UCSF School of Medicine

Applications for New Pathogen Detection and Limitations of Genomic Data

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NAS Workshop: ”Accelerating the Use of Pathogen Genomics and Metagenomics in Public Health”, July 23rd, 2024

Disclosures

- SURPI+ software, “Pathogen Detection using Next Generation Sequencing” (PCT/US/16/52912), filed by University of California, San Francisco

- Scientific Advisory Board for Mammoth Biosciences, BiomeSense, Poppy Health, Biomeme, Flightpath Biosciences, and Delve Bio and Co-Founder of Delve Bio

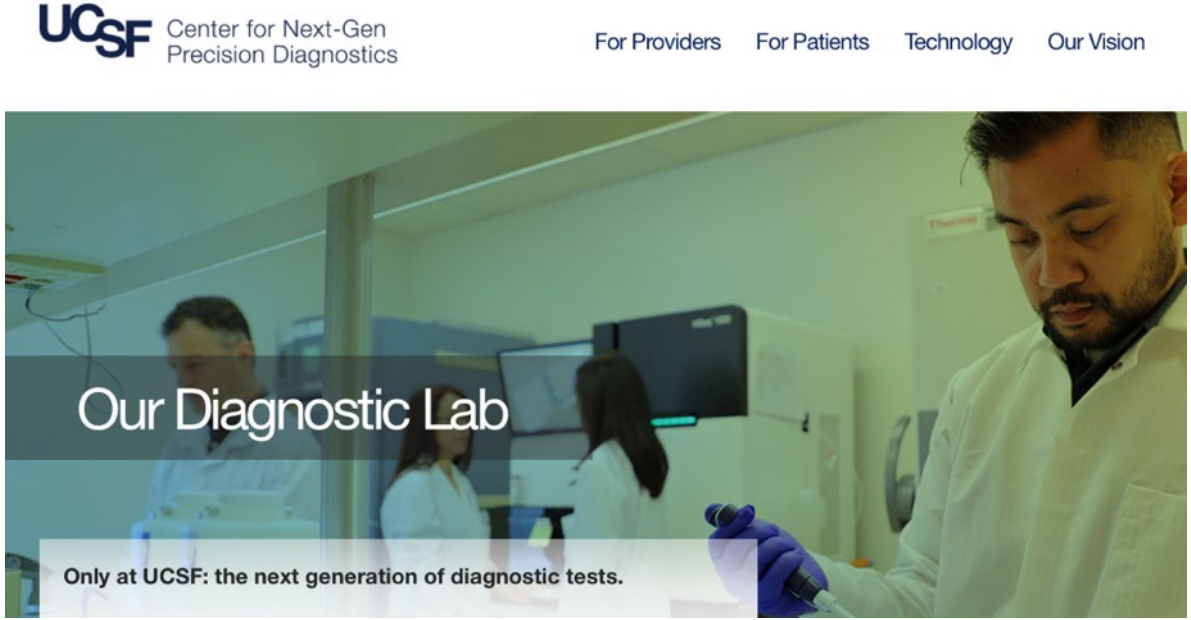
METAGENOMIC NEXT-GENERATION SEQUENCING (mNGS)

1. How it is being used for clinical diagnosis of infections
2. How to optimize mNGS assays and accelerate adoption in public health – *increased automation, increased throughput, decreased turnaround times, and lower cost, identification of clinical use cases*
3. How it is being used in public health for new pathogen detection and characterization
4. How host response can be used to complement mNGS and inform pathogenicity for new pathogens
5. Key limitations and challenges for mNGS in the near future

CLINICAL mNGS ASSAYS AT UCSF

- CSF mNGS*#
- Plasma mNGS* (re-validation in progress with launch in summer)
- Viral Respiratory mNGS*#
- Body fluids mNGS*

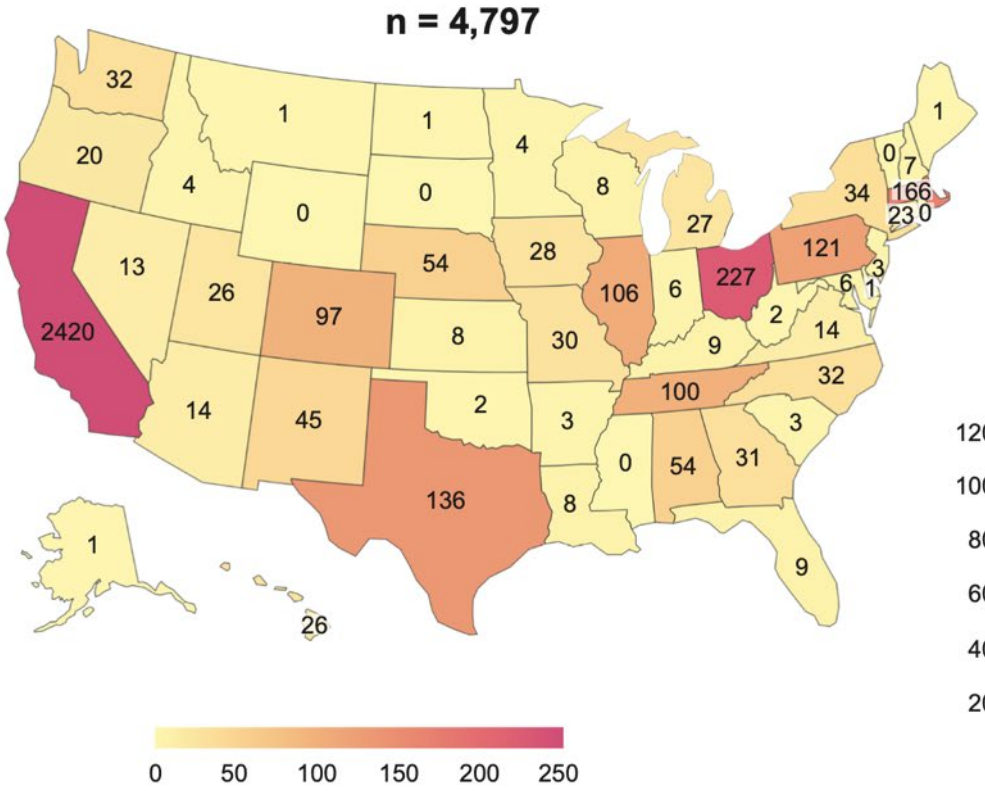
**all tests are LDTs and not FDA-approved IVDs; #granted breakthrough device designation by the FDA*

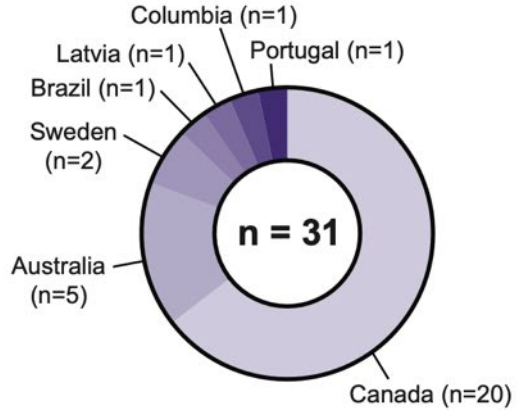


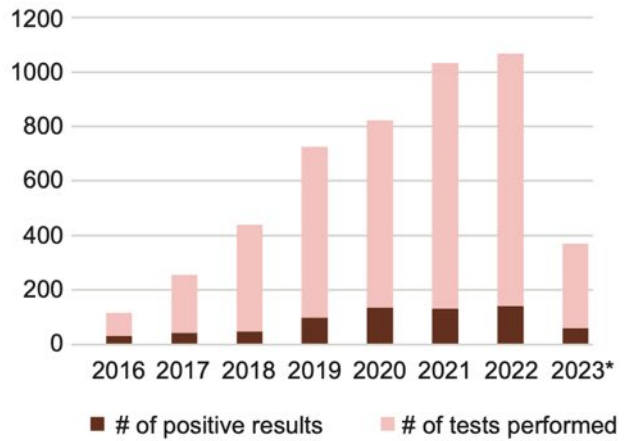
1. Miller, et al., *Genome Research*, 29(5): 831-842.
2. Wilson, et al., *NEJM*, 380(24):2327-2340.
3. Gu, et al., *Nature Medicine*, 27(1):115-124.

http://nextgendiagnostics.ucsf.edu

CSF mNGS TESTING – OUR LONGITUDINAL 7 YEAR EXPERIENCE

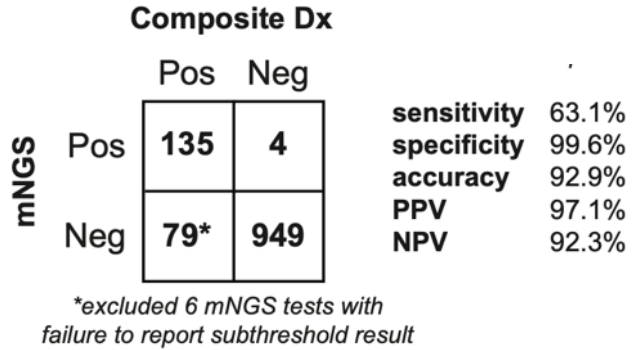


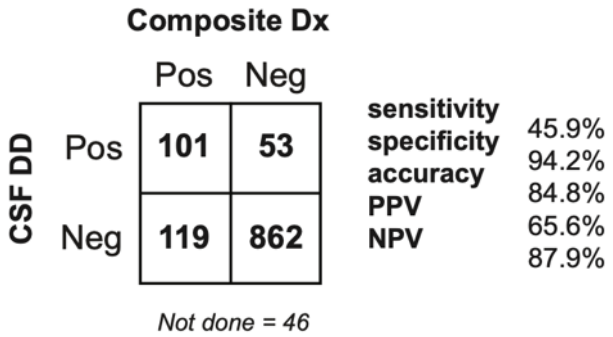


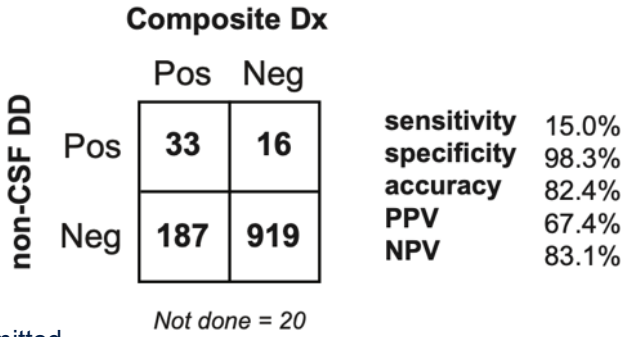


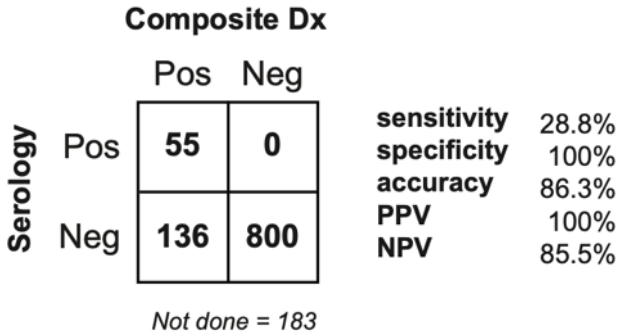
Benoit, et al., 2024, in press

CSF mNGS PERFORMANCE









Benoit, et al., 2024, submitted

48 (21.8%) of 220 infections from 1,053 patients detected only by mNGS

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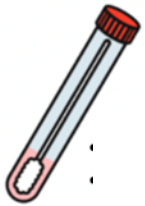
VIRAL RESPIRATORY mNGS TESTING



Clinical sample

Nucleic acid extraction with Depletion of host nucleic acids

cDNA synthesis from RNA (2 hours)

Tecan MagicPrep Instrument (5 hours)

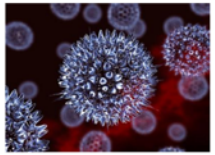
- *48-72 h TAT → 12-24 h TAT; >150 steps → 15 steps*
- *Granted FDA breakthrough device designation August 29, 2023*

5 hours (MiniSeq)

18 hours (NextSeq)

Data Processing

Data Analysis



Pathogen Identification

Project funded by CDC and BARDA
Tan, et al., manuscript in preparation

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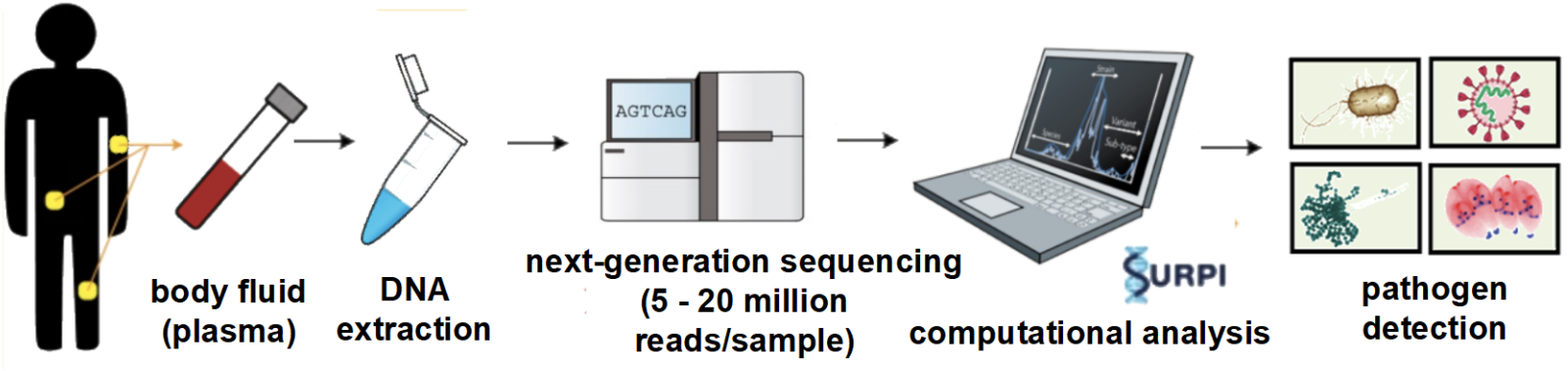
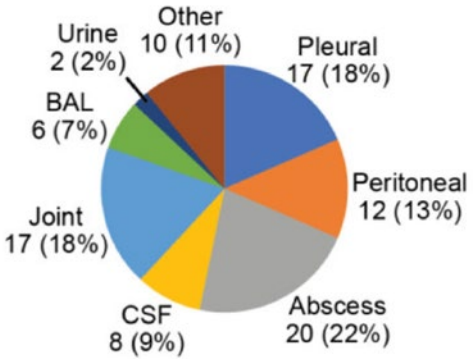
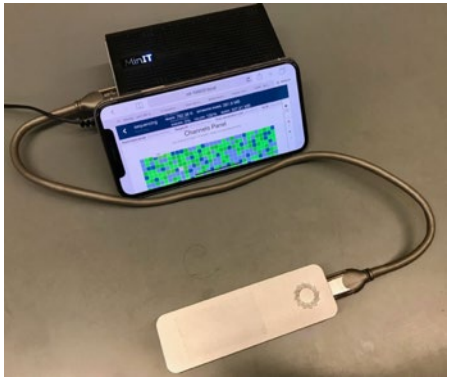
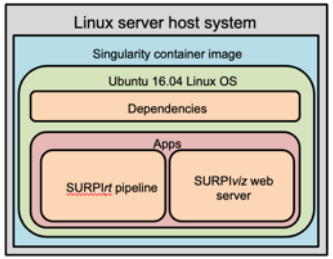
POINT OF CARE NANOPORE METAGENOMIC SEQUENCING







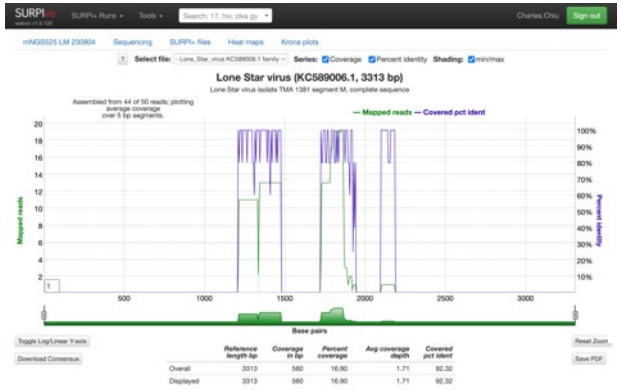


Cloud-based classifiers:

- **SURPI*rt***
- **BugSeq**
- **Kraken**
- **CZ-ID**

- Gu, et al., 2021, *Nature Medicine*, 25:115-124.
- Chandrakumar, et al., 2022, *Communications Biology*, 5(1):151
- Kalantar, et al., 2020, *Gigascience*, 9(10): giaa111
- Lu, et al., 2022, *Nature Protocols*, 17(12):2815-2839

DETECTION OF NOVEL EMERGING VIRAL PATHOGENS BY mNGS



Aedes albopictus **mosquito**

Ambylomma americanum **tick**

Chiu, et al., 2024, unpublished

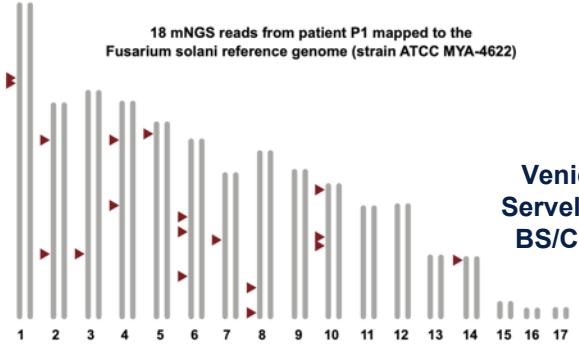
Potosi and Lone Star bunyaviruses
(immunocompromised patients with fatal encephalitis)

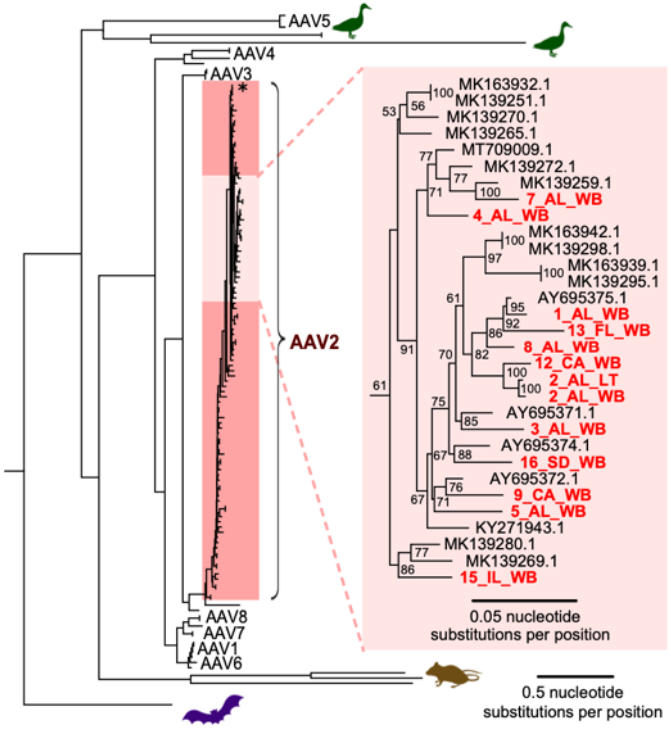
Fusarium solani
(fungal meningitis outbreak in Mexico associated with surgical procedures)

Species	Patient P1
[Nectria] haematococca	
Rhodotorula sphaerocarpa	
Fusarium fujikuroi	
Malassezia restricta	
Penicillium rubens	
Aspergillus niger	
*	

Analytical, DNA prep	
count	13
RPM(pp)	1.4984
RPM(pp) ratio 0.1	14.984
RPM(pp) ratio 0.5	2.9968
RPM(pp) ratio 1.0	1.4984

Smith, et al., 2024, *OFID*, 10(Supplement 2)
Chiu, et al., 2024, unpublished





Venice Servellita, BS/CLS

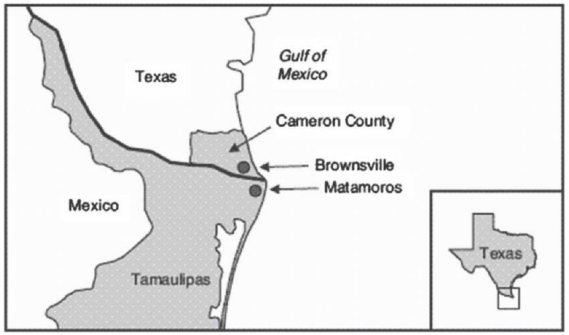
Adeno-associated virus 2
(severe acute pediatric hepatitis)

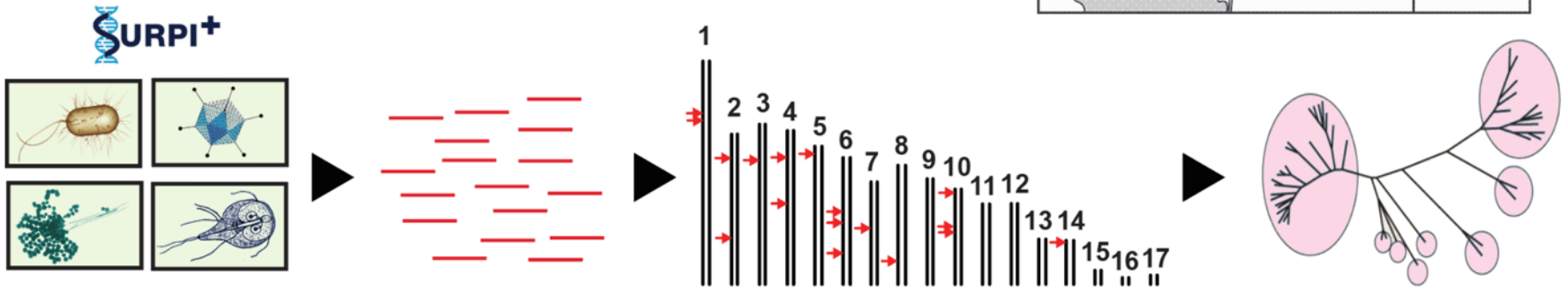
Servellita, et al., 2024, *Nature* 617:574-580

METAMELT (METAGENOMIC MULTIPLE EXTENDED LOCUS TYPING) ANALYSIS

Fungal Meningitis Outbreak Associated with Procedures Performed under Epidural Anesthesia in Matamoros, Mexico

Smith, et al., *CID,* 2023
Ramos, et al., *AJTMH,* 78(3):;364-369





mNGS analysis of patient CSF, plasma, or brain tissue

Identify F. solani reads

Map reads to F. solani reference genome, extract, and concatenate

Phylogenetic analysis of concatenated sequences

Chiu, et al., 2024, submitted

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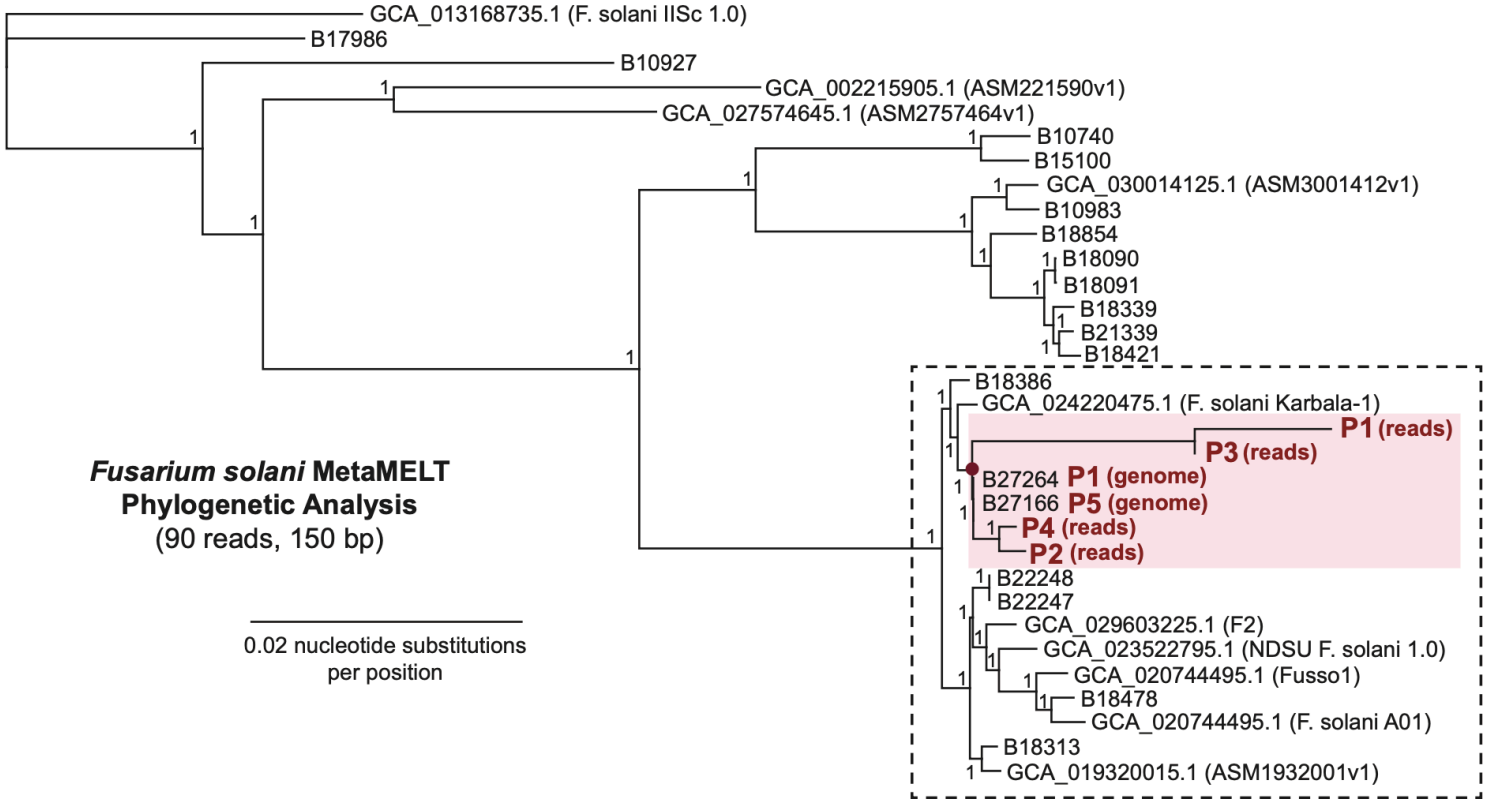
METAMELT IDENTIFIES A LIKELY POINT SOURCE FOR THE OUTBREAK



Chiu, et al., 2024, submitted

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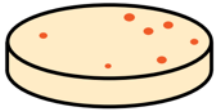
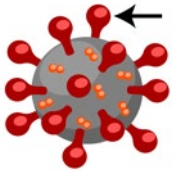
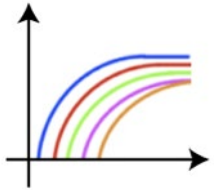
DIRECT VS. INDIRECT DIAGNOSIS OF INFECTION



culture



antigen testing

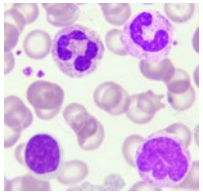


nucleic acid testing (PCR)

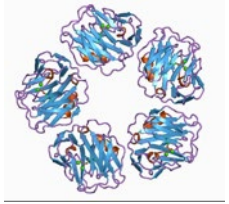
DNA/RNA Sequencing (mNGS)

PATHOGEN

serology



cell count / differential

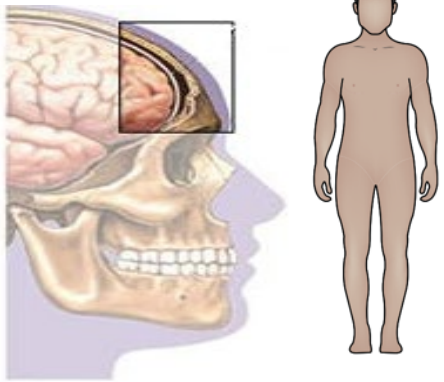


protein expression (CRP, procalcitonin, etc.)

HOST

MACHINE LEARNING CLASSIFER FOR CNS INFECTIONS

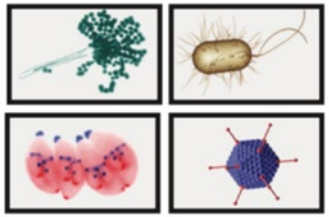
Patient with Meningitis and/or Encephalitis



CSF mNGS

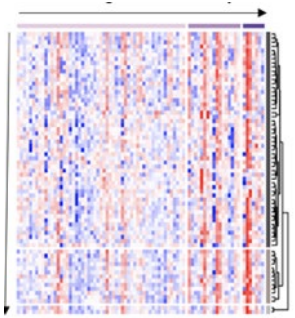
Non-human Reads

Alignment to pathogen database



Human RNA Reads

Host Response



Omura and Chiu, in preparation

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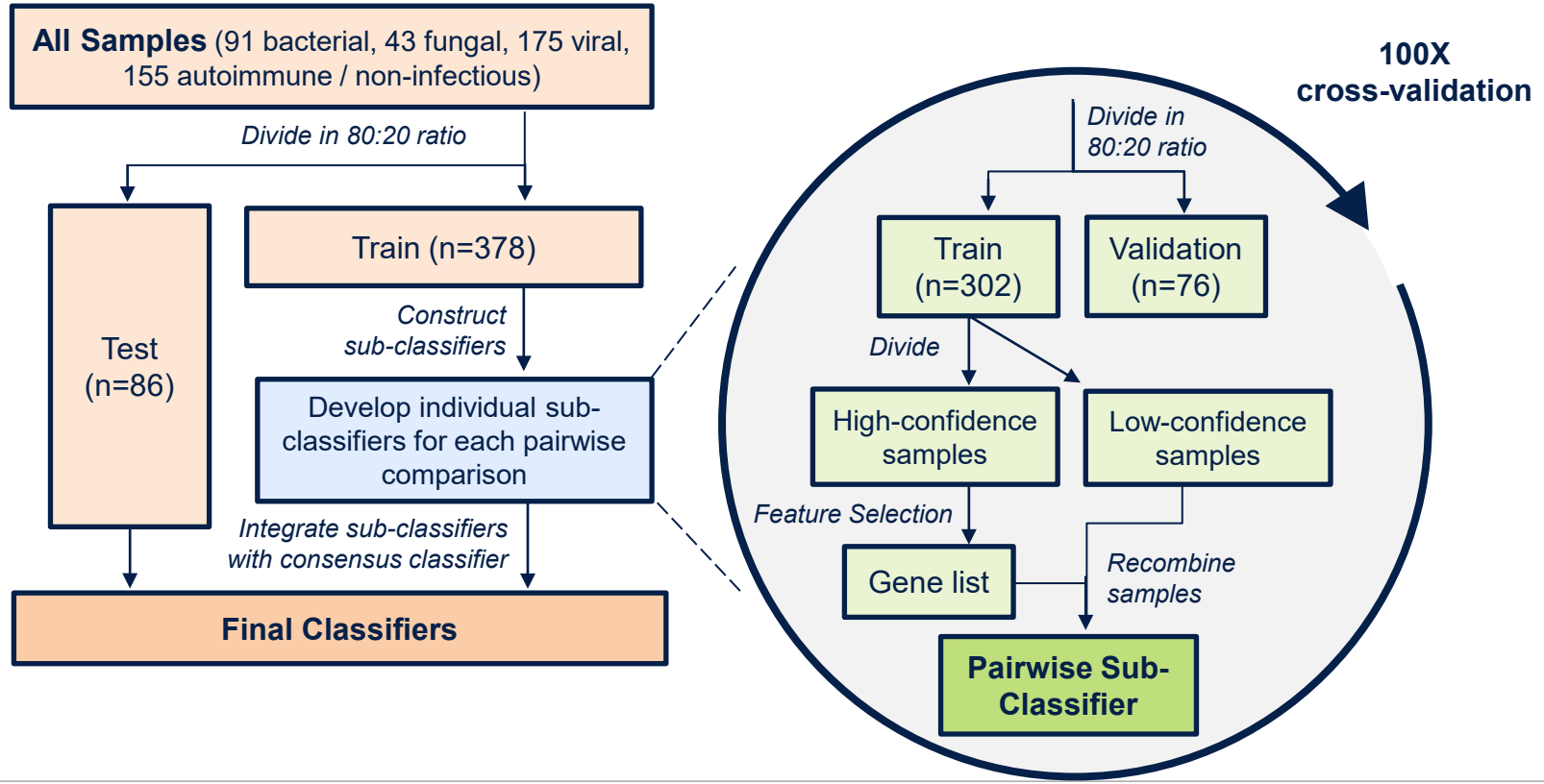
MACHINE LEARNING CLASSIFER FOR CNS INFECTIONS



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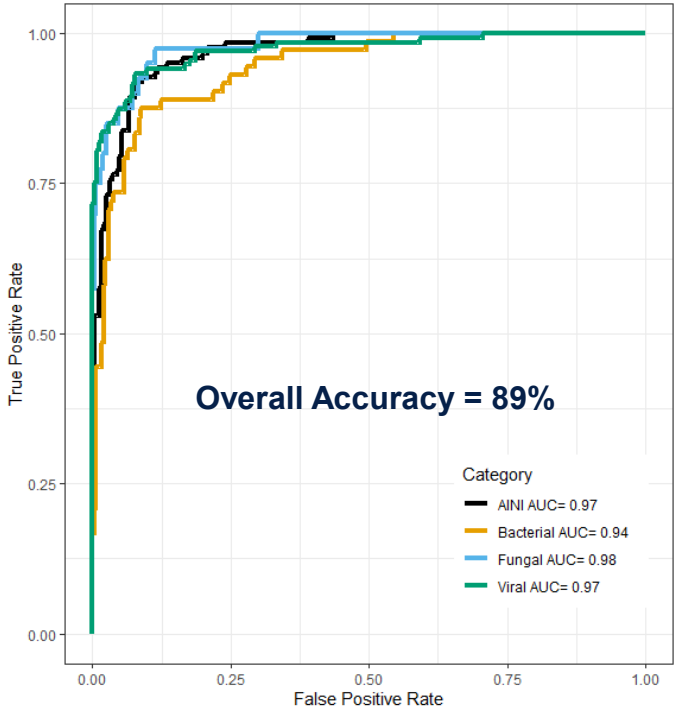
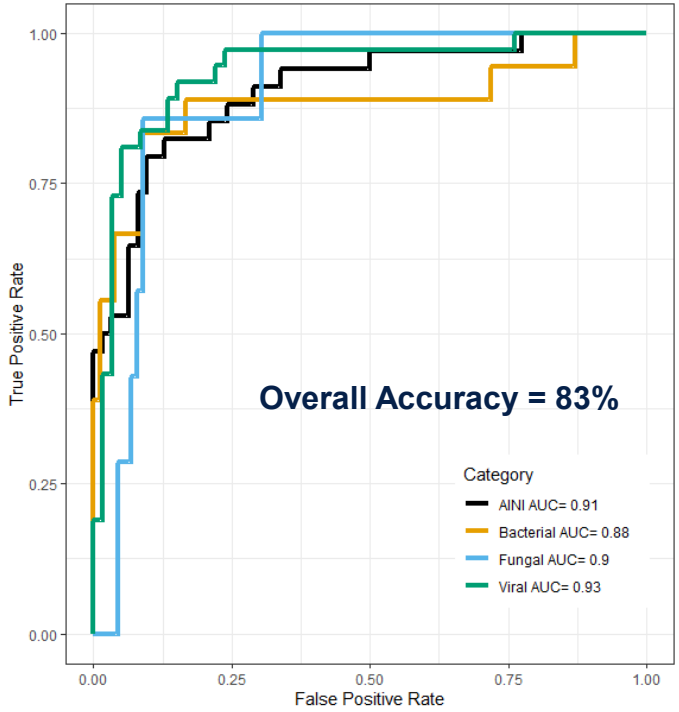
CLASSIFIER PERFORMANCE

Training Set



Test Set



Omura and Chiu, et al, 2024, manuscript in preparation

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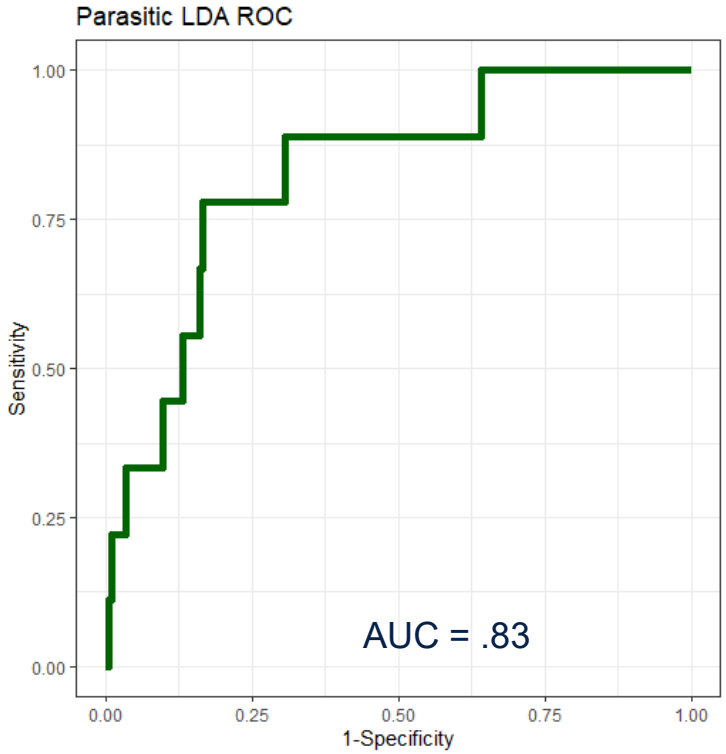
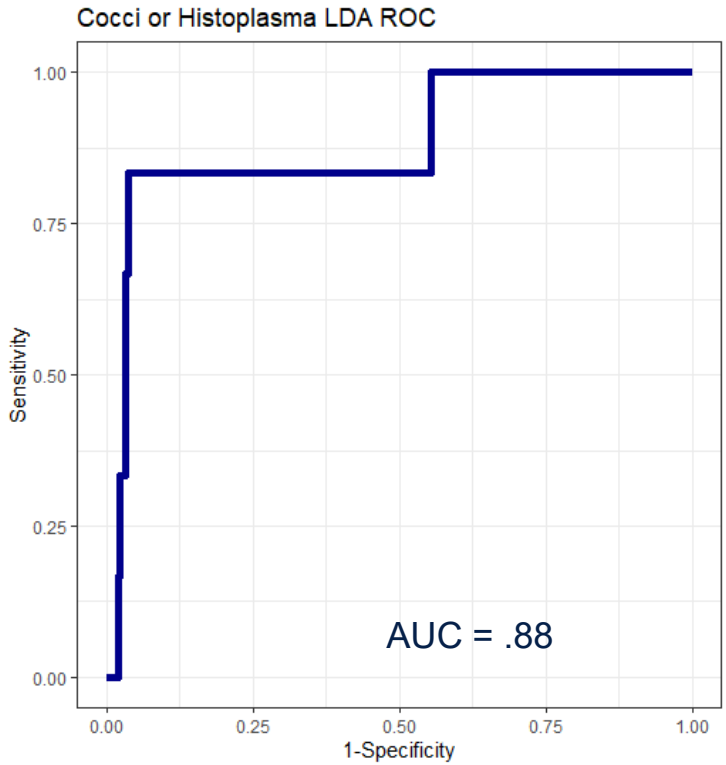
SUBCATEGORY CLASSIFICATION FOR SPECIFIC INFECTIONS





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CLASSIFIER RESULTS (EXAMPLES)

MNC_6532	Score		Signature
Non-infectious	1%		None
Bacterial (typical)	29%		Moderate
Bacterial (atypical)	29%		Moderate
Mycobacterial	21%		Weak
Fungal	25%		Moderate
Dimorphic fungi-related	71%		Strong
Mold-related	3%		None
Viral	1%		None
EV-associated AFM-related	0%		None
Other			
Parasitic	4%		None
Worm-related	0%		None

Coccidioides immitis
(subthreshold mNGS result, RPM ratio=0.1)

Omura and Chiu, 2024, et al, manuscript in preparation

CLASSIFIER RESULTS (EXAMPLES)

MNC_6232	Score		Signature
Autoimmune/Noninfectious	0		No Signature
Bacterial (typical)	0		No Signature
Bacterial (atypical)	10		Strong
Fungal	6		Moderate
Viral	0		No Signature
Parasitic	29%		Possible
Worm	4%		Possible
Flavivirus	13%		Possible
Mycobacterium	24%		Possible
Dimorphic Fungi	9%		Possible
Mold vs rest	2%		Possible
Enterovirus- AFM	0%		Unlikely

Mycobacterium tuberculosis
(culture-negative; subthreshold mNGS result, with RPM ratio=3.1)

CLASSIFIER RESULTS (EXAMPLES)

MNC_5932	Score	Signature
Autoimmune/Noninfectious	10	Strong
Bacterial (typical)	1	Very Weak
Bacterial (atypical)	1	Very Weak
Fungal	0	No Signature
Viral	1	Very Weak
Parasitic	1%	Unlikely
Worm	0%	Unlikely
Flavivirus	2%	Possible
Mycobacterium	0%	Unlikely
Dimorphic Fungi	1%	Unlikely
Mold vs rest	2%	Possible
Enterovirus- AFM	3%	Possible
Amyloid	21%	Possible
Lupus	0%	Unlikely
Solid Organ Cancer	0%	Unlikely

CNS amyloidosis

Hospitalized with altered mental status, encephalopathy, fatigue, and neutrophilic pleocytosis; brain biopsy performed after discharge consistent with cerebral amyloid angiopathy

Omura and Chiu, et al, 2024, manuscript in preparation

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LIMITATIONS OF GENOMIC DATA

1. Provides only indirect insights into pathogenicity – serology, animal models
2. Does not yield functional information – for example, prediction of antimicrobial resistance from genomic data alone is consistent
3. Not useful without annotated metadata
4. Limited by information available in incomplete and biased reference databases
5. Much consider privacy and confidentiality considerations (HIPAA-protected data)
6. Lack of standardization in how the data is generated and analyzed
7. Still too expensive, too slow, too complex, and has an unclear role in clinical microbiology or public health
8. Role of the stakeholders in geneerating, analyzing, and maintaining data is unclear, as is how these efforts will be funded across public health, academia, and industry
9. Microbial genomics is focused on the pathogen and not on the patient (host) response

TAKE-HOME MESSAGES

1. mNGS is an agnostic approach that does not rely on targeted primers or probes so is particularly well-suited for emerging pathogen surveillance
2. Host response profiling is complementary to mNGS and may yield insights into pathogenicity and be useful in enhancing diagnostic yield of mNGS and differential diagnosis of non-infectious syndromes
3. Most genomic studies for non-viral pathogens requires culturing the organism, which is slow and laborious; other approaches such as deeper sequencing or capture probe enrichment are needed
4. Useful information can be extracted from mNGS data despite poor recovery of the genome
5. Public health applications of mNGS include clinical pathology, wastewater analysis, sterility testing of biologics, veterinary, forensic applications
6. Host response (or omics profiling in general) can expand the utility of mNGS by characterization of the host response to an infection to better understand the pathogenicity of an emerging microorganism
7. mNGS testing is beginning to make an impact on patient care – potential for expansion from clinical to public health applications

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- California Initiative to Advance Precision Medicine

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https://chiulab.ucsf.edu