UCSF School of Medicine

Metagenomic Sequencing and Host Profiling for Diagnosis of Infections in Immunocompromised Hosts

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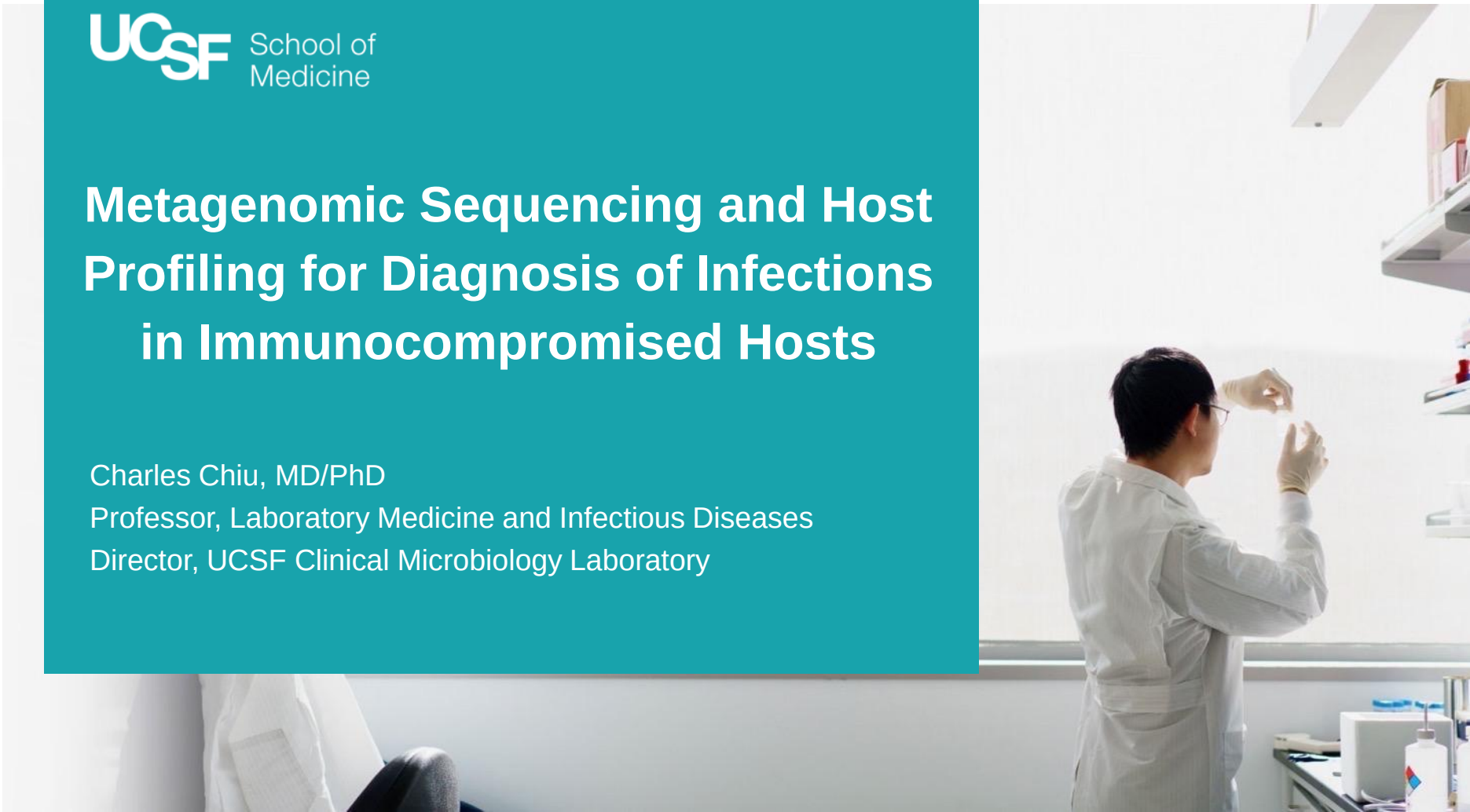


DISCLOSURES

- 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

SUMMARY OF PRESENTATION

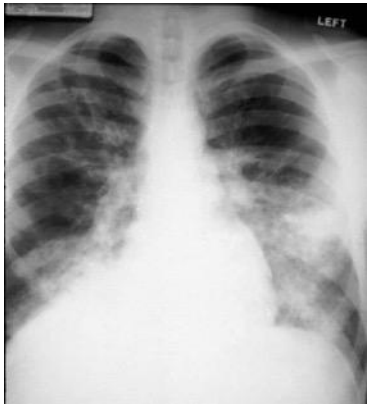
1. Clinical metagenomic sequencing of body fluids

2. Diagnostic yield and clinical utility of mNGS testing
    - Cerebrospinal fluid mNGS
    - Plasma mNGS

3. Incorporation of RNA host response profiling for precision diagnosis of infections

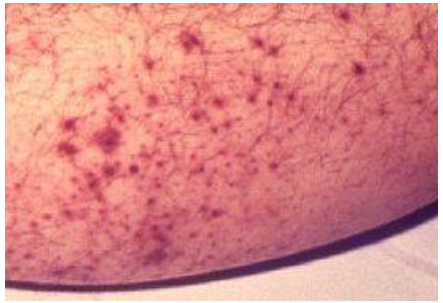
4. Case studies

DIAGNOSTIC CHALLENGES IN INFECTIOUS DISEASES



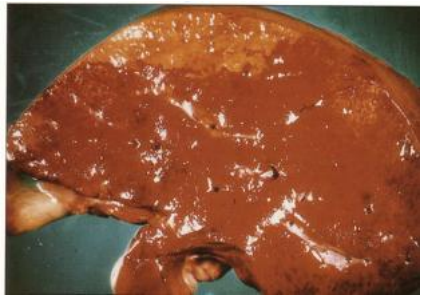
Pneumonia

15 – 60% unknown cause



Fever / Sepsis

20-40% unknown cause

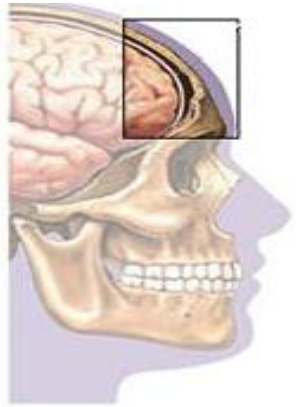


Hepatitis

30 – 50% unknown cause

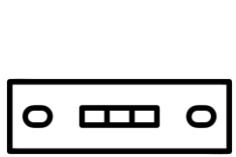
Meningitis / Encephalitis

40 – 60% unknown cause



1. Van Gageldonk-Lafeber, (2005) *CID* 41:490-497
2. Louie, et al., (2005) *CID* 41:822-828
3. Ewig, et al. (2002) *Eur Respir J* 20:1254-1262
4. Jain, et al., (2015) *NEJM* 373(5)
5. Glaser, et al., (2006) *CID* 43:1565-1577
6. Vora, et al., (2010) *Neurology* 82:443-451
7. Eber, et al. (2010) *Arch Intern Med* 170:347-353
8. Vincent, et al. (2015) *Critical Care Med* 43(11)
9. Fulton, et al., (2020) *PloS ONE* 15(1):e0226895
10. Squires, et al., (2006) *J Pediatr* 148:652-8
11. Alonso, et al., (2017) *Hepatology* 65(3):1026-1037.

DIAGNOSTIC TESTS IN MICROBIOLOGY



Antigen Testing

Culture

Multiplex PCR panels

Metagenomic next-generation sequencing (mNGS)

Most specific "biased / targeted"

Least specific "unbiased / agnostic"

PCR

Serology

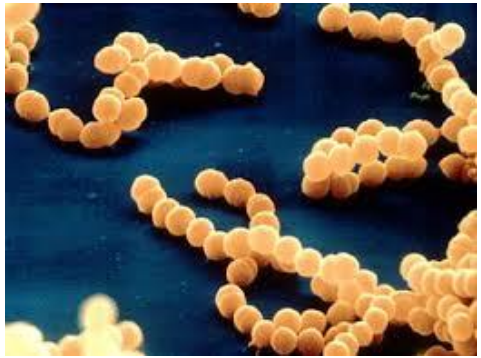
Histopathology

Universal PCR

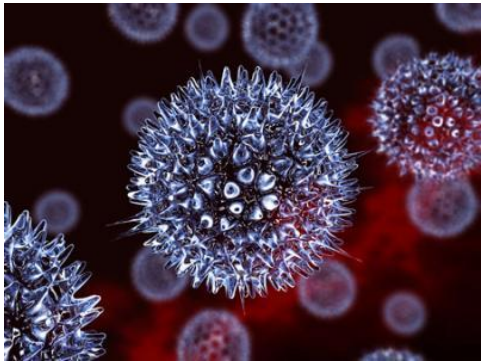
- bacteria: 16S RNA
- fungi: 18S rRNA and ITS
- mycobacteria: *rpoB, hsp65*

METAGENOMIC NEXT-GENERATION SEQUENCING (mNGS)

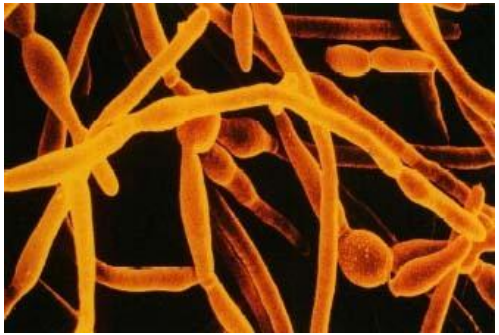
Bacteria



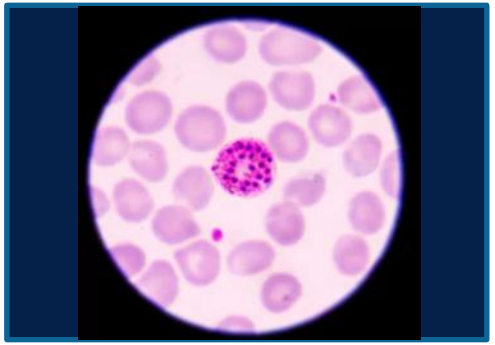
Viruses



Fungi



Parasites



Pan-diagnosis of infections using mNGS without a priori clinical suspicion

Chiu, et al., 2019, *Nature Review Genetics, 20:341-355.*

CLINICAL mNGS ASSAYS AT UCSF

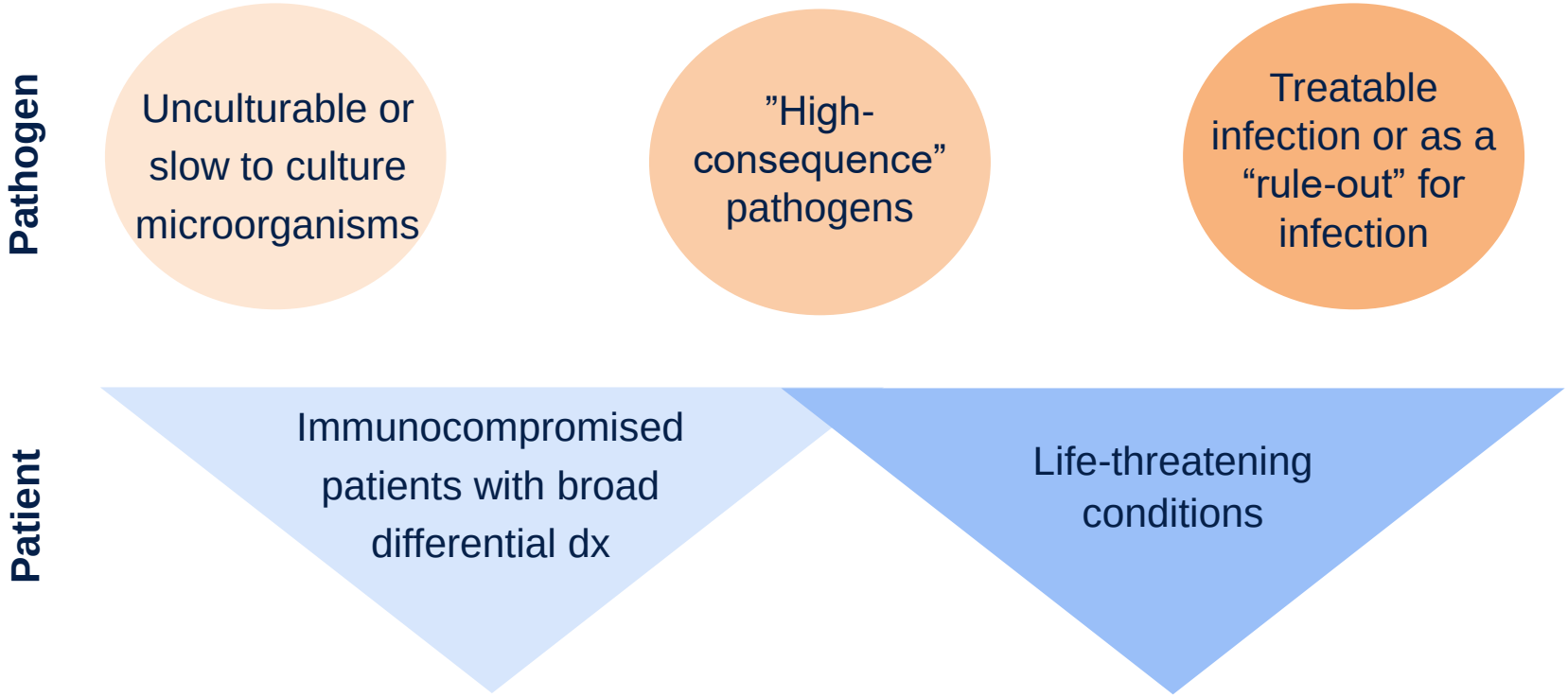
- CSF mNGS*# (since 2016)
- Plasma mNGS* (2019, relaunched Jan 2025)
- Viral Respiratory mNGS*#
- Body Fluids mNGS*

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

1. Miller, et al., 2019, *Genome Research*, 29(5): 831-842
2. Wilson, et al., 2019, *NEJM*, 380(24):2327-2340
3. Gu, et al., 2021, *Nature Medicine*, 27(1):115-124
4. Tan, et al., 2024, *Nature Communications,* 15:9016.

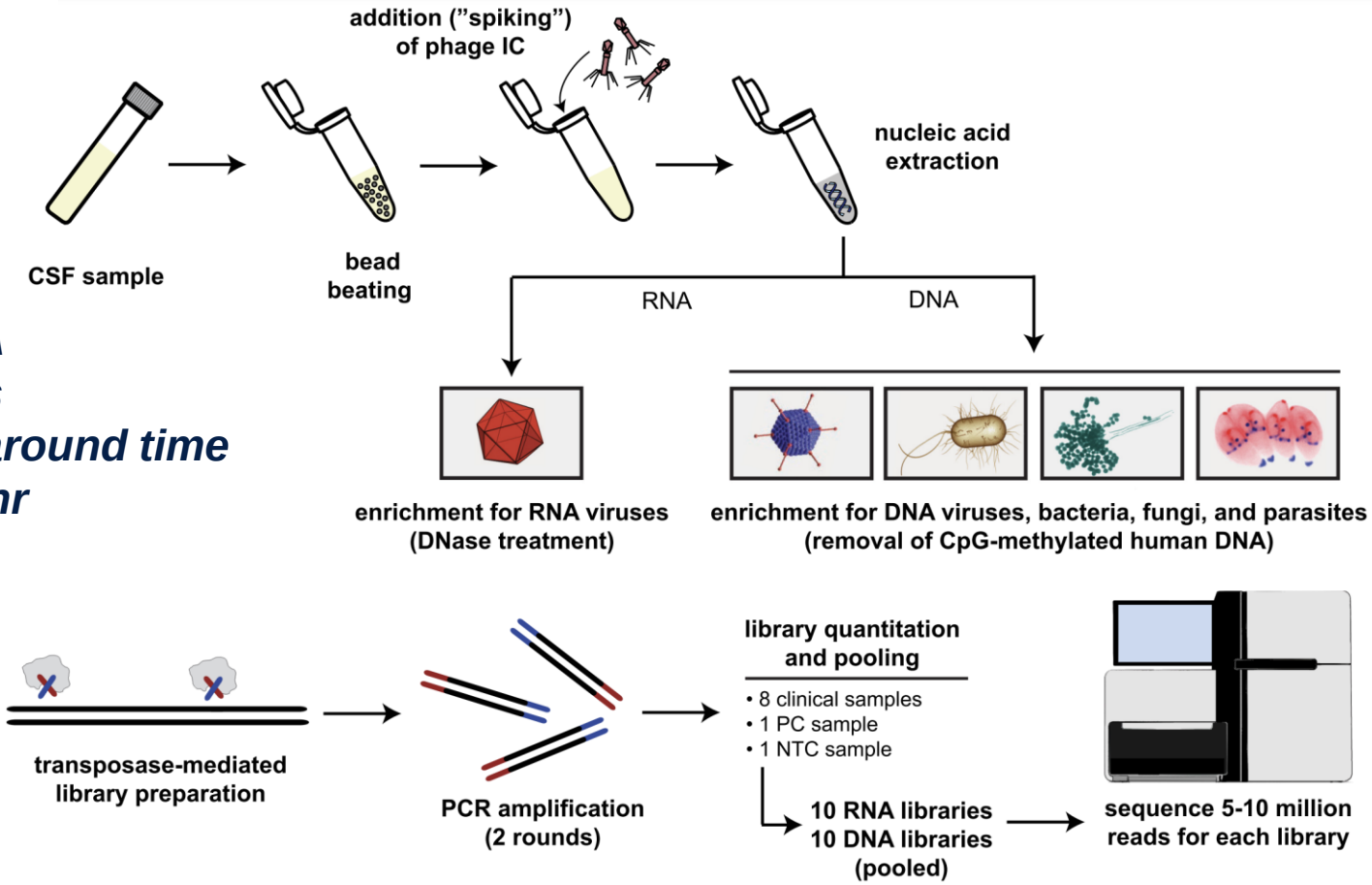
http://nextgendiagnostics.ucsf.edu

POTENTIAL CLINICAL INDICATIONS FOR mNGS TESTING



UCSF CSF mNGS TESTING (2016, MANUAL PROCESSING)

- *DNA/RNA*
- *250 steps*
- *Lab turnaround time of 48-72 hr*

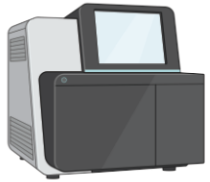


UCSF PLASMA mNGS TESTING (2024, AUTOMATED PROCESSING)

plasma sample

Qiagen EZ1 DNA Extraction

Tecan MagicPrep Instrument

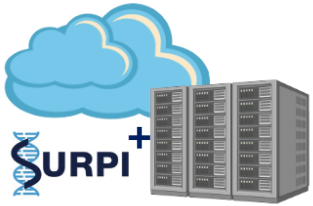


Sequencing on Illumina NextSeq 550 Dx

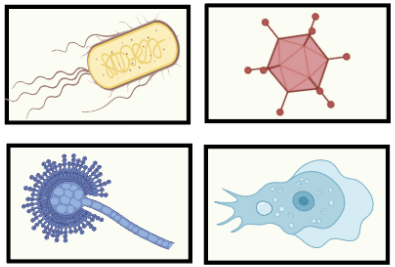
SAMPLE PROCESSING (6 hr)

SEQUENCING (15 hr)

- ***DNA only***
- ***~50 steps***
- ***Lab turnaround time <24 hours***



Computational Analysis



ANALYSIS AND PATHOGEN IDENTIFICATION (2 hr)

REPORTING (1 hr)

Gu, et al., 2021, *Nature Medicine*, 27(1):115-124
Tan, et al., 2024, *Nature Communications,* 15:9016.
Streithorst, et al., 2025, in preparation

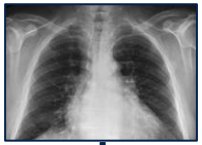
DIAGNOSTIC YIELD AND CLINICAL IMPACT IN INFECTIOUS DISEASES

Medical history

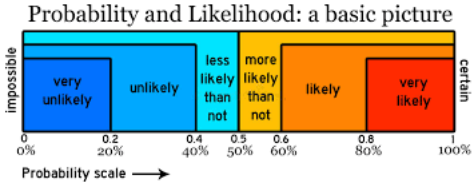
Physical exam

Rapid lab tests

Imaging



Differential diagnosis



Empirical therapy

(pathogen not identified)

Diagnostic testing

POS | NEG

Stop antimicrobials
Non-infectious therapy

(no pathogen | rule-out)

Targeted therapy

(pathogen identified)

Management change

Management change also includes:

- Public health investigation (outbreaks)
- Infection control intervention (isolation)
- Cancel further invasive testing (biopsy)

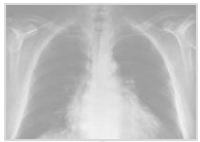
POTENTIAL DIAGNOSTIC YIELD AND CLINICAL IMPACT OF mNGS?

Medical history

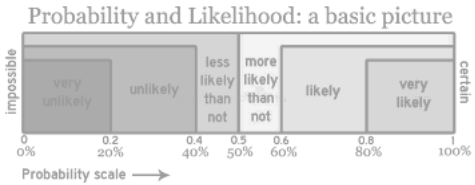
Physical exam

Rapid lab tests

Imaging



Differential diagnosis



Can we bypass or expedite earlier steps in the diagnostic process with mNGS?

Empirical therapy

(pathogen not identified)

Diagnostic testing

mNGS

POS | NEG

Management change

Stop antimicrobials
Non-infectious therapy

(no pathogen | rule-out)

Targeted therapy

(pathogen identified)

Management change also includes:

- Public health investigation (outbreaks)
- Infection control intervention (isolation)
- Cancel further invasive testing (biopsy)

DIAGNOSTIC YIELD OF CSF mNGS

nature medicine

Article

https://doi.org/10.1038/s41591-024-03275-1

Seven-year performance of a clinical metagenomic next-generation sequencing test for diagnosis of central nervous system infections

Received: 21 April 2024

Accepted: 28 August 2024

Published online: 12 November 2024

Check for updates

A list of authors and their affiliations appears at the end of the paper

Metagenomic next-generation sequencing (mNGS) of cerebrospinal fluid (CSF) is an agnostic method for broad-based diagnosis of central nervous system (CNS) infections. Here we analyzed the 7-year performance of clinical CSF mNGS testing of 4,828 samples from June 2016 to April 2023 performed by the University of California, San Francisco (UCSF) clinical microbiology laboratory. Overall, mNGS testing detected 797 organisms from 697 (14.4%) of 4,828 samples, consisting of 363 (45.5%) DNA viruses, 211 (26.4%) RNA viruses, 132 (16.6%) bacteria, 68 (8.5%) fungi and 23 (2.9%) parasites. We also extracted clinical and laboratory metadata from a subset of the samples ($n = 1,164$) from 1,053 UCSF patients. Among the 220 infectious diagnoses in this subset, 48 (21.8%) were identified by mNGS alone. The sensitivity, specificity and accuracy of mNGS testing for CNS infections were 63.1%, 99.6% and 92.9%, respectively. mNGS testing exhibited higher sensitivity (63.1%) than indirect serologic testing (28.8%) and direct detection testing from both CSF (45.9%) and non-CSF (15.0%) samples ($P < 0.001$ for all three comparisons). When only considering diagnoses made by CSF direct detection testing, the sensitivity of mNGS testing increased to 86%. These results justify the routine use of diagnostic mNGS testing for hospitalized patients with suspected CNS infection.

Meningitis, encephalitis and/or myelitis associated with infections of the central nervous system (CNS) can cause severe and often life-threatening illness[1,2]. Timely diagnosis and treatment are paramount to improve clinical outcomes for these infections, and delays have been associated with increased morbidity and mortality[3,4]. A comprehensive infectious workup requires a combination of culture-based, serologic and nucleic acid amplification testing. However, it is estimated that the cause of meningoencephalitis remains unknown in approximately 50% of cases, which hinders clinical management and the initiation of appropriate and effective therapy[5,6].

In recent years, clinical metagenomic next-generation sequencing (mNGS) has emerged as a comprehensive approach for infectious disease diagnosis, enabling simultaneous detection of a wide range of microorganisms, including bacteria, viruses, fungi and parasites, without targeting any specific pathogen a priori[7,8]. This agnostic, hypothesis-free method can be particularly useful in CNS infections for which the differential diagnosis is broad, with overlapping clinical manifestations, and for which cerebrospinal fluid (CSF) and brain biopsy tissue samples are often limited in volume and availability[9–11].

The University of California, San Francisco (UCSF) CSF mNGS test, referred henceforth as 'mNGS test/testing', was developed in 2016 as a clinically validated DNA/RNA metagenomic sequencing assay performed by the Clinical Laboratory Improvement Amendments-certified UCSF clinical microbiology laboratory[11]. A prior prospective study

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PEDIATRICS

15.2%

Patients <18 years of age

INPATIENT

87.7%

Inpatient, including 38.7% ICU
12.3% Outpatient

DISEASE SEVERITY

10.2%

Death at 60 days

12 [5, 25]

Median length of stay (days)

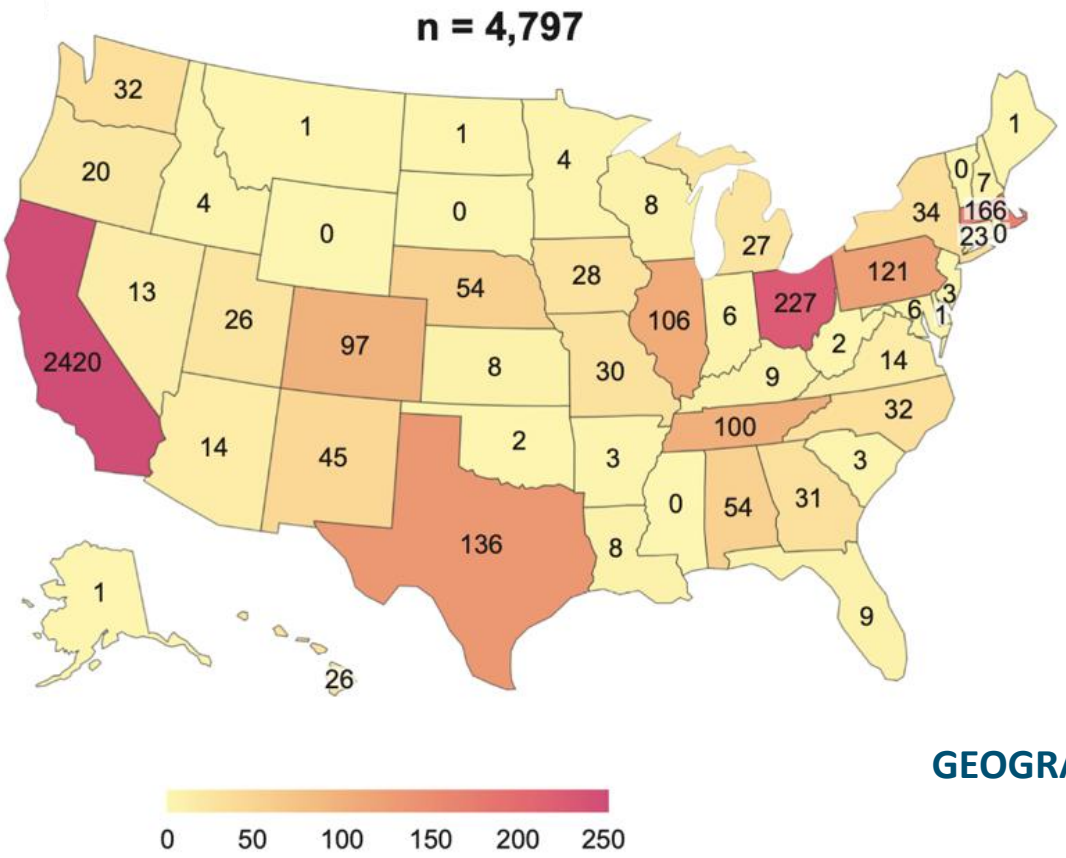
IMMUNOCOMPROMISED

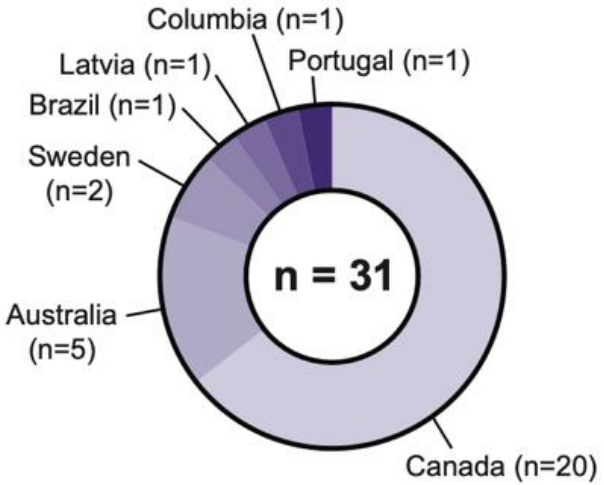
35.8%

Patients with immunosuppression

Benoit, et al., 2024, *Nature Medicine*, 30:3522-3533

CSF mNGS TESTING – OUR LONGITUDINAL 7 YEAR EXPERIENCE



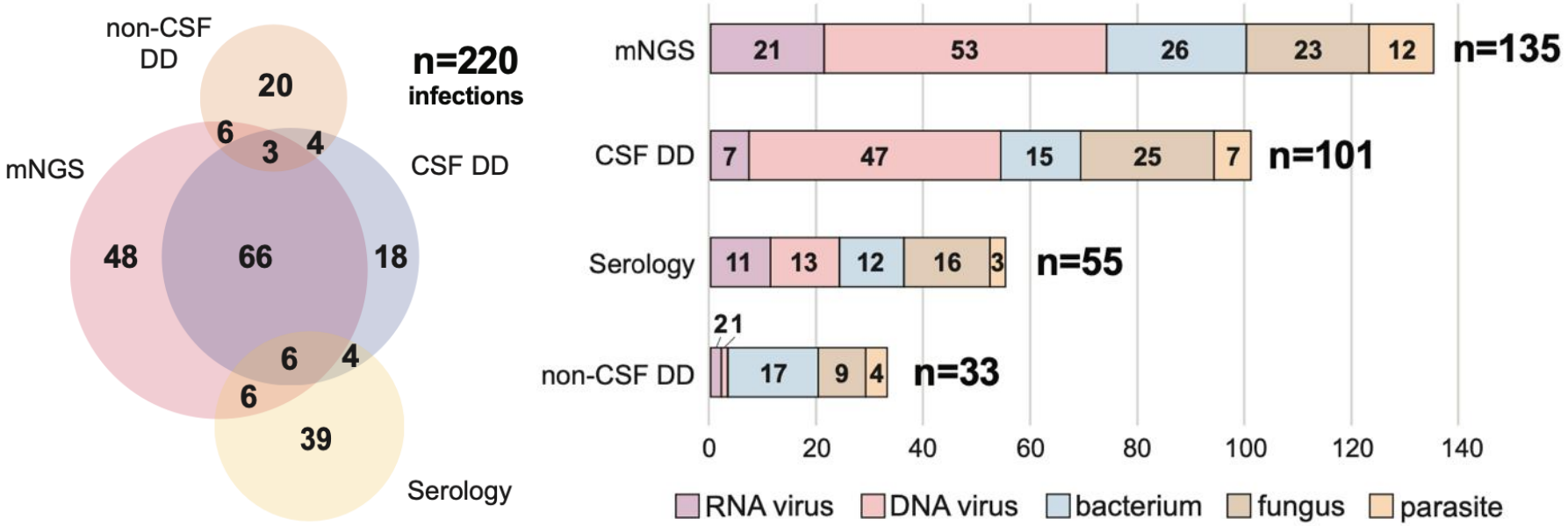


GEOGRAPHIC ORIGIN FOR CSF mNGS SAMPLES

Benoit, et al., 202, *Nature Medicine,* 30(12):3522-3533

CSF mNGS DIAGNOSTIC YIELD VERSUS OTHER TESTS

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



DD = direct detection methods (culture, antigen, and/or PCR testing)

Benoit, et al., 2024, *Nature Medicine*, 30:3522-3533

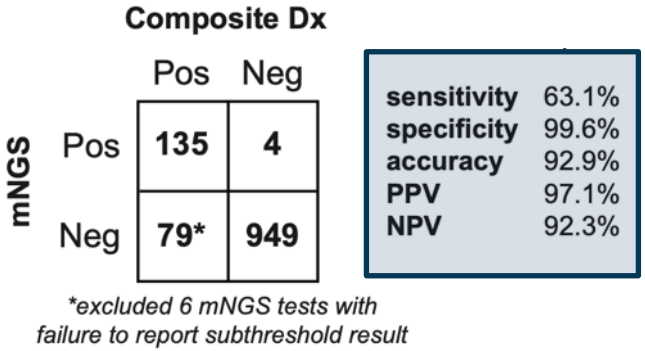
SUBTHRESHOLD DETECTION OF LOW-TITER, HIGH-CONSEQUENCE PATHOGENS

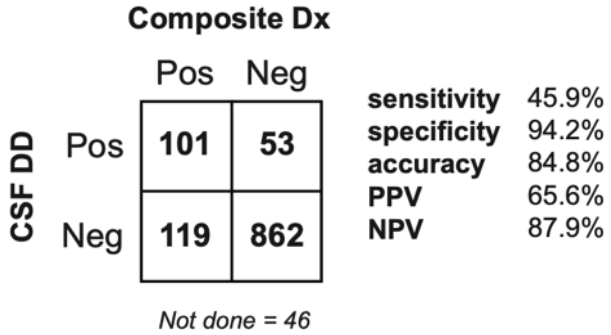
	Sequencing results[d]			
	Total (n = 4828 samples)	UCSF (n=1130 samples)	Non-UCSF (n=3698 samples)	
Positive samples, excluding possible or likely contaminant	697 (14.4%)	183 (16.2%)	514 (13.9%)	P = 0.05
Samples with positive subthreshold results[g]	98 (2%)	32 (2.8%)	66 (1.8%)	P = 0.03
Samples with single organism, possible contamination	164 (3.4%)	23 (2%)	141 (3.8%)	P = 0.004
Samples with Multiple bacterial/fungal taxa, probable contaminant	348 (7.2%)	61 (5.4%)	287 (7.8%)	P = 0.007
Number of detected organisms (after exclusion of contamination)	797	222	575	

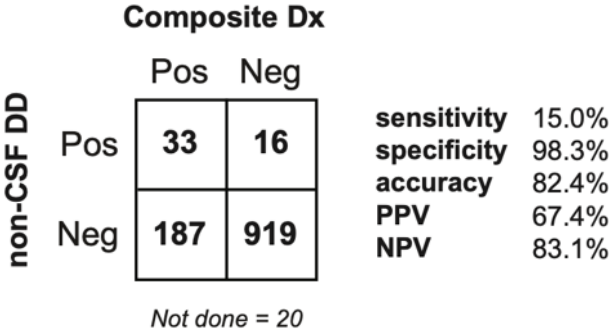
93% of *Coccidioides sp.*, 92% of *Mycobacterium tuberculosis*, 66% of *Balamuthia mandrillaris*, 50% of *Histoplasma capsulatum,* 29% of West Nile virus, and 21% of Powassan virus detection were subthreshold

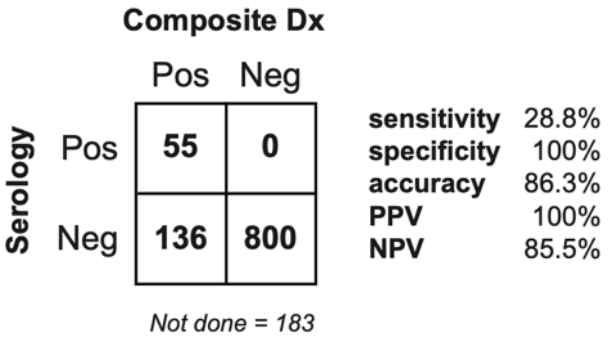
Benoit, et al., 2024, *Nature Medicine*, 30:3522-3533

CSF mNGS PERFORMANCE









63.1% sensitivity (increases to 86% when considering only direct detection modalities) and 99.6% specificity

Benoit, et al., 2024, *Nature Medicine*, 30:3522-3533

REPRESENTATIVE CSF mNGS POSITIVE CASES

Acanthamoeba sp.
Angiostrongylus cantonensis
Aspergillus sp.
Astroviruses
Balamuthia mandrillaris
Borrelia burgdorferi
Brucella melitensis
Cache Valley bunyavirus
Candida sp.
Chlamydia psitacci
Colorado tick fever virus
Coxiella burnetti
Cryptococcus neoformans
Enterobacter aerogenes
Enterovirus A71/D-68
Fusarium solani
Hepatitis E virus
Human herpesviruses 1-8
JC polyomavirus
Leptospira sp.
MW polyomavirus
Mycobacterium sp.
Mycoplasma pneumoniae
Naegleria fowleri
Neisseria meningitidis
Parvovirus B19
Potosi virus
Powassan virus
Rubella virus
St. Louis encephalitis virus
Scedosporium sp.
Streptococcus agalactiae
Tropheryma whipplei
Trypanosoma cruzi
Ureaplasma urealyticum
Yellow fever virus

437 different pathogens detected over 7 years (n=4,828)

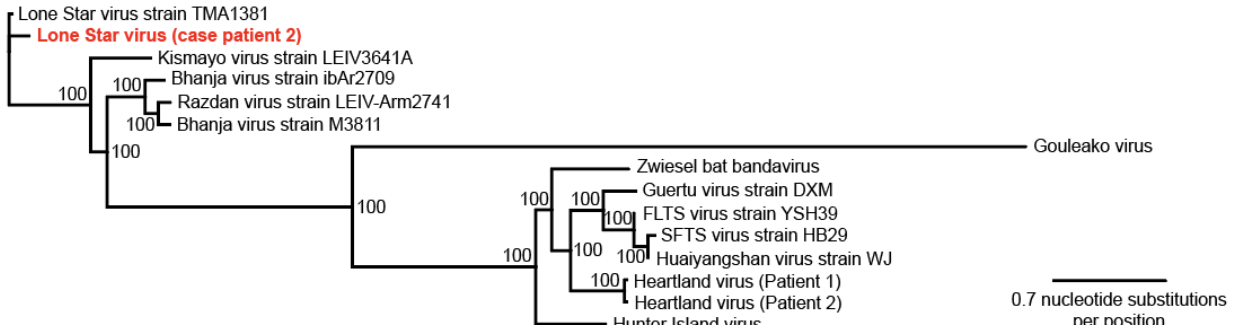
Benoit, et al., 2024, *Nature Medicine*, 30:3522-3533

POTOSI AND LONE STAR VIRUS BUNYAVIRUS MENINGOENCEPHALITIS

Lone Star virus

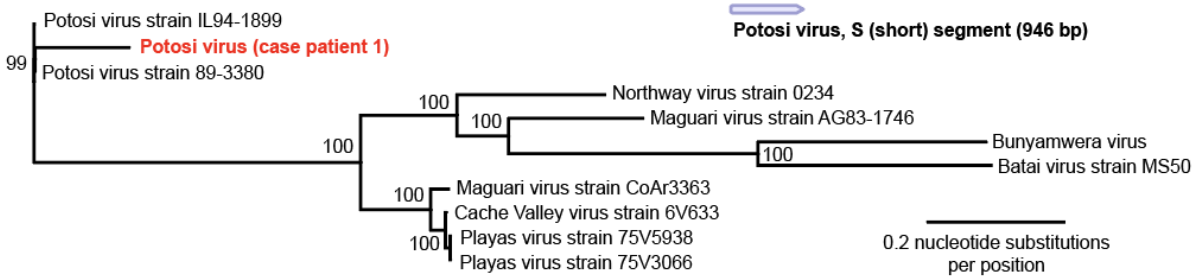
(in CSF from a 60 y/o male with common variable immunodeficiency, avid hiker admitted with florid encephalopathy; virus with 70-90% nucleotide homology to Lone Star virus)

Ambylomma americanum **tick**



Potosi virus

(in CSF from a 70 y/o man with a chronic meningoencephalitis who lives in the rural Midwest**)**

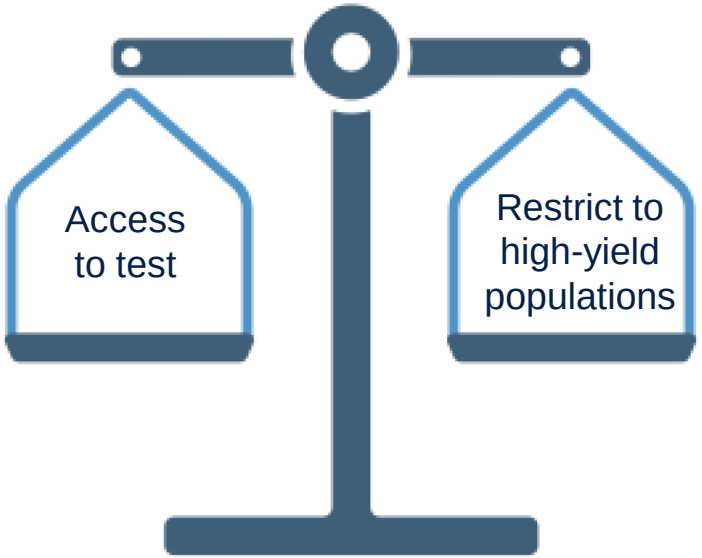


Patients passed away from fulminant encephalitis 26 and 57 days after admission.

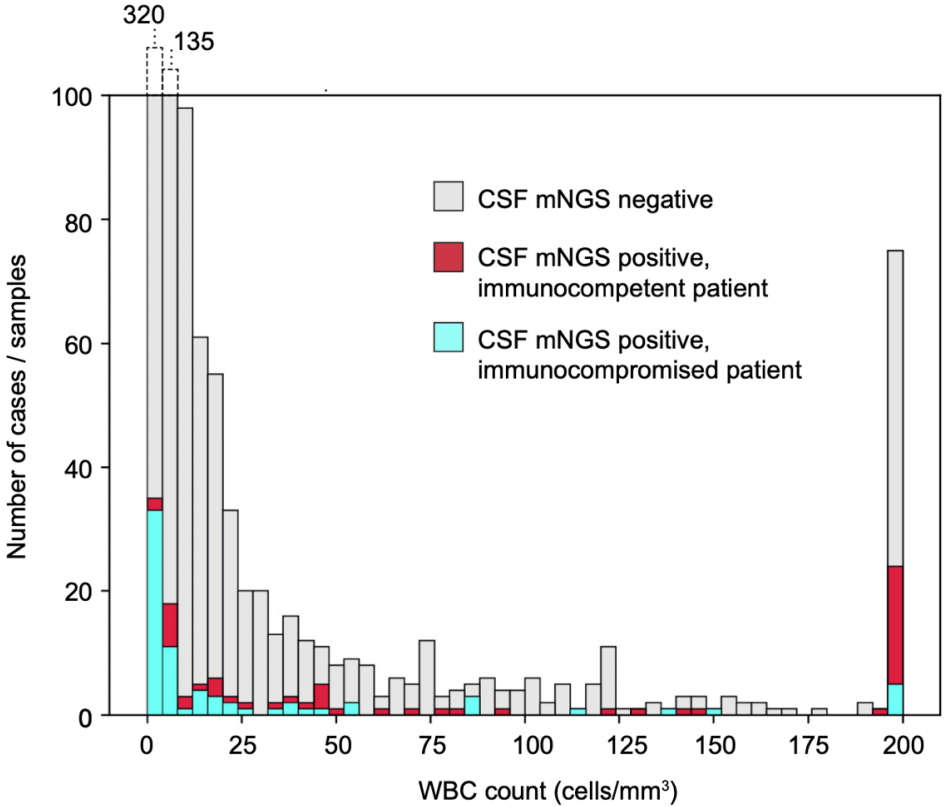
Chiu, et al., 2025, Emerg Infect Dis, 31:2

CLINICAL PREDICTORS OF CSF mNGS POSITIVITY

- Positive mNGS results have the most impact (~15% in the CSF mNGS cohort (Benoit, et al., 2024).
- If you allow testing only if certain conditions are met (diagnostic stewardship), it is possible to increase this yield of positive mNGS results and/or clinically actionable mNGS results, whether positive or negative
- Part of a rational diagnostic stewardship strategy is to optimize the use of the test

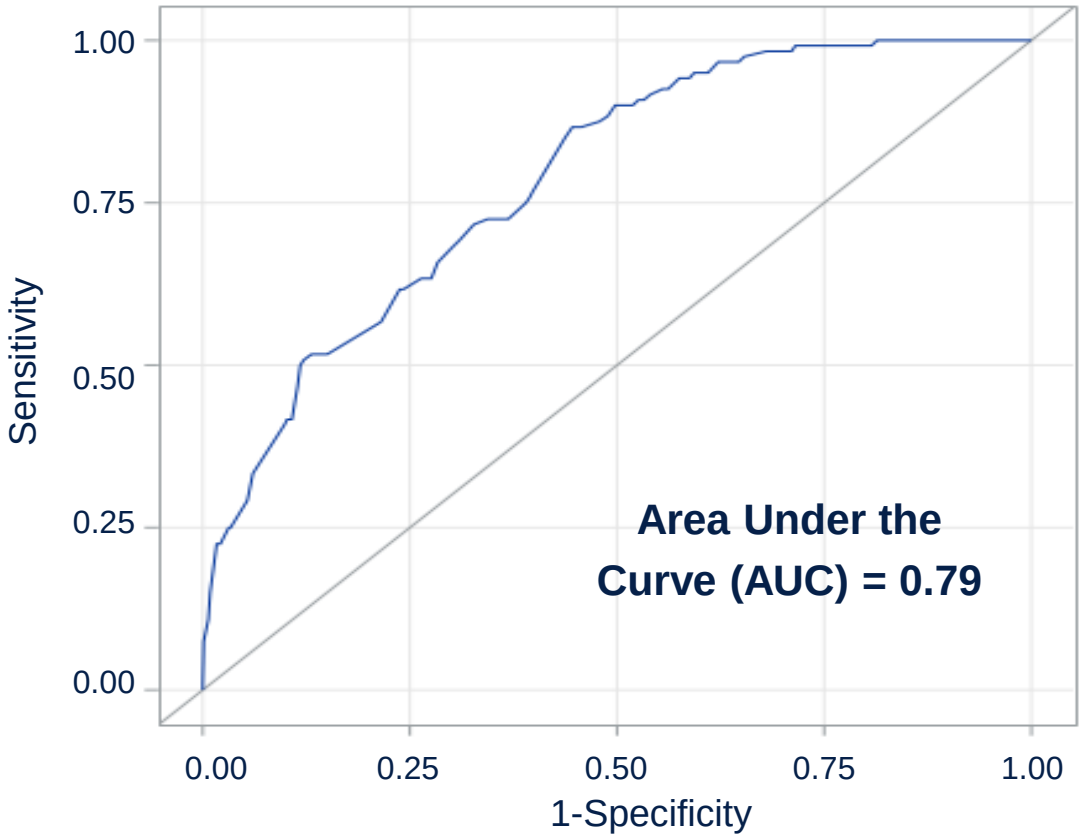


INTERACTION BETWEEN IMMUNOCOMPROMISED STATUS AND CSF PLEOCYTOSIS



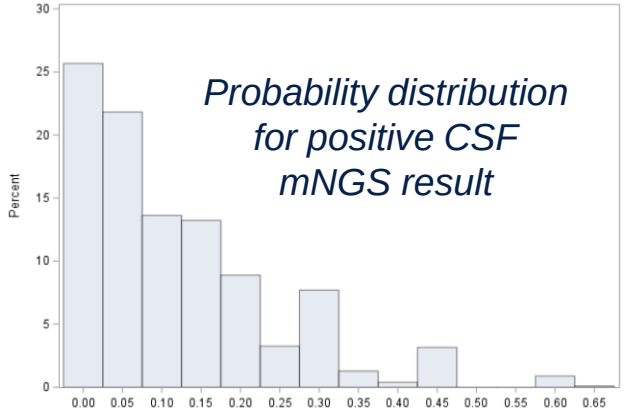
Benoit, et al., 2026, Open Forum Infectious Diseases, 2025, 12(Supplement 1):ofae631.089.

PREDICTIVE REGRESSION MODEL FOR POSITIVE mNGS TESTING



- ❑ Immunocompromised state with CSF pleocytosis interaction term
- ❑ ICU *(versus non-ICU)*
- ❑ Meningoencephalitis/Meningitis *(versus Encephalitis or Myelitis)*
- ❑ Abnormal MRI imaging *(versus Unremarkable MRI imaging)*
- ❑ No history of non-infectious CNS syndrome *(versus history of non-infectious CNS syndrome)*

Probability Score *(likelihood of positive diagnostic yield and/or clinical impact)*



Benoit, et al., 2026, Open Forum Infectious Diseases, 2025, 12(Supplement 1):ofae631.089.

PLASMA mNGS TESTING

Potential High-yield diagnostic scenarios

- Diagnosis of invasive fungal/anaerobic/parasitic infections
- Infections from fastidious or unculturable organisms
- Culture-negative endocarditis
- Neutropenic fever
- Other infections in immunocompromised patients
- Lesions seen on diagnostic imaging (e.g., lung nodules, abdominal abscess)
- Atypical pneumonia

- Kaur, et al., Infect Control Hosp Epidemiol, 2025, 46(5):1-8
- Dong, et al., J Appl Lab Med, 2024, 9(1):14-27
- Lehman, et al., JPIDS, 2023, 12(1), S25.
- Niles DT, J Clin Microbiol, 2020, 58(11):e00794-20
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- Hoenigl, et al., J Clin Microbiol, 2023, 61:e01859-22
- Linder, et al., OFID, 2023, 10(8)
- Benamu, CID, 2022, 74(9):1659-1668

PLASMA mNGS FIRST LINE INDICATIONS (concurrently with other immediate tests)

1. Severely immunocompromised* patient with pneumonia, especially if:

- High concern for atypical infection such as IFI, Nocardia, Legionella
- Not responding to standard care

2. Fulminant CNS infection *and* sampling of CSF/CNS is not feasible or delayed

- High concern for CNS infection that reflects disseminated illness
- CNS/CSF sampling not technically possible

3. Fulminant infection *and* strong epidemiologic concern for atypical infection:

- High specific concern for disseminated *M. tuberculosis*, *Legionella, Nocardia, Coxiella /* Q fever, *Brucella*, tularemia; invasive fungal infection

*Severely immunocompromised = bone marrow transplant within a year, solid organ transplant within a year, primary severe immunodeficiency, HIV with CD4 <200, B cell depleting therapy

UCSF Consensus mNGS Guidelines 2025 (with Natasha Spottiswoode and Monica Fung

PLASMA mNGS SECOND LINE INDICATIONS (after initial round of diagnostic tests)

1. Culture negative endocarditis *and*

- Negative first line workup (blood cultures, serological tests/antibodies as indicated)

2. Fever of unknown origin *and*

- Negative first line workup (blood cultures, imaging, malignancy/autoimmune workup)
- If patient is on empiric antibiotics, a trial of stopping them should be considered

3. Deep seated lesions/abscesses (epidural, hepatic, splenic, peri-renal, pleural effusion) *and*

- Blood cultures negative
- Sampling of site unfeasible and/or unrevealing

4. Persistent febrile neutropenia *and*

- Negative first line workup (blood cultures, cross-sectional imaging)

UCSF Consensus mNGS Guidelines 2025 (with Natasha Spottiswoode and Monica Fung

PICKUP STUDY: PLASMA mNGS FOR PNEUMONIA IN IMMUNOCOMPROMISED PATIENTS

- Key study: PICKUP
  - Multicenter, prospective, observational study comparing plasma mNGS to standard of care in immunocompromised adults*
  - All patients had pneumonia of unclear etiology

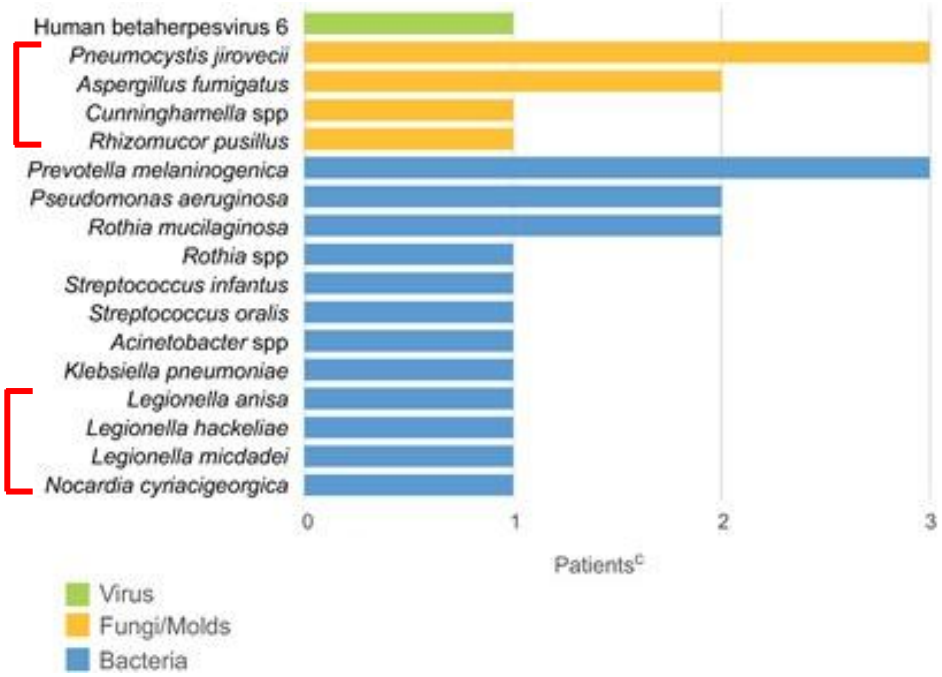
Bergin et al. *Clinical Infectious Diseases*. 2024.

*Immunocompromised = hematopoietic stem cell transplant or hematologic malignancy

PICKUP STUDY: PLASMA mNGS FOR PNEUMONIA IN IMMUNOCOMPROMISED PATIENTS

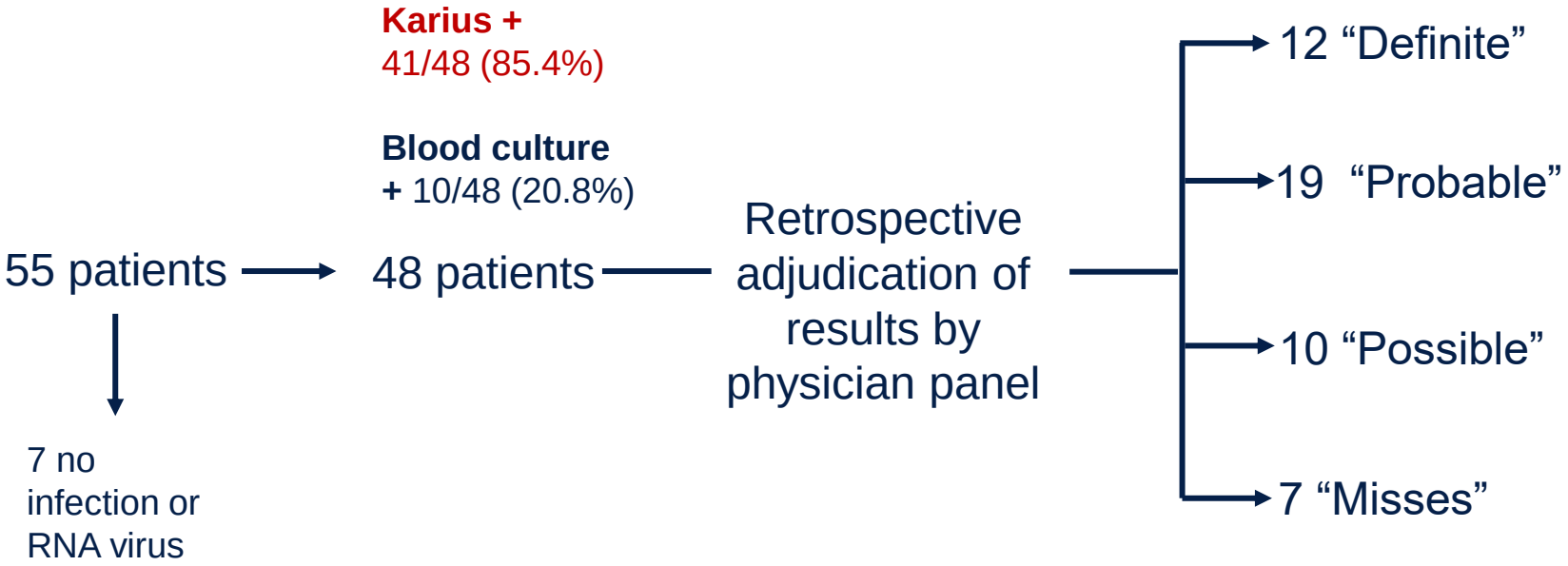
- 30.1% of patients received a diagnosis through usual care
- 42.2% of patients received a diagnosis through a composite of UC and Karius testing
- 21/173 (12.1%) patients exclusively diagnosed by mNGS
- 17/123 (9.8%) of diagnoses could have changed management

Pathogens detected only by mNGS



Bergin et al. *Clinical Infectious Diseases*. 2024.

PLASMA mNGS FOR NEUTROPENIC FEVER

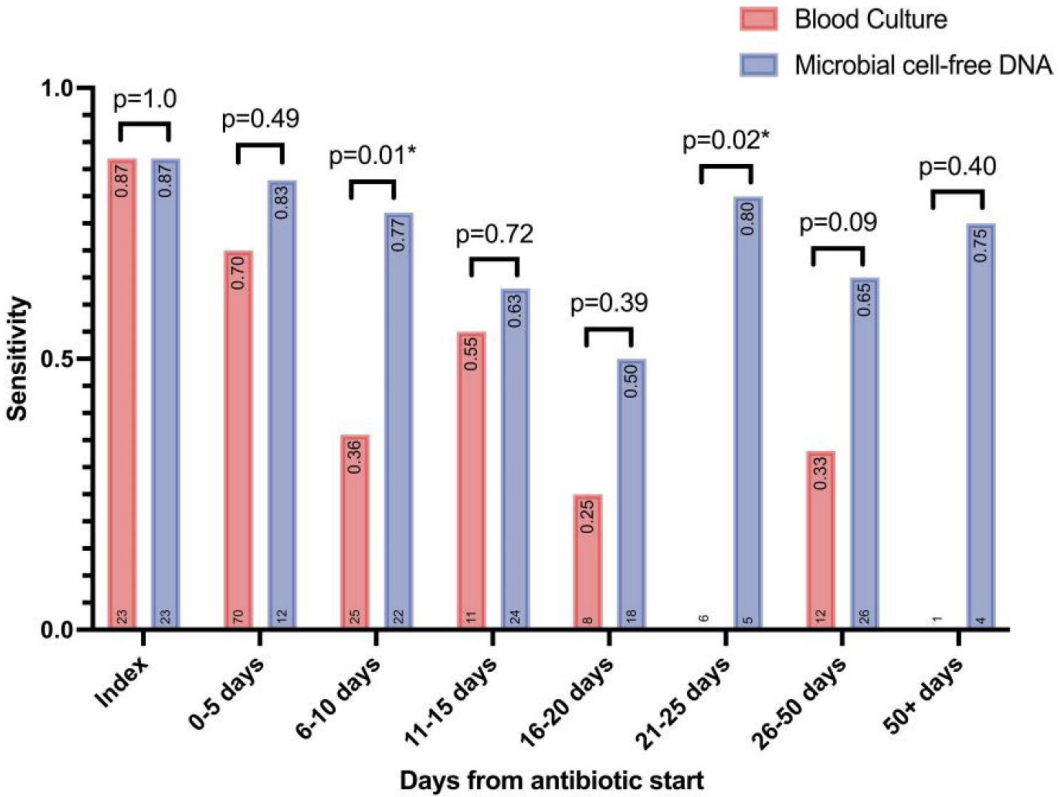


Antimicrobial management might have changed in 26 (47.3%) but assumes de-escalation based on negative results

Benamu et al. *Clinical Infectious Diseases*. 2022.

PLASMA mNGS FOR ENDOCARDITIS WITH BACTEREMIA

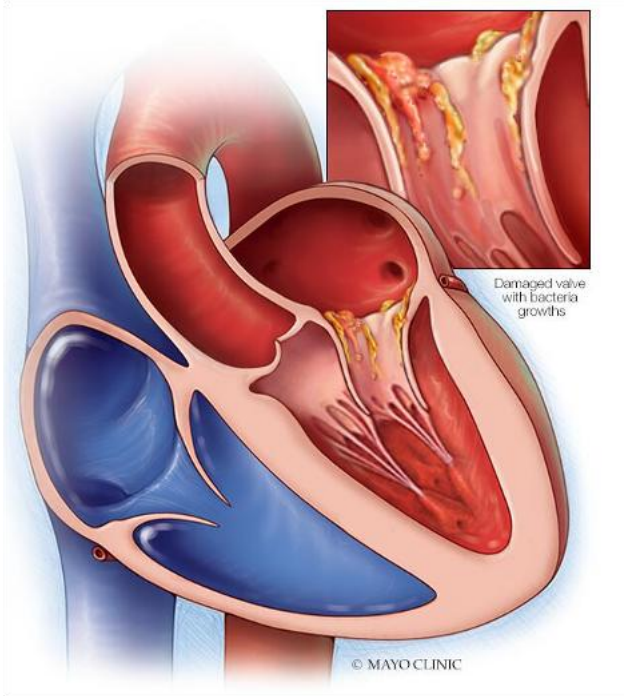
- N=23 patients with IE; 22 of whom had blood cultures+
- mNGS and cultures both 87% sensitive; mNGS remains positive longer



KARIUS®

Eichenberger et al. *Clinical Infectious Diseases*. 2023.

PLASMA mNGS FOR CULTURE-NEGATIVE ENDOCARDITIS



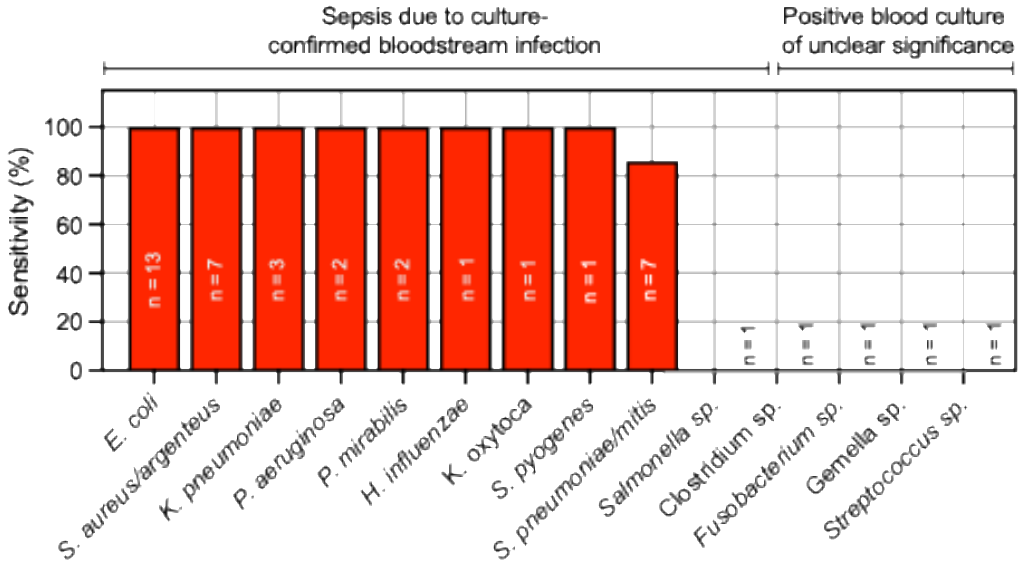
- Culture-negative IE can occur if:
  - Pathogen dead (prior antibiotics)
  - Pathogen hard to culture (AACEK)
  - Pathogen doesn't grow on culture (*Bartonella, Coxiella, Tropheryma, Legionella*)
  - Pathogen not bacteria (fungi)
  - Not a Pathogen (non-infectious mimics)

- Small case series show high sensitivity of mNGS for *Bartonella* IE

- Large-scale UCLA study suggests that mNGS may be particularly high-yield in culture-negative IE

Kaur, et al., *Infect Dis Hosp Epi,* 2024.
Baddour et al. *Circulation.* 2015
Degner et al. *Journal of Pediatrics Infectious Disease Society.* 2021.

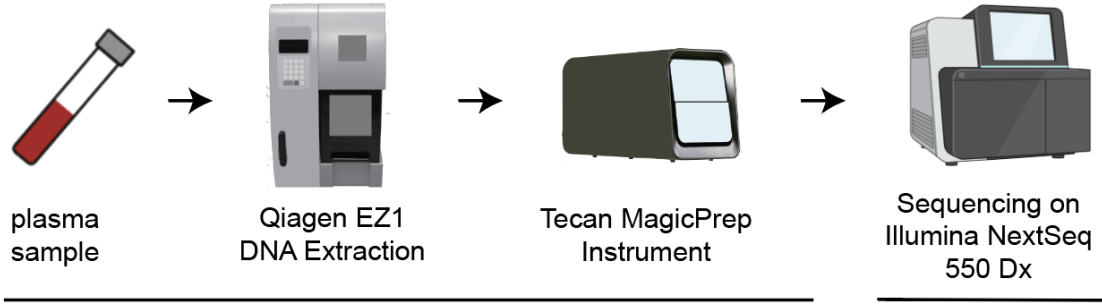
PLASMA mNGS FOR DIAGNOSIS OF SEPSIS

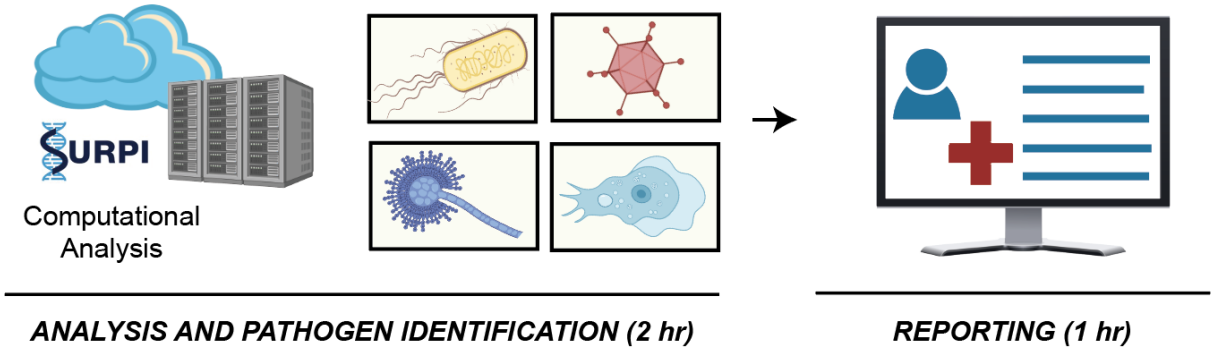


- Sensitivity 83-100% for most common sepsis pathogens
- Specificity 73% measured in patients with non-infectious SIRS
- Possible pathogens in 42% of patients with culture negative sepsis

Spottiswoode and Neyton et al. *AJRCCM*. 2025.; Kalantar and Neyton et al. *Nature Microbiology*. 2022.

UCSF PLASMA mNGS TESTING



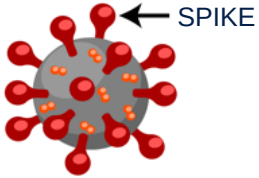


Microbiology lab turnaround time ~24 hours

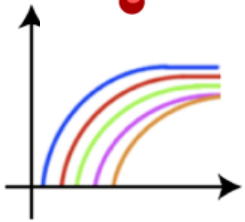
Gu, et al., 2021, *Nature Medicine*, 27(1):115-124
Tan, et al., 2024, *Nature Communications,* 15:9016.
Streithorst, et al., 2025, in preparation

DIRECT VS. INDIRECT DIAGNOSIS OF INFECTION

culture



antigen testing

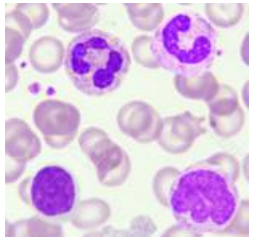


nucleic acid testing (PCR)

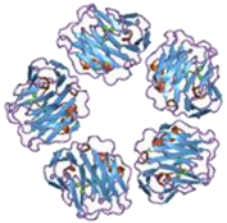
DNA/RNA Sequencing (mNGS)

PATHOGEN

serology



cell count / differential



protein expression (CRP, procalcitonin, etc.)

HOST

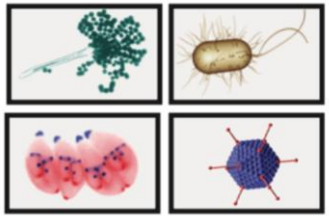
REPURPOSING OF mNGS DATA FOR RNA HOST RESPONSE PROFILING

Patient with Meningitis and/or Encephalitis

CSF mNGS

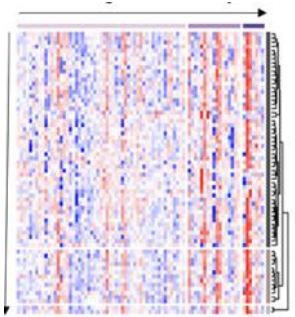
Non-human Reads

Alignment to pathogen database



Human RNA Reads

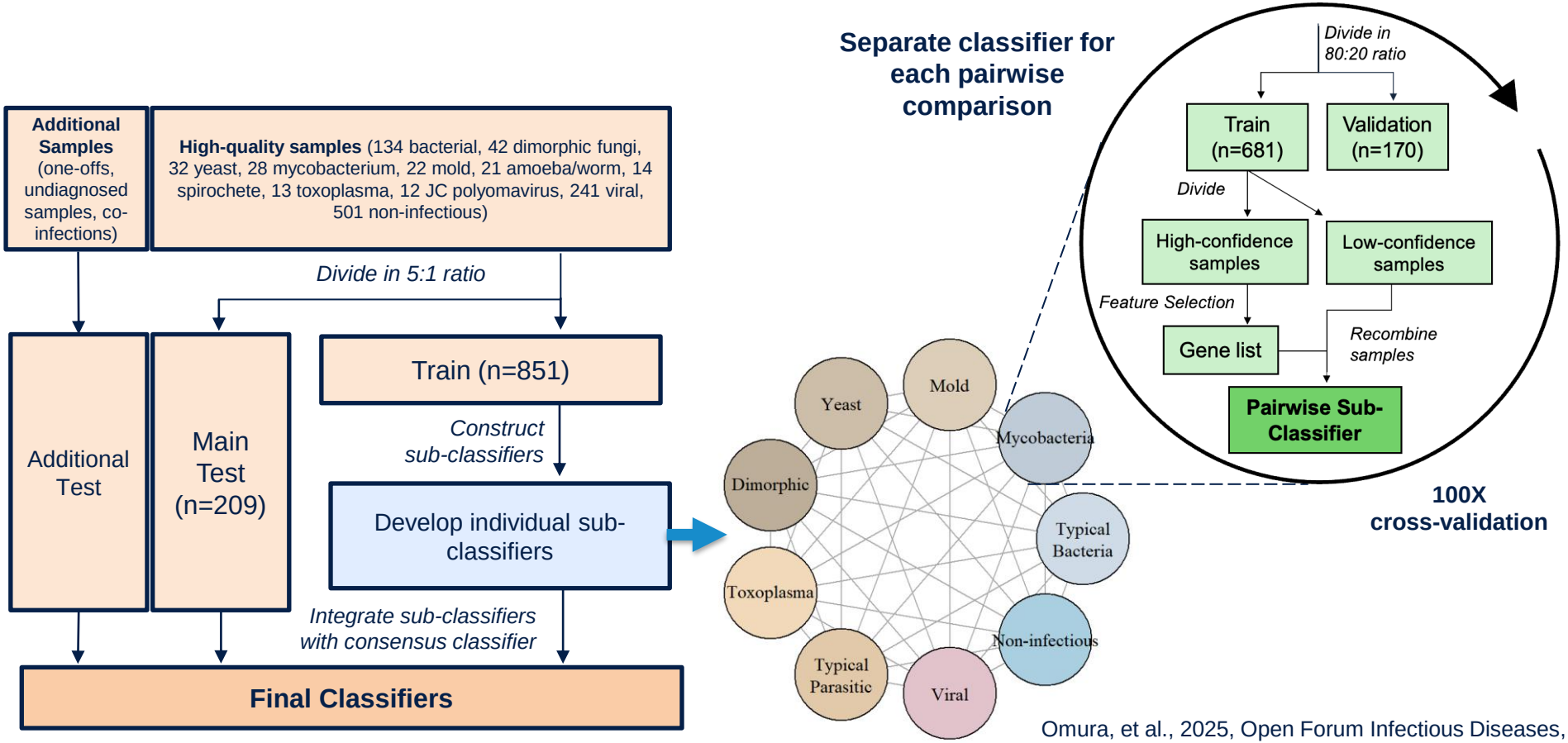
Host Response



MACHINE LEARNING / AI

Omura, et al., 2026, in preparation

MACHINE LEARNING BASED CLASSIFIER FOR NEUROLOGIC ILLNESS



Omura, et al., 2025, Open Forum Infectious Diseases, 12(Supplement 1), ofae631.088.

PERFORMANCE OF THE CSF HOST RESPONSE CLASSIFIER

Non-infectious

Classifier \ Composite Dx	Pos	Neg
Pos	68	7.5
Neg	8	195.5

Sensitivity: 0.8947
Specificity: 0.9631
Accuracy: 0.9444

Bacterial

Classifier \ Composite Dx	Pos	Neg
Pos	39	7.5
Neg	8	195.5

Sensitivity: 0.8298
Specificity: 0.9631
Accuracy: 0.938

Fungal

Classifier \ Composite Dx	Pos	Neg
Pos	18	4.5
Neg	0	256.5

Sensitivity: 1
Specificity: 0.9828
Accuracy: 0.9839

Parasitic*

Classifier \ Composite Dx	Pos	Neg
Pos	36	4.5
Neg	15	223.5

Sensitivity: 0.7059
Specificity: 0.9803
Accuracy: 0.9301

Viral

Classifier \ Composite Dx	Pos	Neg
Pos	78	13.5
Neg	14	279

Sensitivity: 0.8478
Specificity: 0.9538
Accuracy: 0.9285

Overall Performance

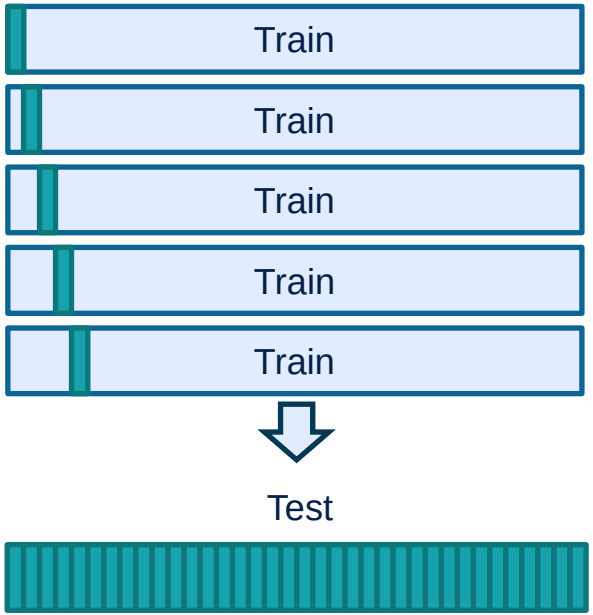
	Composite Dx Pos	Composite Dx Neg
Pos	63	7
Neg	10	63

Sensitivity: 0.863
Specificity: 0.9
Accuracy: 0.8811

Omura, et al., 2025, Open Forum Infectious Diseases, 12(Supplement 1), ofae631.088.

SUBCATEGORY CLASSIFICATION USING “LEAVE-ONE-OUT” FOR VALIDATION

“Leave-One-Out” Approach



Subcategory	Sensitivity	Specificity	n
parasitic	0.778	0.835	20
HSV1/2	0.750	0.806	11
Dimorphic fungi	0.833	0.962	13
Mycobacterium tuberculosis	0.800	0.986	8
Cryptococcus neoformans	0.846	0.837	13

Subcategory	Specificity	n
Leukemia/lymphoma	0.748	22
Amyloidosis	0.791	8
Lupus	0.861	7
Neurosarcoidosis	0.854	15
Solid Organ Cancer	0.801	9
Toxic-Metabolic Syndrome	0.772	20

Omura, et al., 2025, Open Forum Infectious Diseases, 12(Supplement 1), ofae631.088.

CASE STUDY (ASTROVIRUS INFECTION)

- Young patient with XLA and chronic astrovirus infection presenting as headaches and cerebral atrophy on MRI
- Two hospitalizations over the past year associated with progressive
- CSF mNGS x 3 positive for chronic astrovirus encephalitis with persistent low-level lymphocytic pleocytosis
- Started on IV leniuolisib and molnupiravir followed by pembrolizumab

Category	Z-score	Test Results	Training n	LR+	LR-	Sensitivity	Specificity	Interpretation
Fungal								
Dimorphic	0.46	Negative	42	-	0.19	0.87	-	Moderate Rule-Out
Yeast	0.39	Negative	32	-	0.04	0.97	-	Strong Rule-Out
Mold	-0.78	Negative	22	-	0.18	0.96	-	Moderate Rule-Out
Bacterial								
Bacterial (Typical)	-1.35	Negative	134	-	0.01	1.00	-	Strong Rule-Out
Mycobacterium	-0.57	Negative	28	-	0.07	0.98	-	Strong Rule-Out
Spirochete	0.87	Negative	14	-	0.56	0.55	-	Minimal Rule-Out
Viral								
Viral	2.22	Positive	241	24.1	-	-	0.99	Strong Rule-In
JC polyomavirus	-0.87	Negative	12	-	0.38	0.93	-	Weak Rule-Out
Parasitic								
Non-Toxoplasma-Parasitic	0.04	Negative	21	-	0.22	0.88	-	Weak Rule-Out
Toxoplasma	0.59	Negative	13	-	0.54	0.61	-	Minimal Rule-Out
Non_infectious	0.90	Negative	501	-	0.08	0.93	-	Strong Rule-Out

Positive Likelihood Ratios (LR+)		Negative Likelihood
Range	**Interpretation**	**Range**
>20	Strong Rule-In	<0.1
10 to 20	Moderate Rule-In	0.1 to 0.2
5 to 10	Weak Rule-In	0.2 to 0.5
1 to 5	Minimal Rule-In	0.5 to 1
1	Indeterminate	1

Omura, et al., 2025, Open Forum Infectious Diseases, 12(Supplement 1), ofae631.088.

CASE STUDY (POLYMICROBIAL BACTERIAL MASTOIDITIS WITH A PARAMENINGEAL FOCUS)

- 35 y/o male admitted with left ear pain, discharge, and headache found on workup to have cholesteatoma with mastoiditis and lymphocytic meningitis with WBC=950
- Tissue cultures positive for *Staphylococcus epidermidis, Proteus mirabilis, Corynebacterium sp.,* and *Bacteroides fragilis*

Category	Z-score	Result	n	LR+	LR-	Sensitivity	Specificity	Interpretation
Bacterial								
• Bacteria (typical)	2.72	Positive	134	51.88	-	-	99.7%	Strong Rule-In
• *Mycobacterium* sp.	-0.08	Negative	28	-	0.13	93.9%	-	Moderate Rule-Out
• Spirochete	-0.55	Negative	14	-	0.21	93.8%	-	Weak Rule-Out
Fungal								
• Dimorphic	2.60	Positive	42	33.73	-	-	99.5%	Strong Rule-In
• Mold	1.17	Positive	22	3.51	-	-	87.8%	Minimal Rule-In
• Yeast	-0.32	Negative	32	-	0.01	99.6%	-	Strong Rule-Out
Parasitic								
• *Toxoplasma gondii*	-0.57	Negative	13	-	0.27	92.4%	-	Weak Rule-Out
• Worm/Amoeba	0.58	Negative	21	-	0.35	74.6%	-	Weak Rule-Out
Viral								
• Virus, non-JC polyomavirus	-0.69	Negative	241	-	0.03	99.3%	-	Strong Rule-Out
• JC polyomavirus	0.20	Negative	12	-	0.60	65.0%	-	Minimal Rule-Out
Non-infectious								
• Non-infectious	-0.30	Negative	501	-	0.01	99.7%	-	Strong Rule-Out

Positive Likelihood Ratios (LR+)		**Negative Likelihood**
Range	**Interpretation**	**Range**
>20	Strong Rule-In	<0.1
10 to 20	Moderate Rule-In	0.1 to 0.2
5 to 10	Weak Rule-In	0.2 to 0.5
1 to 5	Minimal Rule-In	0.5 to 1
1	Indeterminate	1

Omura, et al., 2025, Open Forum Infectious Diseases, 12(Supplement 1), ofae631.088.

CASE STUDY (INVASIVE MOLD INFECTION FROM *SCEDOSPORIUM SP.*)

- Elderly immunocompromised patient with brain abscess with cultures positive for *Scedosporium apiospermum*.
- Treated with antifungal therapy for 3 months
- Returns with fever, headaches, and recurrence of abscess

Category	Z-score	Test Results	Training n	LR+	LR-	Sensitivity	Specificity	Interpretation
Fungal								
Dimorphic	1.77	Positive	31	11.294	-	-	0.95	Moderate Rule-In
Yeast	0.00	Negative	26	-	0.00	1.00	-	Strong Rule-Out
Mold	3.69	Positive	17	24.458	-	-	1.00	Strong Rule-In
Bacterial								
Bacterial (Typical)	2.36	Positive	114	29.492	-	-	0.98	Strong Rule-In
Mycobacterium	0.20	Negative	22	-	0.15	0.91	-	Moderate Rule-Out
Spirochete	-2.50	Negative	11	-		1.00	-	
Viral								
Viral	0.13	Negative	196	-	0.14	0.91	-	Moderate Rule-Out
JC polyomavirus	-0.53	Negative	9	-	0.53	0.83	-	Minimal Rule-Out
Parasitic								
Non-Toxoplasma-Parasitic	-1.73	Negative	18	-	0.00	1.00	-	Strong Rule-Out
Toxoplasma	-2.03	Negative	10	-	0.00	1.00	-	Strong Rule-Out
Non infectious	-0.02	Negative	397	-	0.06	0.97	-	Strong Rule-Out

Positive Likelihood Ratios (LR+)		**Negative Likelihood**
Range	**Interpretation**	**Range**
>20	Strong Rule-In	<0.1
10 to 20	Moderate Rule-In	0.1 to 0.2
5 to 10	Weak Rule-In	0.2 to 0.5
1 to 5	Minimal Rule-In	0.5 to 1
1	Indeterminate	1

Omura, et al., 2025, Open Forum Infectious Diseases, 12(Supplement 1), ofae631.088.

CASE

- 15-year-old presenting with headache, vomiting, epistaxis for presumptive migraines. Admitted to the hospital with CSF lumbar puncture showing opening pressure of 40, CSF lymphocytic pleocytosis, high protein, and low glucose.
- All microbiologic tests negative and all autoantibody tests negative (including from the Mayo Clinic)
- Brain MRI unremarkable but patient with persistent CSF pleocytosis and
- Started empirically on treatment for cryptococcal meningitis without response
- ***CSF and plasma mNGS negative!***

Omura, et al., 2025, Open Forum Infectious Diseases, 12(Supplement 1), ofae631.088.

ATYPICAL TERATOID RHABDOID TUMOR IDENTIFIED BY HOST PROFILING

Category	Z-score	Test Results	Training n	LR+	LR-	Sensitivity	Specificity	Interpretation
Fungal								
Dimorphic	-0.96	Negative	42	-	0.03	0.99	-	Strong Rule-Out
Yeast	-0.77	Negative	32	-	0.00	1.00	-	Strong Rule-Out
Mold	-0.51	Negative	22	-	0.22	0.93	-	Weak Rule-Out
Bacterial								
Bacterial (Typical)	0.88	Negative	134	-	0.23	0.81	-	Weak Rule-Out
Mycobacterium	-0.78	Negative	28	-	0.06	0.99	-	Strong Rule-Out
Spirochete	0.47	Negative	14	-	0.44	0.70	-	Weak Rule-Out
Viral								
Viral	-0.04	Negative	241	-	0.08	0.96	-	Strong Rule-Out
JC polyomavirus	-0.46	Negative	12	-	0.46	0.85	-	Weak Rule-Out
Parasitic								
Non-Toxoplasma-Parasitic	-0.27	Negative	21	-	0.17	0.93	-	Moderate Rule-Out
Toxoplasma	-0.87	Negative	13	-	0.22	0.96	-	Weak Rule-Out
Non_infectious	2.58	Positive	501	87.6	-	-	1.00	Strong Rule-In
Top Non-infectious Subcategories								
Solid Organ Cancer	6.43	Positive	25	5792	-	-	1.00	Strong Rule-In
Structural (epilepsy)	3.03	Positive	16	5.77	-	-	1.00	Weak Rule-In
White Blood Cell Cancer (leukemia)	2.12	Positive	8	1	-	-	0.98	Indeterminate
Vascular (hemorrhage)	2.03	Positive	9	1.85	-	-	0.98	Minimal Rule-In
White Blood Cell Cancer (bone marrow cancer)	1.07	Positive	3	1	-	-	0.86	Indeterminate
Structural (seizures)	0.98	Negative	5	-	1	0.03	-	Indeterminate
HLH	0.92	Negative	5	-	1	0.17	-	Indeterminate
Idiopathic intracranial hypertension	0.87	Negative	4	-	1	0.00	-	Indeterminate

- CSF mNGS host response showed non-infectious etiology, likely cancer.
- Repeat lumbar puncture done 2 weeks later is positive for malignant cells; CSF cfDNA panel confirms atypical teratoid rhabdoid tumor and patient started on chemotherapy.

CONCLUSIONS

- Metagenomic sequencing is powerful tool for infectious disease diagnosis in immnocompromised patients because pathogens can be detected without *a priori* suspicion
- Clinical syndromes for which the utility of plasma mNGS has been demonstrated include pneumonia in immunocompromised patients, culture-negative endocarditis, neutropenic fever, and fever with high clinical and/or epidemiologic suspicion for infection
- CSF mNGS is an extremely useful single diagnostic test for central nervous system infections and is complementary to routine diagnostic laboratory testing
- Host response profiling in the future may provide complementary information to microbiologic testing, be used to “rule out” infection, and enable diagnosis of non-infectious conditions

ACKNOWLEDGEMENTS

UCSF
Alicia Sotomayor-Gonzalez, PhD
Doug Stryke, MS
Venice Servellita, BS/CLS
Charles Omura, BS/MS
Jess Tan, BS/CLS
Manal Ahmed, BS
Nanami Sumimoto, BS
Kafaya Foresythe, BS
Patrick Benoit, MD
Jeffrey Whitman, MD, PhD
Rama Yakubu, MD, PhD
Michael Metrick, MD
Aaron Bodansky, MD/PhD
Joseph DeRisi, PhD
Michael Wilson, MD
Kelsey Zorn, BS
Sukhie Sidhu, BS

UCSF Infectious Diseases
Natasha Spottiswoode, MD
Monica Fung, MD

UCSF Clinical Microbiology Lab
Jessica Streithorst, BS/CLS
Walter Lorizio, BS/CLS
Michael Kwok, BS/CLS
Danielle Ingebrigsten, BS/CLS

Delve Bio
Steve Miller, MD/PhD
Amy Wong, PhD
Brad Murray, PhD
Tim Blicharz, MD
Sudeb Dalai, MD

NIH
Gulbu Uzel, MD

CHIU LAB
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Funding
- CZ Biohub San Francisco
- NIH
- BARDA
- US Centers for Disease Control and Prevention
- Abbott Laboratories
- Boryung
- Biotia
- Delve Bio
- California Initiative to Advance Precision Medicine