV 1+2/VZV (varicella-zoster virus) and TV, all of which are FDA approved. Quidel is also developing additional assays for the Solana platform.

AmpliVue® platform (Quidel Corporation, USA)

Quidel offers the AmpliVue® *C. difficile* assay, which is an in vitro diagnostic test for the direct, qualitative detection of *tcdA* in unformed stool specimens of patients suspected of having *C. difficile*-associated disease (CDAD). The AmpliVue® *C. difficile* assay is intended for use as an aid in diagnosing CDAD. The assay utilizes HDA to amplify a highly conserved fragment of the *tcdA* sequence, and a self-contained disposable amplicon detection device that allows for manual evaluation of assay results.

The AmpliVue® is an "instrument-free" molecular diagnostic platform. Like the Solana® platform, AmpliVue® combines simple specimen processing and HDA technology, but has qualitative lateral-flow detection housed in a cassette. The platform is low cost, as it only requires a heat block for amplification.

The typical workflow for the *C. difficile* assay on the AmpliVue® platform is illustrated in Fig. 38.

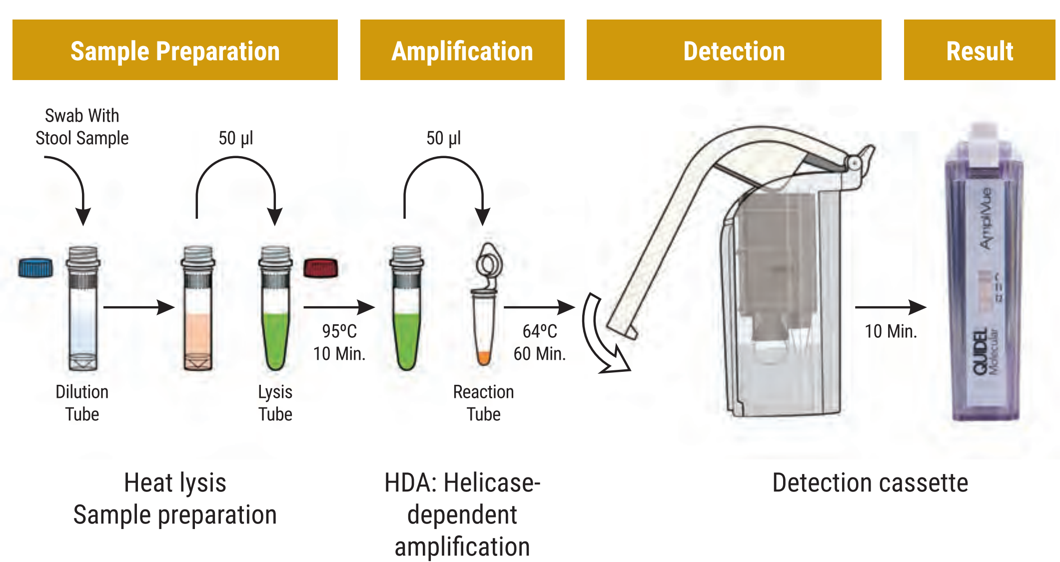
The testing process uses manual specimen preparation (dilution of sample and pipetting of same followed by heat lysis), with the entire process, including heat lysis, taking about 60–90 minutes (depending on the assay).

The first assay developed for the AmpliVue® platform was the *C. difficile* assay, which is both CE marked and FDA cleared. Similarly, five additional assays are CE marked and FDA cleared – assays for herpes simplex (cutaneous and mucocutaneous) lesion specimens (HSV 1+2), Group B *Streptococcus*, Group A *Streptococcus*, TV and *Bordetella pertussis*. The assay for detecting TV in women (using vaginal specimens) was launched in 2015 and is FDA cleared.

VERSANT® kPCR molecular system (Siemens, USA)

The Siemens VERSANT® kPCR system is an automated system which combines extraction of nucleic acids from 96 samples with subsequent real-time PCR. The VERSANT® CT/GC DNA 1.0 kinetic PCR (kPCR) assay detects CT and GC in a multiplex real-time PCR on this automated system, including the recently described new variant of CT (nvCT). The assay is designed for the qualitative detection of CT and NG in symptomatic and asymptomatic individuals from urine specimens from males and females, male urethral and female endocervical swab samples. The assay is CE-IVD marked, but is not available in the United States.

Fig. 38. Typical test workflow on AmpliVue® platform



The VERSANT® CT/GC DNA 1.0 assay is an automated amplification method based on reverse transcription and real-time PCR technology. The system, pictured in Fig. 39, consists of two modules: a sample preparation module used to extract both RNA and DNA from plasma as well as a wide variety of other samples, and an amplification detection module, along with VERSANT® MiPLX software. For the CT/GC assays, the sample preparation is universal for either urine or swabs. The system is flexible and allows for either a "one-room" technology with no need for clean-room operations due to closed-tube processing and other physical and chemical contamination controls or two separate rooms, depending on the individual laboratory's setting.

The VERSANT® kPCR sample preparation module pipettes purified RNA to a PCR plate containing appropriate primer/probe mix and enzymes. The wells are then sealed and transferred to the amplification detection module, where the CT/GC and internal control RNA molecules are reverse transcribed to make cDNA and then simultaneously amplified and detected using the kPCR technique. The system can produce 188 patient results per shift.

Fig. 39. VERSANT® kPCR molecular system

Additional assays available for the VERSANT® kPCR molecular system include the VERSANT® HIV-1 RNA 1.5 assay (kPCR) and HCV RNA 1.0 assay (kPCR).

ARIES® and ARIES® M1 systems (Luminex, USA)

Luminex offers the ARIES® *C. difficile* assay, which is a real-time PCR-based qualitative in vitro diagnostic test for the direct detection of toxigenic *Clostridium difficile* nucleic acid in unpreserved, unformed (liquid or soft) stool specimens obtained from patients suspected of having CDI, i.e., symptomatic patients. The test targets the *Clostridium difficile tcdA* and *tcdB* and is indicated for use as an aid in diagnosing individuals suspected of having CDI. The assay is FDA cleared and CE-IVD marked.

The ARIES® *C. difficile* assay is designed for use on the Luminex ARIES® system, a two-module instrument, or ARIES® M1 system, a single module instrument, pictured in Fig. 40, each of which consists of the associated ARIES software, a stool resuspension kit, an assay-specific test cassette and an assay-specific protocol file. It is a sample-to-result system. The ARIES® *C. difficile* assay cassette is a disposable, single-use device that contains nucleic acid purification reagents, an internal sample processing control (SPC), and an assay-specific master mix for detecting *tcdA* and *tcdB*. Cassettes can be stored at room temperature.

The systems require a universal assay protocol (i.e., identical sample preparation, amplification reagents and conditions) that may enable multiple sample types and up to 12 different IVD assays to be run together in a random batch, which is not the same as random access. TAT for the *C. difficile* assay is approximately 2 hours. The systems are appropriate for moderate-sized laboratories.

Additional FDA-cleared and CE-marked assays for the ARIES® systems include *Bordetella*, Flu A/B & RSV, Group A Strep, Group B Strep and HSV 1&2.

Fig. 40. ARIES® (left) and ARIES® M1 (right) systems

Novodiag® (Mobidiag, Finland)

Mobidiag offers two assays for its Novodiag® platform – the Novodiag® *C. difficile* assay and the Novodiag® Bacterial GE+ assay. The *C. difficile* assay screens for *tcdB* from unformed stool samples, while the Bacterial GE+ assay screens for the most relevant bacteria responsible for diarrhoea, including *Campylobacter coli*, *Campylobacter jejuni*, *Clostridium difficile tcdB*, *Salmonella* spp. and *Shigella* spp. Results are available in just under an hour.

The Novodiag® platform, pictured in Fig. 41, is a four-bay, benchtop, automated sample-in, result-out system for detecting infectious diseases. The platform

combines real-time qPCR and microarray technologies appropriate for screening one or multiple pathogens. The system offers random access for on-demand testing together with automated data analysis and reporting with laboratory information and management system (LIMS) connectivity.

Fig. 41. Novodiag® system

In addition to the Novodiag® *C. difficile* and Bacterial GE+ assays, the company is developing assays for antibiotic resistance, meningitis and parasites.

Respiratory Multiplex Array II/Vivalytic analyser (Randox Laboratories, Ltd., UK/Bosch Healthcare Solutions, Germany)

Bosch Healthcare Solutions has developed the Vivalytic analyser, a universal cartridge-based platform for sample-to-answer molecular diagnostics (pictured in Fig. 42), with the first tests available being the Respiratory Multiplex Assay and the Randox STI Multiplex Array.

Fig. 42. Bosch Vivalytic analyser

The Vivalytic platform accommodates a wide variety of samples and allows for different methods of analysis to run in a fully automated way in a short timeframe, with results from 30 minutes. Single or multiple pathogens can be detected simultaneously in the patient sample. In addition, the Vivalytic platform is an open system that can process molecular diagnostic tests from various assay manufacturers.

The Vivalytic analyser is a small-footprint, fully automated device with no peripherals, capable of quantitative and qualitative PCR procedures with three stable isothermal zones, where rapid microfluidic transfer between these zones achieves fast heating and cooling cycles. The analyser has a universal optical evaluation unit, which enables microarrays, qualitative or quantitative PCR, as well as melting curve analyses to be read out in one system. Four standard colour channels can be evaluated per PCR strand. This corresponds to a degree of multiplexing of up to eight for qualitative or quantitative PCR, or up to 16 in multichannel melting curve analysis. Via geometrical multiplexing with the help of microarrays, a much higher number can be achieved. Up to 100 properties can be examined.

The Vivalytic system has built-in connectivity and can be easily integrated with popular standard Internet technology systems. Further, an analyser device can be networked and combined with many other devices, so that several series of tests can be carried out at the same time.

Randox Laboratories has developed a number of infection arrays that have been adapted for use with the Vivalytic analyser. Of particular interest for this report is the Respiratory Multiplex Array (pictured in Fig. 43), a qualitative assay that simultaneously detects 21 bacterial and viral pathogens from the upper and lower respiratory tract in nucleic acid extracted from a single sputum, lavage or nasopharyngeal sample.

Fig. 43. Randox respiratory tract infection and STI panels

The Respiratory Multiplex Array assay is based on a combination of multiplex PCR and biochip array hybridization. PCR priming technology permits high discrimination between multiple targets. A unique

primer set is designed for each target that will hybridize to a complementary oligonucleotide probe spotted on a biochip discrete test region (DTR). This combination of PCR priming and spatially organized biochip array technology enables enhanced specificity of the assay. Analysis can be completed from template nucleic acid, through PCR, to data readout in about 6 hours.

The STI array, a qualitative assay that is CE-IVD marked, detects 10 of the most important bacterial, viral and protozoan sexually transmitted infections (STIs), providing a comprehensive infection profile from a single swab sample. The test panel includes CT, NG and TV, as well as MG, *Ureaplasma urealyticum*, *Haemophilus ducreyi*, *Mycoplasma hominis*, and HSV 1 and 2.

Each cartridge contains internal controls that indicate successful extraction, amplification, hybridization and detection; all of these must pass acceptance criteria in order for the Vivalytic analyser to return patient results. Further, test results do not require interpretation; positive or negative results are indicated for each target without ambiguity.

STAT-Dx (subsidiary of QIAGEN N.V., Germany)

QIAGEN's STAT-Dx subsidiary offers two CE-IVD-marked panels for syndromic testing: the QIAstat-Dx™ Respiratory Panel V2 and QIAstat-Dx™ Gastrointestinal Panel V2. Each of these is a sample-to-result solution that can be performed in less than an hour on the QIAstat-Dx™ Analyzer 1.0, pictured in Fig. 44, which consists of an analytical module and an operational module. It is appropriate for use at or near POC.

Fig. 44. QIAstat-Dx™ Analyzer 1.0

The QIAstat-Dx™ Analyzer 1.0 utilizes real-time qPCR that is fully integrated and can process up to 48 targets from a variety of sample types, including swabs, tissue and liquids, but not yet whole blood. The system has built-in connectivity capability. The instrument, which is modular and scalable, has a six-wavelength optical sensor. Each cassette has eight possible reaction chambers. The system utilizes a single-use assay cartridge, pictured in Fig. 45, that contains all required sample reagents and has the ability to process samples onboard. It is appropriate for near-patient testing.

Fig. 45. QIAstat-Dx™ Analyzer 1.0

The QIAstat-Dx™ (DiagCORE®) Respiratory Panel V2 simultaneously detects 18 viral and three bacterial pathogens (*Mycoplasma pneumoniae*, *Legionella pneumophila* and *Bordetella pertussis*) from a 300 µL nasopharyngeal sample. The QIAstat-Dx™ (DiagCORE®) Gastrointestinal Panel V2 detects six viruses, 14 bacteria, including diarrhoeagenic *Escherichia coli*, Shiga-like toxin-producing *E. coli*, *Shigella*, EIEC, pathogenic *Campylobacter* spp., *Clostridium difficile* (Toxin A/B) and *Salmonella*, as well as four parasites from 200 µL stool resuspension. Additional syndromic panels in the pipeline include a meningitis panel, a sepsis panel, panel(s) for STIs and a traveller's fever panel.

revogene® (GenePOC™, Canada)

GenePOC, which was recently acquired by Meridian Bioscience (USA), has developed a fully automated platform for NAAT-based testing at POC. The system combines a compact benchtop instrument, the revogene®, pictured in Fig. 46, with a single-use microfluidic cartridge (PIE) that can perform sample homogenization, microorganism lysis, dilution, amplification and detection of target nucleic acid sequences from multiple specimens using fluorescence-based real-time PCR. Manual sample preparation steps are required, however; specimens must be transferred with a disposable transfer loop into a sample buffer tube and subsequently transferred into the PIE using a disposable transfer tool. The PIE must also be manually loaded into, and unloaded from, the revogene® carousel. TAT is approximately 70 minutes. The complexity of the system suggests it would best be used in Level III settings or higher.

Fig. 46. revogene® instrument

The revogene® instrument can process up to eight clinical samples simultaneously, detecting up to 12 genetic targets per sample, but it does not offer random access. Different assays can be run at the same time on the instrument only if sample prep and the programmes for amplification and detection are the same. It should also be noted that the within each PIE, the processed sample is divided into three parts, allowing for unique reactions to be run; however, this "geographic" or "geometric" multiplexing can reduce test sensitivity. The system does not support direct Wi-Fi connectivity, but does support bidirectional LIS connection.

To date, the company has several commercially available assays. Of interest with respect to this report is the GenePOC CDiff assay, which is a qualitative in vitro diagnostic test to detect the *tcdB* gene of toxigenic *Clostridium difficile* direct from unformed (liquid or soft) stool specimens. The assay is intended to aid in diagnosing CDI and is FDA cleared and CE-IVD marked. The workflow for the test is illustrated in Fig. 47.

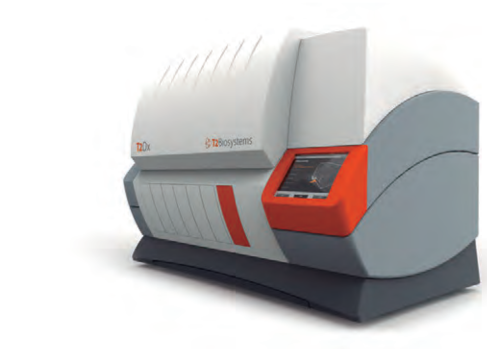
Additional assays available for the platform include two tests for Group B *Streptococcus*: (i) the GenePOC GBS DS test direct from vaginal/rectal swabs, and (ii) the GenePOC GBS LB test from Lim Broth samples. These assays are CE-IVD marked.

GenePOC plans to develop assay panels for gastrointestinal infections and respiratory infections and also plans to include AMR applications.

T2Dx® instrument (T2 Biosystems, USA)

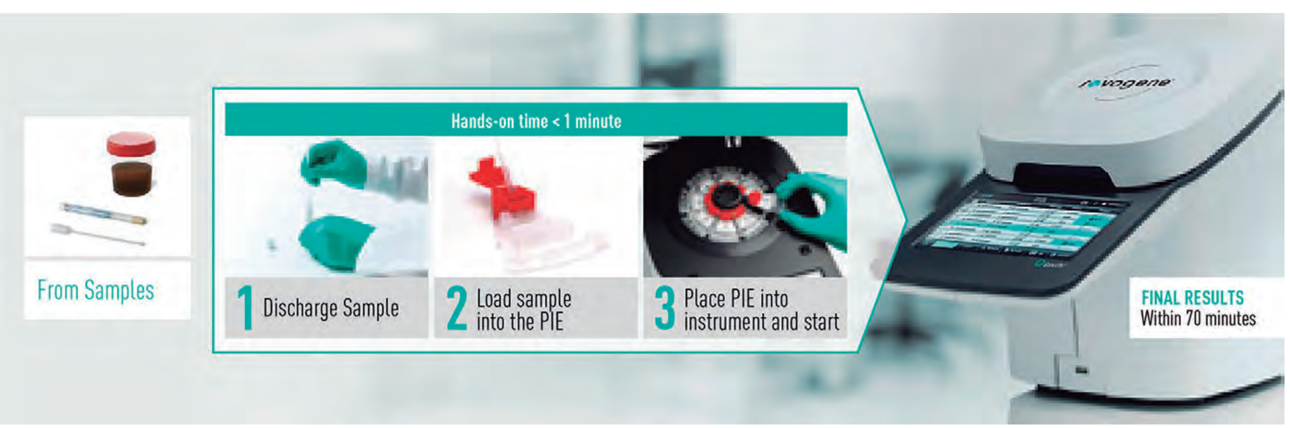
T2 Biosystems offers two multiplex panels for identifying infectious pathogens: the T2Bacteria panel and the T2Candida panel. These assays are performed on the T2Dx® instrument, pictured in Fig. 48.

Fig. 48. T2Dx® instrument



The T2Bacteria panel is a qualitative test using T2 magnetic resonance (T2MR®) to directly detect bacterial species in K2-EDTA human whole blood specimens from individuals suspected of bacteraemia. The T2Bacteria panel identifies five species of bacteria: *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylo-*

Fig. 47. GenePOC CDiff assay workflow on the revogene® platform



coccus aureus. The T2Bacteria panel is to be used as an aid in diagnosing bacteraemia; blood cultures are required to recover organisms for AST or further ID. It is both CE-IVD marked and FDA cleared.

Similarly, the T2Candida panel is a qualitative T2MR® assay for directly detecting *Candida* spp. in K2-EDTA human whole blood specimens from patients with symptoms of, or medical conditions predisposing the patient to, invasive fungal infections. The panel identifies five species of *Candida*. The panel is performed independent of blood culture, although blood cultures are required to recover organisms for AST or further ID. The T2Candida panel is FDA cleared.

Both the T2Bacteria panel and the T2Candida panel are performed on the T2Dx® instrument, which is automated and executes all steps after specimen loading (approximately 4 mL). On the instrument, bacterial DNA is amplified with target-specific primers and amplicons are hybridized to target-specific probes. The hybridization that occurs in individual tubes is analysed in the MR reader, and a signal for each target is generated and detected by the T2MR®, indicating the presence of the target organism. TAT from loading the first specimen on the instrument is approximately 3–5 hours. The results are interpreted by software on the device.

The T2 system is suitable for use in centralized laboratories and is not intended for near-patient testing.

GenomEra® CDX system (Abacus Diagnostica, Finland)

Abacus Diagnostica offers several CE-marked assays for its GenomEra® CDX platform. Of these, the *C. difficile*, MRSA/SA AC (with *mecA* and *mecC*), MRSA/SA Multi Swab and *Streptococcus pneumoniae* assays are of relevance to this report.

- The GenomEra™ CDX *C. difficile* test is a rapid and simple molecular assay for diagnosing toxigenic *Clostridium difficile* directly from unformed stool samples.
- The GenomEra™ CDX MRSA SA AC test is an assay for detecting *Staphylococcus aureus* and MRSA as well as both resistance genes, *mecA* and *mecC*, from a droplet of blood culture or plate samples using the same test kit.
- The GenomEra™ CDX MRSA Multi Swab test simultaneously screens multiple body sites (nose, throat and groin/perineum) for MRSA colonization using swab specimens pooled in liquid medium (eSwab™ MRSA Collection System [Copan]). The same medium can be used for confirmation by culture, thus eliminating the need for collecting additional samples.
- The GenomEra™ CDX *S. pneumoniae* test enables the detection of pneumococcus from positive blood cultures or equivalent liquid bacterial cultures.

All of the above assays are designed for use on the GenomEra® CDX system, pictured in Fig. 49, which comprises (i) a proprietary test chip, (ii) an automated PCR analyser and (iii) a PC-controlled graphical interface with built-in results interpretation technology.

Fig. 49. GenomEra® CDX system: test chips (left and centre), and analyser and PC for test interpretation (right)

Per the company, the system combines highly fluorescent proprietary lanthanide labels with PCR for high detection sensitivity with no signal interference from the clinical samples. The system uses patented multiblock thermal cycling technology that enables rapid PCR and RT-PCR amplification with 45 thermal cycles. The PCR or RT-PCR reagents are lyophilized and preloaded into the GenomEra® test chips. The software loaded on the PC incorporates clear and simple results interpretation technology.

TAT for four patient samples is 50 minutes. Sample preparation of four samples takes about 5–10 minutes depending on the assay kit.

CLART® technology (GENOMICA S.A.U., Spain)

GENOMICA offers a number of CE-IVD-marked assays for infectious disease targets for use with its CLART® technology, which includes genetic amplification and visualization in low-density microarrays. For purposes of this report, the most interesting of these are the CLART® EnteroBac and the CLART® SeptiBac assay panels. CLART® EnteroBac detects the presence in stool samples of the main types of bacteria that produce endotoxins causing diarrhoea; these include *Salmonella* spp., *Shigella* spp., enteropathogenic *Escherichia coli*, *Campylobacter* spp. and *Clostridium difficile* B. Similarly, the CLART® SeptiBac assay detects and types gram-positive and gram-negative bacteria and fungi that cause septicaemia from positive blood culture. These include *Staphylococcus* spp., *Streptococcus* spp. (including

the methicillin-resistance marker *mecA*), *Enterococcus* spp., *Escherichia coli*, *Klebsiella* spp., *Salmonella enterica*, *Enterobacter* spp., *Haemophilus* spp., *Acinetobacter baumannii* and *Pseudomonas*.

The testing process begins with sample preparation, DNA extraction and DNA amplification/labelling of targeted molecules using standard laboratory equipment not provided by GENOMICA. This is followed by specific hybridization (labelled sample incubation) and visualization (conjugation and precipitation of the staining). Finally, colorimetric detection and analysis is done using the CAR (Clinical Array Reader from GENOMICA), pictured in Fig. 50. GENOMICA also offers two instruments that can be used to automate the visualization step: autoclart® and autoclart® plus.

Fig. 50. CAR® (Clinical Array Reader)

Additional assays available for use in association with the CLART® technology include CLART® HPV2, CLART® ENTHERPEX, CLART® PneumoVir, CLART® STIsA, CLART® STIsB, CLART® METABONE, CLART® CMA NRAS·iKRAS, CLART® CMA KRAS·BRAF·PI3K and CLART® CMA EGFR.

Other commercialized molecular amplification assays/platforms

In addition to the integrated NAAT and iNAAT systems described above, and in addition to purely LDTs, there are manufacturers of assay kits for detecting bacterial (and other) pathogens that are validated for use on certain commercially available open systems for sample preparation, nucleic acid extraction and amplification/detection. These include the following:

- Fast Track Diagnostics (Siemens, USA): Siemens recently acquired Fast Track Diagnostics, Ltd. (Malta), which offers a wide array of real-time PCR multiplex IVD kits for syndromic testing. These include test kit panels for respiratory infections (e.g., *Haemophilus influenzae*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Staphylococcus aureus* and *Streptococcus pneumoniae*), gastroenteritis (*Campylobacter coli/jejuni/lari*, *Clostridium difficile*, *Salmonella* spp. and *Shigella* spp.), STIs (NG), eye infections, fever/rash/childhood infections, tropical fever, hepatitis, infections of the immunosuppressed, meningitis infections and human adenovirus. The assays can be used on a range of samples, as appropriate, including tissue from biopsies, blood, faeces, sputum, CSF and swabs of mucosal surfaces. With the exception of kits for HBV/HCV and Rift Valley fever virus (RVFV), all kits are CE labelled for IVD use.

  The assay kits are for use with UgenTec's FastFinder™, a PCR interpretation platform. Depending on the assay, tests can be run on the following real-time PCR platforms: ABI 7500 (Thermo Fisher Scientific, USA), LightCycler® 480 II (Roche, USA) and CFX96™ (Bio-Rad, France).

- SepsiTest™-UMD (Molzym Molecular Diagnostics, Germany): Molzym offers SepsiTest™-UMD, which is a CE-IVD-marked PCR test for detecting bacterial and fungal DNA in 1 mL of K-EDTA (potassium-EDTA) or citrate-treated whole blood; it can also use blood culture, CSF, sputum and nasal swabs among other specimen types. The test is able to identify species from more than 200 genera of bacteria and 65 genera of fungi.

  SepsiTest™-UMD utilizes three processes: extracting and purifying microbial DNA using centrifugation, universal PCR and Sanger sequencing (discussed below). The PCR result can be available in 4 hours. Amplicons from positive samples are then sequenced to confirm the PCR result and to determine which bacterial or fungal species are present. Where readable sequences are available from sequence analysis, bacteria and fungi can be identified using the SepsiTest™-BLAST online tool. Sequencing results are typically available in 3–4 hours, depending on the analyser used, making a total TAT of about 8 hours or slightly more depending on the workflow in the laboratory.

  SepsiTest™-UMD can be performed on a number of real-time PCR instruments, including LightCycler® platforms, CFX96™, AriaMx and Mx3005P (Agilent, USA), ABI 7500 Fast (Thermo Fisher Scientific, USA), and Rotor-Gene® Q (QIAGEN, Germany). For sequencing, SepsiTest™-UMD has been validated for use with the ABI 3730xl and ABI Prism® 310 DNA analysers together with the BigDye® Terminator v3.1 cycle sequencing kit (Thermo Fisher Scientific, USA).

- Cognitor® Minus (Momentum Bioscience Ltd., UK): Momentum has developed an assay, the Cognitor® Minus test, that is designed to provide rapid, universal detection of viable microorganisms without the need for preselection. The test is performed on any standard thermocycler on negative blood culture samples after 12 hours of incubation and is used to confirm a negative BSI result. The technology uses Enzymatic Template Generation and Amplification (ETGA®) to detect microbial DNA polymerase activity common to a wide range of bacteria and fungi from blood culture after amplification by PCR. The assay is CE-IVD marked.

  The company is also planning to develop technology for the detection, ID and AST of positive blood cultures. No timeframe for this is available.

- xTAG® technology (Luminex, USA): Luminex offers a group of in vitro diagnostic assays that combines multiplex reverse transcription and RT-PCR with its proprietary universal tag system that allows easy development and optimization of nucleic acid assays. The assays consist of kit reagents and software. The assay of relevance to this report is the FDA-cleared xTAG gastrointestinal pathogen panel (GPP), which is a multiplexed nucleic acid test intended for simultaneously detecting and identifying 14 viral, bacterial and parasitic nucleic acids in human stool specimens or human stool in Cary-Blair media from individuals with signs and symptoms of infectious colitis or gastroenteritis. In addition to certain viruses and parasites, bacteria in the panel include *Campylobacter* (*C. jejuni*, *C. coli* and *C. lari* only), *tcdA* and *tcdB*, *Escherichia coli*, *Salmonella* and *Shigella*. The assay can aid in diagnosing gastrointestinal infection in conjunction with clinical evaluation.

  The xTAG® technology is not an integrated system. Each sample is pretreated prior to extraction; extraction purification is then done using the bioMérieux NucliSENS® easyMAG® kit. This is followed by multiplex amplification and bead hybridization and detection. Finally, xTag data analysis software for the GPP analyses the data and provides a report summarizing which pathogens are present. Data acquisition and analysis can be done by Luminex MAGPIX® or Luminex 200™ instruments. TAT is approximately 5 hours for 24 samples.

The use of the systems described immediately above is most appropriate for sophisticated laboratories with strong laboratorians and quality assurance in place.

Pipeline molecular systems for identifying pathogens

In addition to the commercialized molecular systems for identifying pathogens detailed above, there are a good number of systems in the diagnostic pipeline. Many of these platforms are currently designed to perform individual tests; others can multiplex. Unless otherwise noted, all of the test systems described below are under development and not approved for sale; their performance characteristics have not yet been established.

- Lucira Health, Inc. (USA) is developing a disposable test kit to detect DNA and RNA of infectious disease pathogens. As pictured in Fig. 51, the platform is a NAAT-based platform designed to look like a lateral flow device. The company has used both RPA and LAMP technologies.

Fig. 51. Lucira test

  The company is developing a test for influenza A and B direct from swabs; additional tests are planned for RSV and strep throat. No additional information is available.

- FAST-ID™ (Qvella, Canada) is developing a rapid, easy-to-use multiplex PCR assay system called FAST-ID™, pictured in Fig. 52. The first panel planned for the system is the FAST-ID™ BSI panel, which, per Qvella, will detect more than 95% of sepsis-causing pathogens direct from whole blood. The technology uses direct aspiration from a closed sample tube, which simplifies test setup and minimizes potential contamination. The system features fully automated intact pathogen recovery integrated with e-lysis™, which results in PCR-ready lysate, eliminating the need for multistep extractions. The test cartridge is disposable. TAT is approximately 1 hour.

Fig. 52. FAST-ID™ PCR system

- NanoDetection Technology (USA) is developing a novel diagnostic system that initially will be applied to detecting human infectious diseases. At the core of the system is a novel biochip technology by which 25 discrete light-sensing diodes enable multiplex testing with high sensitivity. The system, pictured in Fig. 53, allows labs to detect multiple diseases using analyte-specific cassettes.

Fig. 53. NanoDetection instrument

Workflow for the system is simple and requires the user to collect a patient specimen, prepare the sample of the system's cassette and insert the cassette into the instrument, as shown above. The proposed menu of assays for the instrument includes MRSA screening, MRSA/methicillin-susceptible *Staphylococcus aureus* (MSSA) dual screening, sepsis, influenza A and B, HCV, STIs and dengue.

- Biospectrix (3i Diagnostics, Inc. [3iDx], USA) 3iDx is an early-stage company that is dedicated to achieving faster diagnosis of BSIs direct from whole blood. The company's technology, Biospectrix, employs a microfluidics chip with microchannels and reservoirs etched into plastic tags that capture the sample of whole blood. Biospectrix then uses inertial forces to separate blood cells by size. After this separation, blood cells are forced through a nanoscale filter that lyses blood cells into fragments smaller than bacteria, or other microbes, allowing the microbes to collect intact. Blood cell debris is then removed with another filtering process, and excess water is removed to leave concentrated microbes on a surface made transparent for infrared examination. Infrared is used to perform a spectrometry analysis where the absorption of rays by the captured microbes form unique molecular signatures. The company indicates that these signatures allow it to identify a broad range of bacteria.

  The proposed workflow for Biospectrix is simple and can produce results within an hour. 3iDx also indicates that the platform is portable, easy to use and will be cost-effective.

The diagnostic platforms described above are but a few of the potential systems for identifying human infectious diseases in the pipeline. Others are earlier stage, have no plans to develop assays relevant to this report or otherwise are less relevant. These include, but are by no means limited to, the following: BLINK ONE (BLINK DX, Germany), Genedrive® (Genedrive plc, UK), Spartan Cube (Spartan Bioscience, Inc., USA), Accula™ Dock (Mesa Biotech, USA), Puckdx™ (TTP PLC, UK), Polyvalent Analyzer (PAx) platform (ChipCare, CA) and BluBox (BluSense Diagnostics, Denmark). Nonetheless, these and other platforms should be watched as at least some of them could be further developed over the next few years, specifically for use at POC in LMICs.

Sequencing methods

In addition to the molecular hybridization and amplification testing methods discussed above, sequence analyses of genes or the whole genome of pathogens are also being used in microbiology laboratories, primarily for research. Sequencing methods are able to identify bacterial pathogens that cannot be successfully cultured and to detect changing genetic features in evolving pathogens that cannot be detected by molecular testing (*40*).

Nucleic acid sequencing involves methods that determine the exact nucleotide sequence of a microorganism's genome, which is the blueprint for the

organism. As such, sequencing can, among other things (i) identify bacteria by sequence analysis of the 16S rDNA; (ii) detect mutations in viral or bacterial genomes that could lead to resistance against antivirals or antibiotics; (iii) detect and classify previously unknown human pathogens; and (iv) establish the genetic relationship of either bacteria or viruses.

In the early years of sequencing, the most commonly used method was Sanger sequencing. In this method, target DNA is copied many times, making fragments of different lengths; fluorescent "chain terminator" nucleotides mark the end of the fragments and allow the sequence to be determined. However, Sanger sequencing is laborious and slow, and it has largely been replaced by new, automated methods, referred to as next-generation sequencing (NGS). Using NGS, an entire human genome can be sequenced in a single run. While this means that NGS does not require target-specific primers, it does require the preparation of libraries in which fragments of DNA or RNA are fused to adapters and barcodes to distinguish the DNA of the sequenced isolate after sequencing (*40*). Data analysis following sequencing remains a challenge with NGS, as it requires bioinformatics skills and computational resources to analyse large data sets. This analysis is also very time-consuming, taking up to 4–5 days (*40*).

There are currently two sequencer platforms in relatively common use in sophisticated laboratories: Illumina MiSeq™Dx (Illumina, Inc., USA), which has commercially available IVD applications; and Ion PGM™ from Thermo Fisher Scientific (USA), the applications for which are for research use only (RUO). They are discussed briefly below.

MiSeq™ Dx instrument

The MiSeq™Dx instrument, pictured in Fig. 54, uses a fluorescence-based approach to reading the bases in a nucleotide sequence. It is the first FDA-cleared and CE-IVD-marked platform for NGS. The MiSeq™Dx is a compact benchtop sequencer with a relatively simple, three-step workflow and an integrated software design, which enables sample tracking, user traceability and results interpretation. The workflow starts with gDNA extracted from human peripheral whole blood specimens or formalin-fixed, paraffin-embedded (FFPE) tissues. The MiSeq™Dx instrument is built on Illumina sequencing by synthesis, which compared to Sanger sequencing, can provide a broader range of DNA variants in less time and with fewer hands-on steps.

Fig. 54. MiSeq™Dx instrument

Illumina currently offers a small menu of IVD assays and kits. These include the MiSeq™Dx Cystic Fibrosis 139-Variant Assay and the MiSeq™Dx Cystic Fibrosis Clinical Sequencing Assay. The company also offers the TruSeq™ Custom Amplicon Kit Dx, which facilitates LDTs that target the genetic variants most important to any given laboratory.

Ion PGM™ Dx system (Thermo Fisher Scientific, USA)

The Ion PGM™ Dx system is composed of a sequencing instrument that measures the hydrogen ions generated during the incorporation of nucleotides in the DNA sequencing reaction and the ancillary instrumentation necessary for sample processing. More specifically, the system employs Ion Torrent™ technology, which uses pH measurements to read nucleotide sequences using semiconductors, rather than the optics or modified nucleotides used in many other NGS technologies. Ion Torrent™ technology directly translates chemically encoded information (A, C, G, T) into digital information (0, 1) on a semiconductor chip, which per the company is simpler, faster, and more cost-effective and scalable than other NGS methods.

The Ion PGM™ Dx system is used in conjunction with the instrument-specific Ion PGM™ Dx Library Kit, Ion OneTouch™ Dx Template Kit, Ion PGM™ Dx Sequencing Kit, Ion 318™ Dx Chip Kit and data analysis software. The Ion PGM™ Dx system is intended for targeted sequencing of human gDNA derived from peripheral whole blood, and DNA and RNA extracted from FFPE samples. The Ion PGM™ Dx system is not intended for whole genome or de novo sequencing.

Of note with regard to this report is that the Ion S5™ instrument, pictured in Fig. 55, performs targeted sequencing of bacteria, viruses or fungi from biological specimens without the need for culture. It could be of interest if commercialized IVDs become available for the platform.

Fig. 55. Ion S5™ system

The NGS workflow is illustrated in Fig. 56 for the Oncomine™ Immune Response Research Assay (Thermo Fisher Scientific, USA), a precancer gene expression assay that enables quantitative evaluation of the expression of markers associated with tumour progression. The assay contains the reagents for manual library construction using Ion AmpliSeq™ and a single pool of primers used to perform multiplex PCR for preparation of amplicon libraries using the Ion S5™ instrument. The assay is supported by informatics analysis on the Ion Torrent Suite™ plug-ins. Time to results is about 48 hours.

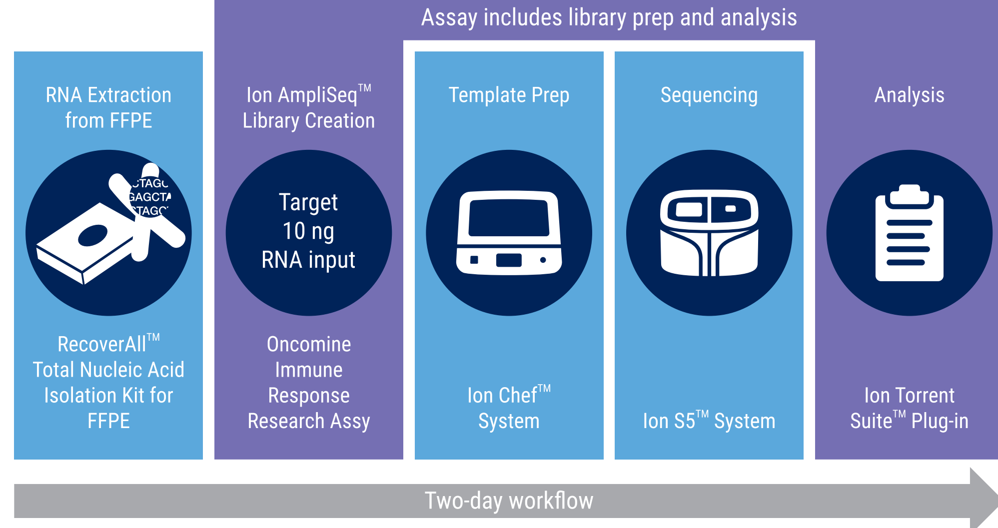
To date, the Oncomine™ assay and other assays for the S5™ Dx instrument are RUO. Laboratories are using the platform and other platforms for NGS to create LDTs. There are no commercially available IVD applications for the Ion S5™.

In their present configurations, these NGS systems are used in highly sophisticated laboratories primarily for research. There are additional systems available, including MinION (Oxford Nanopore Technologies, UK) and Sequel (Pacific Biosciences, USA), but these systems are not yet used in clinical microbiology labs for reasons of affordability, lower quality of sequences and low throughput (*40*).[7] Some studies have shown applicability of NGS in drug-resistance testing (*48*).

Other methods of identifying bacterial pathogens

In addition to the phenotypic, immunoassay and molecular methods of identifying bacterial pathogens discussed above, other techniques are also available. One of these is MS, an analytical technique in which chemical compounds are ionized into charged molecules and the ratio of their mass to charge (m/z) is measured (*49*).[8] In the 1980s, two methods of MS were developed that have brought these methods into the clinical microbiology laboratory: electron spray ionization (ESI) and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) MS. Of the two methods, MALDI-TOF MS has the advantage of being completely automated, allowing for high throughput and speed, which means TATs are typically

Fig. 56. Oncomine™ assay workflow



[7] For detailed information on the future impact of NGS, see Deurenberg, Bathoorn, Chlebowicz et al. (*47*)
[8] For a detailed description of the principles and methodology of MALDI-TOF MS, see Singhal, Kumar, Kanaujia et al. (*49*).

reduced by at least one working day compared to conventional ID methods (*50*). MALDI-TOF is the MS method most often used to identify bacterial species in large clinical laboratories.

MALDI-TOF MS

MALDI-TOF ionizes target pathogen proteins, and a mass profile of the resulting fragments is then created and compared to a large database of organism profiles for ID. It consists of three basic principles: ionization, separation and detection. MALDI-TOF MS is able to identify bacterial and other pathogens most commonly from isolates cultured on solid media, and it has been widely adopted by clinical microbiology laboratories for this purpose (*50*, *51*). Research has shown that MALDI-TOF can also be used for identifying bacteria from other specimen types (e.g., blood, urine, CSF) to detect UTIs, respiratory tract infections and enteric infections (*49*); however, when subculture isolates are used, it means a delay of 24–36 hours from blood culture positivity before ID results can be obtained. MALDI-TOF generally cannot be used alone for AST, but there are some software systems available to allow interface of MALDI-TOF testing modules with certain automated AST systems after pathogen ID (*52*).[9]

There are two well established, commercially available MALDI-TOF systems: (i) Bruker MALDI Biotyper® (Beckman Coulter, a Danaher Corporation, USA) and (ii) VITEK® MS (bioMérieux, France). Both platforms are FDA cleared and approved for use as IVDs. Each of these systems uses a different database, ID algorithm and instrument.

Bruker MALDI Biotyper® system

The Bruker MALDI Biotyper® system identifies microorganisms (bacteria and yeast) using MALDI-TOF MS to determine the unique protein fingerprint of an organism. In particular, the Bruker MALDI Biotyper® system measures highly abundant proteins found in all microorganisms. The characteristic patterns of these proteins are then used to identify a particular microorganism by matching it against a microorganism reference library/database. The Bruker MALDI Biotyper® system has received FDA clearance and CE-IVD marking for the identification of gram-negative and gram-positive bacteria, anaerobic bacteria and yeast cultured from human specimens. The device is to be used in conjunction with other clinical and laboratory findings to aid in diagnosing bacterial and yeast infections. The Bruker MALDI Biotyper® system can identify 333 species or species groups, covering 424 clinically relevant bacteria and yeast species. Per the company, these represent more than 98% of the typical bacterial ID workflow of clinical microbiology laboratories.

The Bruker MALDI Biotyper® system comprises the Bruker MALDI-TOF benchtop microflex™ LT/SH or the microflex™ smart LS (pictured in Fig. 57), software, IVD-labelled reagents, a disposable MBT Biotarget 96 or a reusable 48-spot MALDI target plate, and the FDA-cleared microorganism reference library.

Fig. 57. microflex™ smart LS

The microflex™ LT/SH is a high-performance system designed for basic applications, while the microflex™ LT/SH has been designed for applications where a short TAT is required. It utilizes a fast, solid-state Bruker smartbeam® laser and high-performance vacuum system.

The Bruker MALDI Biotyper® system does not perform AST, but it can be used in conjunction with the MBT STAR-Carba IVD kit and the MBT STAR-BL IVD kit software. Collectively, these allow for rapid microorganism ID and detection of carbapenemase activity in a single workflow.

The MBT-STAR Carba kit utilizes imipenem as the benchmark carbapenem antibiotic. Bacteria from overnight cultures or positive blood cultures are incubated in an imipenem solution. Bacteria with active carbapenemase will inactivate imipenem by hydrolysation of the beta-lactam ring, which is associated with a mass shift. In assays with bacteria without active carbapenemase, only peaks corresponding to the intact antibiotic will be present in the mass spectrum; whereas in assays with bacteria with active carbapenemase, peaks of the intact antibiotic will decrease.

[9] For more information on the use of resistance and/or AST-like methods in combination with MALDI-TOF, see Vrioni, Tsiamis, Oikonomidis et al. (*52*).

The intensity ratio of hydrolysed to intact antibiotic signals indicates the level of carbapenemase activity. The MBT STAR-BL IVD software module monitors the activity on acquired mass spectra of aliquots of the co-incubation solution by automatically calculating the intact imipenem intensity and corresponding ratio of hydrolysed to nonhydrolysed signals.

The MBT-STAR Carba kit can be used to rapidly detect prevalent Class A, B or D carbapenemase activity in gram-negative *Enterobacteriaceae*, *Pseudomonas* spp. and *Acinetobacter* spp.

VITEK® MS

VITEK® MS is a mass spectrometry system using MALDI-TOF MS to identify microorganisms cultured from human specimens. The system is a qualitative in vitro diagnostic device indicated for use in conjunction with other clinical and laboratory findings to aid in diagnosing bacterial, yeast and mould infections.

The VITEK® MS process is as follows. Depending on the isolate culture, the analyte sample may be directly spotted to a target slide; some analyte specimens (e.g., *Mycobacterium*) must first be processed before spotting. The slide is then loaded onto the MS VITEK® MS instrument, pictured in Fig. 58, where a laser targets the sample spot and pulses the isolate spot, resulting in vibrational excitation of matrix and analyte molecules. The matrix transfers protons to the analyte, resulting in a positive charge. The ionized molecules are then accelerated in an electromagnetic field and a grid electrode in the ionization chamber. The time of flight is then measured by the arrival of ions in a particle detector. Based on the time of flight, the m/z ratio of each particle can be determined and a mass spectrum of the analyte sample mixture is generated. The mass spectra are sufficiently unique to allow taxonomic characterization at the genus and species level. The instrument runs up to 192 isolates per run and delivers results in minutes.

Fig. 58. VITEK® MS instrument

The VITEK® MS system has received FDA clearance and CE-IVD marking for identifying an extensive number of bacterial pathogens. These include, but are not limited to, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Shigella* (which are both characterized as *E. coli*), *Klebsiella pneumoniae*, *Clostridium difficile*, NG (for which a confirmatory test is recommended), *Enterococcus faecium*, *Staphylococcus aureus*, *Campylobacter* spp., *Salmonellae* (for which a confirmatory test is recommended), *Streptococcus pneumoniae*, *Haemophilus influenzae* and MTB complex.

In addition to the VITEK® MS instrument, the VITEK® MS system consists of the VITEK® MS preparation station, which is used to prepare the target slides, and the VITEK® MS acquisition station, which is connected to the instrument and displays its status and the spectra from the instrument. The spectra are then sent to MYLA from the acquisition station for analysis. The MYLA® server contains the MYLA® software and is a middleware solution. MYLA® is connected to both the VITEK® MS preparation station and VITEK® MS acquisition station, providing complete integration and traceability of all individual patient samples and their results.

Other MS methods

In addition to MALDI-TOF MS, other methods of MS have been developed. These include electrospray-ionization MS (ESI-MS) and surface-enhanced laser desorption/ionization (SELDI). Both of these are variations on MALDI-TOF MS. ESI-MS uses a multiplex pool of PCR primers that target conserved sequences in bacterial genomes together with high-precision EIS-MS to identify and group organisms. SELDI is a technique for enriching proteins with specific chemical characteristics and combines chromatography with MS (*53*). While these methods are interesting and could be used in clinical microbiology, no currently available commercialized platforms were found.

Conclusion

Immunoassays for bacterial detection are easy to use and fast, with very short TAT. In addition, because the assays generally do not require equipment beyond a test cartridge, the tests can be used at primary care facilities in LMICs. Their utility is somewhat limited, however, because the tests are monoplexes, which can only identify a single bacterial pathogen. In addition, because the performance of some assays has proven to be inadequate, this must be considered with respect to the implementation of the tests.

Molecular-based testing, sequencing and MS, in particular MALDI-TOF MS, are all more rapid IVD pathogen ID methods than traditional phenotypic

methods, and in many cases they can identify certain pathogens, including fastidious or slow-growing bacteria, that cannot be readily identified by culture methods. However, like traditional culture techniques, these methods are most appropriate for use in medium- to high-throughput, sophisticated laboratories with significant infrastructure, consistent electricity, climate control and refrigeration, and well trained laboratory technicians. In resource-limited settings, this would mean that the tests and test platforms would be best positioned in Level III and, more likely, Level IV settings.

Of the platforms described above, there are several diagnostic platforms/systems that have the potential to be used at or near POC. These include the cobas® Liat® system, Solana® platform and revogene®, for which there are no multiplex panels currently available, as well as the Novodiag® system, STAT-Dx and Vivalytic analyser/Bosch Healthcare Solutions, for which multiplex, syndromic testing panels are already available, although not for BSIs. With the exception of the MR platform from T2 Biosystems, none of the platforms can detect BSIs, including *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, from whole blood.

Therefore, for now, there are gaps in the ability of these platforms to identify many of the bacterial pathogens prioritized by WHO. In any event, these platforms do not perform resistance testing and would have to be supplemented with reflex testing to identify genes that directly confer bacterial resistance.

IVDs for AST and antibiotic resistance testing of bacterial pathogens

Given the ability of bacterial and other pathogens to cease to be susceptible to, and/or acquire and express resistance to, the antimicrobial agents used to treat infections, once pathogen ID has been completed, it is important to do an AST profile of the organism in order to confirm susceptibility to empirical antimicrobial agents or to detect resistance in individual bacterial isolates. Phenotypic AST methods are most commonly used for these purposes. Like bacterial ID, AST can be performed manually or using more rapid, growth-dependent automated instruments in the microbiology laboratory. While generally effective, these phenotypic methods are slow and can be costly; they also require a relatively large number of viable microorganisms and have a limited organism spectrum (*54*).

Since all phenotypic traits of microorganisms, including those that render them resistant to antimicrobial agents, are encoded on specific genes, genotypic methods can be used to detect the genes coding antimicrobial resistance (*28*). These methods include molecular methods like PCR and DNA microarrays, as well as, to a limited extent, MALDI-TOF MS and NGS (*48*, *53*). Although faster than phenotypic methods, these methods still have certain downsides. For example, the presence or absence of a gene or a mutation that affects gene function or regulation cannot always accurately predict antibiotic resistance. These methods are also costly.

Phenotypic methods of AST

At the most fundamental level, phenotypic AST methods bring together the antimicrobial agent of interest and the bacterial microorganism in the same in vitro environment to determine the effect of the presence of the antimicrobial on bacterial growth or viability (*28*). The bacterial growth is then measured along with the organism's resistance or susceptibility to the antimicrobial agent. In particular, the minimum inhibitory concentration (MIC) is measured. The MIC is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation (*55*). This is usually translated into a susceptibility testing category based on well-established studies and criteria for an array of antimicrobial agents.[10] The primary interpretive categories, also referred to as break points, are susceptible, intermediate or resistant.

Regardless of the AST method used, certain antimicrobial agents will be selected for use against a particular bacterial isolate. These agents are referred to as the antimicrobial battery or panel. Antimicrobials to which the bacterial isolate is intrinsically resistant are excluded from the test panel (e.g., vancomycin with respect to gram-negative bacilli). By the same token, some antimicrobials have been developed specifically for use against particular bacterial isolates, but not against others (e.g., ceftazidime for use against *Pseudomonas aeruginosa*, but not against *Staphylococcus aureus*) (*28*). These and other considerations guide the selection of the ultimate antimicrobial test battery used.

As indicated above, several methods are available for AST: classical susceptibility testing methods using solid media (e.g., disk diffusion and antimicrobial gradient), liquid media (broth dilution) and chromogenic media, as well as manual and automated commercial susceptibility testing systems, which use various media.

Classical methods of AST

The following manual methods bring together antimicrobial agents and bacterial isolates in solid media. In some cases, commercial methods are available to speed and improve the test procedures. These are noted below.

- Agar dilution: In agar dilution testing, bacteria are inoculated into an agar medium containing various antimicrobial concentrations. The method is laborious as with each doubling dilution of an antimicrobial agent, it is incorporated into a single agar plate, which means that testing a series of six dilutions of one antimicrobial agent requires the use of six plates, plus a positive growth control plate. Following incubation,

[10] Note that in the United States, the Clinical and Laboratory Standards Institute (CLSI) publishes a series of documents on AST that, among other things, provide lists of potential antimicrobial reagents for routine testing and interpretive criteria for an array of antimicrobial agents, which are updated annually.

the plates are examined for growth and the MIC break points are determined and interpreted using CLSI, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) or other established criteria.

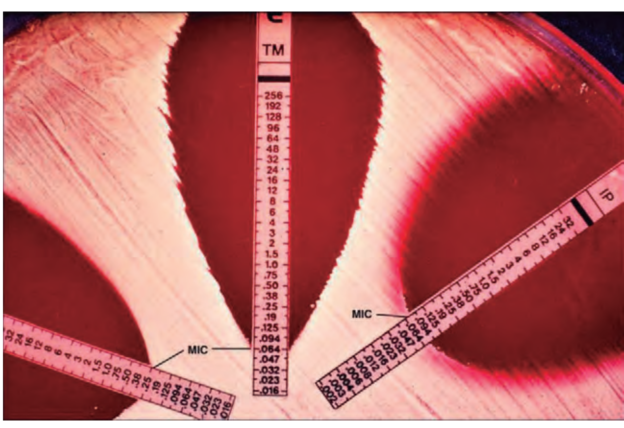
- Disk diffusion test: In disk diffusion AST, which is used to test common, rapidly growing bacterial pathogens (*4*), antibiotic-impregnated filter paper disks (antibiotic discs) are placed on the surface of an agar plate seeded with the bacterial isolate being investigated. With incubation, the bacteria grow on the surface of the plate except where the antibiotic concentration in the gradient around each disk is high enough to inhibit growth. After 16–24 hours of incubation, the diameter of the zone of inhibition around each disk is measured and interpreted using CLSI, EUCAST or other established criteria. While this method is easier, more flexible and less expensive than agar dilution, it provides only qualitative results (susceptible, intermediate or resistant).

  The BIOMIC® V³ microbiology system (Giles Scientific, USA) automates reading, interpreting and expert review of CLSI or EUCAST antibiotic disk diffusion (Kirby-Bauer) tests on 90–150 cm plates. An enlarged plate image with antibiotic disks and zone diameter reading can be displayed on the instrument's screen to provide high-resolution images. It is also possible to combine the results with computer software to produce MIC values. BIOMIC® V³ can also provide antibiotic resistance monitoring following CLSI or EUCAST guidelines, including a variety of antibiogram reports.

- Antimicrobial gradient method: The antimicrobial gradient method is a diffusion method that establishes an antimicrobial concentration gradient in an agar medium in order to determine susceptibility. Unlike the manual disk diffusion test, the antimicrobial gradient method can be used to determine quantitative MICs, which may be necessary in some situations.

  Etest® (bioMérieux, France) is a commercial version of the antimicrobial gradient method. It uses thin plastic strips, one side of which is impregnated with a dried antibiotic concentration gradient and the other side of which contains a numerical concentration scale. Five or six strips may be placed radially on an appropriately inoculated agar plate; this permits multiple antimicrobials to be tested on a single bacterial isolate. Following overnight incubation, the plate is examined and read by viewing the strips from the top of the agar plate. The MIC is determined by reading the number on the concentration scale where the border of growth/inhibition edge intersects with it, which is illustrated in Fig. 59.

Fig. 59. Etest® strips and interpretation scale

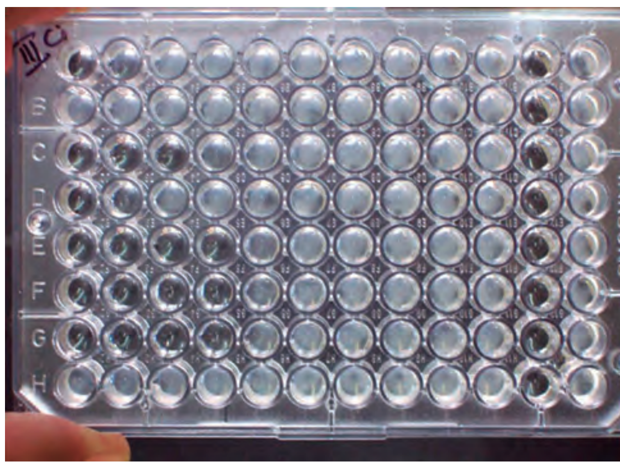


In addition to AST testing using solid media, broth dilution is also an available manual technique.

- Broth dilution: In broth dilution methods, the bacterial organism of interest is challenged with antimicrobial agents in a liquid growth medium. In macrobroth or tube dilution, dilutions of antibiotics are dispensed into test tubes. Each antimicrobial agent is tested using a range of concentrations. Following an incubation time of 20–24 hours, tubes are examined for visible bacterial growth, with the lowest concentration of antibiotic that prevented growth recorded as the MIC. Macrobroth dilution testing produces a quantitative result, but it is laborious and requires considerable space in the laboratory.

  In microbroth dilution testing, the methods are the same as macrobroth dilution, but the total broth volume required is much smaller, effectively miniaturizing the procedure. The testing has been standardized and can be done in small, disposable "microdilution" trays, like the one pictured in Fig. 60, which contain 96 wells and predetermined antibiotic concentrations. Moreover, there are commercially supplied frozen or dried microdilution panels available. These include the following: Sensititre™ (Thermo Fisher Scientific, USA) and MicroScan TouchScan (Siemens, USA).

Fig. 60. Broth microdilution tray



Chromogenic culture media

The use of chromogenic culture media for bacterial pathogen ID and AST is a manual method that utilizes synthetic chromogenic enzyme substrates to specifically identify pathogenic microorganisms based on their enzymatic activity. The majority of chromogenic media, which may be solid or liquid, are both selective and differential, accommodating the inhibition of nontarget microorganisms while enabling target pathogens to grow as coloured colonies due to their metabolism. The method exhibits high specificity.

Although chromogenic media are generally more expensive than traditional media, use of a single chromogenic medium rather than two or three selective ones reduces the cost of sample processing. Further, because only target pathogens should generate colonies of a particular colour, the number of colonies in a polymicrobial culture that require further examination should be reduced. This often results in cost savings from reduced labour time. Because the use of chromogenic media may eliminate certain steps in sample processing (e.g., subculturing and biochemical testing), it can also contribute to quicker confirmation of pathogens and reduce the overall time to result compared with conventional culture methods (*56*).

There are a wide range of chromogenic media commercially available to clinical laboratories from manufacturers, including bioMérieux, Thermo Fisher Scientific and Bio-Rad. These can be used to detect numerous bacterial pathogens. These include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Clostridium difficile*, *Campylobacter* spp., *Salmonella* spp., *Shigella* spp. and Shiga toxin-producing *Escherichia coli*.

Chromogenic media can also be used to screen pathogens with acquired antimicrobial resistance, including VRE, ESBL-producing and carbapenemase-producing *Enterobacteriaceae*, carbapenem-resistant *Acinetobacter* spp. and MRSA.[11]

Culture using chromogenic media, like other culture methods, is used in conjunction with methods of bacterial pathogen ID, including molecular methods and MALDI-TOF MS. The convenience of molecular methods for the ability to screen for a wide variety of pathogens – e.g., enteric and respiratory – simultaneously and effectively is of great value, but the use of chromogenic media to complete pathogen analysis – e.g., for AST following ID by PCR or other molecular methods – is also required. As Perry concludes: “Chromogenic media should no longer be assessed as individual tools but as potentially useful components within diagnostic algorithms” (*56*).

MICRONAUT ID and AST (MERLIN Diagnostika GmbH, a Bruker company, Germany)

MERLIN offers a number of MICRONAUT assays for ID and susceptibility testing of bacteria and yeasts. These assays are primarily manual, but can be combined with some automated instrumentation.

The test principle of MICRONAUT ID systems is based on phenotypic detection of the biochemical properties of microorganisms. Various substrates are placed in dehydrated form into the wells of microtitration plates and are dissolved by adding bacterial suspensions. Following an incubation period of 5 to 24 hours, depending on the test system, the ID plate is measured in a commercially available photometer (not provided by MERLIN) and the test is evaluated by MICRONAUT software (for which an external computer is required).

All MICRONAUT ID systems offer a standardized procedure and optimized software-controlled reading and interpretation of results. Available assays include:

- MICRONAUT-GNE: identifies *Enterobacteriaceae* and other gram-negative bacteria. Identification takes place via 24 biochemical reactions (chromogene substrate, decarboxylases, classical reactions and fermentations after 18–24 hours; 65 different taxa are included in the database.
- MICRONAUT-NF: identifies nonfermenting gram-negative and some glucose-fermenting bacteria via 27 biochemical reactions (decarboxylases, fermentation, assimilations, glucosidases/esterases, classical reactions). TAT is 24 hours; 62 different taxa are included in the database.

[11] For a review a various media available for bacterial pathogens and their performance, see Perry (*56*).

- MICRONAUT-STAPH: identifies relevant staphylococci within 6 or 18–24 hours via 21 biochemical reactions; 21 different taxa are included in the database.
- MICRONAUT-IDS: identifies clinically important *Enterobacteriaceae*, nonfermenters, staphylococci, enterococci and streptococci via 23 biochemical reactions (peptidases, decarboxylases, glucosidases/esterases, fermentations and classical reactions). TAT is 5–6 hours.

In addition to the MICRONAUT ID systems, MICRONAUT also offers AST systems. The test principle of the MICRONAUT system for AST is based on classical phenotypic detection of resistance as expressed by microbial growth in the presence of antibiotic compounds. The microdilution procedure used is a standardized method with accepted reference systems (EUCAST or CLSI) for determining MICs.

For all MICRONAUT AST systems, the various antibiotics are placed with or without broth in a dehydrated form into the wells of microtitration plates supplied by the company and are dissolved by adding bacterial suspensions. After 6 hours (with rapid AST) or 18–24 hours of incubation at 35–37 °C, the AST plate is measured in a photometer (commercially available), and the test is evaluated with the MICRONAUT software or read visually. Users can choose between EUCAST or CLSI standards, and customize antibiograms from more than 200 antibiotics or select from a wide variety of standard layouts.

MERLIN offers MICRONAUT AST microplates for automated or manual susceptibility testing of bacteria, MICRONAUT-S plates and MICRONAUT-SB plates; the latter offers a shorter incubation period of 6 hours for rapid AST. In addition to these two AST systems, there is also a manual MICRONAUT MIC-Strip Colistin test that enables AST against the backup antibiotic colistin by using the broth microdilution method for *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* group. The assay, which is CE-IVD marked, allows phenotypic detection of the *mcr-1* colistin resistance gene using standardized MIC determination of colistin according to EUCAST recommendations in a test strip format.

In addition, the MICRONAUT-UR test system is designed to both identify and detect susceptibility in UTIs. Fifty taxa, including *Acinetobacter* spp., *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella* spp. and *Staphylococcus aureus*, are identified using 24 reactions. Further, AST of all relevant gram-negative bacilli and gram-positive cocci is done using 22 antibiotics, including MRSA detection and ESBL screening. With the exception of automated reading, evaluation and interpretation of results, the test is manual.

MERLIN also offers the MICRONAUT ASTroID, which is an automated workflow combining MALDI-TOF MS for microbial ID and MICRONAUT for AST. Procedures are standardized, and identical samples are used for ID and AST.

Given the manual nature of the MICRONAUT systems, their use is generally most suited to sophisticated laboratories with well trained laboratory technicians. In addition, because the system uses traditional phenotypic methods of microbial ID and AST, results generally take a day or more.

Automated combined ID and AST systems

In addition to conventional manual methods of AST, there are four automated combined ID and AST instruments: VITEK® 2 system (bioMérieux, France), BD Phoenix™ automated microbiology system (BD, USA), MicroScan systems (Beckman Coulter, a Danaher Corporation, USA) and Sensititre™ ARIS™ 2X (Thermo Fisher Scientific, USA). These systems, which are relatively easy to operate and provide a streamlined workflow and quantitative results, are in widespread use in clinical microbiology laboratories in the United States, but are not generally available in resource-limited settings (*4*, *5*). Nonetheless, the automated systems, like current manual AST systems, require the use of cultured bacterial isolates, and AST is based on bacterial growth and turbidity changes. Therefore, even the commercially available, automated systems are plagued by slow TAT and low sensitivity (*4*).

The primary commercialized automated AST systems are described below. They use varying degrees of automation of inoculum preparation and inoculation, varying methods to detect growth, and varying algorithms to interpret and determine MIC values as well as categorical findings (i.e., susceptible, intermediate or resistant).

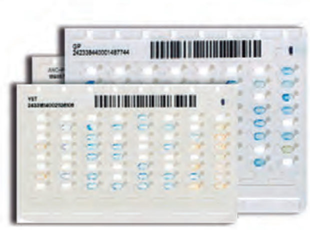
VITEK® 2 system

The VITEK® 2 system is a family of automated instruments (VITEK® 2 Compact, VITEK® 2 and VITEK® 2 XL), which differ with respect to levels of automation and capacity. All three systems use the same compact, colorimetric reagent cards (about the size of a credit card), which are incubated and interpreted automatically. While the VITEK® 2 Compact is primarily for industrial use, it could be used in low- to medium-volume clinical laboratories; the VITEK® 2, pictured in Fig. 61, and VITEK® 2 XL are primarily for use in large clinical microbiology laboratories.

Fig. 61. VITEK® 2 system

The VITEK® 2 system reagent cards, pictured in Fig. 62, have 64 wells that can each contain an individual test substrate. Each card has a pre-inserted transfer tube used for inoculation. Cards also have barcodes that contain information on product type, lot number, expiration date and a unique identifier. There are currently five reagent cards available for identifying different organism classes: (i) gram-negative fermenting and nonfermenting bacilli – 76 antimicrobials and ESBLs; (ii) staphylococci and/or enterococci – 55 antimicrobials, four high-level aminoglycoside screens and an inducible clindamycin resistance (ICR) test; (iii) streptococci – 14 antimicrobials and ICR test and gentamicin synergy; (iv) *Streptococcus pneumoniae* – 23 antimicrobials; and (v) yeasts – six antifungals.

Fig. 62. VITEK® 2 colorimetric ID cards



ID cards are inoculated with microorganism suspensions using an integrated vacuum device, and a test tube containing the microorganism suspension is placed into a special rack (cassette). The ID card is placed in a neighbouring slot. The VITEK® 2 and VITEK® XL cassettes can accommodate up to 15 tests; VITEK® 2 Compact cassettes can accommodate up to 10 tests. Inoculated cards are then incubated using repetitive turbidimetric monitoring of bacteria grown during an incubation period of between 4 and 10 hours; the carousel incubator can accommodate up to 30 or up to 60 cards.

During incubation, the VITEK® 2 systems perform optical readings every 15 minutes to measure the light transmitted through each well. Algorithmic analysis of the growth kinetics in each well is performed by the system's software to derive the MIC results, which are validated with the VITEK® Advanced Expert System™ (AES) software. An interpretation category is assigned, and the organism's antimicrobial resistance patterns are reported.

The VITEK® 2 instruments can be linked with the VITEK® MS ID system using MYLA connectivity. The VITEK® MS ID system and MYLA® are detailed in the previous section of this report.

BD Phoenix™ automated microbiology system

The BD Phoenix™ automated microbiology system is an automated ID and susceptibility system for testing clinically relevant bacterial isolates. It is intended for in vitro rapid ID and quantitative determination of antimicrobial susceptibility by the MIC of certain bacterial pathogens. The system comprises the BD Phoenix™ instrument, pictured in Fig. 63, and software, disposable panels containing biochemicals for organism ID testing and antimicrobial agents for AST determinations, broths for ID and AST, and a susceptibility testing indicator.

Fig. 63. BD Phoenix™ automated microbiology system instrumentation

The BD Phoenix™ system identifies a broad range of gram-positive (including genera *Staphylococcus, Streptococcus* and *Enterococcus*) and gram-negative (15 different genera, including *Acinetobacter*, *Enterobacter*, *Pseudomonas*, *Salmonella* and *Shigella*) bacteria using modified conventional, fluorogenic and chromogenic substrates. The instrument can analyse up to 100 ID and AST combination panels at the same time. The BD Phoenix™ disposable test panel, pictured in Fig. 64, is a sealed, self-inoculating moulded polystyrene tray with 136 microwells containing dry reagents and is available in ID-only, ID/AST and AST-only formats.

Fig. 64. BD Phoenix™ AST panels

Fig. 65. DxM MicroScan WalkAway system

Bacteria for susceptibility testing must be a pure culture and already preliminarily identified as either a gram-negative or gram-positive isolate. The BD Phoenix™ AST method is a broth-based microdilution test that uses a redox indicator to detect organism growth in the presence of an antimicrobial agent. It monitors and reads each panel every 20 minutes using measurements of changes to the indicator as well as bacterial turbidity to determine bacterial growth. The readings are interpreted to provide (i) an ID of the bacteria isolate; (ii) MIC values; and (iii) categorical interpretations (susceptible, intermediate, resistant or not susceptible) of bacterial growth. MIC results are generated in 6–16 hours.

MicroScan systems

Beckman Coulter currently offers three MicroScan systems for ID/AST of clinically relevant bacterial isolates, including gram-positive (*Staphylococcus* and related genera and *Streptococcaceae*) and gram-negative glucose fermenting as well as glucose nonfermenting bacteria. The systems are the DxM MicroScan WalkAway system, pictured in Fig. 65, the MicroScan WalkAway *plus* system, and the autoSCAN-4 system. The DxM MicroScan WalkAway and the MicroScan WalkAway *plus* systems are automated systems, which consist of MicroScan panels (microwells), an inoculator, a MicroScan WalkAway instrument and a LabPro information system. The autoSCAN-4 system is a smaller, manual reading system only, with a separate, stand-alone incubator device. The two automated systems are self-contained incubators/readers that can incubate and analyse 40–96 MicroScan panels at a time.

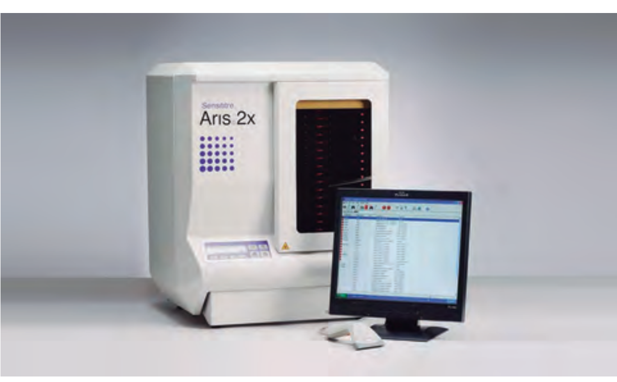
With an incubation time of 16–18 hours, the two automated MicroScan systems are overnight testing systems that offer either a full MIC panel or combination ID and AST panels, which are manually inoculated with bacteria isolated from clinical specimens and then placed in one of the incubator slots in the instrument. The panels contain ID media consisting of preloaded substrates and/or growth inhibitors, which will exhibit colour changes (fluorogenic substrates) or increases in turbidity (if using turbidimetric endpoints). The panels may also contain series of antimicrobial agents in specified concentrations for AST. The instrument incubates the trays for an appropriate time depending on the panel, examining them periodically with either a photometer or fluorometer to determine growth development, and determines the MIC. The company cites as an advantage of their systems that the MIC technology on them detects emerging resistance as it occurs, without reliance on historical data or virtual MIC.

The DxM MicroScan WalkAway and the MicroScan WalkAway *plus* systems are intended for use in medium- to high-capacity laboratories, while the autoSCAN-4 device is intended for small-capacity laboratories. For optimized, high-volume testing, the DxM MicroScan WalkAway system can be paired with the Copan WASP® DT and the Bruker MALDI Biotyper®, both of which were described earlier in this report.

Sensititre™ ARIS™ 2X

The Sensititre™ ARIS™ 2X, pictured in Fig. 66, is an automated benchtop in vitro diagnostic instrument for ID and clinical susceptibility testing of nonfastidious gram-negative isolates (*Enterobacteriaceae*, *Pseudomonas aeruginosa* and other non-*Enterobacteriaceae*) and of nonfastidious gram-positive isolates (*Staphylococcus* spp., *Enterococcus* spp. and beta-haemolytic streptococci other than *Streptococcus pneumoniae*). Additional testing capabilities are for yeasts (*Candida* spp.) and MTB. Not all tests are FDA cleared; some are CE-IVD marked or have RUO designations.

Fig. 66. Sensititre™ ARIS™ 2X instrument



The Sensititre™ ARIS™ 2X is an overnight incubating and reading device that can accommodate 64 MIC, break point or ID plates, for a combination of 192 possible tests on a single instrument. The instrument is part of the Sensititre™ system, which includes the Sensititre™ AIM™ system for automated inoculation, the OptiRead™ automated fluorometric plate reading system, the Vizion™ MIC viewing system, which facilitates reading of all organisms and the Sensititre™ Windows® (SWIN) software system, which combines manual, semi-automated and fully automated read options onto a single software platform.

Inoculation of the Sensititre™ ARIS™ 2X instrument panels (96-well plates) may be done using the automated Sensititre™ AIM™ instrument or manually. The ID system is based on 32 biochemical tests pre-dosed and dried in the Sensititre™ plates; the plates may be read manually or using the automated OptiRead™ instrument. The Sensititre™ ARIS™ 2X is based on fluorescence measurement and detects bacterial growth by monitoring the activity of specific surface enzymes produced by the test organisms. Growth can be measured after 18–24 hours of incubation time.

Novel AST methods

In addition to classical AST methods or automated phenotypic methods of ID/AST, there are interesting new methods, including imaging-based and non-imaging-based techniques, which are designed to deliver faster results. Some of these technologies are already in the market, while some are in the near-term development pipeline. These are discussed below.

Imaging-based ID/AST or AST only

Accelerate Pheno™ system (Accelerate Diagnostics, USA)

The Accelerate Pheno™ system is a relatively new diagnostic device that can perform rapid bacterial ID of organisms that cause BSIs and can provide AST; it is CE marked and FDA cleared. The system provides qualitative ID of organisms utilizing FISH probes targeting organism-specific rRNA sequences and quantitative morphokinetic cellular analysis using time-lapse imaging for AST. The Accelerate Pheno™ system uses electrokinetic concentration technology to direct microbial cells to a surface and hold them in position for image capture and analysis as they grow.

Accelerate Diagnostics offers the Accelerate PhenoTest™ BC, which is a multiplexed in vitro diagnostic test capable of simultaneously detecting and identifying multiple microbial targets followed by susceptibility testing of the appropriate detected bacterial organisms. For each drug-specific AST, a single concentration of antibiotic is used to provide MICs and categorical interpretations (susceptible, intermediate or resistant) per FDA and/or CLSI break points. The PhenoTest™ BC is performed directly on blood culture samples and is intended for use with the Accelerate Pheno system.

The PhenoTest™ BC can identify 16 organisms – six gram-positive and eight gram-negative bacteria, as well as two *Candida* species. The gram-positive bacteria are CNS, *Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus*, *Staphylococcus lugdunensis* and *Streptococcus* spp. The gram-negative bacteria are *Acinetobacter baumannii*, *Citrobacter* spp., *Enterobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas aeruginosa* and *Serratia marcescens*. The *Candida* species are *Candida albicans* and *Candida glabrata*.

The PhenoTest™ BC provides AST data for six gram-positive antimicrobial agents (including ampicillin, ceftaroline, erythromycin, vancomycin and methicillin), two gram-positive resistance phenotype markers (MRSA and MLSb [macrolide-lincosamide-streptogramin B resistance]) and 12 gram-negative antimicrobial agents (including amikacin, ceftazidime, ceftriaxone, ciprofloxacin and gentamicin).

The PhenoTest™ system comprises the Accelerate Pheno™ instrument, pictured in Fig. 67, software, host computer, analysis computer and the Accelerate PhenoTest™ BC kit.

Fig. 67. Sensititre™ ARIS™ 2X instrument

The Pheno™ instrument is a compact benchtop device. The PhenoTest™ BC kit contains a sample vial, a 48-channel disposable test cassette and a reagent cartridge needed to test samples from a blood culture bottle that has been determined to be positive by a continuous monitoring blood culture system. All ID and AST is performed in individual flow cells of the test cassette. The reagent cartridge contains gel electrofiltration stations, FISH probes, antibiotics and reagents for automated sample preparation, ID of bacterial and fungal target organisms, and AST and phenotypic resistance detection testing for bacterial target organisms. The user loads an aliquot of the positive blood culture in the sample vial, places the test cassette, reagent cartridge and sample vial into the Accelerate Pheno™ system device, presses the button on the device to close the door and starts the run. The remainder of the operations are automated. Organism ID is available within 90 minutes, and AST is available in approximately 7 hours from a positive blood culture specimen (*57*).

oCelloScope (BioSense Solutions ApS, Denmark)

The oCelloScope, pictured in Fig. 68, is a small, portable optical imaging instrument that is based on imaging the growth over time of a population of bacterial cells in a fluid sample containing antibiotics in a 96-well microtitre plate. It is essentially an automated microscope using digital time-lapse technology that scans through a fluid sample, generating a series of images.

Fig. 68. oCelloScope instrument and microwell plate

The optical axis is tilted 6.25° relative to the horizontal plane, which facilitates scanning of volumes by recording a series of images to form an image stack. The image acquisition process is repeated every 15 minutes over time (generally a period of 12 hours), and the time-lapse sequence of best-focus images is used to generate a video. All of the images are saved, and the data is processed using imaging algorithms to quantify changes in the area occupied by a growing population of cells. The oCelloScope's UniExplorer software is used for this purpose.

The oCelloScope can be used for real-time monitoring of microbial growth and growth inhibition as well as quantification of morphological features of up to 96 specimens at a time using standard microtitre plates. In a study to evaluate oCelloScope for use in BSIs, AST results were available from blood culture specimens in 1–4.2 hours following incubation, depending on the bacteria-antibiotic combination and whether the bacteria had reached the stationary growth phase prior to testing (*58*).

The oCelloScope is not commercially available. Additional studies of the technology are ongoing.

Sidecar, Alfred 60^{AST} and HB&L systems (Alifax SPA, Italy)

Unlike other AST platforms that rely on specimens cultured for more than 10–12 hours until bacterial colonies form, Alifax manufactures three AST instruments that rely on "rapid bacterial culture", which is based on the concept of monitoring bacterial culture in real time. Alifax claims that this method results in faster TAT, since only the minimum required concentration of bacteria are allowed to grow (rather than a full colony). Alifax makes three instruments, pictured in Fig. 69, that utilize this rapid bacteria culture method:

- Sidecar: a fully integrated system with an automated streaker of solid culture plates, incubator, tube inoculator and turbidity meter;
- Alfred 60^{AST}: a fully automated system that consists of an incubator, inoculator and turbidity meter; and
- HB&L: a semi-automated system consisting of an incubator and turbidity meter.

Each instrument uses turbidity measurements (light scattering) to determine the concentration of bacteria in tubes that have been inoculated with specimens. The concentration of microorganisms is proportional to the amount of light scattered, which is detected by the instruments. Quantitative bacterial count results are reported in culture-forming units (CFU) per millilitre. Turbidity levels in samples are converted to McFarland (McF) standards, and when samples reach a bacterial concentration of 0.5 McF, the sample is then used to inoculate another tube (manufactured by Alifax) containing predefined antibiotics and growth media. The growth within these tubes is then monitored again, using turbidity to assess growth in the presence of the antibiotic. Currently, Alifax manufactures tubes for 33 different EUCAST drugs and 31 CLSI drugs.

Fig. 69. Alifax instruments: Sidecar (left), HB&L (centre) and Alfred 60AST (right)

Alifax instruments do not identify bacterial pathogens, which must be done using other methods – e.g., MALDI-TOF MS.

Tests and applications available for the Alifax systems include:

- urine screening (culture)
- susceptibility test in urine (Uro-Quick)
- susceptibility test in blood culture
- residual antimicrobial activity (RAA)
- human biological liquids bacterial culture
- multidrug-resistant organisms (MRSA, ESBL/AmpC, carbapenem and VRE [vancomycin-resistant enterococci] screening kit [pipeline]).

TAT for results (e.g., urine screening, human biological liquids, bacterial culture and multidrug-resistant organisms) ranges from 3 hours to about 6.5 hours. All assays and systems are CE-IVD marked.

Nonimaging AST

LifeScale® (Affinity Biosensors, USA)

Affinity Biosensors offers the LifeScale®, pictured in Fig. 70, which is an automated system for rapid AST. It uses microelectromechanical systems (MEMS) technology. Unlike optical techniques, which depend on the optical properties of the inoculum to determine microbe concentration, LifeScale® employs the principle of resonant mass measurement to determine microbe mass, and at the same time counts each microbe to yield both the microbe concentration and the mass distribution of the population. The LifeScale®-AST is able to provide a MIC result for most gram-negative organisms in 3–3.5 hours, although some organisms, e.g., *Pseudomonas* spp. may take longer.

Fig. 70. LifeScale® instrument

The LifeScale® instrument is a benchtop direct-from-blood analyser that uses custom-designed Thermo Fisher Sensititre 96-well broth microdilution plates. After blood specimens are prepared for the plates, a simple centrifuge step is added in order to remove red blood cells. LifeScale® generates and automatically measures the MIC for each of the antimicrobials on the panel. Once a species ID has been made, the LifeScale® instrument produces a final report with both MIC and interpretive results, based on either FDA, CLSI or local break points.

LifeScale®-AST is currently RUO; no IVD is available commercially. Initially, LifeScale®-AST will target gram-negative rods from positive blood culture; however, the company indicates that the instrument is capable of determining bacterial growth and, therefore, phenotypic AST from gram-negative and gram-positive species.

In addition, there are novel technologies available for detecting resistance to particularly critical compounds, including phenotypic carbapenemase detection in *Enterobacteriaceae* and nonfermenters. These include:

RAPIDEC® CARBA NP (bioMérieux, France)

RAPIDEC® CARBA NP is a colorimetric phenotypic in vitro diagnostic test for qualitatively detecting carbapenemase enzymes in *Enterobacteriaceae* and *Pseudomonas aeruginosa* colonies that have MIC values to any carbapenem. It is intended as an aid in preventing and controlling infection, in particular, HAIs caused by carbapenemase-producing *Enterobacteriaceae* and *Pseudomonas aeruginosa*, and not to guide or monitor the treatment of these bacterial infections. The test is intended to be used in conjunction with other laboratory tests, including AST.

The RAPIDEC® CARBA NP test, pictured in Fig. 71, is a strip test that can be performed directly from bacterial colonies grown on selective or nonselective agar plates. It comprises five wells prepared with premeasured portions of the necessary substrates for the reactions. Test results are available in 30 minutes to 2 hours. The test is FDA cleared.

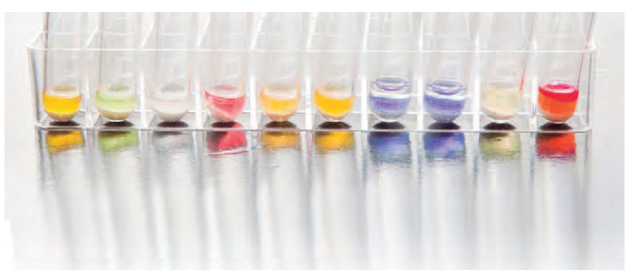
Fig. 71 RAPIDEC® CARBA NP test

Rapid CARB Blue kit (Rosco Diagnostica, Denmark)

Similar to RAPIDEC® CARBA NP, the Rapid CARB Blue test is a colorimetric phenotypic in vitro diagnostic test, pictured in Fig. 72, for qualitatively detecting carbapenemase enzymes in *Acinetobacter* spp., *Enterobacteriaceae* and *Pseudomonas aeruginosa* colonies. The test is based on hydrolysis of imipenem by bacterial colonies (direct inoculation without prior lysis), which is detected by changes in a pH indicator (bromothymol blue). The test is intended for use as an aid in preventing and controlling HAIs. It is CE-IVD marked.

The Rapid CARB Blue kit is in a tablet format; the antimicrobial agent is encapsulated in the tablet. Bacterial suspensions are prepared from isolates grown on agar plates. In two separate tubes, the suspension is vortexed briefly with an imipenem bromothymol Rapid CARB Blue tablet and a Carb Negative Control Blue Diatab, respectively, and further centrifuged. Results are then interpreted after 15 minutes, 30 minutes or 1 hour of incubation at 37 °C, and change in colour (e.g., from blue to yellow) is considered a positive result.

Fig. 72. Rapid CARB Blue suspensions



Two additional kits for detecting bacterial resistance are available from the company: the Rapid ESBL Screen kit and Neo-Rapid CARB kit.

Pipeline technologies for AST

In addition to the emerging AST technologies discussed above, other technologies are being developed and refined that are early-stage systems. Unless otherwise noted, all of the test systems described below are under development and not approved for sale; their performance characteristics have not yet been established. A few of these pipeline technologies focused on rapid AST are described briefly below.

- QMAP (QuantaMatrix, Inc., Korea): QuantaMatrix uses microfluidic agarose channel (MAC) technology to detect MIC and systemic inflammatory response syndrome (SIRS) while observing bacterial growth in real time using a proprietary microfluidic chip. The MAC chip, which is composed of microfluidic channels containing bacteria in agarose and a well to supply antimicrobials and nutrients, is integrated with a 96-well platform for high-throughput analysis. The imaging region is the interface between the liquid medium and the microfluidic channel. The immobilized bacterial cells at the bottoms of the channels are monitored for single-cell morphological analysis (SCMA) by time-lapse bright-field microscopy. SCMA can determine AST by analysing and categorizing the morphological changes in single bacterial cells under various antimicrobial conditions. The company's dRAST system captures an image every

hour and can determine MIC and SIRS after approximately 6 hours from blood culture specimens. The system also has applications for TB.

- QuickMIC™ AST system (Gradientech AB, Sweden): Gradientech is developing a novel and proprietary microfluidic technology solution to create stable substances for rapid AST from blood culture samples. The system, called QuickMIC™, will monitor and quantify bacterial growth of microcolonies within precise antibiotic gradients providing AST within 2 hours. The QuickMIC™ solution can be applied in parallel with, or following bacterial ID, with a system like MALDI-TOF MS. Panels for gram-negative and gram-positive bacteria will also be available. Per the company, QuickMIC™ is a universal AST solution that detects any functional bacterial resistance; it is not based on specific probes or image database comparison.
- Captiver™ system (Astrego Diagnostics AB, Sweden): Astrego is a young diagnostics company that is entirely dedicated to AST solutions. The Astrego technology is based on microfluidics and imaging analysis techniques. In simple terms, a sample is loaded onto a microfluidic chip; bacteria present in the sample are caught in bacteria-sized "traps". Trapping of bacteria is monitored, and the loading time gives an estimate of the bacterial density in the sample. A fraction of the trapped bacteria are exposed to a candidate antibiotic. Bacterial growth is monitored in each trap, both with and without the candidate antibiotic. The average bacterial growth rates, with and without candidate antibiotics, are calculated in real time. Bacteria are considered susceptible if their growth is duly inhibited. To date, Astrego has focused primarily on UTIs using its Captiver™ system, but it is also focusing on AST for sepsis from positive blood cultures.

Also under development are AST products using microcantilevers, plasmonic imaging and tracking, flow cytometry and isothermal microcolorimetry. These and other future technologies for AST are discussed in some detail in Syal and colleagues and van Belkum and Dunne (*4*, *54*).

Conclusion

Phenotypic ID of pathogens and AST are mainstays of the clinical microbiology laboratory. Their advantages over genotypic methods include the ability to predict both drug resistance and drug susceptibility as well as the ability to quantify the level of susceptibility of a bacterial isolate to individual antimicrobial agents (*50*). However, phenotypic pathogen ID typically takes 24 hours, with another 24–48 hours or more for susceptibility testing (Fig. 73). These delays in test results can lead to longer hospital stays, increased cost and patient mortality.

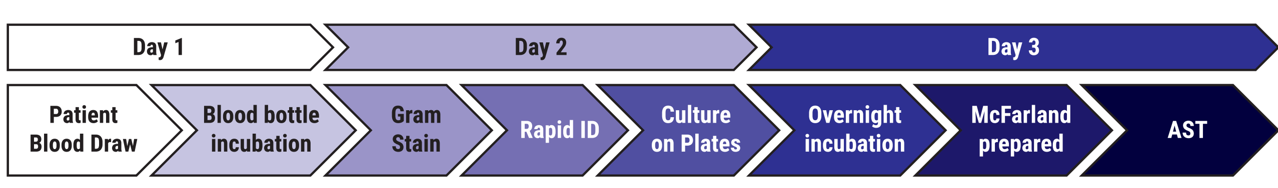
As described above, the introduction of automated phenotypic ID and AST systems have helped to improve TAT, and newer technologies, like the Accelerate Pheno™ system, which combines FISH for ID and multiplexed automated digital microscopy for susceptibility determination, provide both ease of use and faster results. Additional novel phenotypic technologies are under development.

Molecular-based assays are now being used routinely in clinical microbiology laboratories both for ID and resistance testing of bacterial, viral and other pathogens. These methods are discussed below.

Nonphenotypic methods of identifying pathogens and detecting antibiotic resistance

NAAT-based methods, especially PCR, are the genotypic methods that have gained the most widespread acceptance for both pathogen ID and characterization of bacterial resistance. For example, as described earlier in this report, multiplex PCR has made simultaneously detecting multiple AMR genes in bacteria possible. NAAT-based methods that can identify bacterial pathogens and the genes that directly confer bacterial resistance from positive blood cultures are

Fig. 73. Rapid CARB Blue suspensions



an important target of commercial systems. It should be kept in mind that while these methods can provide information on drug resistance, they cannot provide information on possible acquired resistance markers. NAAT-based testing does not provide information on susceptibility nor does it permit quantification of susceptibility to specific antibiotics, i.e., it is not possible to determine MICs (*50*, *51*). As a result, most commercial assays target positive blood cultures yielding growth of cluster-forming gram-positive cocci, as opposed to gram-negative organisms (*50*).

Molecular platforms for identifying pathogens and characterizing bacterial resistance from blood culture

Verigene® (Nanosphere/Luminex, USA)

Luminex offers a number of syndromic test panels for use on its Verigene® system. These include both a Gram-Positive Blood Culture Nucleic Acid (BC-GP) test and a Gram-Negative Blood Culture Nucleic Acid (BC-GN) Test. Both assays are qualitative multiplexed in vitro diagnostic tests for simultaneously detecting and identifying selected gram-positive or gram-negative bacteria, as the case may be, along with resistance markers from blood cultures. The assays are intended to aid in diagnosing BSIs when used in conjunction with other clinical and laboratory findings, but are not used to monitor these infections. Subculturing of positive blood cultures is necessary to recover organisms for susceptibility testing, identify organisms not detected by the assays and differentiate mixed growth.

In addition to certain other gram-positive bacterial pathogens, the BC-GP test detects and identifies the following bacterial genera and species from the WHO priority list: *Staphylococcus aureus* and *Enterococcus faecium*. It also detects the *mecA* resistance marker, inferring *mecA*-mediated methicillin resistance, and the *vanA* and *vanB* resistance markers, inferring *vanA*/*vanB*-mediated vancomycin resistance. Time to result is 2.5 hours.

The BC-GN test detects and identifies the following bacterial genera and species from the WHO priority list: *Acinetobacter* spp., *Enterobacter* spp., *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (but does not distinguish *Escherichia coli* from *Shigella* spp.). The assay also detects and identifies the following resistance markers: CTX-M ($bla_{\text{CTX-M}}$), KPC (bla_{KPC}), NDM (bla_{NDM}), VIM (bla_{VIM}), IMP (bla_{IMP}) and OXA (bla_{OXA}). Time to result is 2 hours.

Additional tests that can be performed on the Verigene® system include a respiratory panel, the Verigene® Respiratory Pathogens Flex (RP-Flex) nucleic acid test, and an enterics panel, the Verigene® Enteric Pathogens (EP) nucleic acid test, as well as a test for *Clostridium difficile*, the Verigene® *Clostridium difficile* (CDF) nucleic acid test.

- Verigene® RP-*Flex*: a qualitative in vitro diagnostic test for detecting and identifying multiple respiratory pathogen nucleic acids in nasopharyngeal swabs collected in viral transport media and obtained from individuals suspected of respiratory tract infections. It detects 13 viruses, including influenza A and B, and RSV A and B; it also detects four bacteria: *Bordetella parapertussis/bronchiseptica*, *Bordetella holmesii* and *Bordetella pertussis*. Time to result is 2 hours.
- Verigene® EP: a qualitative test for simultaneously detecting and identifying common pathogenic enteric bacteria and genetic virulence markers from liquid or soft stool preserved in Cary-Blair media, collected from individuals with signs and symptoms of gastrointestinal infection. The assay identifies the following pathogenic bacteria: *Campylobacter* group (comprising *C. coli*, *C. jejuni* and *C. lari*), *Salmonella* spp., *Shigella* spp. (including *S. dysenteriae*, *S. boydii*, *S. sonnei* and *S. flexneri*), *Vibrio group* (comprising *V. cholerae* and *V. parahaemolyticus*) and *Yersinia enterocolitica*. Time to result is 2 hours.
- Verigene® CDF: a qualitative multiplexed in vitro diagnostic test for rapidly detecting *tcdA*, *tcdB* and *tcdC* sequences of toxigenic strains of *Clostridium difficile* and for presumptive ID of PCR ribotype 027 strains from unformed (liquid or soft) stool specimens collected from patients suspected of having CDI. The CDF test is indicated for use as an aid in diagnosing CDI; detection of PCR ribotype 027 strains of *C. difficile* by the CDF test is solely for epidemiological purposes and is not intended to guide or monitor treatment of *C. difficile* infections. Time to result is less than 2 hours.

All of the above assays are intended to be performed on the Verigene® system, pictured in Fig. 74, which is a benchtop sample-to-result molecular diagnostics workstation that consists of two modules: one or more multiple Verigene® Processor *SP* modules or units and one Verigene® Reader. The Verigene® Processor *SP* automates the steps of (i) sample preparation and, if required, target amplification (i.e., cell lysis and magnetic-bead-based bacterial DNA isolation and nucleic acid amplification); and (ii) hybridization (i.e., detecting and identifying bacterial-specific DNA in a microarray format using gold nanoparticle probe-based technology, called NanoGrid Technology).

Fig. 74. Verigene® system

Once the specimen is loaded by the operator, all other fluid transfer steps are performed by an automated pipette that transfers reagents between wells of trays and finally loads the specimen into a test cartridge for hybridization. The Verigene® test cartridges, pictured in Fig. 75, are single-use, self-contained test units made up of (i) a microfluidic cassette that contains all of the hybridization reagents needed for a single test and captures the waste materials generated during test processing, and (ii) a substrate holder that contains a glass slide that serves as a solid support for the microarray used to capture targeted nucleic acids.

Fig. 75. Verigene® test cassette

To obtain the test results after test processing is complete, the user removes the test cartridge from the Processor *SP* and inserts the substrate holder into the Verigene® Reader for analysis. Light scatter from the capture spots is imaged by the device, and intensities from the microarray spots are used to make a determination regarding the presence or absence of a bacterial nucleic acid sequence/analyte. The determination is automated and is made by means of a software-based decision algorithm in the Verigene® Reader.

The complexity of the overall system suggests that it is only appropriate for use in a sophisticated clinical laboratory. The Verigene® assays described above are all FDA cleared, and all except the *Clostridium difficile* assay are CE-IVD marked.

BioFire® FilmArray® (bioMérieux, France)

BioFire® Diagnostics offers its FilmArray® system, which is a multiplex nucleic acid-based sample-to-answer diagnostic platform with an emphasis on syndromic test panels. Of particular significance for this report is the FilmArray® Blood Culture Identification (BCID) panel, which is intended for use with the FilmArray® instrument for the qualitative in vitro detection and ID of multiple bacterial and yeast nucleic acids and select genetic determinants of antimicrobial resistance. The BCID assay is performed on positive blood culture samples that determine the presence of organisms by a continuous monitoring blood culture system that demonstrates the presence of organisms as determined by Gram stain.

The BCID panel simultaneously tests a single blood culture sample for 24 different organisms and organism groups that cause BSIs as well as three genetic markers that confer AMR. TAT is approximately 1 hour. Note that both gram-positive and gram-negative bacteria are included in the same panel. These are listed in Table 1.

Additional BioFire® test panels intended for use with the BioFire® FilmArray® instrument are the FilmArray® Gastrointestinal (GI) panel, FilmArray® Respiratory panel (RP), FilmArray® Meningitis/Encephalitis (ME) panel and FilmArray® Pneumonia panels. TAT for each of the panels is approximately 1 hour. All of the panels are both FDA cleared and CE-IVD marked, with the exception of one of the pneumonia panels that to date is only CE-IVD marked.

- FilmArray® GI panel: is a qualitative multiplexed in vitro diagnostic test that can simultaneously detect and identify nucleic acids from 22 bacteria, viruses and parasites directly from stool samples in Cary-Blair transport media obtained from individuals with signs and/or symptoms of gastrointestinal infection. The GI panel identifies the following bacteria from the WHO priority list: *Campylobacter* (*C. jejuni*, *C. coli*, *C. upsaliensis*), *tcdA/tcdB*, *Salmonella* and several diarrhoeagenic *Escherichia coli/Shigella* pathotypes. The GI panel is indicated as an aid in diagnosing specific agents of gastrointestinal illness, and results are meant to be used in conjunction with other clinical, laboratory and epidemiological data. The device is not intended to monitor or guide treatment for *Clostridium difficile* infection.
- FilmArray® RP: is a multiplexed in vitro diagnostic for the simultaneous qualitative detection and ID of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs obtained from individuals suspected of respiratory tract infection. The respiratory panel is able to detect 17 viruses and three bacterial pathogens

Table 1. Organisms and organism groups and genetic markers identified by FilmArray® BCID[12]

Gram-positive bacteria	Gram-negative bacteria	Yeast
Enterococcus	*Acinetobacter baumannii*	*Candida albicans*
Listeria monocytogenes	***Enterobacteriaceae***	*Candida glabrata*
Staphylococcus	*Enterobacter cloacae complex*	*Candida krusei*
Staphylococcus aureus	*Escherichia coli*	*Candida parapsilosis*
Streptococcus	*Klebsiella oxytoca*	*Candida tropicalis*
Streptococcus agalactiae	*Klebsiella pneumoniae*	**Antimicrobial resistance genes**
Streptococcus pneumoniae	*Proteus*	*mecA* – methicillin resistance
Streptococcus pyogenes	*Serratia marcescens*	*vanA/B* – vancomycin resistance
	Haemophilus influenzae	KPC – carbapenem resistance
	Neisseria meningitidis (encapsulated)	
	Pseudomonas aeruginosa	

– *Bordetella pertussis*, *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*. The results of the panel are for use as an aid in diagnosing respiratory infection if used in conjunction with other clinical and epidemiological information.

- FilmArray® ME panel: is a qualitative multiplexed nucleic acid-based in vitro diagnostic test for simultaneously detecting and identifying multiple bacterial, viral and yeast pathogens directly from CSF. The panel identifies six bacteria: *Escherichia coli* K1, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis* (encapsulated), *Streptococcus agalactiae* and *Streptococcus pneumoniae*, as well as six viruses and one yeast. Results of the panel are not intended to be used as the sole basis for diagnosis, treatment or other patient management decisions, but are intended as an aid in diagnosis along with other clinical and epidemiological information.
- FilmArray® Pneumonia panels: BioFire® has developed two pneumonia panels, the FilmArray® Pneumonia Panel and the FilmArray® Pneumonia Panel *plus*. The former is FDA cleared, and the latter has recently received CE-IVD marking. Both of the panels detect 18 bacteria and eight viruses, as well as seven genetic markers of antimicrobial resistance, from sputum (including endotracheal aspirate) and bronchoalveolar lavage (including mini-BAL). The *plus* version of the panel also detects MERS-CoV (Middle East respiratory syndrome-coronavirus). The assays yield semi-quantitative levels for 15 of the bacterial targets, including *Acinetobacter calcoaceticus-baumannii* complex, *Klebsiella pneumoniae* group, *Escherichia coli*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pneumoniae*. Antimicrobial resistance genes detected include *mecA/C* and *mec* right extremity junction (MREJ), KPC (bla_{KPC}), NDM (bla_{NDM}), CTX-M ($bla_{CTX\text{-}M}$), VIM (bla_{VIM}) and IMP (bla_{IMP}).

The FilmArray® panels described above are intended to be used with the FilmArray® Torch or FilmArray® 2.0, collectively, the FilmArray® systems. Each panel includes a pouch which contains the freeze-dried reagents necessary to perform nucleic acid purification and nested, multiplex PCR with DNA melt analysis. Certain manual steps are required. A test is initiated by loading hydration solution into one port of the pouches and a sample mixed with the provided sample buffer ampoules into the other port of the pouch, which rehydrates the reagents, and placing it in the FilmArray® instrument. After the pouch is prepared, the FilmArray® software on the FilmArray® systems guides the user through the steps of placing the pouch into the instrument, scanning the pouch barcode, entering the sample ID and initiating the run on the FilmArray® systems.

The FilmArray® instruments (the FilmArray® 2.0 is pictured in Fig. 76) contain a coordinated system of inflatable bladders and seal points that act on the

[12] The organisms/genetic markers highlighted in blue are on the WHO priority list of bacterial pathogens.

pouch to control the movement of liquid between the pouch blisters. Nucleic acid extraction occurs within the pouch using mechanical and chemical lysis, followed by purification using standard magnetic bead technology. After extracting and purifying nucleic acids from the unprocessed sample, a nested multiplex PCR is executed in two stages. During the first stage, the system performs a single, large volume, multiplexed reaction. The products from the first-stage PCR are transferred to an array with approximately 100 wells, prespotted with second-stage PCR primers. The second-stage PCR is performed in each well of the array. The array is then interrogated by melt curve analysis to detect signature amplicons denoting the presence of specific targets. A digital camera placed in front of the array captures fluorescent images of the second-stage PCR reactions, and software interprets the data.

Fig. 76. BioFire® FilmArray® 2.0 instrument

The FilmArray® systems consist of three instruments: the FilmArray® Torch, the FilmArray® 2.0 and the FilmArray® EZ Configuration. The FilmArray® Torch is a larger, fully integrated random access instrument, while the FilmArray® 2.0 is a high-throughput, smaller benchtop system that uses single database management for up to eight instruments per computer. It has LIS-interfacing capabilities. Finally, the EZ Configuration is a CLIA-waived system for near-patient molecular testing. It is currently only used with the Respiratory Panel EZ, which tests for a comprehensive set of 14 respiratory, viral and bacterial pathogens.

iC-System™ (iCubate, Inc., USA)

iCubate offers the iC-GPB Assay™ for use on its iC-System™. The iC-GPC Assay™, which is performed directly on positive blood cultures, is a qualitative, multiplexed in vitro diagnostic test for detecting and identifying potentially pathogenic gram-positive bacteria which may cause BSI. The iC-GPC Assay™ is validated for use on the BACTEC™, BACT/ALERT® and VersaTREK™ blood culture bottles. Together with other clinical laboratory findings, such as blood culture isolate ID and AST, it is intended to aid in diagnosing bacterial BSIs, but not to monitor such infections.

The iC-GPC Assay™ detects an organism's DNA and identifies the following species: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Enterococcus faecalis* and *Enterococcus faecium*. It also identifies the following resistance markers: *mecA*, inferring *mecA*-mediated methicillin resistance, as well as *vanA* and *vanB*, inferring *vanA*/*vanB*-mediated vancomycin resistance.

The iC-GPC Assay™ is intended to be used on the iC-System™, which comprises the iC-Processor™, the iC-Reader™ and assay cartridges, pictured in Fig. 77. A separate iMac computer is also required. The iC-System™ utilizes PCR for multiplex amplification of pathogens and detects amplified targets with microarray hybridization. More specifically, the system utilizes proprietary ARM-PCR (amplicon rescue multiplex PCR) technology; this technology allows multiple targets to be amplified in one reaction, but requires two rounds of amplification. Testing is performed using a self-contained disposable cassette that is processed by the iC-Processor™. After processing, the cassette is transferred to the iC-Reader™, where it is read. Data is then transferred from the iC-Reader™ to an iMac computer on which data is analysed using iC-Report™ software, and a final result is generated. TAT is approximately 4.5 hours. The iC-GPC Assay™ is FDA cleared.

Fig. 77. The iC-Reader™ (left), iC-Processor™ (right rear) and iC assay cartridges (right front)

In addition to the iC-GPC Assay™, iCubate has several assays under development. The iC-GN Assay™ for detecting and identifying gram-negative rods is anticipated to be FDA cleared in 2019. Specifically, the iC-GN Assay™ will detect *Acinetobacter baumannii* complex, *Enterobacter cloacae* complex, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus* spp. and *Serratia marcescens*.

Additional assays in the pipeline include *Mycobacterium*, gastrointestinal and respiratory tests.

Sepsis Flow Chip (Master Diagnóstica, Spain)

Master Diagnóstica offers the Sepsis Flow Chip (SFC) assay, which is an IVD for the simultaneous rapid detection from positive blood culture of 40 bloodstream pathogens in the same assay, including gram-positive and gram-negative bacteria as well as fungi, and for the detection of 20 antibiotic resistance genes, including MRSA, *mecA*, *vanA*, *vanB*, *ESBL* and carbapenems. The SFC assay, which is CE-IVD marked, is based on multiplex PCR using biotinylated primers followed by automatic reverse dot-blot hybridization to a low-density DNA array. Table 2 lists the pathogens and resistance markers detected by the SFC assay.

Table 2. Clinical pathogens and resistance markers detected by SFC assay

Pathogen ID	Genetic resistance markers
Gram-positive bacteria	
Streptococcus pneumoniae	
Streptococcus agalactiae	
Streptococcus spp.	
Staphylococcus aureus	*mecA*
Staphylococcus spp.	
Enterococcus spp.	*vanA/vanB*
Listeria monocytogenes	
Gram-negative bacteria	
Stenotrophomonas maltophilia	bla_{CTX}, bla_{SHV}, bla_{SME}, bla_{KPC}, $bla_{NMC/IMI}$, bla_{GES}, bla_{IMP}, bla_{GIM}, bla_{VIM}, bla_{SPM}, bla_{SIM}, bla_{NDM}, bla_{OXA23}, $bla_{OXA\text{-}24}$, $bla_{OXA\text{-}48}$, $bla_{OXA\text{-}51}$ and $bla_{OXA\text{-}58}$
Serratia marcescens	
Escherichia coli	
Klebsiella pneumoniae	
Morganella morganii	
Proteus spp.	
Enterobacteriaceae	
Acinetobacter baumannii	
Pseudomonas aeruginosa	
Neisseria meningitidis	
Fungi	
Candida albicans	

In addition to the SFC assay, Master Diagnóstica also offers several other CE-IVD-marked test panels. For purposes of this report, the most relevant panel is the Bacterial CNS Flow Chip, which detects *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, *Treponema pallidum*, MTB, *Coxiella burnetii*, *Cryptococcus neoformans* (hongo) and *Borrelia burgdorferi*. Additional assays available are the HPV Direct Flow Chip, Viral CNS Flow Chip and Tick-Borne Bacteria Flow Chip.

The flow chip assays/panels must be run on a combination of instruments that are not integrated, which means the system is suitable only for a sophisticated clinical laboratory. Following Gram staining and organism ID, positive blood culture samples are amplified using a commercially available thermal cycler (e.g., ABI Veriti™ Dx [Thermo Fisher Scientific]). Subsequently, reverse dot-spot hybridization and analysis of the results can be conducted with the fully automated hybriSpot HS24™ platform or semi-automated hybriSpot 12™ instrument from Master Diagnóstica. The TAT to obtain a result from a positive blood culture takes from 30 to 120 minutes on the HS24 platform, which can process up to 24 samples simultaneously. If using the HS12 instrument, 1–24 samples can be done per run in 20–120 minutes.

Unyvero™ system (Curetis GmbH, Germany)

The Unyvero™ system is a cartridge-based molecular diagnostic platform for simultaneously detecting and identifying gram-positive and gram-negative bacteria, mycobacteria and fungi as well as antibiotic resistance markers. It is a qualitative test. The Unyvero™ BCU blood culture application, which is CE-IVD marked, includes 36 analytes covering more than 50 pathogens, and 16 antibiotic resistance gene markers for detection from positive blood culture bottles; Gram stain is not required. TAT is approximately 5 hours. Of interest for this report, the BCU application can detect and identify the bacteria listed in Table 3, among other microorganisms:

Table 3. Select list of clinical pathogens identified by Unyvero BCU application

Group	Pathogen
Gram-positive bacteria	*Staphylococcus aureus*
	Streptococcus spp.
	Streptococcus agalactiae
	Streptococcus pneumoniae
	Streptococcus pyogenes/dysgalactiae
	Enterococcus spp.
	Enterococcus faecalis

(Table 3, continued)

Gram-negative bacteria	*Enterobacteriaceae*
	Escherichia coli
	Klebsiella pneumoniae
	Acinetobacter baumannii
	Pseudomonas aeruginosa
	Haemophilus influenzae

Resistance markers detected and identified by the Unyvero™ BCU application include *mecC* (LGA251), *vanA*, *vanB*, CTX-M ($bla_{\text{CTX-M}}$), KPC (bla_{KPC}), IMP (bla_{IMP}), NDM (bla_{NDM}), OXA-23 ($bla_{\text{OXA-23}}$), OXA-24/40 ($bla_{\text{OXA-24/40}}$), OXA-48 ($bla_{\text{OXA-48}}$), OXA-58 ($bla_{\text{OXA-58}}$) and VIM (bla_{VIM}).

In addition to the BCU application, Unyvero™ offers several other panels for infectious disease, including an FDA-cleared panel for lower respiratory tract infections, the Unyvero™ LRT application.[13] The LRT application is a qualitative nucleic acid multiplex test intended for simultaneously detecting and identifying nucleic acid sequences from certain microorganisms and antibiotic resistance markers in endotracheal aspirates from adult hospitalized patients with suspected lower respiratory tract infection. The LRT application detects and identifies 19 bacteria and fungi, including, but not limited to, the following: *Acinetobacter* spp., *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pneumoniae*. In addition, the panel detects and identifies the following associated resistance markers: *mecA*, CTX-M ($bla_{\text{CTX-M}}$), KPC (bla_{KPC}), NDM (bla_{NDM}), OXA-23 ($bla_{\text{OXA-23}}$), OXA-24/40 ($bla_{\text{OXA-24/40}}$), OXA-48 ($bla_{\text{OXA-48}}$) and VIM (bla_{VIM}).

Similarly, Unyvero™ offers a qualitative in vitro diagnostic test for UTIs. The Unyvero™ UTI application, which was recently CE-IVD marked, is targeted at patients with complicated and severe UTIs, including pregnant women and immunocompromised individuals. The Unyvero™ UTI application can detect 88 pathogens, including a broad range of gram-positive and gram-negative bacteria as well as difficult-to-culture anaerobic bacteria, and the fungus *Candida auris*, which is multidrug resistant.

Unyvero™ UTI also detects 15 genetic markers of antibiotic resistance, including the *mcr-1* antibiotic resistance gene, which results in resistance to colistin, one of the few last-resort antibiotics for gram-negative infections. Similar to other Unyvero™ applications, Unyvero™ UTI allows rapid detection of pathogens and genetic resistance markers in a broad range of routinely available patient sample types, such as midstream urine, catheter urine, suprapubic aspiration and tissue.

Finally, Unyvero™ also offers two additional test panels: the IAI (intra-abdominal infection) application and the ITI (implant and tissue infection) application. Similar to the other Unyvero™ assays, these applications detect and identify a broad range of microorganisms and resistance markers, which will not be discussed in detail in this report.

Each of the above Unyvero™ IVD applications is performed using the Unyvero™ system; the process includes specimen processing (lysis), genomic bacterial DNA isolation and purification, multiplex PCR, and array hybridization and detection. The system comprises three instruments, pictured in Fig. 78: the

Fig. 78. Unyvero™ system instruments: Unyvero™ L4 Lysator (left), Unyvero™ C8 Cockpit (centre) and Unyvero™ A50 Analyzer (right)

[13] Note that, in Europe, Curetis offers a very similar panel called the Unyvero pneumonia application, which is CE-IVD marked. Both the Unyvero LRT application and the pneumonia application are targeted to hospitalized patients.

Unyvero™ L4 Lysator for specimen processing, the Unyvero™ A50 Analyzer (for amplification and reading) and the Unyvero C8 Cockpit controller, which provides the main user interface for the Unyvero™ system, guides the user through the steps to run the applicable Unyvero™ application, and automatically generates and displays test results.

The workflow for the Unyvero system is as follows. It is composed of both manual and automated elements. A specimen is first pipetted into the Unyvero sample tube and closed with the Unyvero sample tube cap. Closing the sample tube automatically adds the lysis reagent and the internal control gene template to the specimen. The sample tube is then placed on the L4 Lysator. After the specimen is lysed in the Lysator, the sample tube and master mix are loaded into the Unyvero cartridge, pictured in Fig. 79, for automated processing and analysis.

Fig. 79. Unyvero™ cartridge

The remainder of the testing steps are automated by the Unyvero™ A50 Analyzer. The lysed specimen is further processed and then transferred onto a DNA purification column for nucleic acid, and DNA is transferred to separate PCR reaction chambers containing multiple primer pairs. After amplification in the Unyvero™ A50 Analyzer, PCR products are hybridized to the corresponding array probes. Results data are then transferred to the Unyvero™ C8 Cockpit for visualization and results printout.

GeneXpert® system (Cepheid, a subsidiary of Danaher Corporation, USA)

The Cepheid GeneXpert® system is a fully automated and integrated system for PCR-based NAAT, which currently has 21 FDA-cleared and 27 CE-IVD-marked assays, including the Cepheid Xpert® MRSA/SA blood culture assay. The assays are performed on the Cepheid GeneXpert® instrument systems, which contain between one and 80 modules, depending on the instrument. The instruments include the GeneXpert® Express, GeneXpert® Dx (the four-module instrument is pictured in Fig. 80), GeneXpert® Infinity-48, GeneXpert® Infinity-48s and GeneXpert® Infinity-80 systems, all of which automate sample preparation, amplification and real-time detection in single-use, disposable cartridges. Select assays available for these instruments relevant to this report are described below.

Fig. 80. Four-module GeneXpert® Dx instrument (left) and cartridge (right)

The Cepheid Xpert® MRSA/SA blood culture assay is a qualitative in vitro diagnostic test intended for detecting *Staphylococcus aureus* and MRSA DNA. The assay utilizes automated real-time PCR for amplifying MRSA/*S. aureus*-specific DNA targets and fluorogenic target-specific hybridization probes for the real-time detection of the amplified DNA. The assay is performed directly on positive blood culture specimens using BD BACTEC™ Plus Aerobic/F blood culture bottles that are determined as gram-positive cocci in clusters (GPCC) or as gram-positive cocci in singles (GPC) by Gram stain. The Cepheid Xpert® MRSA/SA blood culture assay is not intended to monitor treatment for MRSA/*S. aureus* infections. TAT is approximately 1 hour. The assay is CE-IVD marked and FDA cleared.

In addition to the Xpert® MRSA/SA blood culture assay, Cepheid offers three additional cartridges related to testing for MRSA. The MRSA SA Nasal Complete cartridge and the MRSA/SA SSTI cartridge are each a qualitative in vitro diagnostic test for detecting *S. aureus* and MRSA DNA directly from either nasal swabs in patients at risk for nasal colonization (MRSA SA Nasal Complete cartridge) or from skin and soft tissue infection swabs (MRSA SSTI cartridge). Cepheid also offers the Xpert® MRSA NxG (next-generation) assay, which like the MRSA cartridge is a qualitative in vitro diagnostic test for detecting *S. aureus* and MRSA DNA directly from nasal swabs in patients at risk for nasal colonization, but which, per the company, is an improved assay because it expands coverage of the test by using an extensive library of

over 195 MRSA strains from around the world. The assay also adds primers and probes to detect sequences within the *mecA* and *mecC* genes in order to reduce false-positive results due to empty cassettes. The MRSA NxG assay is validated for use with both rayon swabs and eSwab™ (Copan Diagnostics, Italy). TAT is approximately 1 hour. All of these assays are FDA cleared and CE-IVD marked.

Cepheid also offers additional assays related to HAIs. These include the Xpert® *vanA* and Xpert® Carba-R assays, both of which are FDA cleared and CE-IVD marked. The Xpert® *vanA* assay is a qualitative in vitro diagnostic test designed for rapidly detecting the *vanA* gene sequence associated with vancomycin resistance in bacteria obtained from rectal swab specimens from patients at risk for intestinal colonization with vancomycin-resistant bacteria. This assay is intended to aid in recognizing, preventing and controlling vancomycin-resistant organisms that colonize patients in healthcare settings, and not for diagnosing infections caused by vancomycin-resistant bacteria nor to guide or monitor treatment for vancomycin-resistant bacterial infections. TAT is 45 minutes.

The Xpert® Carba-R assay is a qualitative in vitro diagnostic test designed for detecting and differentiating the bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48} and bla_{IMP} gene sequences associated with carbapenem nonsusceptibility. The assay can be performed on carbapenem-nonsusceptible pure colonies of *Enterobacteriaceae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, when grown on blood agar or MacConkey agar. For testing pure colonies, the assay should be used in conjunction with other laboratory tests, including phenotypic AST. The assay can also be performed on rectal and perirectal swab specimens from patients at risk for intestinal colonization with carbapenem-nonsusceptible bacteria. When performed on rectal and perirectal swab specimens, the Xpert® Carba-R assay is not intended to guide or monitor treatment for carbapenem-nonsusceptible bacterial infections nor to determine infection from carbapenem-nonsusceptible bacteria. TAT is less than 50 minutes.

There are two Cepheid assays for *Clostridium difficile* – the Xpert® *C. difficile* assay and the Xpert® *C. difficile*/Epi assay. The Cepheid Xpert® *C. difficile* assay is a qualitative in vitro diagnostic test for rapidly detecting *tcdB* gene sequences from unformed (liquid or soft) stool specimens collected from patients suspected of having CDI. The Xpert® *C. difficile*/Epi assay is a qualitative in vitro diagnostic test for rapidly detecting *tcdB* gene sequences and for presumptive ID of 027/NAP1/BI strains of toxigenic *Clostridium difficile* from unformed (liquid or soft) stool specimens collected from patients suspected of having CDI. Each assay is intended as an aid in diagnosing CDI. Both tests have a TAT of 45 minutes.

Also of relevance to this report are two additional assays for the GeneXpert® system: the Xpert® MTB/RIF assay and the Xpert® CT/NG assay. The Xpert® MTB/RIF assay is a qualitative nested real-time PCR in vitro diagnostic test for detecting *Mycobacterium tuberculosis* complex (MTB complex) DNA in raw sputum or concentrated sediments prepared from induced or expectorated sputum. In specimens where MTB complex is detected, the Xpert® MTB/RIF assay also detects the rifampin-resistance-associated mutations of the beta subunit of RNA polymerase (*rpoB*) gene. TAT is approximately 2 hours.

The Xpert® CT/NG assay is a qualitative in vitro real-time PCR test for automated detection and differentiation of genomic DNA from CT and/or NG. It is CE-IVD marked and FDA cleared. The assay may be used on the following specimens from both asymptomatic and symptomatic patients: female and male urine, endocervical swab, patient-collected vaginal swab (collected in a clinical setting) and rectal and pharyngeal swab specimens. TAT is approximately 90 minutes.

All of the Cepheid assays described above, as well as additional assays in the Cepheid portfolio, are performed on the GeneXpert® system. The GeneXpert® system consists of a GeneXpert® instrument, personal computer and multichambered fluidic cartridges that are designed to complete sample preparation and real-time PCR for detection. The GeneXpert® system integrates and automates sample preparation, amplification and detection in a single-use, self-contained cartridge. Most liquids and dry reagents along with enzymes are prefilled so that pre-analytical steps are minimized, reducing opportunities for sample mix-ups and operational errors. GeneXpert® cartridges can handle a variety of sample volumes (micro- to millilitre volume range) within macrofluidic chambers and then concentrate the target material down to microfluidic volumes, which can increase the sensitivity of the assays, if needed.

Further, the GeneXpert® system is modular. Individual modules contain solid-state circuitry that controls temperature, pressure, rotation of the valve that moves the liquid between reservoirs in the cartridge and the detection software. These individual modules are packaged in cabinets that can hold up to 1, 2, 4, 16, 48 or 80 modules, as mentioned above. The latter two systems (Infinity-48 and Infinity-80) are fully automated, walk-away robotic systems, developed for high-throughput laboratory applications. Additionally, the modules can be removed and replaced individually so that the entire system is not incapacitated if one module fails.

Generally, the GeneXpert® systems are best used at district hospitals and above in the tiered laboratory system in-country. The instruments are not as well suited to use at health centres and below for

reasons including, the need for stable electricity, temperature conditions and calibration requirements. Training, however, is relatively straightforward and can usually be done in less than a day.

Additionally, GeneXpert® Edge, launched in 2018, is Cepheid's new offering based on the existing GeneXpert® instrument family to move molecular testing beyond the laboratory. By including an easy-to-use touch-screen workflow, external battery pack and dust filter, GeneXpert® Edge enables testing in challenging environments.

GenoType assays and FluoroType® system (Hain Lifescience GmbH – a Bruker Company, Germany)

Hain Lifescience has developed a large number of CE-IVD-marked in vitro diagnostic tests, its GenoType and FluoroType® assays, of which the latter can be performed on its FluoroCycler® PCR instrument. Among these are GenoType BC gram-negative and gram-positive test kits, pictured in Fig. 81, which can identify 15 specimens of gram-negative rods and 17 specimens of gram-positive cocci, respectively, taken directly from positive BACTEC™ blood culture bottles. Pathogens identified by the GenoType BC positive kit to the species level include *Staphylococcus aureus*, *Enterococcus faecium* and *Streptococcus pneumoniae*, along with ID of *mecA* and *van* genes. Pathogens identified by the GenoType BC negative kit include *Escherichia coli*, *Enterobacter* spp. (*E. aerogenes*, *E. cloacae* and *E. sakazakii*), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

Fig. 81.GenoType BC test kit

The GenoType BC assays are based on DNA strip technology that uses DNA multiplex amplification with biotinylated primers followed by hybridization to membrane-bound probes. The testing process is quite manual. A drop of positive blood culture is applied to the Hain Lifescience GenoCard, a special membrane device. A small piece of the carrier matrix is then punched out and, following a short drying step, is added to a PCR mixture for amplification. Hybridization and detection are then carried out, for which an automated washing and shaking device may be used. GenoCard strips are then air dried and fixed to a data sheet for evaluation by visual examination. TAT is approximately 5 hours.

Hain Lifescience offers a series of GenoType assays that are based on the same technology used in the BC assays and for which the workflow is the same or similar to that described above. Of relevance to this report, these include GenoType:

- MRSA (for directly detecting MRSA from cultured material);
- Staphylococcus (to detect *Staphylococcus aureus* from cultured material);
- HelicoDR (to identify *Helicobacter pylori* from culture and biopsy samples);
- CDiff (to detect *Clostridium difficile* and ribotype 027 from stool and culture samples as well as rectal smears);
- Enterococcus (to identify and differentiate among *E. faecalis*, *E. faecium*, *E. casseliflavus* and *E. gallinarum*, and to identify vancomycin resistance genes from culture, preferably freshly grown); and
- MTB complex: three assays, including, MTBC VER 1.X (to identify and differentiate MTB complex from liquid and/or solid culture); MTBDRsl VER 1.0/2.0 (to identify MTB complex and its resistance to fluoroquinolones, aminoglycosides/cyclic peptides [and ethambutol]); and MTBDRplus VER 1.0/2.0 (to identify MTB complex and its resistance to rifampicin and/or isoniazid).

In addition to the GenoType assays, Hain Lifescience also offers a series of CE-IVD-marked FluoroType® tests to be performed on its FluoroCycler® system. The assays include FluoroType®:

- MTB (to detect MTB complex from decontaminated pulmonary and extrapulmonary patient specimens);
- MTBDR VER 2.0 (to identify MTB complex and its resistance to rifampicin and/or isoniazid);
- MRSA (to detect methicillin-resistant *Staphylococcus aureus* from swab specimens – nose, throat, skin and wounds);
- CDiff (to detect *Clostridium difficile* and *tcdB* from stool samples); and
- NG (to detect *Neisseria gonorrhoeae* from urethral or cervical swabs, as well as urine).

The Hain Lifescience system includes a 96-well format for nucleic acid extraction, the GenoXtract® 96 instrument, and an amplification instrument, the FluoroCycler® 96, which performs subsequent amplification and detection using qPCR. The company also offers a lower-throughput extraction platform and qPCR cycler, the GenoXtract® 12 and the FluoroCycler® 12, respectively, which can process or amplify up to 12 samples at once. The system is pictured in Fig. 82.

The company uses a novel amplification and probe technology – linear-after-the-exponential (LATE)-PCR combined with fluorescence "lights on/lights off" probes that tile side by side on the target region. LATE-PCR is an optimized form of asymmetric PCR, in which a limiting primer and an excess primer are used for exponential amplification of double-stranded DNA, followed by linear amplification of a single strand. Each single-stranded amplified sample can then be detected in real time. With the lights on/lights off probes, fluorescence is either emitted or suppressed, which is reflected through a characteristic fluorescence pattern in melt curve analysis. This allows larger regions of target DNA to be interrogated compared with the conventional method of using a single probe. Finally, test-specific Fluoro-Software® evaluates the test results and displays them automatically. TAT varies with the assay, but it is approximately 2.5 hours for the MTBDR assay.

ePlex® system (GenMark Diagnostics, USA)

GenMark offers its ePlex® system, which is a clinical multiplex test system using single-use assay cartridges that incorporate digital microfluidics and GenMark's proprietary eSensor detection technology. The system is fully automated.[14]

The ePlex® system is based on competitive nucleic acid hybridization and electrochemical detection of nucleic acids on a microchip in a disposable cassette. Target amplification is done via PCR or RT-PCR. Quantitation is not possible on the system.

The ePlex® system is modular and scalable, and has instrument configurations ranging from 3 to 24 test bays. GenMark recently introduced a small configuration instrument, the ePlex® NP (Near Patient), pictured in Fig. 83, for use at smaller laboratories running as few as 12 patient samples per shift. The ePlex® tower instruments range from a one-tower configuration that can process six cartridges at a time with random access to two-, three- and four-tower configurations containing three to 24 test bays. The system offers bidirectional LIS.

Fig. 83. ePlex® NP platform (left) and test cartridge (right)

GenMark offers a variety of test cartridges. One of these is an FDA-cleared and CE-IVD-marked respiratory pathogen (RP) panel available from GenMark for the ePlex® systems, but the test is targeted primarily at viruses and is not directly relevant to this report. Of relevance to this report are two CE-IVD-marked blood culture assays. These are the ePlex® BCID-GP and ePlex® BCID-GN tests. The ePlex® BCID-GP Panel identifies 26 targets, including *Enterococcus*, *Enterococcus faecium*, *Staphylococcus* and *Staphylococcus aureus*, as well as resistance markers

Fig. 82.Hain Lifescience instruments: GenoXtract® (left), FluoroCycler® (FluoroCycler® 12, centre left; and FluoroCycler® 96, centre right) and separate computer for results display

[14] GenMark also offers a larger system, the XT-8, which performs highly multiplexed post-PCR detection of infectious disease, genetics and pharmacogenetic targets. But the assays for this system are not relevant to this report.

mecA, *mecC*, *vanA* and *vanB*. The ePlex® BCID-GN Panel identifies 29 targets, including *Acinetobacter baumannii*, *Enterobacter* (non-*cloacae* complex and *cloacae* complex), *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella*, as well as resistance markers including CPE (bla_{KPC}, bla_{VIM}, bla_{NDM}, bla_{IMP} and bla_{OXA}) and ESBL ($bla_{CTX\text{-}M}$). Samples are positive blood culture bottles. TAT is approximately 1.5 hours with less than 2 minutes of hands-on time. GenMark has submitted both of its BCID assays to the FDA for clearance.

GenMark also offers a blood culture assay for detecting fungus. In addition, GenMark has a number of assays under development, including (i) a gastrointestinal pathogen panel for bacterial, viral and parasitic targets from stool samples; (ii) a central nervous system panel for bacterial, viral and fungal targets from CSF; and (iii) an HCV genotyping panel from plasma or serum.

Molecular platforms for identifying pathogens and characterizing bacterial resistance from whole blood and other sample types

In addition to the IVD systems described above that can identify bacterial pathogens and genes that directly confer bacterial resistance from blood culture, there are systems available that can similarly detect such pathogens and resistance genes from whole blood and other specimens. These are described below.

Seeplex™, Allplex™, Anyplex™, MagicPlex™ systems (Seegene, Republic of Korea)

Seegene offers numerous highly multiplexed NAAT-based assay kits that use real-time PCR or capillary electrophoresis for amplicon detection. These are the Seeplex™, Allplex™, Anyplex™ and MagicPlex™ test kits, many of which are CE-IVD marked. The company does not, however, supply completely integrated sample-to-result systems. For example, sample preparation is not provided by Seegene; therefore, a product from another manufacturer must be used for this purpose. While DNA extraction and an initial PCR amplification step can be run on Seegene instruments (SEEPREP12™ and SEEAMP™, respectively, pictured in Fig. 84), additional real-time PCR amplification steps or automated electrophoresis, depending on the assay kit, must be done on other systems validated for use with Seegene kits. For real-time PCR, these include the ABI 7500 Real-time PCR (Thermo Fisher Scientific, USA) and the CFX96™ Real-time PCR (Bio-Rad, USA); for electrophoresis, this includes the MultiNA (Shimadzu Corporation, Japan), an automated microchip electrophoresis system that performs automated high-speed electrophoresis separation and fluorescence detection.

Seegene has developed certain proprietary software, Multiple Detection Temperatures Technology (MuDT™), to discriminate between the 10 channels on the CFX96™ platform. It allows simultaneous ID and quantification of multiple pathogen targets in a single channel without melt curve analysis following amplification. In addition, viewer software analyses the raw data to generate test results from the various Seegene assays. The multiplicity of steps and equipment required to perform the Seegene test kits suggests that they should be used only in the most sophisticated laboratory settings.

Examples of Seegene IVD kits include the CE-IVD-marked Magicplex™ Sepsis real-time test, which is notable because it is able to screen for more than 90 sepsis-causative pathogens at genus level and resistance markers using real-time PCR from EDTA whole-blood samples in approximately 3.5 hours, excluding extraction time. The test is done in two different reactions, one for gram-positive bacteria/drug-resistance genes and one for gram-negative bacteria/fungi. Identification of 27 pathogens at the species level, without additional amplification, takes place via a third step. Pathogens include *Streptococcus pneumoniae*, *Enterococcus faecium*, *Staphylococcus aureus*,

Fig. 84. Equipment used to perform the Magicplex Sepsis real-time test (SEEPREP12™, left; SEEAMP™, centre; and CFX96™, right)

Pseudomonas aeruginosa, *Acinetobacter baumannii*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Escherichia coli*. Resistance markers include *vanA*, *vanB* and *mecA*.

Additional Seegene assays are available and include certain drug-resistance tests, among others:

- Seeplex™ assays: Seeplex™ VRE ACE Detection (detects *vanA* and *vanB* genes in enterococci isolated from stool culture) and Seeplex™ *H. pylori*-ClaR ACE Detection (detects two types of permutations causing clarithromycin resistance in *Helicobacter pylori* isolated from a gastric biopsy), as well as CE-IVD-marked multiplex respiratory pathogen assays, STI assays, HSV 1 and 2, HPV (screening and genotyping) and meningitis. Non-CE-marked assays include MTB/nontuberculous mycobacteria (NTM).
- Anyplex™ assays: Anyplex™ VanR Real-time Detection (simultaneously detects *vanA*, *vanB* and *vanC* genes from cultured samples of enterococci). CE-IVD-marked assays include multiplex respiratory pathogen assays, STI assays, HPV genotyping, MDR-TB, XDR-TB and MTB/NTM/MDR-TB. These assays require sample extraction and PCR setup on the Microlab NIMBUS (Hamilton, USA), followed by qPCR analysis on an instrument such as the CFX96™ (Bio-Rad).
- Allplex™ assays: Allplex™ Entero-DR assay (simultaneously detects eight antibiotic-resistance genes, including CPE [bla_{KPC}, bla_{VIM}, bla_{NDM}, bla_{IMP} and $bla_{\mathrm{OXA\text{-}48}}$], VRE [*vanA*, *vanB*] and ESBL [$bla_{\mathrm{CTX\text{-}M}}$]) from rectal swabs. Additional CE-IVD-marked assays include multiplex respiratory pathogen assays, STI assays, a GI assay and a meningitis assay.

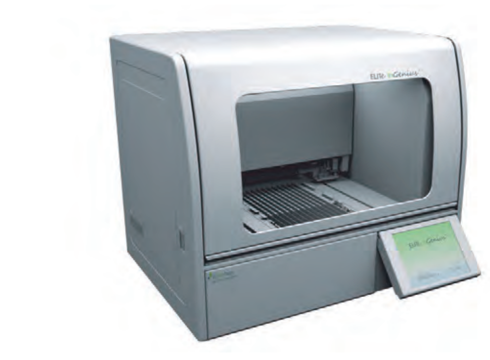
ELITe MGB® kits and panels (ELITechGroup Solutions, France)

ELITechGroup offers a number of IVD kits and panels. Of relevance to this report are several bacterial ID panels as well as several panels solely for identifying bacterial resistance markers. The assays can be performed on the ELITe InGenius® system, pictured in Fig. 85, which is a sample-to-result molecular diagnostics platform.

ELITechGroup offers the following assays for identifying bacterial pathogens primarily for HAIs:

- *C. difficile* ELITe MGB® kit: a real-time PCR assay designed for qualitatively detecting and differentiating *Clostridium difficile tcdA* and *tcdB*, including the NAP1/B1/027 strain, in stool. The assay is CE-IVD marked.

Fig. 85. ELITe InGenius® platform



- STI ELITe MGB® panel: a triplex real-time PCR assay designed to detect and differentiate CT, NG and MG DNAs in urine and cervical swabs (in the pipeline). The assay can be used in combination with the Macrolide-R/MG ELITe MGB® kit for detecting macrolide resistance. The assay is CE-IVD marked.
- MRSA/SA ELITe MGB® kit: a PCR assay for the direct, qualitative detection of *Staphylococcus aureus* and MRSA using DNA purified from human nasal swabs. The MRSA/SA ELITe MGB® is not intended to diagnose, guide or monitor MRSA infections nor to provide results of susceptibility to oxacillin/methicillin. Rather, it is intended to aid in preventing and controlling MRSA infections in healthcare settings. The assay is CE-IVD marked and is FDA cleared (when performed with sample processing on the bioMérieux NucliSENS® easyMAG® and performed on the Thermo Fisher Scientific ABI 7500 FAST Dx system, which consists of the 7500 FAST Dx instrument, a personal computer, 96-well plates and seals).

ELITechGroup also offers several assays for detecting resistance genes in bacterial HAIs:

- CRE ELITe MGB® kit: a multiplex real-time PCR assay designed to detect and differentiate the conserved regions of carbapenem-resistance genes of *Enterobacteriaceae*: bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP} and $bla_{\mathrm{OXA\text{-}48}}$-like genes from rectal swabs and blood culture (in the pipeline). The assay is CE-IVD marked.
- ESBL ELITe MGB® kit: a multiplex real-time PCR assay designed to detect ESBL genes of *Enterobacteriaceae*: CTX-M 1, 9, 14 and 15 groups. The assay can be performed on rectal swabs and blood culture (in the pipeline). The assay is CE-IVD marked.

- Colistin ELITe MGB® kit: a multiplex real-time PCR assay designed to detect conserved regions of mobilized colistin-resistance genes, *mcr-1* and *mcr-2*, of *Enterobacteriaceae* in rectal swabs. The assay is CE-IVD marked.

Each of the assay kits above is designed to be performed on the ELITe InGenius® platform, which is an integrated benchtop instrument that automatically performs extraction, real-time PCR amplification and results interpretation. The platform has bidirectional connectivity, which enables the laboratory to automatically communicate with an LIS to import testing information and export patient results. Overall TAT from extraction to results analysis is approximately 2.5 hours. Hands-on time is about 2 minutes per sample.

The ELITe InGenius® platform offers random access. One to 12 samples can be processed in parallel in independently controlled real-time PCR units. The platform has a minimum sixplex target capability, which is enhanced with melt curve analysis. The user can potentially mix all kinds of sample matrices and use diverse thermal profiles and even different PCR chemistries at the same time.

Amplidiag® system (Mobidiag, Finland)

Based on qPCR test panels, Mobidiag offers multiplex test panels for clinically relevant gastrointestinal bacteria (as well as parasites and viruses) and antibiotic resistance. The system is designed for mid- to large-sized laboratories.

Test panels relevant to this report include the following CE-IVD-marked assays:

- Amplidiag® Bacterial GE: detects eight bacterial pathogens from DNA extracted from stool (without preculture) in a single test in less than 2 hours. Pathogens identified include *Campylobacter*, *Salmonella* and *Shigella*/EIEC, among others.
- Amplidiag® *C. difficile*+027: detects pathogenic *Clostridium difficile* and its hypervirulent 027 ribotype from DNA extracted from stool (without any preculture) in a single test. TAT is less than 2 hours.
- Amplidiag® *H. pylori*+ClariR: detects *Helicobacter pylori* and its clarithromycin resistance directly from stool samples or gastric biopsies. TAT is less than 2 hours.
- Amplidiag® CarbaR+VRE: detects most relevant carbapenemases and vancomycin resistance from DNA extracted from pure culture in a single test. These include bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, $bla_{OXA\text{-}48}$, $bla_{OXA\text{-}181}$, *Acinetobacter* bla_{OXA}, *vanA* and *vanB*. TAT is less than 2 hours.
- Amplidiag® CarbaR+MCR: detects clinically relevant carbapenemases and colistin resistance from DNA extracted from stool samples, rectal swabs or pure culture. These include bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, $bla_{OXA\text{-}48}$, $bla_{OXA\text{-}181}$, *Acinetobacter* bla_{OXA}, *mcr* and bla_{GES}. TAT is less than 2 hours.

Test panels for parasites and viruses are also available.

The Amplidiag® test panels can be run on the Amplidiag® system, which is not integrated. As illustrated in Fig. 86, the system comprises (i) sample preparation; (ii) nucleic acid extraction and PCR plate setup on the Amplidiag® Easy instrument, the NucliSENS® easyMAG® (bioMérieux, France) or MagNA pure 96 (Roche, USA); (iii) real-time PCR on compatible/validated instruments, including the Bio-Rad CFX96™, ABI 7500 Fast, Corbett RotorGene (Thermo Fisher Scientific, USA) and QIAGEN Rotor-Gene® Q; and (iv) automated analysis and reporting using Amplidiag® Analyzer software. Up to 48 samples can be processed in about 2 hours.

Fig. 86. Amplidiag® system workflow

QIAsymphony® SP/AS (QIAGEN N.V., Germany)

QIAGEN has a line of assays relevant to this report. They are:

- artus™ *C. difficile* QS-RGQ kit: an in vitro diagnostic test for qualitatively detecting toxigenic *Clostridium difficile tcdA* and *tcdB* from human liquid or soft stool samples;
- artus™ CT/NG QS-RGQ kit: an in vitro diagnostic test for qualitatively detecting CT plasmid and gDNA, and NG gDNA from vaginal swabs and urine; and
- artus™ VanR QS-RGQ kit: an in vitro diagnostic test for detecting *vanA* and *vanB* vancomycin-resistance genes from human perianal or rectal swabs.

Each of the assays is CE-IVD marked, and the kits come ready to use with all optimized reagents required to run the test. The kits are designed to be used with the automated extraction and sample preparation system (QIAsymphony® SP/AS). The assays must then be run on one of the QIAGEN real-time Rotor-Gene® Q (RGQ) thermocyclers for amplification and detection. An example of a complete QIAsymphony® RGQ system is shown in Fig. 87.

Fig. 87. QIAsymphony® RGQ system

The QIAsymphony® SP/AS instruments provide automated sample preparation and assay setup. The QIAsymphony® SP can process 1–96 samples (in batches of 24) with sample volumes up to 1 mL. It is a ready-to-run instrument that requires minimal installation. The SP can be combined with the QIAsymphony® AS device in a fully integrated system that can automate the entire workflow. To reduce manual handling and minimize the risk of sample contamination, samples processed on the SP can be transferred automatically to the AS, or the two instruments can be operated independently. Despite their ease of use, the QIAsymphony® system and RGQ instruments are designed for use in sophisticated laboratories.

The QIAGEN real-time PCR cycler, the RGQ, offers a unique centrifugal rotary design. Each tube in the instrument spins in a chamber of moving air, which keeps all samples at precisely the same temperature during rapid thermal cycling. Detection is also uniform. When each tube aligns with the detection optics in the instrument, the sample is illuminated and the fluorescent signal is rapidly collected from a single, short optical pathway. Per the company, the thermal and optical uniformity of the system results in sensitive, precise and fast real-time PCR.

In addition to the assay kits described above, artus™ panels for QIAsymphony® RGQ are offered for numerous other assays, including assays for HBV and HCV, as well as assays for detecting and quantifying cytomegalovirus, Epstein-Barr virus, HSV 1 and 2, HIV, varicella-zoster virus and BK virus.

UtiMax™/BsiMax® (GeneFluidics, USA)

GeneFluidics is an early-stage company that is developing a diagnostics system based on electrochemical measurement of bacterial 16S rRNA for detection, ID and AST. The company currently has one CE-IVD-marked UTI assay on the market for performance on the UtiMax™ lab automation system; TAT is approximately 30 minutes for pathogen ID and 120 minutes for AST.

Fig. 88. UtiMax™ lab automation system

The UtiMax™ lab automation system, pictured in Fig. 88, is a fully automated rapid diagnostic system for identifying uropathogens directly from urine samples. Pathogen ID and AST are performed by the UtiMax™ lab automation system with a reagent kit and disposable sensor array chip. UtiMax™ ID/AST

is an electrochemical-based sandwich hybridization test to quantify species-specific ribosomal 16S rRNA. Each sample is lysed chemically prior to hybridization at high stringency. A built-in multichannel potentiostat reads the electrical current from the steady-state enzymatic cycling amplification: the signal is proportional to the bound 16S rRNA content from lysate and reported in ranges of CFU per millilitre through an established calibration curve.

A follow-up product line, BsiMax® (with the additional feature of lysis centrifugation), is in development. The BsiMax panel will include *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter* spp., MRSA, MSSA and *Enterococcus*, among others. The AST panel will include the following antibiotics: gentamicin, ciprofloxacin, cefepime, meropenem, ceftriaxone, ampicillin and piperacillin-tazobactam. The BsiMax® assay can process whole-blood samples for BSIs with a limit of detection (LOD) < 4 CFU/mL with ID in 5 hours and AST in 2 hours. Both UtiMax™ and BsiMax® can be performed using the company's robotic liquid-handling systems, with associated reagent kits and sensor chips.

Per the company, both the BsiMax® and UtiMax™ ID/AST tests can quantify unique species-specific nucleic acid sequences associated with each target pathogen without using PCR, and can conduct AST without the need to obtain a clinical isolate or positive blood or urine culture sample. No peer-reviewed published studies are available on the UtiMax™ ID/AST assay, and the BsiMax® ID/AST assay is still in development.

Nonphenotypic methods of detecting antibiotic resistance

In addition to some of the systems described above, including the GeneXpert® system, Unyvero™ system, FluoroType® system and several systems from Seegene, which can both identify bacterial pathogens and detect resistance genes, other platforms identify multiple genes that directly confer antibiotic resistance but do not identify pathogens.

Molecular methods of detecting antibiotic resistance

Check-Direct and Check-MDR assays (Check-Points, Netherlands)

Check-Points manufactures screening assay kits for rapid AMR detection. There is a family of CE-IVD-marked assays for use on the BD MAX™ system, described earlier in this report. The reagents for these assays come in pre-aliquoted, dried-down format, pictured in Fig. 89, for easy automation on the BD MAX™ system from BD. The company also offers assays available for use on other systems.

Fig. 89. Check-Direct CPE for BD MAX™ reagent pouch

The assays for the BD MAX™ system are:

- Check-Direct CPE for BD MAX™: detects the clinically most prevalent carbapenemases – *bka*$_{KPC}$, *bla*$_{OXA-48}$, *bla*$_{VIM}$ and *bla*$_{NDM}$ – including the emerging *bla*$_{OXA-181}$ variant from culture;
- Check-Direct CPO for BD MAX™: detects the five most prevalent carbapenemase genes (*bka*$_{KPC}$, *bla*$_{OXA-48}$, *bla*$_{VIM}$, *bla*$_{NDM}$ and *bla*$_{IMP)}$ directly from rectal swabs in about 2.5 hours; and
- Check-Direct ESBL screen for BD MAX™: detects ESBL genes (*bla*$_{CTX-M-1}$ group, *bla*$_{CTX-M-2}$ group, *bla*$_{CTX-M-9}$ group, *bla*$_{SHV\ ESBL}$) from rectal swabs or culture.

Check-Points also offers an assay, the Check-Direct CPE, which can be performed using the NucliSENS® easyMAG® for sample preparation and the ABI 7500, CFX96™, LightCycler® 480 system I and II (Roche, USA) and Rotor-Gene® Q. The assay detects the most prevalent carbapenemases – *bka*$_{KPC}$, *bla*$_{OXA-48}$, *bla*$_{VIM}$ and *bla*$_{NDM}$ – from rectal swabs or culture. TAT is approximately 2 hours.

In addition, Check-Points offers a family of microarrays for epidemiology and confirmation. These assays are performed from culture using the following equipment: (i) magnetic bead- or column-based methods for sample preparation; (ii) a validated thermocycler, vortex mixer and mini-centrifuge, pre-PCR; and (iii) a validated thermocycler, vortex mixer, mini-centrifuge, thermomixer with active cooling, Check-Points Tube Reader, including E-Ads software, computer with USB drive and Internet connection and barcode reader (optional), post-PCR. The assays offered are:

- CHECK-MDR CT101: permits investigation of the epidemiology of suspected CTX-M ESBLs, discriminates directly between ESBL and non-ESBL variants of TEM and SHV, and identifies presumptive mobile AmpCs.

- CHECK-MDR CT102: detects the clinically most prevalent carbapenemases and ESBLs in *Enterobacteriaceae*, and discriminates directly between ESBL and non-ESBL variants of TEM and SHV.
- CHECK-MDR CT103 XL: a CE-IVD-marked assay that identifies carbapenemase and ESBL targets, including emerging types. It can identify carbapenemases typically identified in *Acinetobacter baumannii* and carbapenemases and ESBLs found in *Pseudomonas aeruginosa*. The assay also discriminates directly between carbapenemase and ESBL variants of GES-type beta-lactamase (GES).

eazyplex® (AmplexDiagnostics GmbH, Germany)

AmplexDiagnostics offers the eazyplex® lyophilized ready-to-use amplification system for which there are numerous test kits available, most of which identify multiple genes that directly confer antibiotic resistance from various specimen types. Tests are validated to be run on the Genie® II instrument (OptiGene, UK), pictured in Fig. 90, for target isothermal amplification and detection. It is a fully portable, compact and lightweight platform designed for use at or near POC.

Fig. 90. Genie® II platform

The eazyplex® tests are qualitative in vitro molecular diagnostic tests to detect bacterial DNA in no more than 30 minutes. No DNA/RNA extraction is required, and eazyplex® test kits can be stored at ambient temperature. Test kits generally consist of eight-microtube test strips containing freeze-dried, ready-to-use reagents for amplifying seven resistance genes and one internal control. The test strips are used with the Genie® II platform, which uses a single-channel fluorescence excitation and detection system, to carry out LAMP of targeted resistance genes. The platform is mains powered, but can be used with a battery as well.

The test process is as follows. Samples are suspended in resuspension and lysis fluid (RALF) buffer solution and incubated for 2 minutes with thermal lysis. Twenty-five millilitres of the RALF suspension is then added to each tube of the strip containing ready-to-use mastermix. The test strip is then immediately placed into the Genie® II instrument, where it is incubated at 66 °C for 30 minutes with fluorescent monitoring. Isothermal amplification is indicated by a strong increase in fluorescence signal in the form of a typical amplification curve. Different colours are given to each of the tested gene variants. Genie® II has two heating blocks, each of which can process a single eight-microtube test strip. The blocks can be controlled independently or run together to process up to 16 samples.

In addition to the Genie® II platform, OptiGene has introduced the Genie® III, pictured in Fig. 91, which has been developed for use with the eazyplex® test kits. It is smaller and lighter than the Genie® II, and per the company, is suitable for use in demanding environments. The platform includes dual-channel fluorescence measurement to allow use of internal controls and multiplexed assays. It also has positional information through GPS and offers wireless connectivity in the form of Bluetooth and Wi-Fi. Genie® III incorporates a rechargeable lithium-polymer battery that can support operation of the instrument for an 8-hour day. The instrument has a single heating block, which can process a single eight-microtube strip.

Fig. 91. Genie® III platform

Eazyplex test kits of relevance to this report include the following, all of which are validated for use on the Genie platforms. The assays are CE-IVD marked.

SuperBug® tests include three assays that determine the presence of carbapenemase-producing organisms (CPOs) and ESBL genes in people for whom colonization with these organisms is suspected. These are:

- SuperBug® complete A: a qualitative IVD for directly detecting carbapenemase-producing bacteria and culture confirmation directly from rectal swabs taken with eSwab™ (Copan) or

bacterial isolates from agar plates. The following carbapenemases are detected: KPC (*bla*$_{KPC}$), NDM (*bla*$_{NDM}$), VIM (*bla*$_{VIM}$) and OXA (*bla*$_{OXA-23,40,48,58}$).

- SuperBug® complete B: a qualitative IVD for directly detecting carbapenemase-producing bacteria and culture confirmation directly from rectal swabs taken with eSwab™ (Copan) or bacterial isolates from agar plates. The following carbapenemases are detected: KPC (*bla*$_{KPC}$), NDM (*bla*$_{NDM}$), VIM (*bla*$_{VIM}$) and OXA (*bla*$_{OXA-23,40,48,181}$).
- SuperBug® CRE: a qualitative IVD for directly detecting carbapenemase-producing bacteria and culture confirmation directly from bacterial isolates from agar plates, blood culture media from positive flagged blood culture bottles, rectal swabs taken with eSwab™ or urine. The following carbapenemases are detected: KPC (*bla*$_{KPC}$), NDM (*bla*$_{NDM}$), VIM (*bla*$_{VIM}$) and OXA (*bla*$_{OXA-48,181}$). In addition, the following ESBL genes are detected: *bla*$_{CTX-M-1}$ group and *bla*$_{CTX-M-9}$ group.

There are two additional eazyplex® SuperBug® test kits:

- SuperBug® mcr-1: a qualitative IVD for confirming *mcr-1*, which confers resistance to colistin (polymyxin B), in gram-negative bacteria from culture; and
- SuperBug® AmpC: a qualitative IVD for confirming AmpC beta-lactamases from AmpC-positive *Enterobacteriaceae* in culture.

Eazyplex® also offers two VRE assays. These are:

- eazyplex® VRE: a qualitative IVD for detecting VRE from rectal swabs or blood culture. The assay detects *vanA* and *vanB* and has three integrated controls.
- eazyplex® VRE basic: a qualitative IVD for confirming VRE directly from agar plates or from positive blood culture. The assay confirms *vanA* or *vanB* in 20 minutes.

Finally, eazyplex® offers two IVDs for MRSA screening, one for screening for MRSA based on detection directly from nasal swabs, and one for confirmation based on culture media. It also offers a family of *Clostridium difficile* test kits and a selection of CSF tests for detecting bacteria and viruses from CSF.

Carbaplex® IVD PCR (Bruker, Germany)

Bruker offers the Carbaplex® IVD PCR assay for qualitatively detecting CPEs. The test, which is a multiplex real-time PCR assay, detects and differentiates the five most prevalent carbapenemase genes from a single rectal swab sample. These are KPC (*bla*$_{KPC}$), NDM (*bla*$_{NDM}$), VIM (*bla*$_{VIM}$), OXA (*bla*$_{OXA-48,181}$) and IMP (*bla*$_{IMP}$). The test can also be used for confirmation testing from suspected culture isolates. The assay is CE-IVD marked. TAT is less than 3 hours.

Carbaplex is provided in an easy-to-use master mix format and is designed for use with existing laboratory equipment in large laboratories, including the ABI 7500 and ABI QuantStudio 5, both from Thermo Fisher Scientific, the CFX detection systems (Bio-Rad), and the Rotor-Gene® Q.

Antibiotic resistance line probe assays (AUTOIMMUN DIAGNOSTIKA GmbH, Germany)

AUTOIMMUN DIAGNOSTIKA offers several antibiotic resistance line probe assays (LPAs) for infectious disease. These assays are designed to be run on end-point PCR equipment, which requires a sophisticated and well equipped laboratory. Especially well trained technicians are important as the system is not integrated.

In addition to several assays for TB, which are not covered in this report, the assays include the following CE-IVD-marked tests for use on automated systems, which are relevant to this report:

- AID ESBL: an assay for rapidly detecting ESBL genes, including *bla*$_{TEM}$, *bla*$_{CTX-M}$, *bla*$_{SHV}$ and *bla*$_{KPC}$ within 5 hours from culture and clinical specimens.
- AID carbapenemase: an assay for initial screening of the most frequent carbapenemases from bronchoalveolar lavage, sputum, wound swabs or bacterial culture. The test detects 13 different carbapenem resistances, including *bla*$_{KPC}$, *bla*$_{VIM}$, *bla*$_{NDM}$ and *bla*$_{OXA-48}$. TAT is less than 4 hours.
- AID MRSA combi: an assay to detect the most frequent resistance genes of staphylococci, including *mecA* and *mecC*, and to differentiate *Staphylococcus aureus* and CNS. TAT is approximately 4 hours.

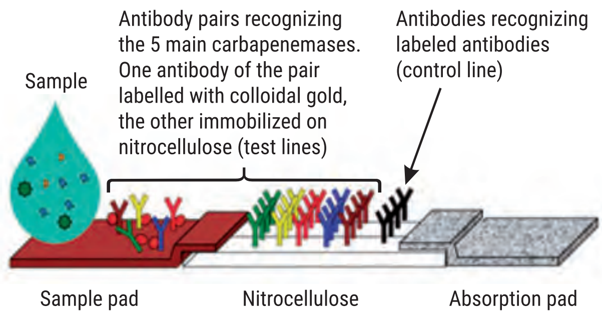
Immunoassays and other methods for detecting antibacterial resistance

Antimicrobial lateral flow immunoassays (LFIAs) (NG Biotech, Netherlands)

NG Biotech offers several in vitro LFIAs for detecting/confirming resistance genes from culture. These are NG-Test CARBA 5, NG-Test CTX-M and NG-Test MCR-1. The assays are qualitative lateral flow strip tests, all of which are CE-IVD marked. TAT is 10 to 15 minutes.

Fig. 92. Test protocol for NG Biotech LFIAs[15]

1- Structure of the strip



2 - Immunological detection

Sample flow: capillarity

3 - Result

✓ The control line appears: the test is correct

✓ One or several test lines appear: positive test for the corresponding carbapenemase(s)

✓ No test line appears: negative test for the 5 carbapenemases

- NG-Test CARBA 5 detects the five main carbapenemases – NDM (bla_{NDM}), KPC (bla_{KPC}), NDM (bla_{NDM}), VIM (bla_{VIM}) and OXA-48 ($bla_{\mathrm{OXA\text{-}48}}$) – from cultured bacterial isolates.
- NG-Test CTX-M detects the ESBL CTX-M ($bla_{\mathrm{CTX\text{-}M}}$) from cultured bacterial isolates.
- NG-Test MCR-1 detects and confirms *mcr-1*, which confers colistin resistance, in gram-negative bacteria from culture.

The test protocol for these assays is illustrated in Fig. 92.

RESIST assays (Coris BioConcept, Belgium)

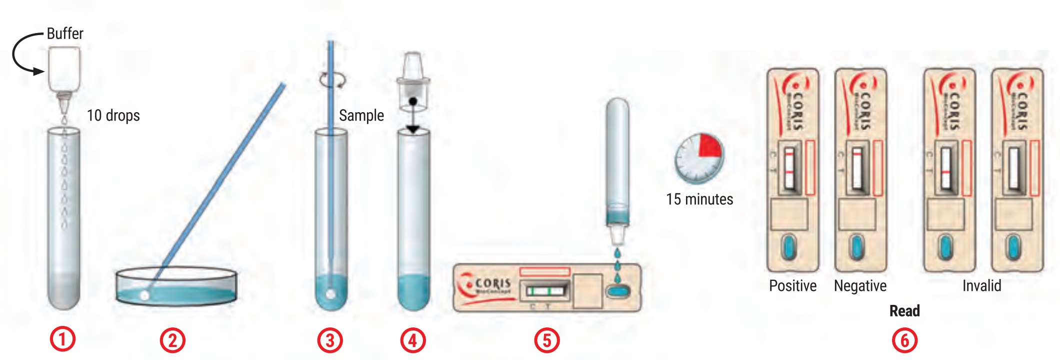
Coris BioConcept offers a range of CE-IVD-marked in vitro cartridge-based LFIAs for detecting/confirming select carbapenemase resistance genes from cultured bacterial isolates. The tests are branded "RESIST" and consist of the following:

- OXA 48 *K*-SeT for detecting $bla_{\mathrm{OXA\text{-}48}}$
- KPC *K*-SeT for detecting bla_{KPC} only
- RESIST-3 O.K.N. *K*-SeT for detecting $bla_{\mathrm{OXA\text{-}48\text{-}like}}$, bla_{KPC} and bla_{NDM}
- RESIST-3 O.O.K. *K*-SeT for detecting $bla_{\mathrm{OXA\text{-}48\text{-}like}}$, bla_{KPC} and $bla_{\mathrm{OXA\text{-}163}}$
- RESIST-4 O.K.N.V. for detecting $bla_{\mathrm{OXA\text{-}48\text{-}like}}$, bla_{KPC}, bla_{NDM} and bla_{VIM}.

TAT for each of the tests is approximately 15 minutes. The procedure is illustrated in Fig. 93.

In addition to the RESIST line of assays, Coris BioConcept also offers a family of CE-IVD-marked in vitro diagnostic tests for rapidly detecting various

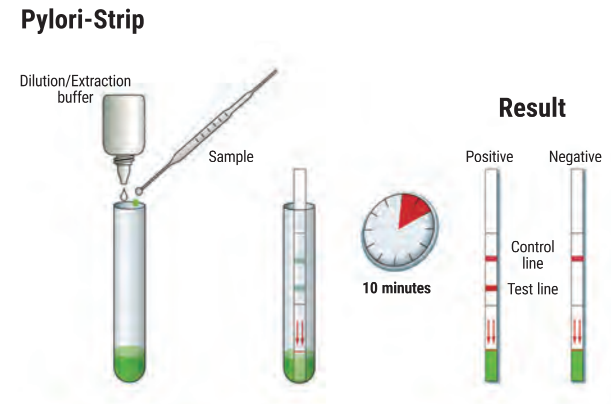
Fig. 93. Test procedure for RESIST range of LFIAs from Coris BioConcept



[15] Reproduced from Boutal H, Vogel A, Bernabeu S, Devilliers K, Creton E, Cotellon G et al. A multiplex lateral flow immunoassay for the rapid identification of NDM-, KPC-, IMP- and VIM-type and OA-48-like carbapenemase-producing *Enterobacteriaceae*. J Antimicrob Chemo. 2018;73(4):909–15.

pathogens (bacteria, virus, parasites). These immunochromatographic assays come in both strip/dipstick and cassette format. Of relevance to this report are the assays for *Helicobacter pylori*, *Escherichia coli* and *Clostridium difficile*. All of the assays require stool specimens, which in the case of the *E. coli* assay must have been broth enriched. TAT ranges from 10 minutes (*H. pylori*) to 15 minutes (for *E. coli* and *C. difficile*). The test procedure is similar for all three of the assays and is illustrated in Fig. 94 for the *H. pylori* assay.

Fig. 94. Coris BioConcept *Helicobacter pylori* strip test procedure



Finally, Coris BioConcept is developing a fully automated system, the TRAPIST V6 instrument and test cassettes, pictured in Fig. 95, for multiplex diagnostic testing. The platform, which is being designed for use in clinical laboratories, uses disposable microfluidic chip technology that combines both molecular assays and immunoassays. To date, the platform has not been commercialized; no assays have FDA clearance or CE-IVD marking. Initial assays for the TRAPIST system are focused on two sepsis panels – multiplex gram-positive cassette (e.g., *Staphylococcus aureus*); multiplex gram-negative cassette (e.g., *Escherichia coli* and *Pseudomonas aeruginosa*) – and resistance markers (e.g., *vanA*, *vanB*, *mecA* and *mecC*) for gram-positive bacteria. Coris BioConcept aims for the TRAPIST system to return results in less than 1 hour. Additional information on these assays is not available.

Fig. 95. Coris BioConcept TRAPIST V6 instrument (left) and test cartridge (right)

Pipeline technologies for identifying pathogens and/or detecting antibiotic resistance

There are PCR, DNA hybridization, electrochemical detection and other technologies for pathogen ID and detection of genes that directly confer bacterial resistance in the development pipeline. These are discussed below. Unless otherwise noted, all of the test systems described below are under development and not approved for sale; their performance characteristics have not yet been established.

LabDisk (SpinDiag, Germany)

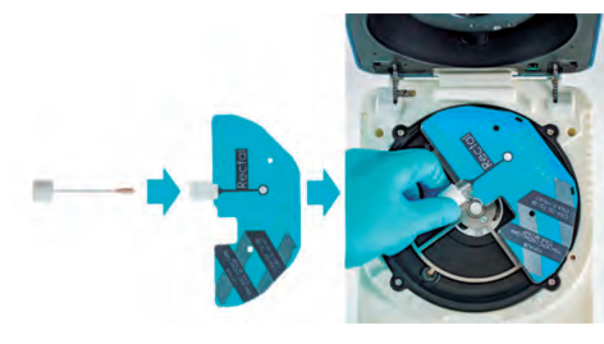
SpinDiag is a start-up company that was a spin-off from the Hahn-Schickard research institute. SpinDiag is developing a benchtop instrument, the LabDisk (pictured in Fig. 96), currently in prototype form, to test for 25 drug-resistant bacterial strains in 30 minutes at low cost. The technology uses centrifugal microfluidics with a disc-based test cartridge. There are no active components inside the instrument, but rather a simple optical reader.

Fig. 96. LabDisk prototype instrument

The LabDisk instrument has a small footprint. As illustrated in Fig. 97, the system also has a simple workflow. Specimen swabs can be directly inserted into the LabDisk cartridge with no manual sample preparation; all dried reagents for sample preparation, amplification and detection are contained in the cartridge. The instrument uses fast, solid-state heating/cooling elements, mechanical lysis for extraction and nested PCR for high sensitivity. It can run 48 PCRs in parallel (composed of 36 parameters from one sample plus 12 internal controls).

To date, having tested 70 samples from 30 patients, the company has only a limited data set for the system. Results have been good, indicating that the instrument can detect down to single pathogens.

Fig. 97. LabDisk workflow

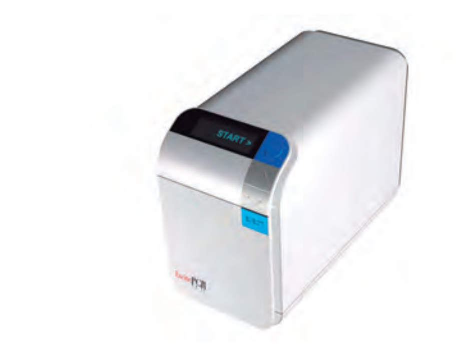


SpinDiag is currently testing for MRSA using nasal swabs and for VRE using rectal swabs. The company expects to be able to launch its first assay, a pan-bacterial test, by 2020. The next assays to be developed will be for respiratory tract infections and STIs. SpinDiag is also testing whether the platform could be used to test whole blood and/or urine specimens. The company believes that quantitation is possible on the LabDisk, but it has not yet been demonstrated.

FireflyDx™ (ExcitePCR, a subsidiary of Positive ID, USA)

ExcitePCR is developing two diagnostic platforms for use at POC in low-resource settings: the FireflyDX-Portable™ and the FireflyDX-Handheld™. Both are in prototype stage. The FireflyDX-Portable™, pictured in Fig. 98, is a lightweight, "bookbag-sized" system utilizing real-time PCR that is designed to provide integrated sample purification, biological analysis and wireless communication of pathogen detection results in 30 minutes or less. The system incorporates single-use, disposable cartridges containing radio-frequency identification (RFID) chips that encode data. Cartridges will be able to process whole blood, nasal swabs and urine, among other specimens. Per the company, the FireflyDX-Portable™ instrument is an open system and will operate with any commercial assay, including those used by CDC and WHO.

Fig. 98. Rendering of the FireflyDX-Portable™ instrument



To date, the FireflyDX-Portable™ instrument has detected the Ebola and Zika viruses, *Escherichia coli*, influenza, MRSA, MSSA and *Clostridium difficile* on its prototype system. No commercialized assays for the system are available.

The other system being developed by ExcitePCR is the FireflyDX-Handheld™, pictured in Fig. 99. Like the FireflyDX-Portable™, it is a sample-in, result-out platform utilizing real-time PCR and single-use disposable cartridges. Because of its small size, the FireflyDX-Handheld™ can be used at bedside.

Fig. 99. Rendering of FireflyDX-Handheld™ instrument

The company plans multiple applications for the two FireflyDX™ systems, including AMR assays.

GeneSTAT® analyser system (DxNA, LLC, USA)

DxNA is developing a sample-in, result-out diagnostic instrument platform, the GeneSTAT® analyser, for use at or near POC. The instrument, pictured in Fig. 100, has a small footprint with only four moving parts. Per the company, it requires minimal maintenance and no calibration. The GeneSTAT® analyser uses real-time PCR technology; test results can be read either on the instrument itself or on an attached laptop computer.

Fig. 100. GeneSTAT® analyser

The GeneSTAT® generates, analyses and transmits results to a connected preconfigured computer for presentation to the user. One single-use test cartridge

can be tested at a time in each GeneSTAT® analyser. Up to four GeneSTAT® analysers can be connected to a single computer.

Each GeneSTAT® single-use cartridge contains all required reagents as well as all the information needed to perform a test. In its present design, the cartridge has the capacity to perform up to four results with three analytical targets and one control. Reagents are lyophilized in each of the reaction wells, and once the specimen is placed into the cartridge, the cartridge becomes a closed, pressurized system. Per the company, this eliminates issues that arise from contamination from PCR products produced in the test process. Needed test information is provided on an RFID tag on each cartridge.

In 2017, DxNA received FDA clearance for its in vitro diagnostic assay for detecting *Coccidioides* spp. (valley fever). Currently, DxNA is developing a diagnostic test for *Staphylococcus aureus* that will both identify and differentiate resistant and nonresistant strains of *S. aureus* and CNS from multiple specimen types. The test uses three separate proprietary targets and a proprietary methodology to determine which type(s) of *Staphylococcus* are present and which carry a bacteria-resistant gene. TAT is about 60 minutes.

ASTar™, ASTrID® (Q-linea AB, Sweden)

Q-linea is in the process of developing two platforms for detecting BSIs: ASTar™ for AST and ASTrID® for pathogen ID.

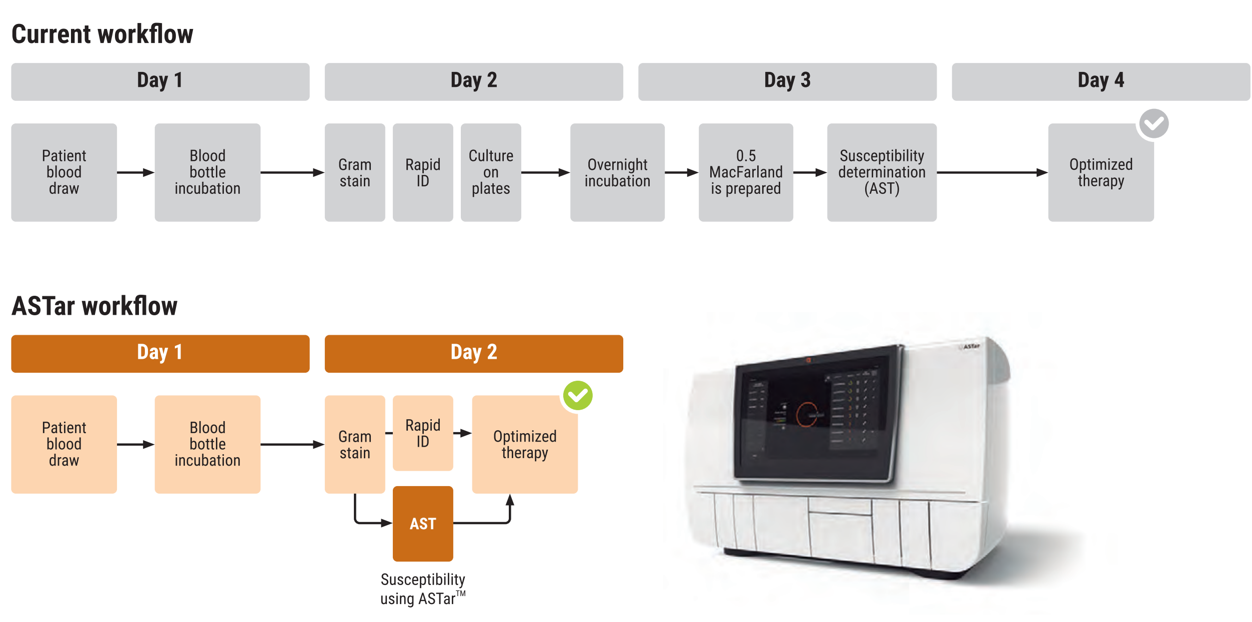
The ASTar™ instrument performs phenotypic AST in about 3–6 hours following pathogen ID by current methods, e.g., MALDI-TOF MS, with which ASTar™ can be combined. The ASTar™ workflow compared to classical phenotypic AST is illustrated in Fig. 101.

The ASTar™ assay is based on broth microdilution and produces reproducible MICs. Currently, the assay takes blood culture samples only, although the company plans to develop an assay for use with whole blood. In internal studies, the ASTar™ assay has shown phenotypic AST results, obtained within 6 hours, with 96% essential agreement and 95% categorical agreement compared to reference broth microdilution with respect to *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Enterococcus faecalis* (*59*).

Q-linea is also developing the ASTrID® platform, pictured in Fig. 102, which will be a fully automated, high-throughput, multiplex benchtop system. The platform is based on Q-linea's core padlock probe technology and circle-to-circle isothermal amplification (C_2CA). More specifically, highly specific and selective padlock probes forming circularized DNA strands are amplified via RCA and subsequent C_2CA. The resulting RCA products are labelled with fluorescence and are detected on a microarray (*60–62*).

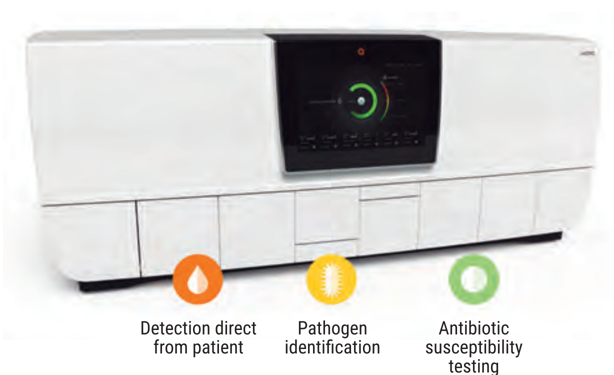
ASTrID® will enable ID of more than 50 sepsis pathogens and selected resistance genes, in addition to phenotypic AST, in 10 hours directly from whole blood. Per the company, the ID panel will cover 95% of relevant pathogens as well as 11 resistance markers; the panel of antibiotic substances will contain more than 30 antibiotics. Susceptibility will be reported as MIC values. Q-linea® has conducted a preclinical study using the prototype ASTrID® instrument that demonstrated that it can deliver pathogen ID directly

Fig. 101. ASTar™ workflow compared to classical phenotypic AST



from clinical blood samples without a positive blood culture, and a susceptibility profile after 6 hours using positive blood cultures.

Fig. 102. ASTrID® platform



Reveal AST™/ID™ (Specific Diagnostics, USA)

Specific Diagnostics is developing a diagnostic system for ID and AST of pathogens. The system will use a proprietary, novel small molecule sensor (SMS) array that responds to metabolic byproducts produced by microorganisms to identify pathogens, detect their growth and assess their antibiotic efficacy. The SMS array detects low (parts per billion) concentrations of volatile organic compounds (VOCs) in very complex mixtures. A high-dimensional printed array of more than 70 colorimetric chemical indicators embedded in a nanoporous matrix has distinct chemical reactivity with volatile microorganisms and changes colour based on exposure to various VOCs and VOC mixtures. When placed in a culture, the resulting pattern of colour changes comprises a high-dimensional fingerprint of the cell type for bacterial species and strain from which bacteria can easily be differentiated.

The technology will permit Reveal ID™ to identify bacteria from blood culture in 4 hours on average directly in the vial, while Reveal AST™ will provide phenotypic MICs in 4 hours, also directly from positive blood culture or from isolate dilutions. Per the company, the technology has been clinically validated. Specific Diagnostics indicates that the system is easy to use and will be low cost.

GeneXpert® Omni (Cepheid, a subsidiary of Danaher Corporation, USA)

Cepheid is developing the GeneXpert® Omni system (pictured in Fig. 103). The system leverages existing Xpert® cartridge technology (described earlier in this report). It should be noted, however, that the Omni platform does not use the same cartridge as that in the original GeneXpert® platforms; therefore, the cartridges are not interchangeable between the two systems.

The GeneXpert® Omni is highly portable, measuring just 9 inches tall (about 23 cm) and weighing 2.2 pounds (about 1 kg). The system is battery operated (with up to 4 hours of operation and a supplemental rechargeable battery with an additional 8 hours of battery life), and is wireless and connectivity enabled. Advanced microfluidics regulate all aspects of the testing process within the test cartridge – from sample preparation and nucleic acid extraction to amplification and detection. Additionally, the platform has solid-state digital electronic architecture, which means it is durable.

Fig. 103. GeneXpert® Omni platform

The GeneXpert® Omni platform will use a dedicated mobile device to control each test module. The provided mobile device can control up to 3 Omni instruments, thus providing scalability and flexibility. The platform will also use a secure, hosted platform that collects and aggregates real-time test and system telemetry information. A single system can store more than 20 000 test results.

The initial assays planned for availability on the system will be the Xpert® MTB/RIF (resistance to rifampicin), Xpert® MTB/RIF Ultra, Xpert® HIV-1 Qual, Xpert® HIV-1 Viral Load and Xpert® HCV Viral Load and Xpert® HPV. Over time, it is Cepheid's intent to have the majority of the Xpert® menu available on the GeneXpert® Omni, which could include ID and molecular resistance cartridges.

binx io® diagnostic system (Binx Health, Inc., formerly Atlas Genetics, UK)

The binx io® platform is a rapid, multiplex, molecular diagnostic system that can deliver laboratory quality results in about 30 minutes. The system consists of a small instrument and disposable cartridge (pictured in

Fig. 104) that contains all reagents necessary to run a test and is designed to be simple and intuitive; the user interacts with the instrument through a touch-screen interface, which then guides the suer through the io system process. Once the raw sample has been added to the cartridge and loaded into the instrument, no further interaction is required. The instrument fits easily on a bench-top and is fully integrated, which eables the movement of a sample and reagents within the cartridge.

The cartridge has three main assay steps: sample preparation to isolate and purify target DNA, ultra-rapid PCR, which amplifies specific regions of DNA from the target organisms, and proprietary electrochemical detection to identify the presence of amplified DNA. Once the test is completed, a qualitative 'Detected/Not Detected' result is available with no clinical or laboratorian interpretation needed.

Fig. 104. binx io® instrument and cartridge from Binx Health

Binx Health's core focus is on STIs and their first application is for CT and NG. The binx test is designed to provide a result directly from an unpurified patient sample in about 30 minutes with equivalent accuracy and performance as current standard of care platforms run in central laboratories (which can take seven or more days).

The dual target CT/NG assay received CE marking for use within Europe in April 2019. Binx Health also recently successfully completed a US-based multicenter study of the platform and has submitted an application to the FDA for 510(k) clearance.

Binx continues to develop additional targets to add to its test menu, including an expansion of its current CT/NG multiplex test to include two other STIs with rapidly increasing prevalence: TV and MG. In addition, the company is also developing a NG resistance assay to detect Ciprofloxacin-sensitive strains. This work funded by the National Institute for Health Research (UK), and in collaboration with St. Georges Hospital in London, will allow greater antibiotic stewardship and open the breadth of treatments available to address this major public health crisis.

Q-POC™ (QuantuMDx Group, UK)

QuantuMDx Group is developing a small benchtop diagnostic device, Q-POC™ and test cassette (pictured in Fig. 105), which can deliver patient results in less than 20 minutes.

Fig. 105. Q-POC™ cassette and device

The Q-POC™ device is a portable sample-to-answer molecular platform that is simple to use and runs end-point PCR chemistries, qPCR chemistries and includes a microarray after the amplification step. It is the first MDx POC platform that combines the ability to quantitate pathogens through its six-channel qPCR and additionally perform multiplex detection of approximately 50 markers with its integrated microarray. Because it uses a rapid microfluidic thermal cycler that performs a 35-cycle end-point PCR in a few minutes, the device can run raw sample-to-answer assays in as little as 7–20 minutes, depending on the complexity of the assay.

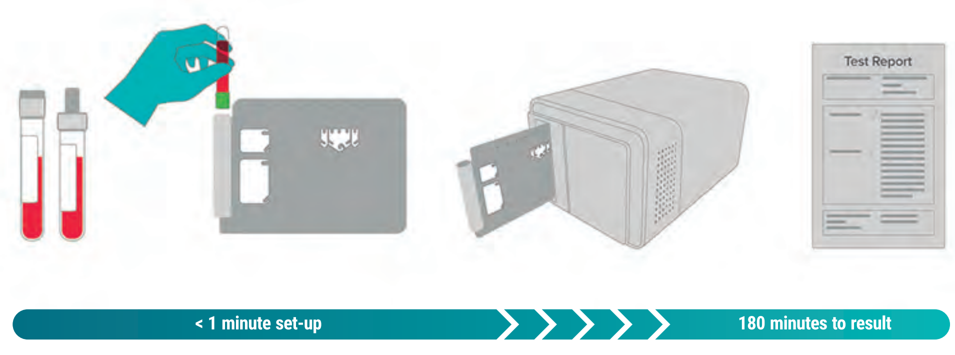
The first assay being developed on the Q-POC™ platform is an HPV genotyping assay that provides individual genotypes for 13 high-risk HPV subtypes in under 20 minutes, direct from a swab sample. The assay is presently in clinical field studies to demonstrate the clinical utility in screen-and-treat programmes in LMICs. The next assay planned for the Q-POC™ platform is a CT/NG/TV triplex assay, also run direct from swab samples in under 15 minutes. The company is developing an AMR NG assay to complement its triplex STI detection assay.

LiDia® (DNA Electronics, Ltd. [DNAe], UK and USA)

DNAe is developing its LiDia® system for diagnosing infectious diseases using semiconductor genomic analysis. The target assay for its first IVD is the detection of sepsis from whole blood by direct sequencing. Tests for antimicrobial resistance testing (e.g., *mecA*), as well as tests for influenza and liquid biopsies, will follow.

The LiDia® technology uses immunomagnetic beads to capture only intact bacterial (and fungal) pathogens from specimens. Bead cell complexes are

Fig. 106. Workflow for sepsis assay on LiDia® platform



separated from background sample matrices, and human DNA is removed. Pathogens are then lysed, and purified DNA is eluted. Primers are bound onto transistors on a pH-sensing semiconductor chip. The purified pathogen nucleic acid is then amplified in a multiplexed PCR reaction containing primers specific for multiple pathogen targets; the multiplex mixture is split into separate reaction vessels, each with a specific nested PCR target assay. Detection of changes in pH associated with the release of hydrogen ions during PCR provides a real-time readout of detection events. The technology requires no optics or fluorescent labels.

The planned workflow with respect to the sepsis assay, illustrated in Fig. 106, consists of inserting a 10 mL blood Vacutainer® or Monovette® into a disposable cartridge, loading the cartridge into the LiDia® instrument and reading the result in about 3 hours. The company indicates that only about 1 minute of setup time is required.

Smarticles™ technology (Roche, USA)

In 2015, Roche acquired GeneWeave Biosciences, which was developing Smarticles technology, a class of molecular diagnostics that can quickly identify multidrug-resistant pathogens and can assess AST directly from clinical samples without the need for traditional enrichment, culture or sample preparation processes. GeneWeave was also developing a platform called vivoDx, a fully automated random-access system.

Smarticles™ are nonreplicative transduction particles (phages) that bind specifically to bacteria and deliver DNA that contains a reporter luciferase gene that is expressed in the bacteria. It is complex technology in which each organism requires a unique phage.

Roche has indicated that its first assay for its cobas® vivoDx platform will be for MRSA; assays for CRE and VRE are in development. No additional information is available.

Conclusion

There are numerous nonphenotypic assays and platforms for identifying bacterial pathogens as well as for identifying the genes that directly confer antibiotic resistance. Most of the platforms, especially those for BSIs, are systems best used in sophisticated laboratory settings with strong infrastructure and well trained laboratory staff. A few platforms, including the GeneXpert® system and FilmArray® EZ Configuration, can be used in near-patient settings, including Level II facilities in LMICs, but not for assays requiring culture. All of the platforms offer faster results than phenotypic methods.

Molecular and immunoassay platforms for detecting antibiotic resistance only, including Check-Direct and eazyplex®, as well as lateral flow assays from NG Biotech, are suitable for near-patient testing in LMICs. They do not, however, provide pathogen ID, which must be done on separate instrumentation.

Finally, a number of molecular systems in the development pipeline are designed for use in LMICs. The systems are smaller and simpler to use than conventional systems designed for use in large laboratories. Some of these pipeline diagnostics provide pathogen ID as well as resistance testing capabilities; some do not. Some perform monoplex testing only and some will only process sample matrices such as swabs and urine, which limits the pathogens they are able to detect. Some systems will process complex matrices, including whole blood, which would offer the possibility of avoiding culture. However, detecting and identifying bacteria direct from whole blood with performance at least equivalent to blood culture has proven to be very difficult. It is a challenge that has not yet been met.

Host response assays

The diagnostic systems mapped in this report so far have focused on specific pathogen ID approaches. There are other rapid, easy-to-use diagnostics that might have value in combating ABR that are suitable for use at Level I and Level II settings in LMICs that are not direct pathogen detection methods. These include host immune response assays, including tests to detect blood-based host-derived biomarkers. Some of these tests are already commercialized; some are in the development pipeline.

Host-derived biomarkers of infection include white blood cell levels, erythrocyte sedimentation rate, CRP, PCT, presepsin, CD64 and proADM, among others (*63*). Of particular interest for this report are CRP and PCT, the levels of which increase with bacterial infection. CRP is a nonspecific, inflammation-related protein that is produced in the liver and regulated by plasma interleukin-6 (IL-6). It increases with bacterial infections, postoperative conditions or tissue injury (*64*). PCT is a glycoprotein with no hormonal activity. It demonstrates high sensitivity to viral and bacterial infections. These host-derived biomarkers may reflect the severity of the infection/condition (e.g., immune activation), but cannot determine etiology. Nonetheless, in the appropriate clinical context, such host response assays can help guide appropriate antibiotic use by ruling in or ruling out a serious bacterial infection (*65*).

CRP tests

The use of CRP assays to guide antibiotic treatment has been examined extensively (*66*). Studies have generally focused on the use of CRP testing in the context of sepsis, particularly in hospital settings, or for both lower and upper acute respiratory tract infection (ARI) in the context of primary care settings; most studies have been done in HICs (*64*, *66*). The majority of studies on CRP have shown statistically significant differences in CRP levels in patients with bacterial infections as opposed to those with nonbacterial infections (*66*). A recent Cochrane review found that the use of CRP at POC can significantly reduce prescription of antibiotics in ARIs (*64*). However, as Cooke and colleagues caution, CRP is "not a substitute for a proper clinical examination" (*67*).

There are commercialized rapid diagnostic CRP tests that can be used at or near the point of patient care. These include qualitative and semi-quantitative lateral flow tests as well as fully quantitative test systems. Some of these tests are described below.

Qualitative or semi-quantitative tests

- DTS233 (Creative Diagnostics, USA): a qualitative (single threshold of 10 mg/L), disposable rapid test for detecting CRP abnormality in whole blood, serum or plasma, as an aid in the clinical diagnosis of CRP. It is based on the principle of colloidal gold immunochromatography. It is a one-step test with a TAT of 10–15 minutes. Test results are read visually without any instrument. The test is currently RUO.
- WD-23 (Assure Tech, China): a CE-marked, semi-quantitative (four CRP concentration ranges: <10 mg/L; 10–30 mg/L; 30–80 mg/L; >80mgL) rapid diagnostic immunoassay for detecting CRP in whole blood, serum or plasma specimens. The assay utilizes a combination of colloidal gold conjugate and anti-CRP antibodies. TAT is between 5 and 7 minutes. Assure Tech also offers a similar CE-marked rapid diagnostic assay for PCT.
- bioNexia® CRPplus (bioMérieux, France): a semi-quantitative (four CRP concentration ranges: <10 mg/L; 10–40 mg/L; 40–80 mg/L; ≥80 mg/L) rapid lateral flow diagnostic test for detecting inflammatory reaction from whole blood. TAT is 5 minutes.
- Actim® CRP (Medix Biochemica, Finland): a semi-quantitative (three concentration ranges: 10–40 mg/L; 40–80 mg/L; >80 mg/L rapid assay for detecting inflammatory reaction from fingerstick blood (EDTA, citrate or heparin blood can also be used). TAT is 5 minutes. The test requires no laboratory equipment.

Assuming the tests above are available for in vitro use and are easy to use, they would be suitable for use in primary care settings. Performance would also be a factor in their selection and implementation.

Quantitative tests

- Alere Afinion™ CRP (Abbott, USA): a CE-marked in vitro diagnostic test to determine the amount of CRP in human whole blood, serum

or plasma. It is a solid-phase immunochemical assay that uses a membrane coated with anti-human CRP antibodies, which react with CRP in the sample. The test is intended to be performed on the Afinion™ AS100 analyser, pictured in Fig. 107, which measures the colour intensity of the membrane and is proportional to the amount of CRP in the sample. The test cartridge contains all the reagents needed to measure CRP in a blood sample. The CRP concentration is displayed on the Afinion™ AS100 analyser within 4 minutes. The measurement range is 5–200 mg/L for whole blood and 5–160 mg/L for serum and plasma samples.

Fig. 107. Alere Afinion™ AS100 analyser and cartridges

The Afinion™ system is appropriate for use at or near POC. In addition to the Alere Afinion™ CRP assay, two other assays are available: (i) Alere Afinion™ ACR for detecting albumin, creatinine and albumin/creatinine ratio (ACR) in human urine; and (ii) Alere Afinion™ HbA1c for quantitatively determining glycated haemoglobin (HbA1c) in human whole blood.

- QuikRead go CRP (Orion Diagnostica Oy, Finland): an FDA-cleared and CE-IVD-marked in vitro diagnostic test for determining the level of CRP from fingerstick blood, venous blood, plasma or serum of people who present with symptoms of infection. The CRP measurement range is 5–200 mg/L. The assay is a particle-enhanced immunoturbidimetric assay that uses nanoparticles coated with anti-human CRP fragments, which react with CRP in the sample. The QuikRead go CRP assay is intended to be run on the QuikRead go instrument, pictured in Fig. 108, which is a photometer that is calibrated for both photometric and turbidimetric measurement. The instrument measures the change in turbidity of the sample solution and converts the value into a concentration value on the basis of preset test calibration data. TAT is 2 minutes. Results are automatically stored in the instrument's memory, along with user and patient ID. The QuikRead go instrument also features both unidirectional and bidirectional connectivity.

Fig. 108. QuikRead go instrument

Orion also offers a combined CRP + Hb assay for use on the QuikRead go instrument and offers a CRP assay for use on its QuikRead 101 instrument, pictured in Fig. 109, a portable device that works on the same principle as the QuikRead go instrument. The QuikRead 101 instrument and CRP test are CE-IVD marked, but not FDA cleared.

Fig. 109. QuikRead 101 instrument

- AQT90 FLEX CRP (Radiometer Medical ApS, Denmark): an in vitro CE-marked diagnostic test for determining the concentration of CRP from venous whole blood or plasma in people presenting with symptoms of infection. The CRP measurement range is 5–200 mg/L. The test is designed to be run on the AQT90 FLEX immunoassay analyser, pictured in Fig. 110, which is based on time-resolved fluorescence using a

europium chelate as the fluorescent label. The instrument can process up to 30 samples per hour, has full connectivity capabilities and, per the company, can be used in near-patient settings. TAT is less than 13 minutes per test. Radiometer also offers additional assays for the system, including PCT, troponin and D-dimer.

Fig. 110. AQT90 FLEX analyser

- iChroma™ CRP (Boditech Med, Korea): an in vitro diagnostic test to determine the concentration of CRP from whole blood (EDTA whole blood or capillary blood), serum or plasma in people presenting with symptoms of infection. The test is based on laser-induced immunofluorescence and uses a method of immunodetection which, by mixing whole blood with a detection buffer in a test tube, binds fluoresced anti-CRP antibodies in the buffer to the CRP antigen in the blood sample. The CRP measurement range is 2.5–300 mg/L. The test can be read on the iChroma™ II, pictured in Fig. 111. The instrument is a semi-automated or automated portable desktop fluorescence scanning device/reader that measures the fluorescence intensity of the test sample in a test cartridge. The device calculates the concentration of the analyte according to a preprogrammed calibration equation and displays the result. TAT for each CRP test is approximately 3 minutes.

Fig. 111. iChroma™ II immunoassay reader

The iChroma™ II is suitable for use in near-patient settings. It is easy to use with simple user interface and backup battery power. It has a built-in printer and wired or wireless connectivity.

Additional tests that can be performed on the iChroma™ II include PCT, antistreptolysin O (ASO), as well as a variety of viruses and cardiac markers. All iChroma assays are CE-IVD marked, and the iChroma™ II reader and CRP assay are FDA cleared.

- NycoCard™ CRP (Abbott, USA): a CE-marked in vitro immunochemical assay for quantitatively determining CRP in whole blood, serum and plasma. The assay uses a dilution liquid to make cells soluble, a membrane-bound antibody that binds CRP and a gold-conjugated antibody for making the bound CRP visible. The CRP measurement range from whole blood is 8–200 mg/L; from serum/plasma it is 5–129 mg/L. TAT is less than 3 minutes. The test is read on the NycoCard™ Reader II, pictured in Fig. 112, which is a small battery-powered instrument. It comprises two units: the instrument box, which is the operational and calculating unit, and the reader pen, which detects the signal.

Fig. 112. NycoCard™ Reader II

The NycoCard™ system is suitable for use in primary care settings. Additional tests for the system include HbA1c, U-albumin (urine albumin) and D-dimer.

- CRP test kit (Eurolyser Diagnostica GmbH, Germany): Eurolyser offers a number of test kits for use on its CUBE and Smart analysers. One of these is a CE-marked in vitro assay for the kinetic determination of CRP from whole blood and serum. The test is an immunoturbidimetric assay that uses photometric measurement at 546 nanometres (nm) or 700 nm of

antigen-antibody reaction between antibodies to human CRP bound to polystyrene particles and CRP present in the sample. The test has two ranges: (i) serum: 0.5–120 mg/L for 546 nm and 1.0–120 mg/L for 700 nm; and (ii) whole blood: 2.0–240 mg/L for 546 and 700 nm.

The Eurolyser CRP test can be run on any of several instruments available from the company. For purposes of providing rapid testing at primary care, the CUBE-S instrument, pictured in Fig. 113, is of most interest. It is a lightweight, easy-to-use sample-in, result-out device that the company refers to as a "pocket-sized laboratory". It employs RFID technology and Android app-based operation. It is Bluetooth and USB enabled with data transfer to a printer or host. With respect to the CRP assay, the CUBE-S automatically includes a patient's individual haematocrit values when calculating CRP. In addition to CRP, multiple CE-marked tests can be run on the CUBE-S. These include ASO, haemoglobin, HbA1c and D-dimer, among others.

Fig. 113. Eurolyser CUBE-S instrument

- CRP IS - InnovaStar® (DiaSys Diagnostic Systems GmbH, Germany): a CE-marked in vitro immunoturbidimetric assay for quantitatively determining CRP in whole blood or plasma. The CRP measurement range from whole blood is 5–400 mg/L; from plasma it is 2–160 mg/L. TAT is approximately 7 minutes. The test can be run on the company's InnovaStar® clinical chemistry analyser, pictured in Fig. 114. The instrument is a compact sample-in, result-out benchtop analyser with fully automated measurement. For ease of use, the system uses precalibrated methods and prefilled unit dose reagents. Additional assays available for the InnovaStar measure HbA1c and glucose/haemoglobin, both of which are CE marked.

Fig. 114. InnovaStar instrument

- spinit® (biosurfit, Portugal): biosurfit has developed a multiplex, multianalyte diagnostic platform. The system is a centrifugal microfluidic platform that employs three different technologies: (i) immunoassays performed with surface plasmon resonance using a polarized laser beam; (ii) clinical chemistry performed by measuring absorbance at multiple wavelengths using LEDs; and (iii) haematology performed using an integrated microscopy module and standard dyes. The spinit® instrument is a compact sample-in, result-out platform that is suitable for use in near-patient testing.

  Several CE-IVD-marked assays are available for use on the spinit® platform, pictured in Fig 115. One of these is a quantitative in vitro diagnostic for measuring CRP in whole blood (venous and capillary) or, alternatively, in serum and plasma. The CRP measurement range from whole blood is 2–180 mg/L. The spinit® test cartridge, also pictured in Fig. 115, is a microfluidic disc (similar to a DVD), and allows automated sample processing and assay performance based on antibody-antigen reaction on the spinit® instrument. The instrument uses an optical-based (photometry) detection system. CRP concentration is determined from reaction data. TAT is less than 4 minutes.

Fig. 115. spinit® instrument and cartridges

PCT tests

Like CRP, the use of PCT as a host-derived biomarker to determine bacterial infection has been extensively examined (*66*, *68*, *69*). Higher levels of PCT are generally found in severe bacterial infections, but remain relatively low in nonspecific inflammatory diseases. Studies have demonstrated that PCT may be used to support clinical decision-making with respect to starting and/or stopping antibiotic therapy in various types of infections in a variety of settings, including primary care, emergency rooms and hospital wards (*70*, *71*). PCT is generally more specific for bacterial infections than other inflammatory markers, including CRP (*69*). A recent Cochrane review found that the use of PCT to guide initiation onto, and duration of, antibiotic treatment of ARIs "results in lower risks of mortality, lower antibiotic consumption and lower risk for antibiotic-related side effects" (*68*). Rhee concludes that the use of PCT to guide antibiotic therapy is most useful in two contexts: (i) noncritically ill patients with suspected or proven ARI, and (ii) critically ill patients with suspected infection/sepsis (69).

Commercial diagnostic PCT tests are available. Of these, at least two assays are disposable rapid diagnostic tests, one from Assure Tech and one from Cortez Diagnostics, Inc. (USA). There is no available performance data on these assays. In addition to the rapid diagnostic assays, a number of quantitative and semi-quantitative assays are available for use on various instrument systems, most of which are designed for use in sophisticated clinical laboratories. However, a few systems could be used for near-patient testing. Some of these assays and systems are described below.

- B·R·A·H·M·S PCT™ direct assay (Thermo Fisher Scientific, USA): an automated in vitro immunochromatographic sandwich assay for determining PCT in human whole blood (capillary or venous). The assay is intended to be performed on the B·R·A·H·M·S™ direct reader, pictured in Fig. 116, which is designed for use at POC. The reader offers data input via a scanner and reader connectivity to an LIS. The assay takes only 20 µL of whole blood. TAT is about 20 minutes. The assay is CE marked, but not FDA cleared.

Fig. 116. B·R·A·H·M·S™ direct reader

- Thermo Fisher Scientific offers a number of PCT assays, including the B·R·A·H·M·S PCT™ sensitive KRYPTOR™ assay, for use on its large, high-throughput instruments, including the B·R·A·H·M·S KRYPTOR™ Gold. It should be noted that Thermo Fisher Scientific has a patent for using PCT as a biomarker for sepsis. Other companies, including Abbott, Siemens, bioMérieux, Roche and DiaSorin, license the use of PCT and its antibodies from Thermo Fisher Scientific. All of the commercial quantitative B·R·A·H·M·S PCT™ assays, including the B·R·A·H·M·S PCT™ sensitive KRYPTOR™ (Thermo Fisher Scientific), ADVIA Centaur® and ATELLICA® IM B·R·A·H·M·S PCT™ assays (Siemens), ELECYS® B·R·A·H·M·S PCT™ assay (Roche), LIAISON® B·R·A·H·M·S PCT™ assay (DiaSorin, Italy) and VIDAS® B·R·A·H·M·S PCT™ assay (bioMérieux), use the same sandwich ELISA principle to quantify PCT by forming antibody-PCT-antibody complexes. The primary difference among these assays is the mechanism of detection of the complexes. All of the instrument systems for which the assays above are described are designed for use in relatively large clinical laboratories and would be best used in Level III and Level IV laboratories in LMICs. They are not described in detail in this report. The assays/systems from Thermo Fisher Scientific and its licensing partners are FDA cleared.
- B·R·A·H·M·S PCT™ LIA (Thermo Fisher Scientific): an immunoluminescence assay used to determine the concentration of PCT in human serum and plasma. The B·R·A·H·M·S PCT™ LIA is intended for use in conjunction with other laboratory findings and clinical assessments to aid in assessing risk for progression to severe sepsis and septic shock of critically ill patients on their first day of admission into an intensive care unit (ICU). The assay uses a coated tube system with two monoclonal antibodies (sandwich principle). The PCT concentration is quantified by measuring the luminescence signal using a luminometer and B·R·A·H·M·S™ Basiskit LIA reagents and calculating the resolves from the standard curve. TAT is 60 minutes at room temperature. The assay is CE marked and FDA cleared.
- B·R·A·H·M·S PCT-Q (Thermo Fisher Scientific): a semi-quantitative immunochromatographic assay for determining the concentration of PCT in human serum and plasma. The assay is a cartridge-based, one-step test that uses the sandwich principle with immunogold labelling. TAT is 30 minutes at room temperature. The user

determines the PCT concentration range of the sample by comparing the colour intensity of the best band on the cartridge with colour blocks on the reference card, illustrated in Fig. 117. The assay is CE marked, but not FDA cleared.

Fig. 117. B·R·A·H·M·S PCT-Q test cassette and reference card

- Diazyme PCT assay (Diazyme Laboratories, Inc., USA): an in vitro latex-particle-enhanced immunoturbidimetric assay intended for quantitatively determining PCT in human serum, EDTA or lithium heparin plasma. The test is intended for use on the first day of ICU admission for progression to severe sepsis and septic shock. TAT is 10 minutes.

  The Diazyme PCT assay is intended to be used on validated chemistry analysers, including the Olympus AU 400 instrument (Beckman Coulter). The test can be run on the Diazyme CUBE-A and SMART instruments. With respect to the CUBE-A and SMART systems, the instrument calculates the PCT concentration of a patient sample by utilizing a lot-specific calibration curve that is stored on an RFID card provided with each instrument kit. The assay is FDA cleared for use on FDA-approved instruments. The assay is CE marked for use on the CUBE-A and SMART systems.

As indicated above in the description of commercially available quantitative CRP assays, quantitative PCT assays are also available for the AQT90 FLEX immunoassay analyser (Radiometer) and for the iChroma™ II analyser.

Novel host response tests

In addition to tests for CRP and PCT, some diagnostic tests are available that measure novel host-derived biomarkers, a combination of host biomarkers, or combinations of protein biomarkers and gene classifiers. These are described below.

- ImmunoXpert™ (MeMed BV, Israel): an in vitro diagnostic test to distinguish between bacterial and viral infections. The assay measures three human immune system biomarkers in serum: (i) tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), (ii) interferon gamma-induced protein-10 (IP-10) and (iii) CRP. A computer algorithm is then used to compute a score indicating the likelihood of a bacterial versus viral (or other nonbacterial) immune response. The company emphasizes that the test is not intended as a stand-alone diagnostic tool and should be used in conjunction with other clinical data; in addition, the test is not intended to distinguish between infectious and noninfectious etiologies.

  The ELISA format version of ImmunoXpert™ is CE marked, but not FDA cleared. TAT is 99 minutes. ImmunoXpert™ is suitable for use in centralized laboratories, although the company is developing the ImmunoPoc™ device, pictured in Fig. 118, which would be suitable for use in near-patient settings.

Fig. 118. Prototype ImmunoPoc™ device and cartridge

  Also in the pipeline from MeMed are two additional assays: (i) MeMed Sepsis™ and (ii) MeMed Neo™ for bacterial infections in neonates.

- FebriDx® (RPS Diagnostics, USA): an in vitro single-use, qualitative disposable rapid test to identify patients who have a clinically significant underlying infection and to help differentiate a clinically significant immune response to viral and/or bacterial ARI from fingerstick blood. The test detects elevated levels of myxovirus resistance A (MxA), a nonspecific inflammatory protein, which is a derivative of interferon. MxA becomes elevated in the presence of acute viral infection, and CRP. TAT is approximately 10 minutes. The assay is CE marked, but is not FDA cleared.

The assay is contained in an all-in-one plastic cassette housing, pictured in Fig. 119, that includes a built-in safety lancet, blood collection and delivery system, and integrated push-button buffer delivery features, which make the test easy to use. The FebriDx® assay requires no additional equipment to perform the test or to interpret results, making it appropriate for use at POC.

Fig. 119. FebriDx® test cassette

- SeptiCyte™ LAB (Immunexpress, USA): an in vitro gene expression assay using real-time, reverse-transcription PCR to quantify the relative expression levels of four host response genes (*CEACAM4*, *LAMP1*, *PLAC8* and *PLA2G7*) isolated from whole blood collected in PAXgene blood RNA tubes. The SeptiCyte™ LAB assay is used in conjunction with clinical assessments and other laboratory findings as an aid in differentiating infection-positive (sepsis) from infection-negative systemic inflammation in patients suspected of sepsis on their first day of admission into ICU. The SeptiCyte™ LAB assay generates a score (SeptiSCORE™) that falls within one of four discrete Interpretation bands based on the increasing likelihood of infection-positive systemic inflammation; it uses a binary cut-off of 3.1 to classify patients as high or low risk for sepsis. TAT is approximately 6 hours.

  The SeptiCyte™ LAB sepsis assay is FDA cleared and validated for use on the ABI 7500 Fast. As such, it is appropriate for use in centralized clinical laboratories. However, in January 2018 Immunexpress entered into an agreement with Biocartis Group NV (Belgium) to develop and commercialize the Immunexpress SeptiCyte™ assay for use on Biocartis's sample-to-result Idylla™ platform, pictured in Fig. 120. The platform is a fully automated molecular system using real-time PCR to identify up to 30 targets. It features minimal hands-on time of approximately 2 minutes. TAT is between 90 and 150 minutes depending on the assay.

Fig. 120. Idylla™ platform

- abioSCOPE® (Abionic, Switzerland) is a multiplex immunoassay platform. One of the assays for the abioSCOPE® platform measures a sepsis risk biomarker, pancreatic stone protein, in whole blood (capillary or venous) or serum/plasma samples. TAT is 5 minutes. The assay is CE-IVD marked, but has not yet been commercialized.

  The abioSCOPE® platform uses fluorescent nanofluidic immunoassay technology. Fluorescent molecular complexes are formed on a nanosensor. These complexes are then detected and quantified optically using an integrated microscope laser. Because of the nanofluidic configuration of the platform, biomolecular interactions are accelerated, leading to quick TATs. The system uses disposable "capsules" (cartridges) that are placed into a disc-shaped mounting plate and then inserted into the instrument.

  The abioSCOPE® instruments, pictured in Fig. 121, come in three configurations for use in different settings – pharmacy, hospital/ICU and physician's office. The instruments all use the same technology, but have different displays and assay availability.

Fig. 121. abioSCOPE® instruments

In addition to the sepsis assay, Abionic has two additional CE-IVD-marked assays, one for allergy and one for iron deficiency (ferritin). Additional assays, including CRP and D-dimer, are in the development pipeline.

Finally, there are two interesting host response assays in development:

- HostDx sepsis and fever assays (Inflammatix, USA) is developing two assays that will use a host response marker, specifically an mRNA signature, to identify acute infection. They are the HostDx Sepsis and HostDx Fever assays. The tests will use quantitative multiplex gene expression to analyse a patient's immune system – the host response – rather than directly identifying pathogens. The assays will be run on a molecular, multiplex platform from white blood cells. The HostDx Sepsis assay will be targeted at hospitalized patients with acute infection, while the HostDx Fever assay will help clinicians decide whether to administer antibiotics for patients presenting with fever in primary care settings. Inflammatix indicates that it is working with partners that are already building platforms suitable for integrating its assays. The company is in the in vivo assay development phase in which it is developing its assays (wet lab) based on a locked set of genes and algorithms and working with instrument partners to finalize the assays. No timeline for launch of either the HostDx Sepsis or HostDx Fever assay is known.
- UTRiPLEX (Mologic, UK) is developing a qualitative lateral flow test, UTRiPLEX, in dipstick format that uses the presence of three biomarkers to rule out UTIs. TAT is 6 minutes. The test is not commercially available; it is currently undergoing clinical evaluation.

Numerous additional host-derived biomarkers are being studied for use in diagnostic assays. These include CH13KI plus CRP, haptoglobin-related protein (Hpr), Lpc-2 plus Hpr, heparin-binding protein and more. Most of this work is being done in academic institutions and has not yet been translated into diagnostic products.

Conclusion

Some assays, in particular disposable RDTs to detect CRP, could be used in primary care settings (Level I) to help target the need for antibiotics in patients presenting with febrile and respiratory illness. There are also a few instrument-based platforms that could be implemented in Level II settings. These include the iChroma™ CRP test and immunoassay reader and the NycoCard™ CRP test and reader. Unlike the RDTs available for CRP testing, there are very few such tests available for PCT testing, although a few platforms, like the B·R·A·H·M·S PCT™ direct assay and reader and the B·R·A·H·M·S PCT-Q, which requires no instrumentation but does require a serum or plasma sample, could be used in Level II settings. In all cases, tests would need to be used in an appropriate context and algorithm.

In addition, some interesting novel host response tests/platforms that are commercially available or in the pipeline could also be used to help make an initial determination as to whether an infection is bacterial or nonbacterial for patients presenting with symptoms of infection. For example, the FebriDx single-use qualitative test could be used in Level I settings to help distinguish a clinically significant host immune response to viral and/or bacterial ARIs, which should help determine whether antibiotics are needed. In general, these tests utilizing new host-derived biomarkers or combinations of such biomarkers have not been widely studied, and performance data is still lacking.

Finally, none of these tests is a complete solution, because none of the tests can identify bacterial pathogens nor can they determine AST. Further testing would be required.

Discussion

This landscape report maps current diagnostic methods for identifying bacterial pathogens, including manual and automated phenotypic methods primarily done at higher levels of the healthcare system in LMICs, with a primary focus on commercialized platforms. The landscape also maps current immunoassay, molecular and other methods of identifying bacterial pathogens, with a focus on commercial systems. Emerging and pipeline diagnostics are also considered.

The landscape maps current and emerging phenotypic methods of AST, including both manual and automated methods, as well as commercial platforms combining bacterial ID and AST. It considers pipeline AST technologies that may be used in primary and secondary care settings, particularly in LMICs. The landscape then maps commercially available and pipeline nonphenotypic methods for simultaneous pathogen ID and detection of ABR or methods for identifying genes that directly confer antibiotic resistance only. In both cases, pipeline technologies are reviewed.

The landscape therefore provides a reasonably comprehensive picture of the test systems for combating ABR available at all levels of the healthcare system and what is in the pipeline; these are summarized in Annex II. This provides a backdrop from which to identify gaps in diagnostics in the context of, and in consideration of, certain key parameters of interest to WHO:

- diagnostics that target high-priority drug-resistant bacterial pathogens identified by WHO, CDC and ECDC and set out in Annex I;
- the need for diagnostics to improve clinical/syndromic management of patients to reduce overprescribing of antibiotics, i.e., in the context of antimicrobial stewardship;
- the need for IVDs that can be performed at primary and secondary healthcare facilities (Level I and Level II laboratories) in LMICs (as described in this report); and
- given the needs in primary care facilities, focuses on priority bacterial pathogens that are primarily community acquired, including *Escherichia coli*, NG, *Helicobacter pylori*, *Campylobacter* spp., *Salmonella* spp., *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Shigella* spp. and *Staphylococcus aureus*.

As noted earlier in this report, TB is a high-priority multidrug-resistant pathogen. However, extensive landscaping of diagnostics and drug-resistance testing for MTB has already been performed, and a number of TPPs have been developed for priority diagnostic needs. Given the work that has already been done with respect to diagnostics for MTB, they are not a focus of this report. Nonetheless, because of the importance of TB, the report highlights priorities for TB diagnostics R&D as well as priorities for other targeted bacterial pathogens.

In brief, the question that frames this report is, What are the gaps in diagnostics to combat ABR for prioritized drug-resistant bacterial pathogens, with an emphasis on CAIs, at Levels I and II of the healthcare system in LMICs? Based on this landscape report, the short answer is that there are many commercially available test systems, both phenotypic and nonphenotypic, for identifying and/or performing AST/resistance testing with respect to virtually all priority bacterial pathogens. But most systems are predicated on sophisticated, well equipped laboratories with well trained laboratory staff. Therefore, most test systems are only of practical use at Level III and Level IV of the laboratory systems in LMICs. This is shown in Annex III, which summarizes diagnostic platforms for combating ABR that are potentially suitable for Level I and/or Level II in LMICs.

Despite the availability of test systems for sophisticated clinical laboratories, classical phenotypic test methods utilizing culture and AST are considered to be too slow and cumbersome, especially when done manually. This is particularly problematic for patients suspected of sepsis, where time is of the essence in diagnosis.

The introduction of automated phenotypic ID and AST systems has helped to reduce time to result; and newer technologies, like the Accelerate Pheno system, which combines FISH for microorganism ID and multiplexed automated digital microscopy for susceptibility determination, provide both ease of use and faster results. Some of these platforms are compact, easy to use and robust, but they still require samples from culture before AST can be performed. In particular, blood culture is still the gold standard for detecting BSIs and AST (*71*). However, since culture is generally only performed at Level III and Level IV in LMICs, this means that the systems are not appro-

priate for use in primary and most secondary public healthcare facilities.

It has been generally agreed that simpler and faster methods of bacterial pathogen ID and AST are needed at all levels of the healthcare system, particularly at Level II, and nonphenotypic methods of bacterial ID and/or AST/resistance testing have helped to make this possible. As shown in this report, there are a myriad of such systems, including molecular-based testing, NGS and mass spectrometry, in particular MALDI-TOF MS, all of which are more rapid bacterial pathogen ID methods than traditional phenotypic methods.

Some of these diagnostic systems are only for bacterial ID and perform no resistance testing. In this category are several diagnostic platforms/systems that have the potential to be used in secondary, or possibly primary, care settings. These include the cobas® Liat® system, Solana® platform and revogene®, which are monoplexes, identifying one pathogen at a time, as well as the Novodiag® system, QIAstat-Dx™ and Randox assays for the Vivalytic platform for which multiplex, syndromic testing panels are already available. At Level II, for example, these platforms can be useful in identifying multiple gastrointestinal and respiratory pathogens from stool and swabs. However, with the exception of the platform from T2 Biosystems, which is suited only to centralized laboratories, none of the platforms can detect priority BSIs, including *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, from whole blood, i.e., independent of culture.

There are also nonphenotypic systems that combine bacterial ID and AST/resistance testing capabilities. For example, the GeneXpert® system and FilmArray® EZ Configuration can be used in near-patient settings, including Level II facilities in LMICs, but only for assays not requiring culture samples. Alternatively, diagnostic systems can be combined in the laboratory. A common example in HICs is to combine a high-speed, completely automated bacterial ID platform, like a MALDI-TOF MS platform, with a rapid, automated AST system. However, for reasons of required infrastructure and complexity, among others, most of these combined, nonintegrated systems are appropriate for use only in Level III and, in some cases, only in Level IV settings.

Finally, there are diagnostic platforms that perform resistance testing or AST only. Some of these, including RESIST assays from Coris BioConcept, are LFIAs that require no equipment. However, in many cases, a culture sample is required, which for the most part limits the use of these tests in LMICs to Level III settings and above.

There are interesting and novel diagnostic products in the pipeline targeted for use at Levels I and II. Some of these will perform both pathogen ID and AST or resistance testing (ASTriD®, ASTar™ and GeneXpert® Omni); some will only perform ID (FireflyDx™, Qvella); some will only perform AST (LabDisk, LiDia® and Astrego). Generally speaking, however, most of these platforms will process only swabs and urine, which limits the breadth of bacterial pathogens covered; some are targeting ID from whole blood (ASTriD® and LiDia®). At this point, none of these platforms has proven capabilities, especially for ID and resistance testing, from whole blood.

At the other end of the spectrum, considering diagnostic tests currently available for use at Level I and Level II, some immunochromatographic and other RDTs are available for a number of individual bacterial pathogens that do not require culture specimens. For HAIs, these include tests for *Clostridium difficile*; for CAIs, these include tests for *Helicobacter pylori*, NG, *Salmonella typhi*, *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Escherichia coli*. Some of these tests have not exhibited adequate performance (sensitivity/specificity), which can limit their efficacy and needs to be taken into consideration before such tests are adopted.

Of particular concern in this regard are RDTs for NG. In 2016, WHO estimated that approximately 87 million new cases of NG were diagnosed worldwide (*72*). Yet, many researchers have concluded that although current RDTs for NG often have specificities >90%, sensitivities are often 50% or lower, and as such, they do not perform adequately to be used as screening tests (*73*, *74*). In addition, AMR in NG is particularly problematic. With resistance to both cephalosporins, including third-generation extended spectrum cephalosporins, as well as fluoroquinolones, NG is a multidrug-resistant pathogen. Resistance appears to be outpacing new antibiotics for NG. Although the Xpert® CT/NG assay, described earlier in this report, is FDA cleared, its cost is a limiting factor to uptake. In addition, it is targeted at Level II settings, while patients presenting with symptoms of NG generally come to primary healthcare settings for diagnosis and treatment. Improved RDTs for NG and AST are needed at Level I.

There are also a good number of RDTs available to measure the presence of certain biomarkers reflective of host response, including CRP. Assuming adequate performance, these assays can be useful at Level I, in particular, for initial screening to determine whether a patient is likely to have a bacterial or nonbacterial infection. The ability to classify infection has the potential to enhance antibiotic stewardship. Although there are currently few, if any, RDTs available for PCT, there is at least one platform, B.R.A.H.M.S PCT™ direct assay, that could be used at Level II and possibly Level I.

Lastly, some interesting novel host response assays/platforms are commercially available or in the pipeline that could also be used to help make an initial determination as to whether an infection is bacterial or not for patients presenting with symptoms of infection. For example, the FebriDx® is interesting in this respect. However, none of these tests can identify bacterial pathogens nor can they determine AST. Further patient testing would be required.

What emerges from this assessment of available diagnostics to combat ABR, as well as from additional work by WHO with respect to TB, is that there are significant gaps in tests and testing platforms for use at Level I and Level II in LMICs. These include:

- inadequate near-patient testing for (i) biomarker-based, non-sputum-based detection of TB; (ii) patient triage evaluation for TB; (iii) sputum-based replacement for AFB smear microscopy; and (iv) TB drug susceptibility (see https://www.who.int/tb/publications/tpp_report/en/);
- little or no ability to perform simplified phenotypic bacterial ID or AST to enable definitive therapeutic decision-making at Level III, and potentially Level II, in LMICs, especially in the context of BSIs, in particular sepsis;
- inadequate near-patient testing options for ID and susceptibility testing for multidrug-resistant NG;
- Few RDTs or easy-to-use, robust diagnostic platforms for use in primary (or secondary) healthcare settings that can reliably distinguish bacterial and nonbacterial infections from accessible, minimally invasive clinical specimens (e.g., whole blood, urine, stool and nasal swabs);
- no multiplex platform suitable for Level II and/or Level I settings to detect bacterial pathogens, including BSIs, from whole blood (no culture required) with AST/resistance testing done on a separate platform or combined with AST/resistance testing on the same platform; and
- no simple, easy-to-use test/platform suitable for use in Level II and/or Level I settings for AST from whole blood or other sample matrices (urine, stool and respiratory specimens) for which culture is not required.

Priority diagnostics for R&D

The AMR diagnostic gaps listed above suggest the following R&D priority diagnostics against AMR for primary and secondary healthcare facilities over the next 3–5 years for which consensus TPPs to stimulate product development are proposed:

- Improved near-patient testing for TB. Globally, a third of all TB cases are not notified, and the samples of many patients are not tested for drug susceptibility. In order to achieve the global strategy for TB prevention, care and control, new diagnostics are needed. Based on diagnostic needs expressed by the TB community, WHO has developed consensus-driven TPPs to enable POC assays capable of (i) detecting all forms of TB by identifying characteristic biomarkers or biosignatures in specimen(s) other than sputum; (ii) low-cost patient triage by first-contact healthcare providers to identify those patients who need further testing; (iii) replacing AFB smear microscopy for detecting pulmonary TB; and (iv) determining first-line regimen-based therapy via DST that can be used at the microscopy-centre level of the healthcare system.

  Suggested action: Consensus TPPs have been developed. No further action is required.

  For detail, see https://www.who.int/tb/publications/tpp_report/en/.

- Simplified phenotypic ID and AST. As indicated above, phenotypic methods of bacterial pathogen ID and AST are the gold standard. In particular, for identifying bacterial pathogens associated with BSIs, which are a common cause of morbidity and mortality worldwide, with an estimated mortality rate of 15–30% (*75*), blood culture is essential. Further, ABR is a particular problem for ESBL *Escherichia coli* and *Klebsiella* spp. and CRE. Yet, in LMICs, culture and AST are generally only performed at Level III and Level IV laboratory facilities. In a recently published study by Dailey and colleagues, specialists in Africa and Asia (e.g., infectious disease doctors, public health/clinical microbiologists, clinical researchers and technology experts) suggested that blood culture should be available at district hospitals (i.e., at Level II laboratories in LMICs) to support both patient management and surveillance (*36*). Expanding phenotypic methods to Level II laboratories would require a simpler culture system. Dailey and colleagues have put forward a proposed TPP for a diagnostic test for such a simplified blood culture system (*36*). The minimal standard is a culture system for detecting the culture positivity and gram status of bacterial pathogens suitable for Level III settings. The optimal standard is a culture system for pathogen ID and AST for key resistance categories (e.g., ESBL and CRE) targeted at Level II settings. The target population is any individual presenting with fever.

  Suggested action: Review existing TPP for a simplified blood culture system to address BSIs, in particular sepsis, for definitive therapeutic decision-making at Level II and higher facilities, and consider whether revisions and a broader consensus process are required.

- Improved diagnostics and AST for NG. As discussed earlier in this report, NG is an STI that results in significant morbidity and mortality. Yet, the ability to diagnose the infection, and to diagnose and distinguish it from CT effectively, is limited to molecular testing, at best available at Level II settings and above. Similarly, there is essentially no ability to perform AST, despite the fact that NG is a multidrug-resistant bacterial pathogen. In addition to being on the WHO priority pathogen list (Annex I), it is also a priority organism for AMR monitoring in GLASS as well as GARDP. WHO, FIND and GARDP have identified key TPPs to be developed with respect to NG. These comprise two TPPs: (i) a simple diagnostic test to detect CT/NG infection in symptomatic patients (minimally) and asymptomatic patients (optimally) for use at Level I, and (ii) a comprehensive test that would confirm NG infection for patients presenting with treatment failure (minimally) for use at Level II and detect both NG and CT infection and identify AST for NG (optimally) for use at Level I. The importance of such tests is critical for the effort to protect a next-generation antibiotic for NG due to be launched in 2020.

  Suggested action: Since a TPP for a CT/NG rapid test for use in primary care settings and a second TPP for a comprehensive test that would both confirm NG infection and enable genotypic

resistance testing of NG infection are already being developed from the combined efforts of WHO, FIND and GARDP, assuming alignment with these efforts, support this work as needed, but do not undertake a separate initiative.

- Host response tests. To date, there is still not an RDT to reliably distinguish between bacterial and nonbacterial infections for use at primary healthcare settings, a test that is often referred to as the Holy Grail. There are, however, RDTs in the form of host response tests that incorporate specific host-derived biomarkers, including CRP, PCT and a few tests combining such biomarkers, that show promise in distinguishing between bacterial and nonbacterial infections. Assuming adequate performance, these tests could be an important way to triage patients presenting with fever at Level I healthcare settings. A consensus TPP for a rapid biomarker-based test, which was the result of a Delphi process, was published in 2016 (*76*).

  Suggested action: Consider whether the existing TPP for a rapid biomarker-based test should be refined/revised. If not, bring renewed attention to the existing TPP.

- Multiplex diagnostic platform to identify bacterial pathogens and perform AST/resistance testing independent of culture. There are effectively no multiplex diagnostic platforms that can be used at Level II of the laboratory system to simultaneously identify bacterial pathogens causing BSIs from whole blood (without culture). Such a system could detect *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter* spp., MRSA and *Enterococcus*, among others, on a single assay panel. As illustrated in Annex III, there are a few multiplex systems that can identify respiratory pathogens or gastrointestinal panels from swabs (nasal or stool), but culture is required in the context of BSIs. Similarly, there are multiplex platforms that can identify a number of drug-resistant bacterial strains from nasal and stool swabs, but not from whole blood. There are a few platforms in the pipeline that are striving to perform both ID and AST/resistance testing, but they are not yet a reality.

  Even if a simplified phenotypic and AST system were developed for use at district hospitals, it would still be important to have a platform suitable for near-patient testing that could identify a broad range of bacterial pathogens from whole blood, as well as from other accessible, minimally invasive clinical specimens (e.g., urine, stool and nasal swabs), independent of culture – and that ideally could perform AST/resistance testing on the same platform. Such a system would have value at Level III as well.

  Suggested action: Develop a consensus-driven TPP for a multiplex diagnostic platform suitable for use at Level II that can simultaneously identify multiple bacterial pathogens, including bacterial pathogens associated with BSIs, from whole blood and other sample matrices without culture (minimum standard), and that can both identify multiple bacterial pathogens, including bacterial pathogens causative of BSIs, and can perform AST/resistance testing from whole blood and other sample matrices (optimal standard) without culture.

- Simple AST assay from whole blood. While there are existing, and a few pipeline, phenotypic and nonphenotypic AST/resistance-only platforms commercially available, they are generally not designed for Level II settings and below, and to date all require culture samples for AST/resistance testing of BSIs. It would be useful to encourage development of such a platform that minimally could perform AST from accessible, minimally invasive clinical specimens (e.g., urine, stool and nasal swabs), but that optimally could perform AST from whole blood.

  Suggested action: Develop a consensus TPP for a simple stand-alone AST device not requiring culture isolates for use following bacterial pathogen detection on a separate instrument or RDT.

Annex I – ABR prioritization

Bacteria	Gram stain	HAI or CAI[16]	WHO (*11*)	US CDC (*12*)	EU (ECDC) (*13*)
Mycobacterium tuberculosis, drug resistant[17]	N/A	CAI/HAI	Global priority http://apps.who.int/medicinedocs/en/m/abstract/Js23298en/	Serious	
Acinetobacter baumannii, carbapenem resistant	Neg	HAI	Critical	Serious	✓
Pseudomonas aeruginosa, carbapenem resistant	Neg	HAI	Critical	Serious	
Enterobacteriaceae, carbapenem resistant/ESBL producing	Neg	HAI/CAI ***Klebsiella pneumoniae*** (HAI); ***Escherichia coli*** (HAI/CAI)	Critical	Critical/ Serious	✓ (***Klebsiella pneumoniae***, ***Escherichia coli***)
Clostridium difficile	Pos	HAI		Critical	
Neisseria gonorrhoeae, cephalosporin-resistant, fluoroquinolone-resistant	Neg	CAI	High	Serious	
Enterococcus faecium, vancomycin-resistant	Pos	HAI	High	Serious	✓
Staphylococcus aureus, methicillin-resistant/ vancomycin-intermediate and resistant	Pos	HAI/CAI	High	Serious/ Concerning	✓
Helicobacter pylori, clarithromycin-resistant	Neg	CAI	High		
***Campylobacter* spp.**, fluoroquinolone-resistant	Neg	CAI	High	Serious	
***Salmonella* spp.**, fluoroquinolone-resistant	Neg	CAI	High	High	
Streptococcus pneumoniae, penicillin-non-susceptible	Pos	CAI	Medium	Serious	
Haemophilus influenzae, ampicillin-resistant	Neg	CAI	Medium		
***Shigella* spp.**, fluoroquinolone-resistant	Neg	CAI	Medium	Serious	

[16] Indicates the resistant pathogen is most often nosocomial or community acquired, as the case may be. Some pathogens are found commonly in both settings.

[17] In the 2017 WHO report *Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis*, MTB was listed as a global priority for R&D (https://www.who.int/medicines/areas/rational_use/prioritization-of-pathogens/en/, accessed 19 June 2019).

Appendix II – Diagnostic platforms for all levels of healthcare system

Diagnostic platforms to combat ABR suitable for all levels of the healthcare system				
Phenotypic methods				
Automated Gram staining				
Manufacturer	**Platform**	**Minimum laboratory level**	**Technology**	**Assays**
bioMérieux	PREVI® COLOR GRAM	I/II	Automated Gram staining via spray technology	Pan-bacteria
ALL.DIAG - Biosynex	MULTISTAINER®	I/II	Gram stain and fast staining	Pan-bacteria
ELITechGroup Solutions	Aerospray® Gram series 2	I/II	Gram stain	Pan-bacteria
Hardy Diagnostics	QuickSlide™ GramPRO 1™	I/II	Gram stain	Pan-bacteria
Automated specimen processing and inoculation of media				
BD	BD™ Innova automated microbiology specimen processor	III/IV	Processing of liquid specimens and automated streaking	Pan-bacteria
BD	BD Kiestra™ InoqulA+™	III/IV	Processing of liquid and nonliquid specimens	Pan-bacteria
bioMérieux	PREVI® Isola	III/IV	Processing of liquid and nonliquid (e.g., swabs) specimens	Pan-bacteria
Beckman Coulter	Copan WASP® DT: Walk-Away Specimen Processor	III/IV	Accommodates most specimen types, including swabs, urine, faeces, sputum, body fluids and pre-enrichment broths	Pan-bacteria
Phenotypic bacterial ID				
Automated culture systems				
BD	BD BACTEC™ FX	III/IV	Automated culture using fluorescent sensing of CO_2 production	Pan-bacteria, fungi, yeast
bioMérieux	BACT/ALERT® 3D	III/IV	Automated culture using colorimetric sensing of CO_2 production	Pan-bacteria, fungi, yeast
bioMérieux	BACT/ALERT® VIRTUO®	III/IV	Automated culture using colorimetric sensing of CO_2 production	Pan-bacteria, fungi, yeast
Thermo Fisher Scientific	VersaTREK™	III/IV	Automated culture that senses all microorganism gas production, including CO_2	Pan-bacteria, fungi, yeast and mycobacteria

(Appendix II, continued)

Manual biochemical bacterial ID systems				
Manufacturer	**Platform**	**Minimum laboratory level**	**Technology**	**Assays**
bioMérieux	API®	Level III/IV	Detects enzymatic activity or fermentation of carbohydrates	API gram-negative ID: API 20E – *Enterobacteriaceae* and other nonfastidious gram-negative bacteria API Rapid 20E – *Enterobacteriaceae* API 20NE – gram-negative non-*Enterobacteriaceae* API NH – *Neisseria/Haemophilus* API gram-positive ID: API Staph – staphylococci and micrococci RAPIDEC® Staph – commonly occurring staphylococci API 20 Strep – streptococci and enterococci API anaerobe ID: API 20A – anaerobes Rapid ID 32A – anaerobes
BD	BBL™ Crystal™	Level III/IV	Utilizes miniaturized fluorogen and/or chromogen-linked substrates to detect enzymes that bacteria use to metabolize a variety of substrates	Enteric/nonfermenter (E/NF): clinically significant aerobic gram-negative *Enterobacteriaceae* isolates and nonfermenting gram-negative rods Rapid stool/enteric (RS/E): clinically significant aerobic gram-negative bacteria of the *Enterobacteriaceae* family as well as most pathogens isolated from stool specimens *Neisseria/Haemophilus* (N/H): *Neisseria*, *Haemophilus* and other fastidious bacteria Gram-positive ID system: both gram-positive cocci and bacilli Rapid gram-positive ID kit: gram-positive bacterial isolated from clinical specimens Anaerobe ID kit: clinically significant anaerobic organisms
Thermo Fisher Scientific	RapID™ systems	Level III/IV	Detects enzymatic activity	RapID™ ONE system: over 70 medically important, oxidase-negative, gram-negative bacilli RapID™ ANA II: over 90 clinically important anaerobes RapID™ NH system: 30 taxa, including *Neisseria*, *Moraxella*, *Haemophilus* and related microorganisms RapID™ NF PLUS system: over 70 clinically important oxidase-positive, gram-negative bacilli, including *Vibrio* spp. RapID™ STAPH PLUS system: 40 different staphylococci and related genera RapID™ STR system: streptococci and related genera RapID™ SS/u system: commonly isolated urinary tract pathogens in 2 hours

(Appendix II, continued)

Manufacturer	Platform	Minimum laboratory level	Technology	Assays
Thermo Fisher Scientific	Oxoid™ Microbact™ biochemical systems	Level III/IV	Measures pH changes in various substrates and substrate utilization tests	Microbact™ GNB kit: *Enterobacteriaceae* and common miscellaneous gram-negative bacilli Oxoid™ Microbact™ Staphylococcal 12S kit: staphylococci, including *Staphylococcus aureus* and CNS
		Automated biochemical bacterial ID systems		
Biolog, Inc.	Biolog microbial ID systems	Level III/IV	Uses oxidation-reduction chemistry	Broad range of gram-positive and gram-negative bacteria
MIDI, Inc.	Sherlock™ microbial ID system	Level III/IV	Uses GC analysis of extracted FAME	Broad range of gram-positive and gram-negative bacteria
BacterioScan	216Dx UTI system	Level III/IV (culture)	FLLS	Qualitative diagnosis of bacteriuria (UTIs)
		Nonphenotypic methods of bacterial ID		
		Immunoassays		
Thermo Fisher Scientific	Oxoid™ PBP2' latex agglutination test	Level III (culture)	Latex slide agglutination test	MRSA and CNS from culture
Abbott	Clearview Exact PBP2a test	Level III (culture)	Immunochromatographic test	MRSA from culture
Thermo Fisher Scientific	*RAPID*™ Hp StAR™	Level I	Immunochromatographic test	*Helicobacter pylori* from stool
Otsuka Pharmaceutical Co., Ltd.	RAPIRUN® *H. pylori* antibody detection kit	Level I	Immunochromatographic test	*Helicobacter pylori* from stool
Meridian Bioscience, Inc.	Immuno*Card* STAT!® CAMPY	Level I	Immunochromatographic test	*Campylobacter* antigens (*C. jejuni* and *C. coli*) from stool
Abbott	*C. DIFF QUIK CHEK COMPLETE*®	Level I	Immunochromatographic test	*Clostridium difficile* (TcdA and TcdB) from stool
Thermo Fisher Scientific	Xpect® *C. difficile* Toxin A/B test	Level I	Immunochromatographic test	*Clostridium difficile* (TcdA and TcdB) from stool
Meridian Bioscience, Inc.	Immuno*Card*® Toxins A&B	Level I	Horizontal flow enzyme immunoassay	*Clostridium difficile* (TcdA and TcdB) from stool
Thermo Fisher Scientific/ BioStar	BioStar® OIA GC	Level I	Optical immunoassay	NG from female endocervical swabs and male urine specimens
Abbott	BinaxNOW® *Streptococcus pneumoniae*	Level I	Immunochromatographic assay	*Streptococcus pneumoniae* from urine
BIOSYNEX	BIOSYNEX *S. pneumoniae*	Level I	Immunochromatographic test	*Streptococcus pneumoniae* from urine and CSF
Malaysian Biodiagnostic Research	Typhidot®	Level II, possibly Level I	ELISA	*Salmonella typhi* in serum
IDL Biotech	TUBEX®	Level I	IMBI	*Salmonella typhi* in serum
Thermo Fisher Scientific	Wellcogen™ Haemophilus influenzae b Rapid latex agglutination test	Level I	Latex diagnostic test	*Haemophilus influenzae* type b from CSF, serum, urine or blood cultures
Coris BioConcept	O157 Coli-Strip	Level III (broth dilution)	Immunochromatographic test	*Escherichia coli* in stool (after broth enrichment)

(Appendix II, continued)

Manufacturer	Platform	Minimum laboratory level	Technology	Assays
Meridian Bioscience, Inc.	Immuno*Card* STAT! ® E. coli 0157 Plus	Level I	Horizontal-flow enzyme immunoassay	*Escherichia coli* in stool or culture
		Molecular methods		
		Hybridization methods		
OpGen	AdvanDx PNA FISH® *Quick*FISH®	Level III/IV	FISH with PNA probes	AdvanDx PNA FISH®: *Staphylococcus aureus*/CNS, *Enterococcus faecalis*/OE (other enterococci), gram-negative organisms and *Candida* QuickFISH®: *Staphylococcus*, *Enterococcus*, gram-negative organisms and *Candida* *mecA XpressFISH*: *mecA* mRNA from MRSA
		Amplification methods – integrated systems		
Abbott	Abbott *m*2000 System	Level III/IV	Multiplex real-time PCR followed by fluorescent probe-based detection	CT/NG
Roche	cobas® 6800/ cobas® 8800 systems	Level III/IV	Real-time PCR	CT and/or NG
	cobas® 4800 system	Level III/IV	Real-time PCR and nucleic acid hybridization	CT/NG, *Clostridium difficile*, MRSA
	cobas® Liat® system	Level II/ possibly Level I if cold chain	Real-time PCR	*Clostridium difficile*
Hologic	Hologic Panther® Aptima Combo 2 system	Level III/IV	TMA followed by fluorescent probe-based detection	CT and/or NG
	Hologic Panther Fusion® system	Level III/IV	TMA and PCR	MRSA
BD	BD ProbeTec™ ET system	Level III/IV	ET	CT and GC DNA
	BD Viper™ system	Level III/IV	SDA and ET	NB/CT
	BD MAX™ system	Level III/IV	Real-time PCR	CT/GC/TV, *Clostridium difficile*, enteric bacterial panel, extended enteric bacterial panel, MRSA, StaphSR (for surveillance)
Vela Diagnostics	Great Basin analyser system	Level III/IV	Hot-start PCR and hybridization probes	Stool panel: *Campylobacter* (*C. coli* and *C. jejuni*), *Salmonella*, *stx1*, *stx2*, *Escherichia coli* serotype 0157 and *Shigella* Blood culture panel: *Staphylococcus aureus*, *S. lugdunensis*, various *Staphylococcus* spp. and *mecA* gene *Clostridium difficile*, Shiga toxin, *Bordetella pertussis*, Group B Strep
Meridian Bioscience, Inc.	Alethia™ platform	Level III/IV	iNAAT using LAMP	*Clostridium difficile*, Group A Strep, Group B Strep

(Appendix II, continued)

Manufacturer	Platform	Minimum laboratory level	Technology	Assays
Quidel Corporation	Solana® platform	Level II	HDA	*Clostridium difficile*, Group A Streps, Group B Strep
	AmpliVue® platform	Level II	HDA and lateral flow	*Clostridium difficile*, Group A Strep, Group B Strep
Siemens	VERSANT® kPCR molecular system	Level III/IV	Real-time PCR	CT, NG
Luminex	ARIES® and ARIES® M1 systems	Level II	Real-time PCR	*Clostridium difficile*, Group A Strep
Mobidiag	Novodiag® platform	Level II	Real-time qPCR and microarray	*Clostridium difficile*, Bacterial GE+ assay
Randox Laboratories/ Bosch Healthcare Solutions	Respiratory Multiplex Array II/Vivalytic analyser	Level III/IV	Multiplex PCR and biochip array hybridization	Respiratory multiplex array, STI array (CT, NG and others)
QIAGEN NV	QIAstat-Dx™	Level II	Real-time PCR with optical sensor	Respiratory panel, gastrointestinal panel, sepsis panel
GenePOC	revogene®	Level III	Fluorescence-based real-time PCR	*Clostridium difficile*, Group B Strep
T2 Biosystems	T2Dx® instrument	Level IV	T2 magnetic resonance	Bacteria panel (*Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*); *Candida* panel
Abacus Diagnostica	GenomEra™ CDX system	Level III/IV	Fluorescent lanthanide levels, thermal cycling, RT PCR	*Clostridium difficile*, MRSA SA SC (with *mecA* and *mecC*), MRSA/ SA Multi Swab and *Streptococcus pneumoniae*
GENOMICA S.A.U.	CLART® technology	Level III/IV	Genetic amplification and visualization	CLART® EnteroBac: *Salmonella* spp., *Shigella* spp., *Escherichia coli* enteropathogenic, *Campylobacter* spp. and *Clostridium difficile* CLART® SeptiBac: *Staphylococcus* spp., *Streptococcus* spp. (including methicillin-resistance marker *mecA*), *Enterococcus* spp., *Escherichia coli*, *Klebsiella* spp., *Salmonella enterica*, *Enterobacter* spp., *Haemophilus* spp., *Acinetobacter baumannii* and *Pseudomonas*
Amplification systems – not integrated				
Siemens	Fast Track Diagnostics	Level III/IV	Real-time PCR	Respiratory infections: e.g., *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Staphylococcus aureus* and *Streptococcus pneumoniae* Gastroenteritis: *Campylobacter coli/jejuni/lari*, *Clostridium difficile*, *Salmonella* spp. and *Shigella* spp. STIs: NG
Molzym Molecular Diagnostics	SepsiTest™-UMD	Level III/IV	Universal PCR, Sanger sequencing	200 genera of bacteria

(Appendix II, continued)

Manufacturer	Platform	Minimum laboratory level	Technology	Assays
Momentum Bioscience Ltd.	Cognitor® Minus	Level III/ IV (requires culture)	ETGA® to detect microbial DNA polymerase activity	Confirms negative blood BSI result Pipeline: assay for detection, ID and AST of positive blood cultures
Luminex	xTAG® technology	Level III/IV	RT-PCR	Gastrointestinal panel: *Campylobacter jejuni*, *C. coli* and *C. lari* only, *tcdA* and *tcdB*, *Escherichia coli*, *Salmonella* and *Shigella*
		Molecular methods – pipeline		
Lucira Health	Disposable (no name)	Level I/II	RPA and LAMP	CT and NG, *Streptococcus* (swabs)
Qvella	FAST-ID™	Level II	PCR	Sepsis pathogens (whole blood)
NanoDetection Technology	No name	Level II (?)/ Level III	Biochip	MRSA screening, MRSA/MSSA dual screening, sepsis, influenza A and B, HCV, STIs and dengue
Biospectrix	3iDx	Level II/ Level I (?)	Microfluidics and nanotechnology	BSIs (whole blood)
		Sequencing methods		
Illumina, Inc.	MiSeq™ Dx instrument	Level IV	NGS	LDTs; no bacterial pathogen kits
Thermo Fisher Scientific	Ion PGM™ DX system	Level IV	NGS	RUO; no commercialized IVDs
		MS		
Beckman Coulter	Bruker MALDI Biotyper® system	Level IV	MS	424 clinically relevant bacteria and yeast species
bioMérieux	VITEK® MS	Level IV	MS	*Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Shigella* (which are both characterized as *E. coli*), *Klebsiella pneumoniae*, *Clostridium difficile*, NG (for which a confirmatory test is recommended), *Enterococcus faecium*, *Staphylococcus aureus*, *Campylobacter* spp., *Salmonellae* (for which a confirmatory test is recommended), *Streptococcus pneumoniae*, *Haemophilus influenzae* and MTB complex – among others
		Phenotypic methods of ID and AST		
		Semi-automated classical phenotypic methods		
MERLIN Diagnostika GmbH	MICRONAUT ID and AST (semi-automated)	Level IV	Phenotypic biochemical methods and photometry	*Enterobacteriaceae* and other gram-negative bacteria; nonfermenting gram-negative and some glucose-fermenting bacteria; *Staphylococcus*, among others

(Appendix II, continued)

Automated classical phenotypic methods				
Manufacturer	**Platform**	**Minimum laboratory level**	**Technology**	**Assays**
bioMérieux	VITEK® 2 system	Level III/IV	Automated phenotypic biochemical methods	Gram-negative fermenting and nonfermenting bacilli: 76 antimicrobials and ESBL Staphylococci and/or enterococci: 55 antimicrobials, four high-level aminoglycoside screens and inducible ICR test Streptococci: 14 antimicrobials and ICR test and gentamicin synergy *Streptococcus pneumoniae*: 23 antimicrobials andyeasts – six antifungals
BD	BD Phoenix™ automated microbiology system	Level III/IV	Broth-based microdilution method	Gram-positive bacteria (including genera *Staphylococcus*, *Streptococcus* and *Enterococcus*) and gram-negative bacteria (15 different genera, including *Acinetobacter*, *Enterobacter*, *Pseudomonas*, *Salmonella* and *Shigella*)
Beckman Coulter	MicroScan systems	Level III/IV	Automated phenotypic biochemical methods	Gram-positive (*Staphylococcus* and related genera and *Streptococcaceae*) and gram-negative glucose fermenting as well as glucose nonfermenting bacteria
Thermo Fisher Scientific	Sensititre™ ARIS™ 2X	Level III/IV	Automated phenotypic biochemical methods using fluorescence measurement	Nonfastidious gram-negative isolates (*Enterobacteriaceae*, *Pseudomonas aeruginosa* and other non-*Enterobacteriaceae*) and of nonfastidious gram-positive isolates (*Staphylococcus* spp., *Enterococcus* spp. and beta-haemolytic streptococci other than *S. pneumoniae*); additional testing capabilities are for yeasts (*Candida* spp.) and MTB
Imaging-based ID/AST or AST only				
Accelerate Diagnostics	Accelerate Pheno™ system	Level III/IV	FISH probes targeting organism-specific rRNA and quantitative morphokinetic cellular analysis using time-lapse imaging for AST	BSIs: Gram-positive bacteria (CNS, *Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus*, *Staphylococcus lugdunensis* and *Streptococcus* spp.); gram-negative bacteria (*Acinetobacter baumannii*, *Citrobacter* spp., *Enterobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas aeruginosa* and *Serratia marcescens*); *Candida* species (*C. albicans* and *C. glabrata*)
BioSense Solutions ApS	oCelloScope	Level III/IV	Automated microscopy using digital time-lapse technology	BSIs: ID and AST NOT COMMERCIALLY AVAILABLE
Alifax SPA	Sidecar, Alfred 60AST and HB&L Systems	Level III/IV	Bacterial culture in real time using light-scattering technology	Urine screening (culture); susceptibility test in urine; susceptibility test in blood culture; RAA; human biological liquids bacterial culture Pipeline: multidrug-resistant organisms (MRSA, ESBL/AmpC, carbapenem and VRE screening kit)

(Appendix II, continued)

Manufacturer	Platform	Minimum laboratory level	Technology	Assays
Nonimaging AST only				
Affinity Biosensors	LifeScale®	Level III/IV	MEMS	AST for gram-negative bacteria; RUO
bioMérieux	RAPIDEC® CARBA NP	Level III/IV	Colorimetric assay from suspensions of isolates on agar plates	Detection of carbapenemase enzymes in *Enterobacteriaceae* and *Pseudomonas aeruginosa* colonies
Rosco Diagnostica	Rapid CARB Blue kit	Level III/IV	Colorimetric assay from suspensions of isolates on agar plates	Detection of carbapenemase enzymes in *Acinetobacter* spp., *Enterobacteriaceae* and *Pseudomonas aeruginosa* colonies; Rapid ESBL Screen kit; Neo-Rapid CARB kit
Pipeline AST technologies				
QuantaMatrix	dRAST™	Level III/IV (culture)	MAC technology	Detection of SIRS
Gradientech	QuickMIC™	Level III/IV (culture)	Microfluidics	Panels for gram-negative and gram-positive bacteria will be available; universal AST solution
Astrego	Captiver™ system	Level II/ Level I (?)	Microfluidics and imaging	AST for UTIs
Nonphenotypic methods of identifying pathogens and detecting antibiotic resistance				
Molecular platforms for identifying and characterizing bacterial resistance from blood culture				
Nanosphere/Luminex	Verigene®	Level III/IV	Target DNA hybridization to microarray-based oligonucleotides with visualization based on gold nanoparticle oligonucleotide probes	Gram-positive blood culture nucleic acid test and gram-negative blood culture nucleic acid test for ID and resistance; respiratory panel; enterics panel; *Clostridium difficile*
bioMérieux	BioFire® FilmArray®	Level III/ IV; Level II for panels other than BCID and pneumonia (?)	Nested PCR and real-time PCR; detection using fluorescent intercalation dye within separate array module (1 target per well within array)	Multiple panels: blood culture ID, gastrointestinal, respiratory, meningitis/encephalitis, pneumonia
iCubate, Inc.	iC-system™	Level III/IV	PCR and microarray hybridization	Gram-positive bacteria panel: *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus pneumoniae*, *Enterococcus faecalis* and *E. faecium* Resistance markers: *mecA* resistance as well as *vanA* and *vanB* Pipeline: gram-negative bacteria (*Acinetobacter baumannii* complex, *Enterobacter cloacae* complex, *Escherichia coli*, *Klebsiella oxytoca*, *K. pneumoniae*, *Pseudomonas aeruginosa*, *Proteus* spp. and *Serratia marcescens*); *Mycobaterium*, gastrointestinal and respiratory tests

(Appendix II, continued)

Manufacturer	Platform	Minimum laboratory level	Technology	Assays
Master Diagnóstica	Sepsis Flow Chip	Level III/IV	Multiplex PCR using biotinylated primers followed by reverse dot-blot hybridization	40 bloodstream pathogens in the same assay, including gram-positive and gram-negative bacteria as well as fungi, and 20 antibiotic resistance genes, including MRSA, *mecA*, *vanA*, *vanB*, ESBL and carbapenems
Curetis GmbH	Unyvero™ system	Level III/IV	Multiplex PCR followed by array hybridization	BSI: 36 gram-positive and gram-negative analytes covering more than 50 pathogens; and 16 antibiotic resistance gene markers, including *mecC* (LGA251), *vanA*, *vanB*, CTX-M (*bla*$_{\text{CTX-M}}$), KPC (*bla*$_{\text{KPC}}$), IMP (*bla*$_{\text{IMP}}$), NDM (*bla*$_{\text{NDM}}$), OXA-23 (*bla*$_{\text{OXA-23}}$), OXA-24/40 (*bla*$_{\text{OXA-24/40}}$), OXA-48 (*bla*$_{\text{OXA-48}}$), OXA-58 (*bla*$_{\text{OXA-58}}$) and VIM (*bla*$_{\text{VIM}}$) LRT application: 19 bacteria and fungi, including *Acinetobacter* spp., *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pneumoniae*; and resistance markers *mecA*, CTX-M (*bla*$_{\text{CTX-M}}$), KPC (*bla*$_{\text{KPC}}$), NDM (*bla*$_{\text{NDM}}$), OXA-23 (*bla*$_{\text{OXA-23}}$), OXA-24/40 (*bla*$_{\text{OXA-24/40}}$), OXA-48 (*bla*$_{\text{OXA-48}}$) and VIM (*bla*$_{\text{VIM}}$) UTI panel: 88 pathogens and 15 resistance markers
Cepheid	GeneXpert® system	Level III/IV; Level II for panels not requiring culture	Real-time PCR and fluorogenic target-specific hybridization	BSI: MRSA/SA, MRSA/SA SSTI, MRSA Nasal Complete and MRSA NxG from swabs Bacterial resistance: *vanA* assay and Carba-R assay *Clostridium difficile* assays; MTB/RIF assay; and CT/NG assay
Hain Lifescience	GenoType assays and FluoroType® system	Level III/IV	LATE-PCR with fluorescence “lights on/lights off” probes	BSI: Gram-negative – *Escherichia coli*, *Enterobacter* species (*E. aerogenes*, *E. cloacae* and *E. sakazakii*), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* – and gram-positive (*Staphylococcus aureus*, *Enterococcus faecium* and *Streptococcus pneumoniae*, along with the ID of *mecA* and *van* genes) test kits Other kits: MRSA, *Staphylococcus aureus*, *Helicobacter pylori*, *Clostridium difficile*, *Enterococcus* and assays for MTB complex

(Appendix II, continued)

Manufacturer	Platform	Minimum laboratory level	Technology	Assays
GenMark Diagnostics	ePlex® system	Level III/IV	Competitive nucleic acid hybridization and electrochemical detection of nucleic acids on a microchip	BSI: Gram-positive panel – *Enterococcus*, *E. faecium*, *Staphylococcus* and *S. aureus*, as well as resistance markers *mecA*, *mecC*, *vanA* and *vanB*. Gram-negative panel – *Acinetobacter baumannii*, *Enterobacter* (non-*cloacae* complex and *cloacae* complex), *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella*, as well as resistance markers including CPE (bla_{KPC}, bla_{VIM}, bla_{NDM}, bla_{IMP} and bla_{OXA}) and ESBL ($bla_{CTX\text{-}M}$) Blood culture assay for fungus
Molecular platforms for ID and resistance from whole blood and other matrices				
Seegene	Seeplex™, Allplex™, Anyplex™, and MagicPlex™	Level III/IV	Real-time PCR on validated systems not provided by Seegene	MagicPlex™ Sepsis panel: ID of 27 gram-positive and gram-negative pathogens at the species level from whole blood, including *Streptococcus pneumoniae*, *Enterococcus faecium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Escherichia coli*. Resistance markers include *vanA*, *vanB* and *mecA* Variety of Seeplex™, Anyplex™ and Allplex™ assays, including drug-resistance tests
ELITechGroup Solutions	ELITe MGB® kits and panels	Level II (?), Level III/IV	Real-time PCR on ELITe InGenius® platform	ID: *Clostridium difficile* kit; STI panel (CT/NG and MG); *Staphylococcus aureus* and MRSA Resistance: carbapenem resistance kit; ESBL gene kit; and colistin resistance kit
Mobidiag	Amplidiag® system	Level III/IV	Real-time PCR on nonintegrated instruments	ID and resistance testing for gastrointestinal bacteria (parasites and viruses) and antibiotic resistance Bacterial gastroenteritispanel (*Campylobacter*, *Salmonella* and *Shigella*/EIEC, among others); *C. difficile*+027 panel; *H. pylori*+ClariR (*Helicobacter pylori* and clarithromycin resistance); CarbaR+VRE (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, $bla_{OXA\text{-}48}$, $bla_{OXA\text{-}181}$, *Acinetobacter* bla_{OXA}, *vanA* and *vanB*); CarbaR + MCR (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, $bla_{OXA\text{-}48}$, $bla_{OXA\text{-}181}$, *Acinetobacter* bla_{OXA}, *mcr* and bla_{GES})
QIAGEN N.V.	QIAsymphony® SP/AS	Level III/IV	qPCR and real-time qPCR amplification of nucleic acids; detection using fluorescence-labelled oligonucleotide probes	Kits for *Clostridium difficile*; CT/NG; vanR for detecting *vanA* and *vanB* vancomycin-resistance genes

(Appendix II, continued)

Manufacturer	Platform	Minimum laboratory level	Technology	Assays
GeneFluidics	UtiMax™/BsiMax®	Level II (?); Level III/IV	Electrochemical-based sandwich hybridization method to measure bacterial 16S rRNA	UtiMax™: detection and AST of uropathogens from urine Pipeline: BsiMax®: (detection of BSIs from whole blood); ID panel (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter* spp., MRSA, MSSA and *Enterococcus*, among others); AST antibiotics (gentamicin, ciprofloxacin, cefepime, meropenem, ceftriaxone, ampicillin and piperacillin-tazobactam)
Nonphenotypic methods of detecting antibiotic resistance				
Molecular methods of detecting antibiotic resistance				
Check-Points	Check-Direct and Check-MDR assays	Level III/IV	Assays only for use on BD MAX™ real-time PCR system and other systems	For BD MAX™: CPE kit (carbapenemases – bka_{KPC}, bla_{OXA-48}, bla_{VIM} and bla_{NDM}); CPO kit for BD MAX™ (carbapenemase genes – bka_{KPC}, bla_{OXA-48}, bla_{VIM}, bla_{NDM} and bla_{IMP}); ESBL screen kit (ESBL genes: $bla_{CTX-M-1}$ group, $bla_{CTX-M-2}$ group, $bla_{CTX-M-9}$ group, $bla_{SHV\ ESBL}$) For use on other instruments: CPE kit for carbapenemases (bka_{KPC}, bla_{OXA-48}, bla_{VIM} and bla_{NDM}) Microarray assays for epidemiology and confirmation
AmplexDiagnostics GmbH	eazyplex®	Level II (assays not requiring culture), Level III/IV	iNAAT on Genie® II or Gene® III platform	SuperBug® complete A: carbapenemases – KPC (bla_{KPC}), NDM (bla_{NDM}), VIM (bla_{VIM}) and OXA ($bla_{OXA-23,40,48,58}$) SuperBug® complete B: carbapenemases – KPC (bla_{KPC}), NDM (bla_{NDM}), VIM (bla_{VIM}) and OXA ($bla_{OXA-23,40,48,181}$) SuperBug® CRE: KPC (bla_{KPC}), NDM (bla_{NDM}), VIM (bla_{VIM}) and OXA ($bla_{OXA48,181}$) SuperBug® mcr-1: confirmation of *mcr-1* gene (colistin resistance) SuperBug® AmpC: confirmation of AmpC beta-lactamases eazyplex® VRE: *vanA* and *vanB* eazyplex® VRE basic: *vanA* or *vanB* from positive blood culture
Bruker	Carbaplex® IVD PCR	Level III/IV	Multiplex real-time PCR on validated platforms not from Bruker	CPE: carbapenemases – KPC (bla_{KPC}), NDM (bla_{NDM}), VIM (bla_{VIM}), OXA ($bla_{OXA48,181}$) and IMP (bla_{IMP})
AUTOIMMUN DIAGNOSTIKA	Antibiotic resistance line probe assays	Level III/IV	LPA on end-point PCR equipment not provided by AUTOIMMUN	AID ESBL: ESBL genes (bla_{TEM}, bla_{CTX-M}, bla_{SHV} and bla_{KPC}) AID Carbapenemase: 13 different carbapenem resistances, including bla_{KPC}, bla_{VIM}, bla_{NDM} and bla_{OXA-48} AID MRSA combi: *mecA* and *mec*, and differentiation of *Staphylococcus aureus* and CNS

(Appendix II, continued)

Immunoassays and other methods for detecting antibacterial resistance				
Manufacturer	**Platform**	**Minimum laboratory level**	**Technology**	**Assays**
NG Biotech	Antimicrobial lateral flow immunoassays	Level III/ IV (culture required)	LFIA using colloidal gold-labelled antibodies; no equipment required	Detection/confirmation of resistance genes from culture: CARBA 5 – carbapenemases: NDM (*bla*$_{NDM}$), KPC (*bla*$_{KPC}$), NDM (*bla*$_{NDM}$), VIM (*bla*$_{VIM}$) and OXA-48 (*bla*$_{OXA-48}$) CTX-M – ESBL (*bla*$_{CTX-M}$) MCR-1 – *mcr-1* gene
Coris BioConcept	RESIST assays	Level III/ IV (where culture required); Level II/I for identifying pathogens from stool on LFIA	LFIA; no equipment required Pipeline: TRAPIST V6: microfluidic chip technology using both molecular and immunoassay modalities	RESIST assays: carbapenemases – OXA-48 *K*-SeT for detecting *bla*$_{OXA-48}$; KPC *K*-SeT for detecting *bla*$_{KPC}$ only; O.K.N. *K*-SeT for detecting *bla*$_{OXA-48-like}$, *bla*$_{KPC}$ and *bla*$_{NDM}$; O.O.K. *K*-SeT for detecting *bla*$_{OXA-48-like}$, *bla*$_{KPC}$ and *bla*$_{OXA-163}$; and O.K.N.V. for detecting *bla*$_{OXA-48-like}$, *bla*$_{KPC}$, *bla*$_{NDM}$ and *bla*$_{VIM}$ Assays for bacterial pathogen ID from stool: *Helicobacter pylori*, *Escherichia coli* and *Clostridium difficile* TRAPIST pipeline: sepsis panels – multiplex gram-positive cassette (e.g., *Staphylococcus aureus*); multiplex gram-negative cassette (e.g., *Escherichia coli* and *Pseudomonas aeruginosa*); and resistance markers (e.g., *vanA*, *vanB*, *mecA* and *mecC* for gram-positive bacteria)
Pipeline molecular technologies for identifying pathogens and/or detecting antibiotic resistance				
SpinDiag	LabDisk	Level II/ Level I (?)	Nested PCR; microfluidics with disc-based test cartridge	Test 25 drug-resistant bacterial strains. Prototype tests: MRSA from nasal swabs and VRE from rectal swabs. Additional assays planned for RTIs and STIs.
ExcitePCR (Positive ID)	FireflyDx™	Level II/ Level I (?)	Real-time PCR and single-use disposable cartridges	Plan to process whole blood, nasal swabs and urine samples, among others. Have tested MRSA, MSSA and *Clostridium difficile* on prototype system. Resistance assays planned.
DxNA, LLC	GeneSTAT® analyser system	Level III/IV	Real-time PCR	Pipeline test: identify and differentiate *Staphylococcus aureus* and CNS
Q-linea	ASTar™, ASTrID®	Level IV (when combined with MALDI-TOF MS); possibly Level III for ASTrID®	ASTar™: automated microdilution for AST ASTrID®: padlock probe technology and amplification via RCA and subsequent C_2CA; RCA products labelled with fluorescence probes and detected on a microarray	ASTar™: phenotypic AST from culture after ID (e.g., via MALDI-TOF MS). Currently in prototype form. ASTrID®: will enable ID of more than 50 sepsis pathogens and select resistance genes as well as phenotypic AST in 10 hours directly from whole blood.
Specific Diagnostics	Reveal AST™/ID	Level III/IV (culture required)	SMS arrays that detect low concentrations of VOCs	Bacterial pathogen ID from blood culture; AST, including phenotypic MICs, from blood culture or from isolate dilutions
Cepheid	GeneXpert® Omni	Level II for tests not requiring culture	Real-time PCR and fluorogenic target-specific hybridization	Xpert® CT/NG and eventually other relevant assays available on the larger GeneXpert® platforms (e.g., MRSA)

(Appendix II, continued)

Manufacturer	Platform	Minimum laboratory level	Technology	Assays
Binx Health	io® diagnostic system	Level I/II	iNAAT using electrochemical DNA-detection technology based on differential pulse voltammetry	STIs: CT/NG assay and possible ciprofloxacin-sensitive NG resistance test
QuantuMDx Group	Q-POC™	Level I/II	End-point and qPCR chemistries and microarray after amplification	STIs: CT/NG/TV and possible NG antimicrobial resistance to accompany this assay
DNA Electronics (DNAe)	LiDia®	Level I/II	Semiconductor genomic analysis and multiplexed PCR and nested PCR	Sepsis assay from whole blood
Roche	Smarticles™	Level II, Level I (?)	Molecular technology using “Smarticles™”, nonreplicative transduction phages that bind to bacteria	MRSA; CRE and VRE in development
Host response and biomarker detection assays				
CRP				
Qualitative or semi-quantitative tests				
Creative Diagnostics, Inc.	DTS233	N/A	Colloidal gold immunochromatography	Qualitative CRP detection; RUO
Assure Tech	WD-23	Level I	Colloidal gold conjugate and anti-CRP antibodies	Semi-quantitative CRP concentration ranges
bioMérieux	bioNexia® CRPplus	Level I		Semi-quantitative CRP concentration ranges
Medix Biochemica	Actim® CRP	Level I		Semi-quantitative CRP concentration ranges
Quantitative tests				
Abbott	Alere Afinion™ CRP	Level I/II	Solid-phase immunochemical assay	Quantitative determination of CRP levels
Orion Diagnostica Oy	QuikRead go and QuikRead 101 instruments	Level I/II	Particle-enhanced immunoturbidimetric assay	Quantitative determination of CRP levels
Radiometer Medical ApS	AQT90 FLEX CRP	Level I/II	Time-resolved fluorescence using a europium chelate as the fluorescent label	Quantitative determination of CRP levels
Boditech Med	iChroma™ CRP	Level I/II	Fluorescence immunofluorescent assay	Quantitative determination of CRP levels
Abbott	NycoCard™	Level I/II	Immunochemical assay	Quantitative determination of CRP levels
Eurolyser Diagnostica GmbH	CRP test kit	Level I using CUBE-S; Level II	Immunoturbidimetric assay using photometric measurement	Quantitative determination of CRP levels
DiaSys Diagnostic Systems GmbH	CRP IS - InnovaStar®	Level I/II	Immunoturbidimetric assay	Quantitative determination of CRP levels
biosurfit	spinit®	Level I/II	Spectrophotometry	Quantitative determination of CRP levels

(Appendix II, continued)

PCT				
Manufacturer	**Platform**	**Minimum laboratory level**	**Technology**	**Assays**
Thermo Fisher Scientific	B·R·A·H·M·S PCT™ direct assay	Level II, Level I (?)	Immunochromatographic sandwich assay	Quantitative determination of PCT
	B·R·A·H·M·S PCT™ LIA	Level III/IV	Immunoluminescence sandwich assay	Quantitative determination of PCT
	B·R·A·H·M·S PCT-Q	Level II (requires serum)	Immunochromatographic sandwich assay with immunogold labelling	Semi-quantitative determination of PCT
Diazyme Laboratories	Diazyme PCT assay	Level III/IV	Latex particle enhanced immunoturbidimetric assay	Quantitative determination of PCT
Tests using novel biomarkers, a combination of host biomarkers, or combinations of protein biomarkers and gene classifiers				
MeMed BV	ImmunoXpert™	Level III/IV	Measures three human immune system biomarkers in serum	Qualitative assay to distinguish between bacterial and viral infection Pipeline: MeMed Sepsis™ and MeMed Neo™ (neonates); ImmunoPoc™ device
RPS Diagnostics	FebriDx®	Level I	Disposable immunoassay assay that detects CRP and MxA, an inflammatory protein	Qualitative assay to help differentiate a clinically significant immune response to viral and/or bacterial ARI
Immunexpress	SeptiCyte™	Level III/IV Level II for use on Idylla™	Real-time RT-PCR	Quantitative assay to differentiate infection-positive (sepsis) from infection-negative systemic inflammation
Abionic	abioSCOPE®	Depending on instrument, Level II, III, IV	Nanofluidic immunoassay technology; fluorescent molecular complexes are formed on a nanosensor	Quantitative determination of sepsis risk through measurement of pancreatic stone protein NOT COMMERCIALLY AVAILABLE
Pipeline host biomarker tests				
Inflammatix	HostDx Sepsis and HostDx Fever	Level III/ IV (HostDx Sepsis Level II (HostDx Fever)	Quantitative multiplex gene expression using molecular, multiplex platform	Assay to quantitate acute infection (HostDx Sepsis) and assay to quantitate whether fever is caused by bacteria or virus (HostDx Fever)
Mologic	UTRiPLEX	Level 1/II	Qualitative lateral flow assay detecting host biomarkers	Assay to rule out bacterial infections from urine

Annex III – Diagnostic platforms suitable for Level I/Level II

Diagnostic platforms for combating ABR suitable for Level I and/or Level II				
Automated Gram staining				
Manufacturer	**Platform**	**Minimum laboratory Level**	**Technology**	**Assays**
bioMérieux	PREVI® COLOR GRAM	I/II	Automated Gram staining via spray technology	Pan-bacteria
ALL.DIAG - Biosynex	MULTISTAINER®	I/II	Gram stain and fast staining	Pan-bacteria
ELITechGroup Solutions	Aerospray® Gram series 2	I/II	Gram stain	Pan-bacteria
Hardy Diagnostics	QuickSlide™ GramPRO 1™	I/II	Gram stain	Pan-bacteria
Automated specimen processing and inoculation of media				
None				
Phenotypic bacterial ID				
None				
Automated culture systems				
None				
Manual biochemical bacterial ID systems				
None				
Automated biochemical bacterial ID systems				
None				
Nonphenotypic methods of identifying bacteria				
Immunoassays				
Thermo Fisher Scientific	*RAPID*™ Hp StAR™	Level I	Immunochromatographic test	*Helicobacter pylori* from stool
Otsuka Pharmaceutical Co., Ltd.	RAPIRUN® *H. pylori* antibody detection kit	Level I	Immunochromatographic test	*Helicobacter pylori* from stool
Meridian Bioscience, Inc.	Immuno*Card* STAT!® CAMPY	Level I	Immunochromatographic test	*Campylobacter* antigens (*C. jejuni* and *C. coli*) from stool
Abbott	*C. DIFF QUIK CHEK COMPLETE®*	Level I	Immunochromatographic test	*Clostridium difficile* (TcdA and TcdB) from stool

(Annex III, continued)

Manufacturer	Platform	Minimum laboratory Level	Technology	Assays
Thermo Fisher Scientific	Xpect® *Clostridium difficile* Toxin A/B test	Level I	Immunochromatographic test	*Clostridium difficile* (TcdA and TcdB) from stool
Meridian Bioscience, Inc.	Immuno*Card* Toxins® A&B	Level I	Horizontal flow enzyme immunoassay	*Clostridium difficile* (TcdA and TcdB) from stool
Thermo Fisher Scientific/ BioStar	BioStar® OIA GC	Level I	Optical immunoassay	NG from female endocervical swabs and male urine specimens
Abbott	BinaxNOW® *Streptococcus pneumoniae*	Level I	Immunochromatographic assay	*Streptococcus pneumoniae* from urine
BIOSYNEX	BIOSYNEX *S. pneumoniae*	Level I	Immunochromatographic test	*Streptococcus pneumoniae* from urine and CSF
Malaysian Biodiagnostic Research	Typhidot®	Level II, possibly Level I	ELISA	*Salmonella typhi* in serum
IDL Biotech	TUBEX®	Level I	IMBI	*Salmonella typhi* in serum
Thermo Fisher Scientific	Wellcogen™ *Haemophilus influenzae* b Rapid latex agglutination test	Level I	Latex diagnostic test	*Haemophilus influenzae* type b from CSF, serum, urine or blood cultures
Meridian Bioscience, Inc.	Immuno*Card* STAT!® E. coli O157 Plus	Level I	Horizontal-flow enzyme immunoassay	*Escherichia coli* in stool or culture
		Molecular methods		
		Hybridization methods		
		None		
		Amplification methods – integrated systems		
Roche	cobas® Liat® system	Level II/ possibly Level I if cold chain	Real-time PCR	*Clostridium difficile*
Quidel Corporation	Solana® platform	Level II	HDA	*Clostridium difficile*; Group A Strep, Group B Strep
	AmpliVue® platform	Level II	HDA and lateral flow	*Clostridium difficile*; Group A Strep, Group B Strep
Luminex	ARIES® and ARIES® M1 systems	Level II	Real-time PCR	*Clostridium difficile*; Group A Strep
Mobidiag	Novodiag® platform	Level II	Real-time qPCR and microarray	*Clostridium difficile*; Bacterial GE+ assay
QIAGEN NV	QIAstat-Dx™	Level II	Real-time PCR with optical sensor	Respiratory panel; gastrointestinal panel; sepsis panel
		Amplification systems – not integrated		
		None		
		Pipeline molecular methods		
Lucira Health	Disposable (no name)	Level I/II	RPA and LAMP	CT and NG, Strep (swabs)
Qvella	FAST-ID™	Level II	PCR	Sepsis pathogens (whole blood)

(Annex III, continued)

Manufacturer	Platform	Minimum laboratory Level	Technology	Assays
NanoDetection Technology	No name	Level II (?)/ Level III	Biochip	MRSA screening, MRSA/ MSSA dual screening, sepsis, influenza A and B, HCV, STIs and dengue
3iDx	Biospectrix	Level II/ Level I (?)	Microfluidic and nanotechnology	BSIs (whole blood)
Sequencing methods				
None				
MS				
None				
Phenotypic methods of ID and AST				
Semi-automated classical phenotypic methods				
None				
Automated classical phenotypic methods				
None				
Imaging-based ID/AST or AST only				
None				
Nonimaging AST only				
None				
Pipeline AST technologies				
Astrego	Captiver™ System	Level II/ Level I (?)	Microfluidics and imaging	AST for UTIs
Nonphenotypic methods of identifying pathogens and detecting antibiotic resistance				
Molecular platforms for identifying and characterizing bacterial resistance from blood culture				
bioMérieux	BioFire® FilmArray®	Level III/ IV; Level II for panels other than BCID and pneumonia (?)	Nested PCR and real-time PCR; detection using fluorescent intercalation dye within separate array module (one target per well within array)	Multiple panels: blood culture ID panel, gastrointestinal, respiratory, meningitis/encephalitis, pneumonia
Cepheid	GeneXpert® system	Level III/IV; Level II for panels nor requiring culture	Real-time PCR and fluorogenic target-specific hybridization	BSI: MRSA/SA; MRSA/SA SSTI, MRSA Nasal Complete and MRSA NxG from swabs Bacterial resistance: *vanA* assay and Carba-R assay *Clostridium difficile* assays; MTB/RIF assay; and CT/NG assay

(Annex III, continued)

Molecular platforms for ID and resistance from whole blood and other matrices				
Manufacturer	**Platform**	**Minimum laboratory Level**	**Technology**	**Assays**
ELITechGroup Solutions	ELITe MGB® kits and panels	Level II (?), Level III/IV	Real-time PCR on ELITe InGenius® platform	ID: *Clostridium difficile* kit, STI panel (CT/NG and MG); *Staphylococcus aureus* and MRSA Resistance: carbapenem resistance kit; ESBL gene kit and colistin resistance kit.
GeneFluidics	UtiMax™/BsiMax®	Level II (?); Level III/IV	Electrochemical-based sandwich hybridization method to measure bacterial 16S rRNA	UtiMax™: detection and AST of uropathogens from urine Pipeline: BsiMax® – detection of BSIs from whole blood. ID panel: *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter* spp., MRSA, MSSA and *Enterococcus*, among others. AST antibiotics: gentamicin, ciprofloxacin, cefepime, meropenem, ceftriaxone, ampicillin and piperacillin-tazobactam
Nonphenotypic methods of detecting antibiotic resistance				
Molecular methods of detecting antibiotic resistance				
None				
Immunoassays and other methods for detecting ABR				
None				
Pipeline molecular technologies for identifying pathogens and/or detecting antibiotic resistance				
SpinDiag	LabDisk	Level II/ Level I (?)	Nested PCR; microfluidics with disc-based test cartridge	Tests 25 drug-resistant bacterial strains. Prototype tests: MRSA from nasal swabs and VRE from rectal swabs. Additional assays planned for RTIs and STIs.
ExcitePCR (Positive ID)	FireflyDx™	Level II/ Level I (?)	Real-time PCR and single-use disposable cartridges	Plan to process whole blood, nasal swabs and urine samples, among others. Have tested MRSA, MSSA and *Clostridium difficile* on prototype system. Resistance assays planned.
Binx Health	io® diagnostic system	Level I/II	iNAAT using electrochemical DNA detection technology based on differential pulse voltammetry	STIs: CT/NG assay and possible ciprofloxacin-sensitive NG resistance test
QuantuMDx Group	Q-POC™	Level I/II	End-point and qPCR chemistries and microarray after amplification	STIs: CT/NG/TV and possible NG antimicrobial resistance to accompany this assay
DNA Electronics (DNAe)	LiDia®	Level I/II	Semiconductor genomic analysis and multiplexed PCR and nested PCR	Sepsis assay from whole blood

(Annex III, continued)

Manufacturer	Platform	Minimum laboratory Level	Technology	Assays
Roche	Smarticles™	Level II, Level I (?)	Molecular technology using "Smarticles™", nonreplicative transduction phages that bind to bacteria	MRSA; CRE and VRE in development
Host response and biomarker detection assays				
CRP				
Qualitative or semi-quantitative tests				
Assure Tech	WD-23	Level I	Colloidal gold conjugate and anti-CRP antibodies	Semi-quantitative CRP concentration ranges
bioMérieux	bioNexia® CRPplus	Level I		Semi-quantitative CRP concentration ranges
Medix Biochemica	Actim® CRP	Level I		Semi-quantitative CRP concentration ranges
Quantitative tests				
Abbott	Alere Afinion™ CRP	Level I/II	Solid-phase immunochemical assay	Quantitative determination of CRP levels
Orion Diagnostica Oy	QuikRead go and QuikRead 101 instruments	Level I/II	Particle-enhanced immunoturbidimetric assay	Quantitative determination of CRP levels
Radiometer Medical ApS	AQT90 FLEX CRP	Level I/II	Time-resolved fluorescence using a europium chelate as the fluorescent label	Quantitative determination of CRP levels
Boditech Med	iChroma™ CRP	Level I/II	Immunofluorescent assay	Quantitative determination of CRP levels
Abbott	NycoCard™	Level I/II	Immunochemical assay	Quantitative determination of CRP levels
Eurolyser Diagnostica GmbH	CRP test kit	Level I using CUBE-S; Level II	Immunoturbidimetric assay using photometric measurement	Quantitative determination of CRP levels
DiaSys Diagnostic Systems GmbH	CRP IS -InnovaStar®	Level I/II	Immunoturbidimetric assay	Quantitative determination of CRP levels
biosurfit	spinit®	Level I/II	Spectrophotometry	Quantitative determination of CRP levels
PCT				
Thermo Fisher Scientific	B·R·A·H·M·S PCT™ direct assay	Level II, Level I (?)	Immunochromatographic sandwich assay	Quantitative determination of PCT
	B·R·A·H·M·S PCT-Q	Level II (requires serum)	Immunochromatographic sandwich assay with immunogold labelling	Semi-quantitative determination of PCT
Tests using novel biomarkers, a combination of host biomarkers or combinations of protein biomarkers and gene classifiers				
RPS Diagnostics	FebriDx®	Level I	Disposable immunoassay that detects CRP and MxA, an inflammatory protein	Qualitative assay to help differentiate a clinically significant immune response to viral and/or bacterial ARI

(Annex III, continued)

Manufacturer	Platform	Minimum laboratory Level	Technology	Assays
Immunexpress	SeptiCyte™	Level II for use on Idylla	Real-time RT-PCR	Quantitative assay to differentiate infection-positive (sepsis) from infection-negative systemic inflammation
		Pipeline host biomarker tests		
Mologic	UTRiPLEX	Level I/II	Qualitative lateral flow assay detecting host biomarkers	Assay to rule out bacterial infections from urine

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