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Defining the Need for Rapid Diagnostics

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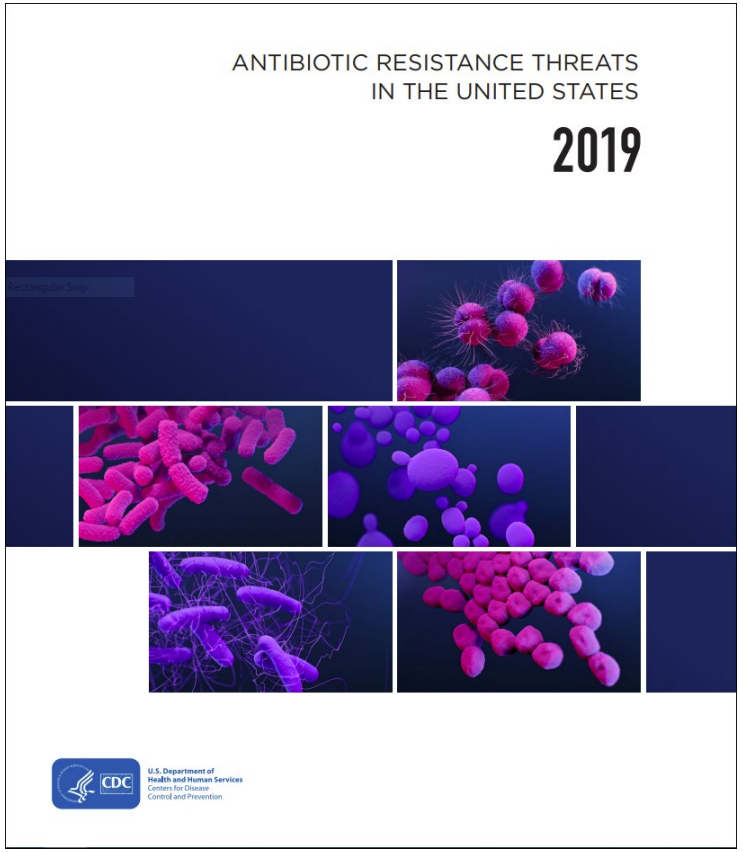
Objectives

- Examine the current state of rapid diagnostic development
- Consider gaps that rapid diagnostics may address
- Discuss what “success” might look like in the future

Background

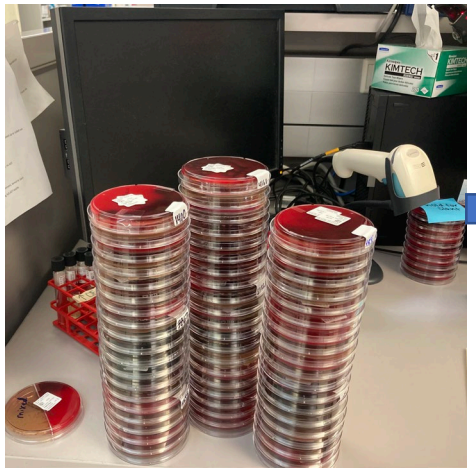
- Antimicrobial resistance (AMR)—ranked by WHO as one of top 10 global public health threats facing humanity
- Globally
  - Mortality projected to rise to 10 million by 2050
  - Burden to global economy due to loss of productivity projection: $100 trillion
- CDC Report
  - 2.8 million antibiotic-resistant infections in USA each year
  - 35,000 deaths
- Urgent threats
  - MDR Gram-negative bacteria (carbapenem resistant *Acinetobacter*, *Enterobacterales*)
  - Drug-resistant *Neisseria gonorrhoeae*
  - *Candida auris*
  - *Clostridium difficile*
- SARS CoV-2 pandemic has exacerbated global AMR crisis



Centers for Disease Control and Prevention (CDC). Antibiotic Resistance Threats in the United States, 2019. CDC; 2019. doi: 10.15620/cdc:82532
WHO. Antibiotic Resistance. https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance. O'Neill J. 2016. Review on antimicrobial resistance, London, UK

Current Clinical Microbiology Laboratory Landscape: Dependence on Traditional Techniques

- Very slow!
- Advantages
  - Cost effectiveness
  - Extensive clinical validation
- Limitations of newer methods
  - Limited spectrum of pathogens detected
  - Variable sensitivity and specificity
  - Lack of differentiation between living and dead cells
  - Cost
- Lack of *a priori* knowledge of causative pathogen in many cases
- Importance of phenotypic susceptibility testing for AMR detection



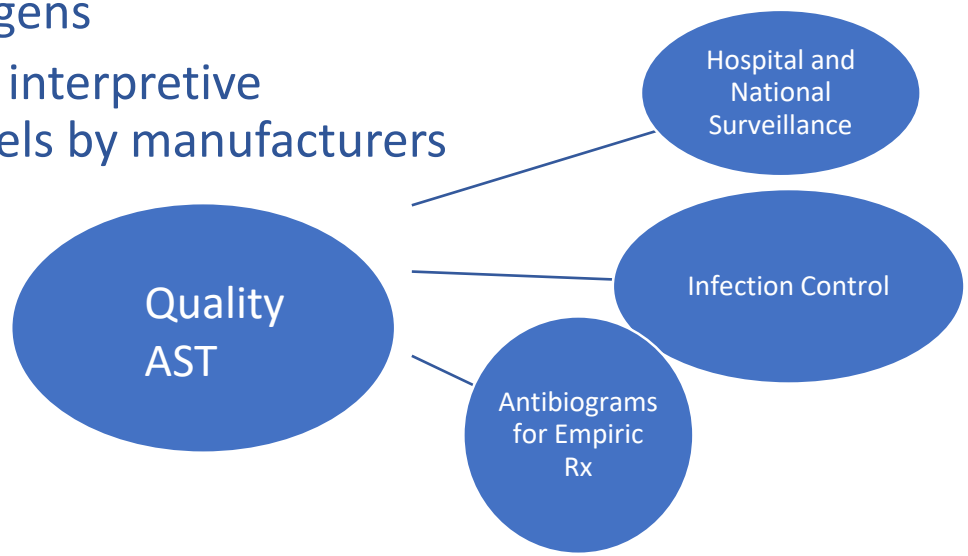
Disk Diffusion

BD Phoenix M50

Doern CD, et al. *J Clin Microbiol* 2022:60 (7): e0009222. Miller MB et al. *J Clin Microbiol* 2019;57:e00495-19.

Antimicrobial Susceptibility Testing

- Semi-automated and automated devices use microbroth dilution methods, a reference standard
- Advantages: standardized, quantitative, interpretive guidelines exist (CLSI, EUCAST)
- Disadvantages:
  - slow (18 h)
  - not amenable for testing all bacterial pathogens
  - lag between availability of new agents, new interpretive breakpoints and incorporation into AST panels by manufacturers



Simner PJ, et al. *Open Forum Infect Dis* 2022 Feb 7;9(3):ofac007; Humphries RM, et al. *Clin Infect Dis* 2018; 66:1061-67. Humphries RM, Hindler JA. *Clin Infect Dis* 2016;63:83-8. National Academies of Sciences, Engineering, and Medicine. 2022. *Combating antimicrobial resistance and protecting the miracle of modern medicine*. Washington, DC: The National Academies Press. https://doi.org/10.17226/26350.

Progress with Rapid Phenotypic Susceptibility Testing Methods

- Proof of concept demonstrated from positive blood cultures using disk diffusion
- One commercial FDA-approved platform to date; others in the pipeline

Accelerate Pheno®
Used with permission from André Gressieux, Accelerate Diagnostics, Inc.

- Advantages
  - Very rapid < 8 h
  - Improved antimicrobial stewardship
  - Affordable if using disk diffusion
- Barriers
  - Technical barriers for some drug/bug combinations
  - Limited to certain antibiotics
  - Additional costs
  - Financial risk to industry

Progress with Rapid Detection of Resistance Mechanisms

- DNA amplification methods targeting specific genes
- Immunochromatographic assays
- Antibiotic degradations assays

Cepheid
Sunnyvale CA

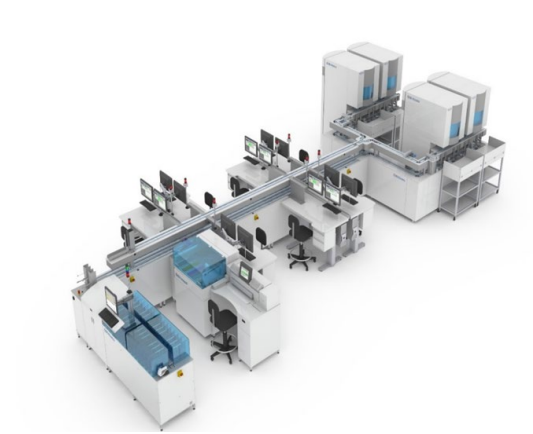
CARBA-5 NG TEST
Hardy Diagnostics
Santa Maria, CA

- Advantages
  - Very rapid 15 min-1 h
  - Confirmation of resistance
  - Improves antimicrobial stewardship
  - Enhances infection control
- Limitations
  - Negative result does not imply susceptibility
  - Positive resistance marker does not necessarily confer phenotypic resistance
  - Limited to certain antibiotics
  - Additional associated costs

Evolution of Diagnostic Methods in Clinical Microbiology Laboratories

Disruptive technologies have advanced diagnosis in clinical labs over the last several decades

- Multiplexed molecular syndromic panel tests
- Proteomics using Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS)
- DNA-microarray based hybridization technology
- T2 magnetic resonance
- Rapid phenotypic/genotypic susceptibility testing
- Total laboratory automation combined with artificial intelligence
- Next generation sequencing

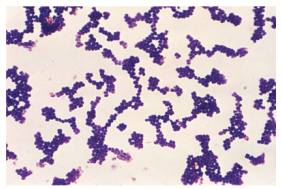


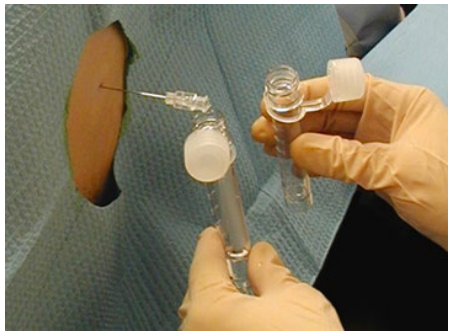
Cherkaoui A, Schrenzel J. *Front Cell Infect Microbiol* 2022;12:807668. Miller MB et al. *J Clin Microbiol* 2019;57:e00495-19.

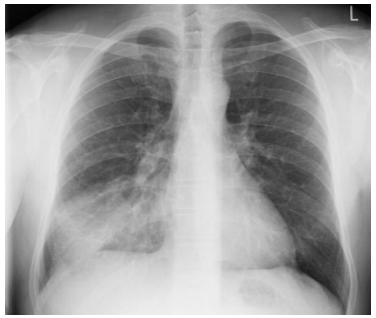
Progress to Date
Syndromic Panel Tests

Combine organism detection and resistance determinants (bacterial pathogens)

- Respiratory viruses
- Positive blood culture bottles
- Enteric pathogens
- Meningitis/encephalitis
- Bacterial lower respiratory infections
- Prosthetic joint/septic arthritis







Ramanan P, et al *Clin Micro Rev 2018.*31:e00024-17. Gonzalez MD, et al *Infect Dis Clin N Amer* 32:19-34. Dumkow LE et al. *J Antimicrob Chemo* 2021;76 (suppl 3).

Advantages and Challenges of Syndromic Panel Tests

Advantages	Challenges
Comprehensive	Potential to contaminate raw specimens
Moderate complexity allows near patient testing	FDA approved for limited sample types; user must validate other specimens
More sensitive than culture and DFA	Interpretation of coinfections (as high as 33%)
More specific than antigen tests	Costs
Reduced TAT Guides antiviral care Improves targeted antibacterial/yeast therapy Reduces antibacterial use	Limited studies on patient outcomes Most studies show variable impact on LOS, mortality Need accepted guidelines for appropriate utilization
Recognition of outbreaks	Mutations may impact assay sensitivity over time
Detection of pathogens not considered	Prolonged shedding of some pathogens may complicate interpretation; determination of colonization vs. infection

Banerjee R, et al. *Clin Infect Dis* 2015;61:1071-80. Other references available upon request.

Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry

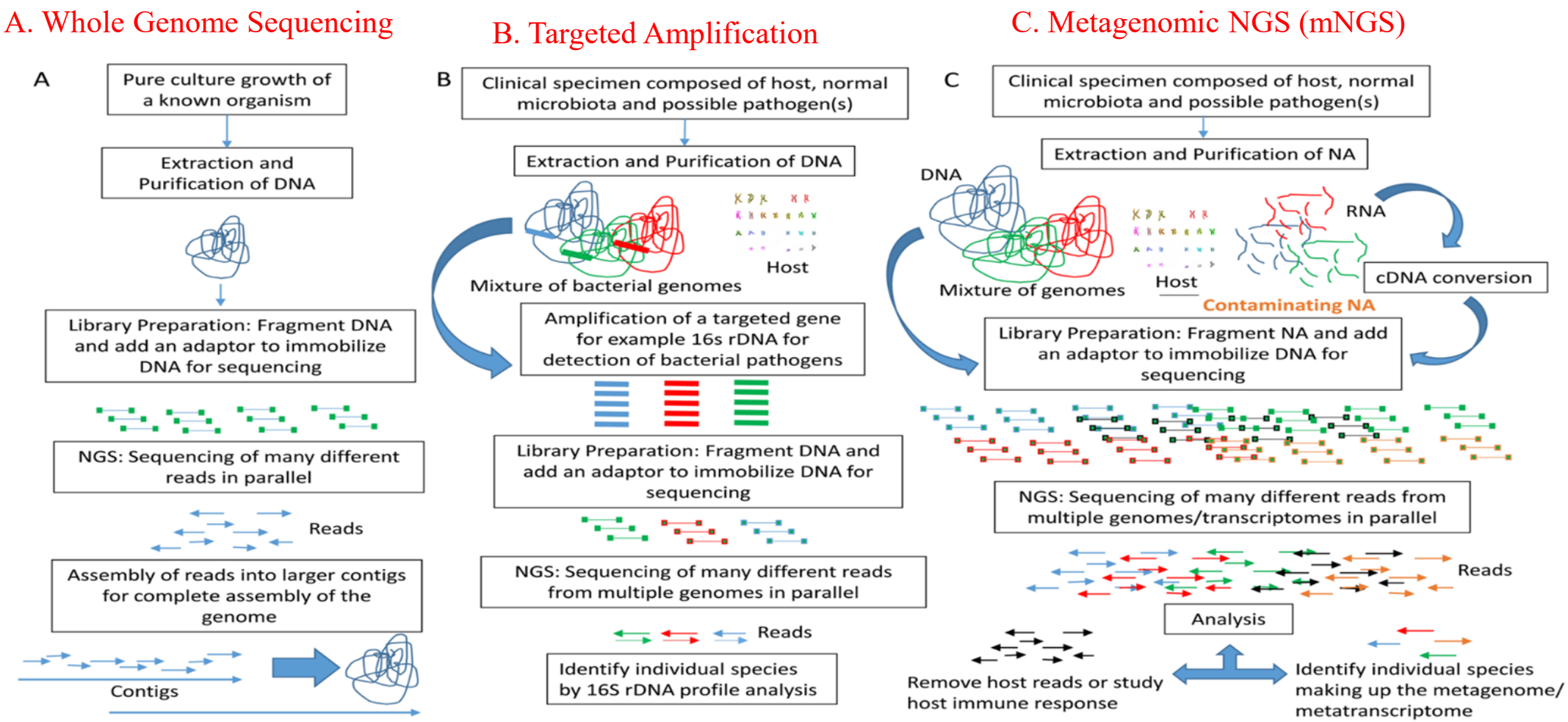
- Identifies organisms based on unique protein profiles
- Rapid, inexpensive, broad-based, cost-effective, highly accurate
- Medical impact
  - Reduction in time to therapy
  - Reduction in length of stay
  - Cost savings
- Direct testing from urine, positive blood cultures (Sepsityper)
- Beyond identification
  - Susceptibility testing
  - Strain typing for epidemiology

Bruker MS Biotyper (Bruker Daltonics, Inc. Billerica, MA)

Vitek MS (bioMerieux, Durham, NC)

Patel R. 2013; *Clin Infect Dis* 57:564. Tan KE, et al 2012; *J Clin Microbiol* 50:3301; Tran A, et al 2015. *J Clin Microbiol* 53:2473; Tamma PD, et al 2013; *PLoS One* 34:990; Vlek ALM, et al. 2012. *PloS One*7:e32589; Rapp E, et al. 2018. *J Microbiol Methods* 146:37; Dortet L. et al. 2018. *J Antimicrob Chemother* 73:2352; Neonakis IK, et al. 2019; *EJCMID* 38:1795. Welker M, et al. 2019. *Front Microbiol* 10:2711

Applications NGS in Clinical Microbiology



Slide compliments of Patricia Simner, PhD

Metagenomic Next-generation Sequencing: Utility

- A large proportion of samples are culture-negative.
  - Pre-treatment
  - Uncultivatable pathogens
  - Pathogens requiring specialized handling or prolonged incubation (fungi, mycobacteria)
- Useful in scenarios that require detection of a broad range of pathogens e.g. immunocompromised patients
- Currently used as a method of last resort.

Wilson MR et al *N Engl J Med* 2019; 380L2327-2340. Mitchell SL, Simner PJ. *Clin Lab Med* 2019; 39:405-18.

What is Needed to Make NGS more Available?

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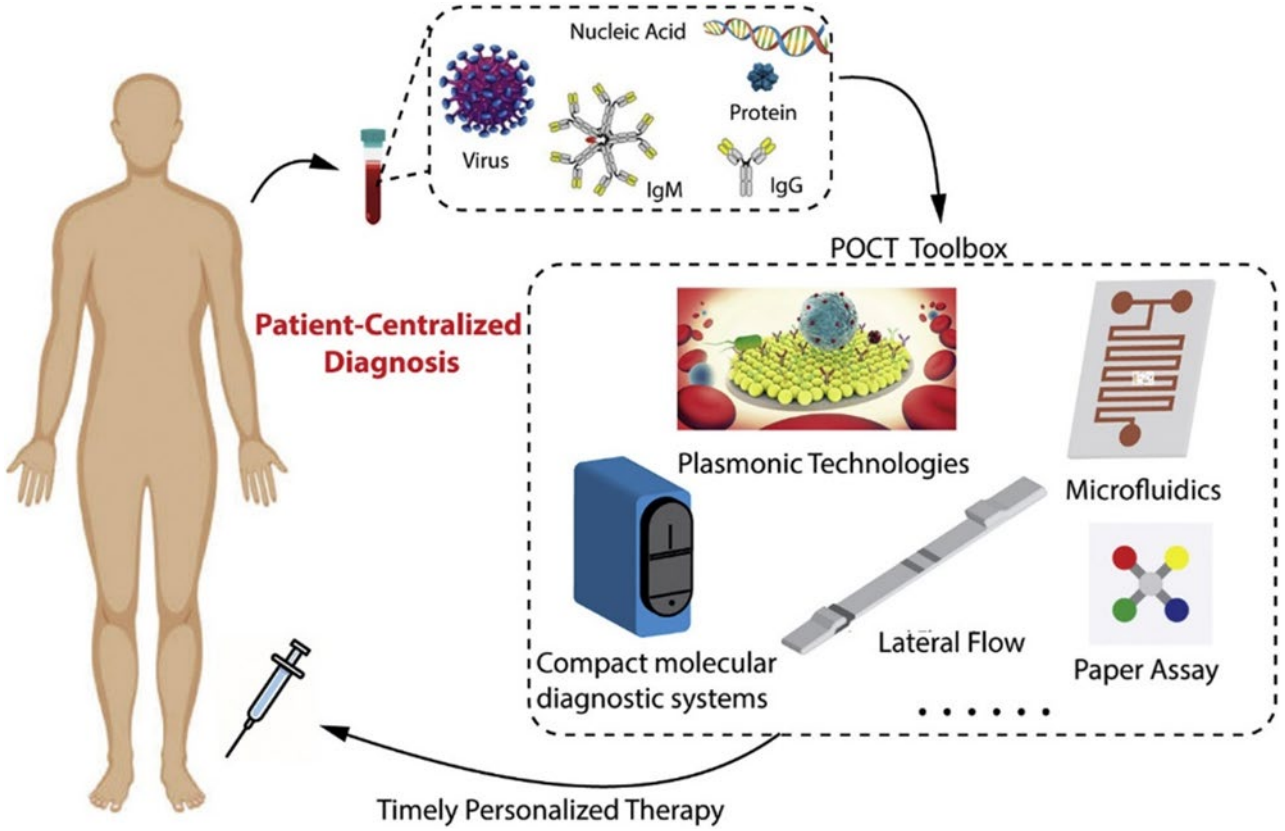
Current Challenges	Potential Solutions
Requires investment in laboratory infrastructure • Information technology; database storage • Separate sample prep/library prep areas • Specialized equipment • Unique validation processes • Specialized personnel • Variable sensitivity and specificity caused by unbiased approach (host and all organisms) are sequenced	Use of existing molecular workflows • Validation of user-friendly specialized commercialized and free software • High quality databases • Sequencing negative controls; removal of post-sequencing contamination • Quantification of pathogen abundance • Ultraclean nucleic acid extraction kits
Costs	Use of commercially available systems Limit use to diagnostic dilemmas Prospective cost-effectiveness studies
Not amenable to immediate to fast TAT (5 days on average) • Deeper sequencing limits number of samples per run	• Implementation of newer technologies that can speed up actionable results (e.g. Oxford Nano-pore technology) • Transition to POC environment  MinION (Oxford Nanopore, UK)
Complicated validation (Laboratory developed tests, no reliable reference method)	Use of published protocols More universal well-standardized metrics needed

Clinical Laboratories: Current Challenges

- In spite of technological advances there are many challenges in clinical laboratories
  - Workforce shortages
  - Lack of institutional investment in clinical laboratories
  - Needed support for diagnostic stewardship defined as implementation of guidelines to ensure appropriate test utilization to optimize patient care
  - Regulatory impediments
    - Testing for new agents
    - Prompt verification/implementation of AST breakpoint changes

Doern CD, et al. *J Clin Microbiol* 2022:60 (7): e0009222

Point of Care Diagnostics



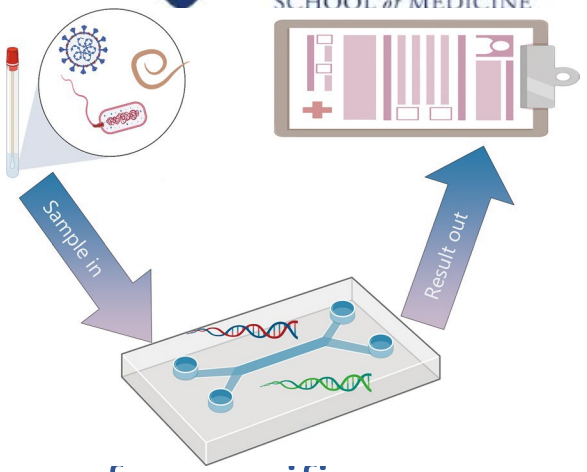
Used with permission by Elsevier, Inc. Chen H, et al 2019. *Clinica Chimica Acta* 493:138-147

Reassured Criteria for POC Testing

2019 –real-time connectivity and ease of specimen collection were added: REASSURED

WHO 2003, 2006 and Land KJ et al. *Nat Microbiol* 2019;4:46-54

Emerging Technologies



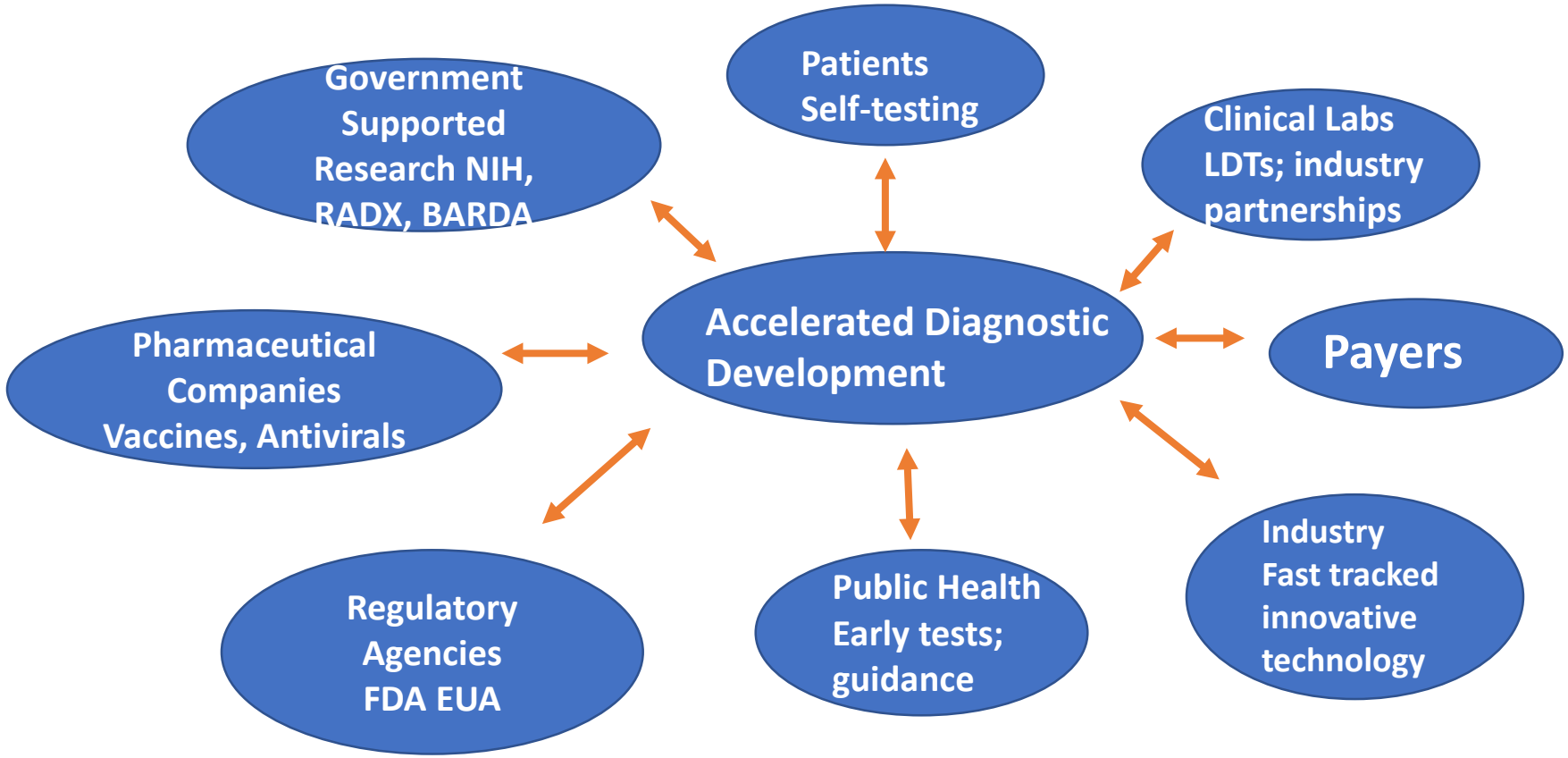
- Microfluidics
  - Manipulation/analysis of fluid within micrometer-sized channels
  - Offers fast thermocycling (< 30 min), high sensitivity at the POC
- Biosensors
  - Biomolecules immobilized on a physiochemical transducer for detection of a specific

Remaining challenges: more research needed on realizing REASSURED criteria.
Excessive costs associated with translating to clinical environments.

- Digital Droplet PCR—PCR in ultra small volumes
- Paper-based devices

Alamolhoda SZ, et al. *Adv Pharm Bull* 2022;12:58-76. Harpaldas H, et al *Lab Chip* 2021;21:4517-48. Bhardwaj T et al *Biosensors* 2022;12:357

Impact of SARS CoV-2



Otoo JA, Schlappi TS. 2022. REASSURED Multiplex Diagnostics: A Critical Review. *biosensors* 12,124.

Examples of Innovative Technologies-SARS CoV-2 POC Tests Using Microfluidics

POC Test	Assay Chemistry	Sample Type	Fluid Activation/Control	Signal Detection	Connectivity
Cue Health https:// cuehealth.com/ 	Isothermal	Nasal swab	Capillary/wax valves Fluid mixing by sonication	Electrochemical	Portable Bluetooth connected reader/mobile app
Visby Medical https://www.visbymedical.com/ 	RT-PCR	Nasal swab	Gear motor/rotary on chip valves	Colorimetric (LFA)	None
Abbott ID Now https://www.abbott.com/ 	Isothermal	Nasal/NP/ throat	Manual/Manual	Fluorescence	Portable instrument with LCD screen

Priority Diseases for POC Testing

- Sexually transmitted infections (STIs)
- Tuberculosis
- Urinary Tract Infections
- Respiratory Infections
- Malaria
- Neglected tropical diseases

Sexually Transmitted Infections (STIs)

- More than 2.4 million U.S. cases of syphilis, gonorrhea and chlamydia were reported to the CDC in 2018
- Globally, resistance among *Neisseria gonorrhoeae* isolates is high
  - Annual economic burden in the USA--$133.4 million
- Other STIs of interest: emerging *Mycoplasma genitalium*, Human papilloma virus, *Trichomonas vaginalis* and herpes simplex 1 and 2
- Impact of SARS CoV-2 pandemic on STI case surveillance is unknown
  - National Coalition of STD Directors report:
    - 83% of STIs programs deferred services or field visits
    - 66% reported a decrease in screening capacity
    - 60% reported reduced capacity to treat STIs

Kersh EN, et al 2021. At-home specimen self-collection and self-testing for sexually transmitted infection screening demand accelerated by the COVID-19 pandemic: a review of laboratory implementation issues. *J Clin Microbiol* 59:e02646-20. National Coalition of STD Directors 2020. COVID-19 and the state of the STD field.

Current and Future POC for STIs

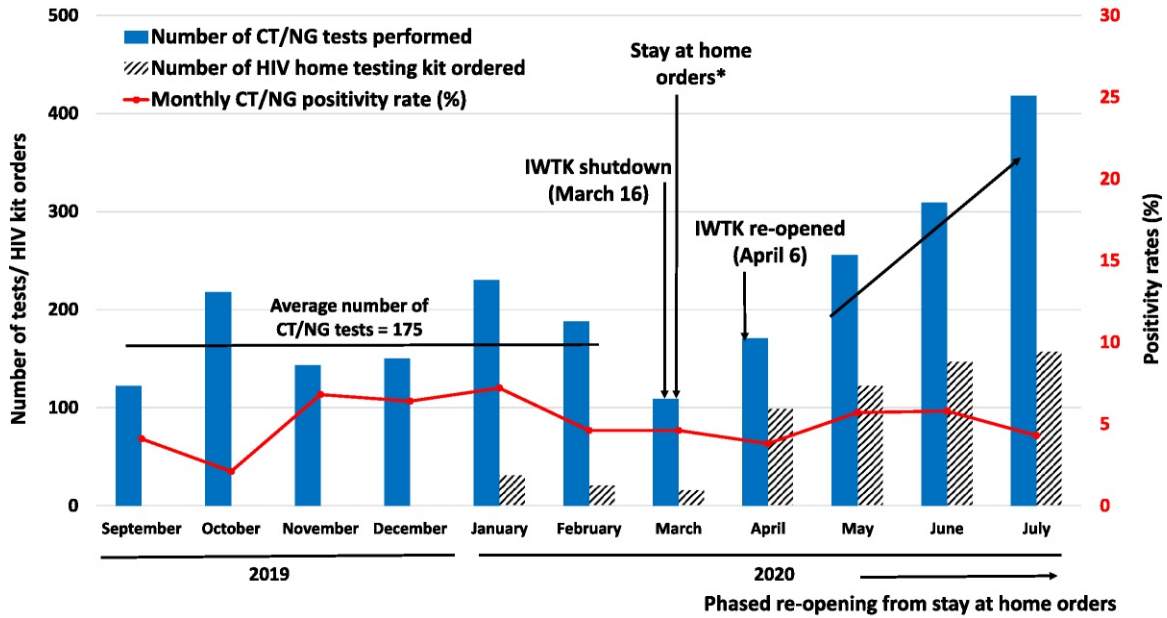
Test	Description	Regulatory Status
Binx io (Binxhealth, Cambridge MA) GC/CT	NAAT; 30 min to results; male and female urine; female vaginal swabs	CLIA waived
Visby Medical Sexual Health Test GC/CT/*Trichomonas vaginalis*	NAAT; 30 min; portable, handheld, disposable device; vaginal swabs	CLIA waived
Cepheid GeneXpert NAAT GC/CT (Cepheid Sunnyvale, CA)*	NAAT; 90 min; near-patient test; instrument need; vaginal, cervical; male, female urine; rectal, oropharyngeal	Not CLIA waived
MobiNAAT Platform Prompt Diagnostics (Baltimore)	NAAT plus droplet magnetofluidics; cartridge based < 15 min; CT; NG plus cipro	Not FDA cleared
Novel Microdevices LLC (Baltimore, MD)	LAMP plus microfluidics CT/NG assay; < 30 min; Vaginal swabs; urine	Not FDA cleared

*XpertXpress CT/NG in development

Gaydos CA, et al. *Sex Trans Dis* 2021; 48:S71-77. Karellis A, et al *Lancet Microbe* 2022; 3:e303-15

Sexually Transmitted Infections (STIs) Self-Collected Samples

- Prior to pandemic, studies demonstrated ability of patients to reliably self-collect vaginal, pharyngeal, penile meatal swabs for mail in testing.
- Several successful programs:
  - I WANT THE KIT (IWTK)
  - I KNOW
  - TAKEMEHOME
- During early days of COVID pandemic, STD clinics reverted to home collection with mail-in of swabs for CT/NG testing combined with telemedicine.



Sexually transmitted infection testing and distribution of HIV home testing kits by IWTK before and during the COVID-19 pandemic. CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*. *Stay at home orders for Maryland; Washington, DC; and Alaska. Melendez JH, et al *Sex Transm Dis* 2021; 48:e8-e10. Used with permission by Wolters Kluwer Health, Inc.

Kersh EN, et al. *J Clin Microbiol* 2021; 59: e02646-20.
Gaydos C. *Sex Transm Dis* 2018;45:278-9

Benefits and Challenges with Self-Collection/Self-Testing

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Benefits

- Convenient
- Private (overcomes stigma)
- Cost-effective
- As accurate as provider collected
- Readily accepted by patients
- Increased testing means increased detection and treatment

Challenges/Priorities

- Is it feasible for the patients most at need? Language or literacy barriers.
- Low specimen return rates (65% IWTK)
- Concerns regarding false positives, false negatives.
- How to accomplish surveillance and contact tracing?
- Linkage to care in limited access areas
- Regulatory issues in some states
- Verification/validation of existing in-laboratory high-throughput testing platforms for self-collected specimens

*Melendez JH, et al. *Sex Transm Dis* 2021: 48:e8-e10; Hobbs MM, et al. *Sex Transm Dis* 2008; 35; Gaydos CA. *Sex Transm Dis* 2018;45:278-9.

Summary

- Unprecedented technological advancements hold promise for enhanced diagnostics in labs and at the point of care
- Variety of hurdles need to be addressed to optimize implementation of rapid diagnostics
  - Technical barriers and costs to mass production/commercialization
  - Better outcomes studies to understand patient impact
  - Studies to understand workflow barriers in clinics and laboratories
  - Quality management considerations—contamination; poor user performance
  - Data management
  - Regulatory and reimbursement issues for laboratories and industry

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