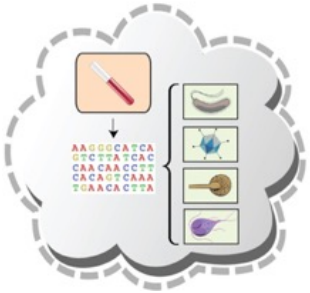
University of California
San Francisco



Clinical Metagenomic Sequencing for Diagnosis of Infections

Charles Chiu, MD / PhD

Professor, Department of Laboratory Medicine and Medicine / Infectious Diseases

Director, UCSF-Abbott Viral Diagnostics and Discovery Center

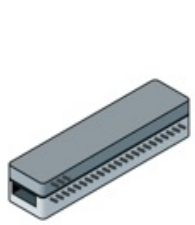
Associate Director, UCSF Clinical Microbiology Laboratory

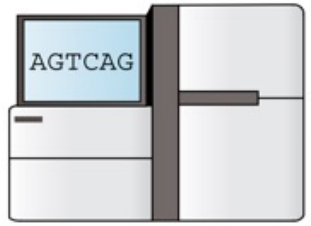
University of California, San Francisco

ICCMg Meeting, 2021

Clinical Metagenomic Sequencing for Diagnosis of Infectious Diseases

Clinical Laboratory





Metagenomic Next-Generation Sequencing (**mNGS**)

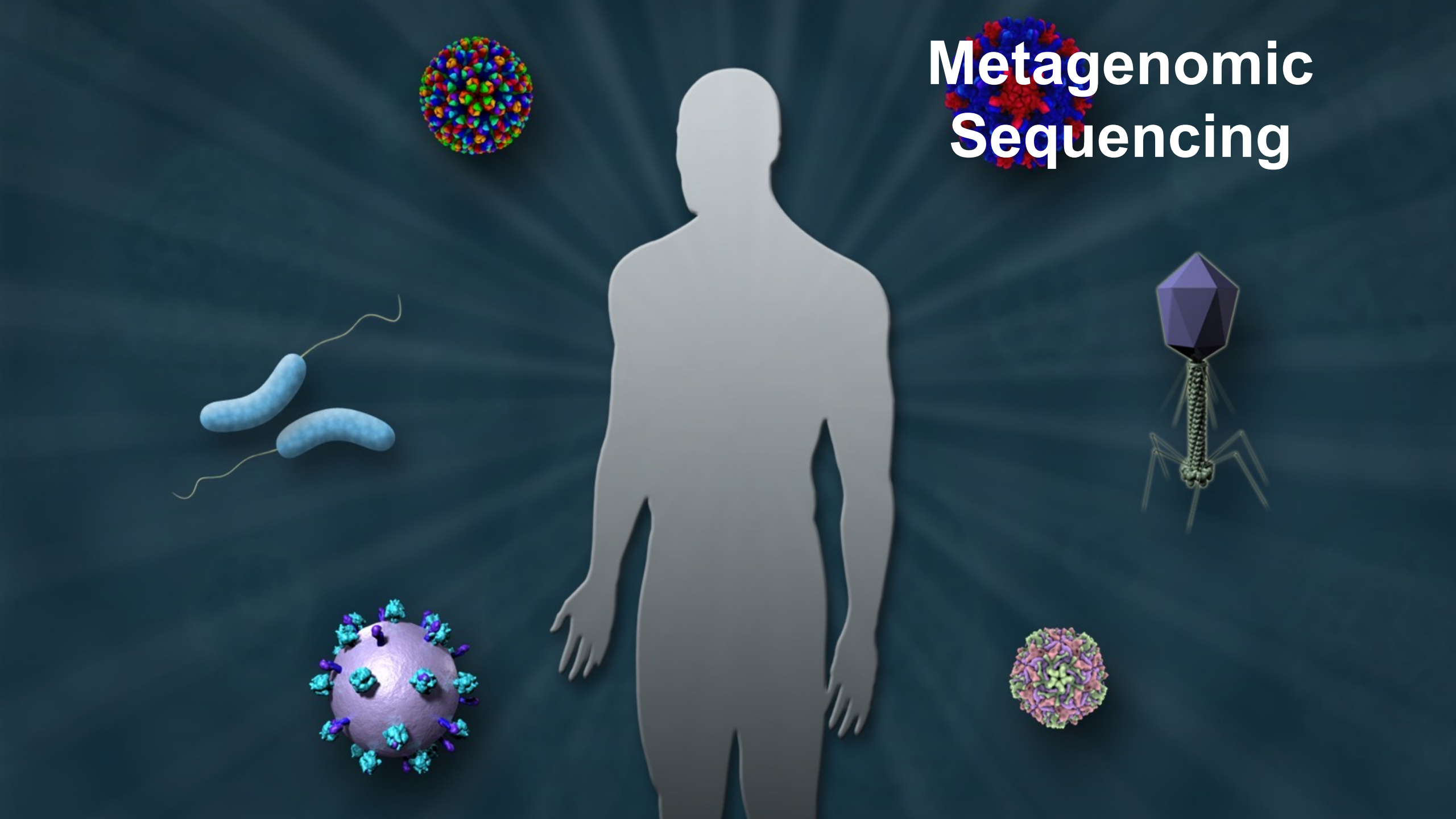
Bioinformatics

Clinical Dx

Cost-effective and actionable information for early treatment

Lower healthcare costs
Improved patient outcomes

Turnaround time: hours – days (versus days – weeks)


Metagenomic
Sequencing

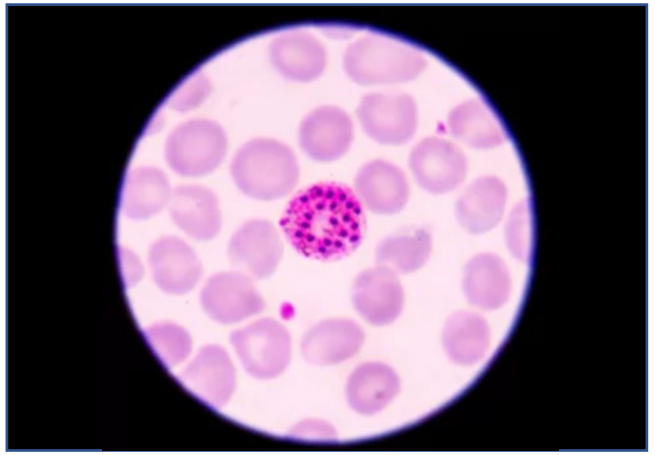
Nearly All Microbes can be Uniquely Identified by Metagenomic Next-Generation Sequencing (mNGS)

Bacteria

Viruses

Fungi

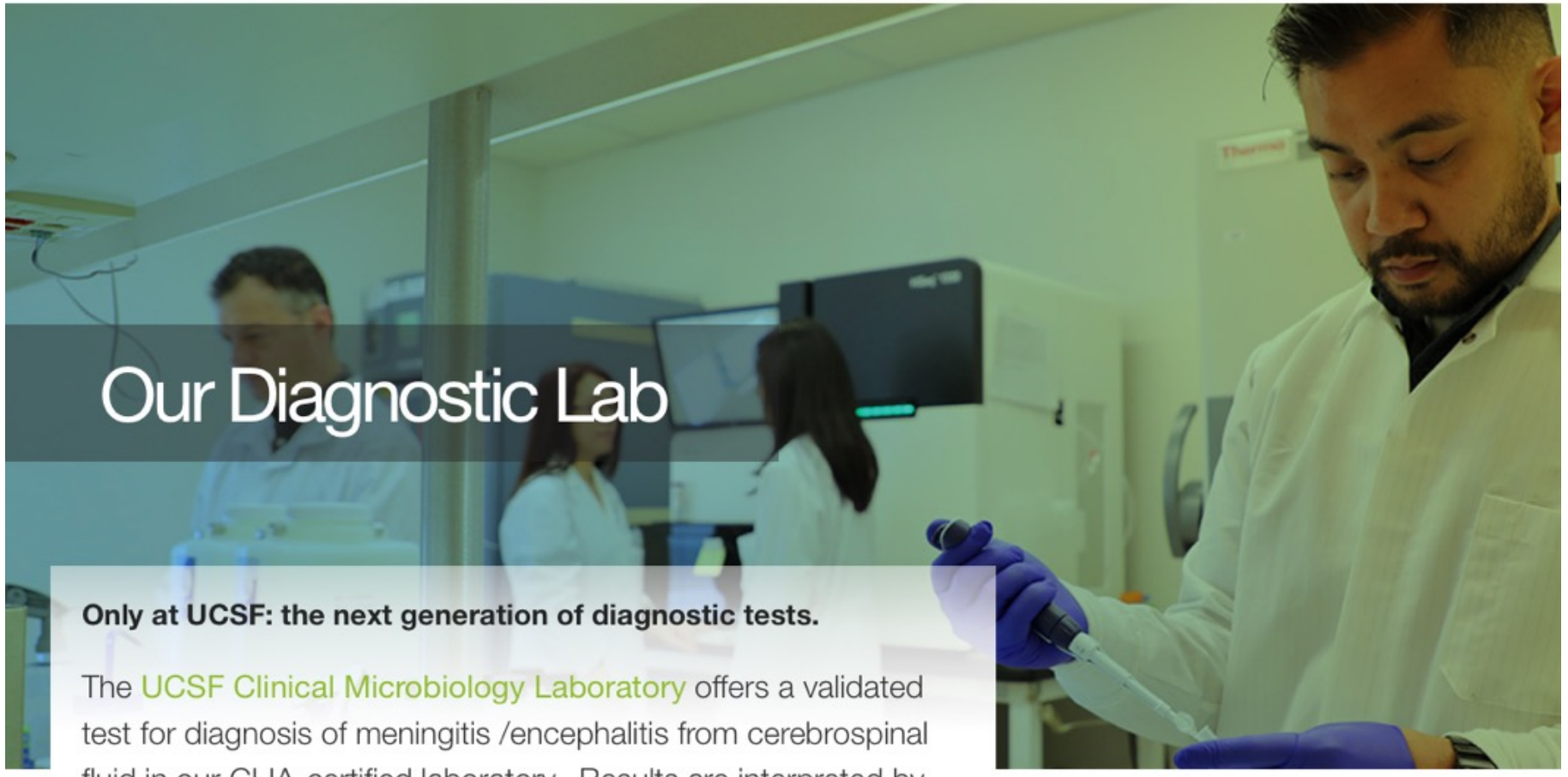
Parasites



CLIA-Validated mNGS Assay at UCSF

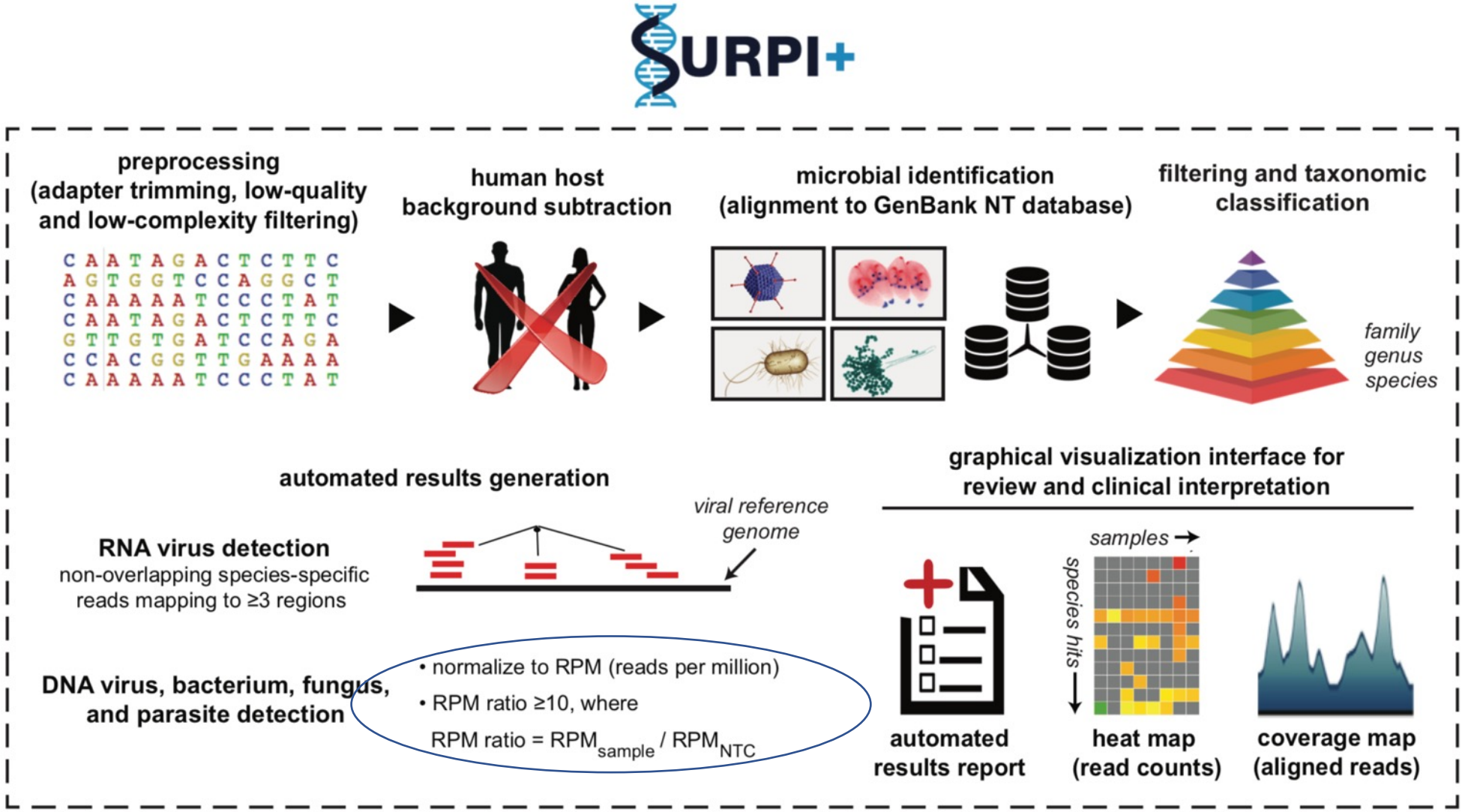
UCSF Center for Next-Gen Precision Diagnostics

For Providers | For Patients | Technology | Our Vision



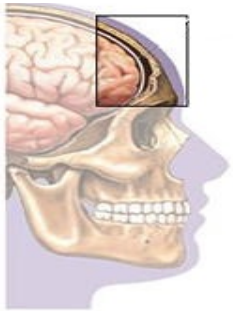
http://nextgendiagnostics.ucsf.edu

SURPI (Sequence-Based Ultra-Rapid Pathogen Identification)



Miller and Chiu, et al., *Genome Research*, 2019

The Precision Diagnosis of Acute Infectious Diseases (PDAID) Study



8 hospitals in CA and nationwide
Enroll/consent patients
204 total completed study
CSF collected; clinical chart review

40-60% unknown cause
Meningitis/Encephalitis

SURPI+

mNGS assay validated in CLIA lab
86% analytic sensitivity, 98% specificity

Clinical microbial sequencing board

Clinical report in patient EMR

Clinical Dx*
(direct detection CSF testing)

mNGS	Pos	Neg
Pos	32	3
Neg	8	164

sensitivity = 80.0% ***PPV = 91.4%***
specificity = 98.2% ***NPV = 95.3%***

Clinical Dx*
(direct detection CSF testing)

Conventional testing (direct detection CSF testing)	Pos	Neg
Pos	27	1#
Neg	13	164

sensitivity = 67.5% ***PPV = 96.4%***
specificity = 99.4% ***NPV = 92.7%***

Quest Diagnostics
UCSF
UC DAVIS
Berkeley UNIVERSITY OF CALIFORNIA
Children's Hospital Colorado
UCLA
Children's National
St. Jude Children's Research Hospital
Children's Hospital LOS ANGELES We Treat Kids Better

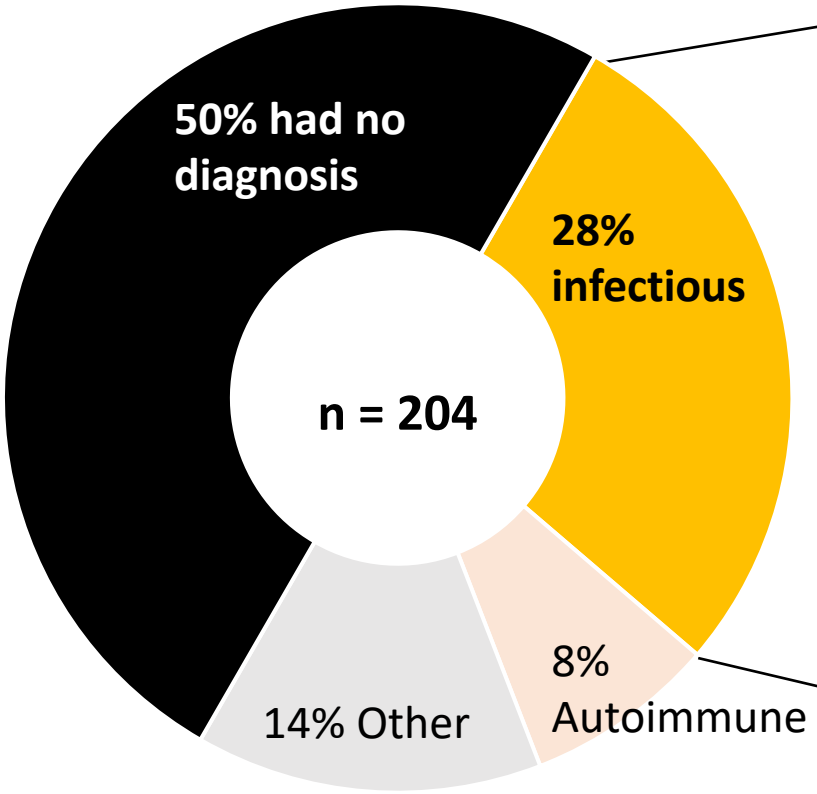
Wilson *et al*., 2019 *N Eng J Med* **380**:2327-2340

Clinical Utility

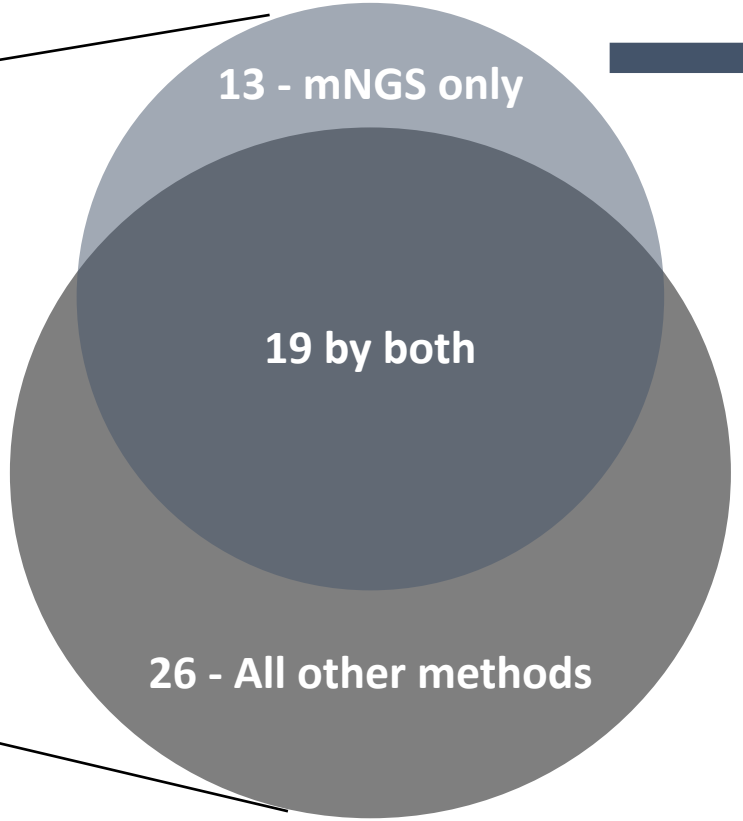
Clinical false negatives by mNGS (n=26)

- Diagnosed by serology (n=11)
- Diagnosed by samples other than CSF (n=7)
- Low pathogen titer in CSF (n=8), 6 of 6 bacterial/ fungal cases were "sub-reporting threshold" detections

PDAID Trial Summary



Diagnoses (n=58)



Clinical Utility

8 cases with direct clinical impact

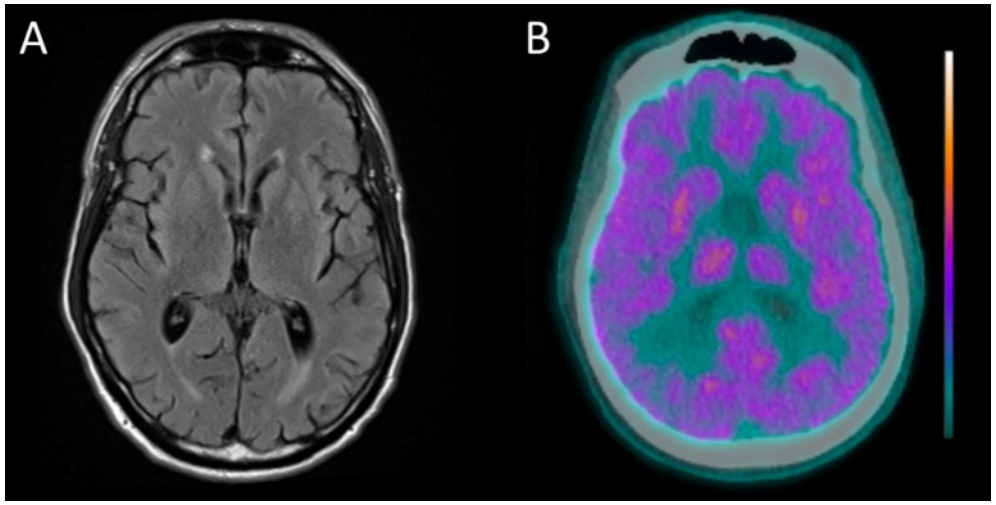
- *Neisseria sp.* – changed antibiotics
- *Nocardia farcinica* – changed antibiotics
- *Candida tropicalis* – changed antibiotics, discontinued antifungals
- Hepatitis E virus – antiviral, stopped liver transplant
- *Enterobacter aerogenes* – changed antibiotics
- *Enterococcus faecalis* – changed antibiotics
- *Streptococcus mitis* – changed *antibiotics*
- *Streptococcus agalactiae* – changed antibiotics

Highest diagnostic yield results from combination of mNGS of CSF and conventional microbiologic testing (including serology and testing of different specimen sources)

Wilson *et al.*, 2019 *N Eng J Med* **380**:2327-2340

Unknown Case – Patient with Chronic Meningoencephalitis

- 60 y/o male from New England with history of mantle cell lymphoma in remission, on maintenance rituximab since 2014, developed fatigue, arthralgias, and weight loss over 6 months
- He was empirically treated for Lyme disease without improvement, developed progressive insomnia, inattention, and was admitted for workup of rapidly progressive dementia
- On exam, he had impaired arousal and attention (Montreal Cognitive Assessment score was 6 of 30)
- Cranial nerve exam, muscle tone, strength, sensory, and reflex exams all normal. No ataxia.
- Brain MRI unremarkable; PET scan showed global hypometabolism
- CSF from multiple lumbar punctures → mild lymphocytic pleocytosis, elevated protein, and normal glucose
- Extensive laboratory workup was normal.



Solomon, et al., Emerging Infectious Diseases, 2020, in press

Clinical mNGS Testing

UCSF
University of California San Francisco

Microbiology Report

Metagenomic next-generation sequencing

LABORATORY PHYSICIAN INTERPRETATION:

Organism Type:

DNA Viruses:
Not Detected

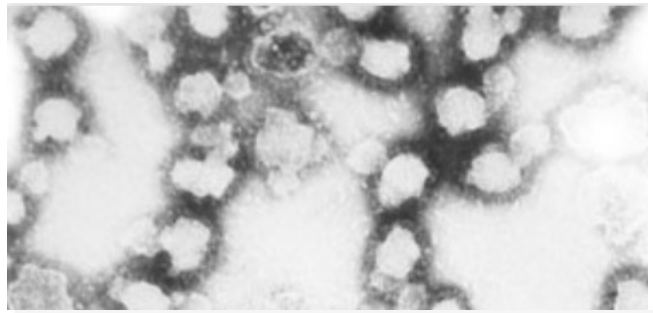
RNA Viruses:
California encephalitis virus**

Bacteria:
Not Detected

Fungi:
Not Detected

Parasites:
Not Detected

**Detected viral sequences most closely match Jamestown Canyon virus, and map to 2 of the 3 segments of the viral genome.



AP AP NEWS

California recall | Coronavirus pandemic | Afghanistan | Politics | Sports | Entertainment | Photog

NH adult infected with Jamestown Canyon Virus dies

August 6, 2021

Click to copy

RELATED TOPICS

Health
Coronavirus pandemic
Canyons

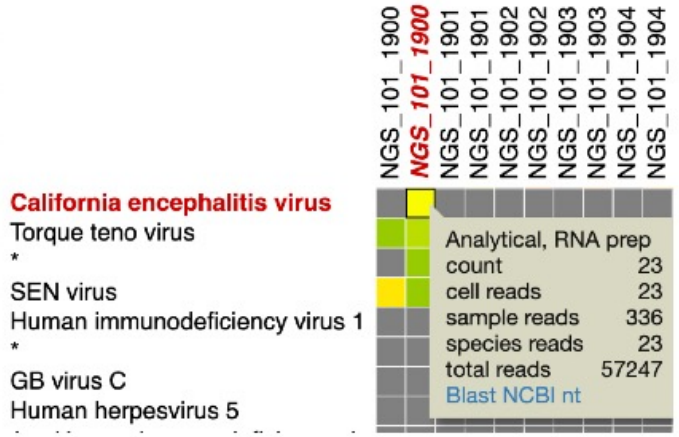
CONCORD, N.H. (AP) — An adult who became infected with the Jamestown Canyon Virus has died, the New Hampshire Department of Health and Human Services said Friday.

The virus is transmitted by the bite of an infected mosquito.

The adult, from Dublin, New Hampshire, was "hospitalized with worsening neurological symptoms and ultimately died," the department said in a news release. "JCV infection was identified as a contributing cause to their death." No further information was released about the person.

The department said this has been the first detection of the virus in a person in the state this year.

The risk of transmission of mosquito-borne diseases to people in Dublin will be increased to high



Solomon, et al., Emerging Infectious Diseases, 2020, in press

- **mNGS testing was positive for California encephalitis virus most closely matching Jamestown Canyon virus (JCV); detected reads mapped to 2 of the 3 segments of the viral genome**

- **Another CSF sample, obtained 3 weeks later, was negative for JCV by RT-PCR; however, serum JCV RT-PCR was positive**

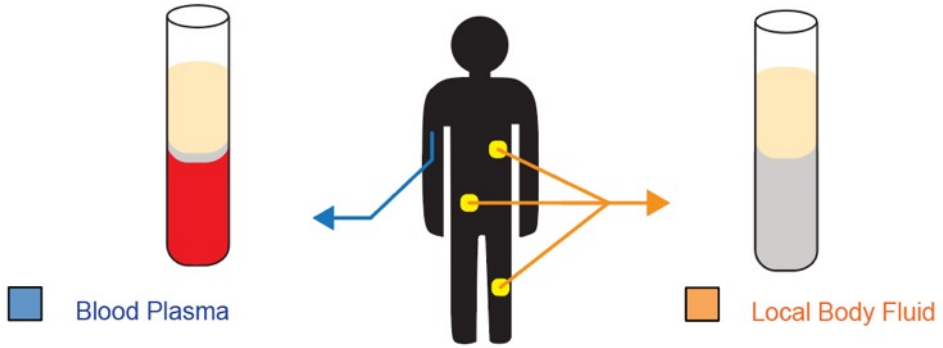
- **JCV IgM and neutralizing antibody testing was negative in both CSF and blood.**

- **Patient was treated with IVIg (2 g/kg) followed by 2-week course of favipiravir (an experimental inhibitor of viral RNA polymerase), without clinical improvement**

- **He became comatose and was transitioned to comfort care and died, approximately 1 year from suspected symptom onset**

Solomon, et al., 2021, Emerging Infectious Diseases 27(1)

Plasma mNGS at UCSF



The UCSF clinical metagenomic next-generation sequencing (mNGS) assay is now available for use with plasma samples. This clinically validated test broadens the offerings from the UCSF Center for Next-Gen Precision Diagnostics, which until now had been restricted for cerebrospinal fluid (CSF) samples only.

Some clinical indications for the mNGS plasma assay may include:

- Culture-negative endocarditis
- Fever of unknown origin (FUO) with suspected infectious etiology, for both pediatric and adult patients
- Difficult-to-biopsy end-organ disease
- Immunocompromised and transplant patients with suspected infections
- ICU patients, most on empiric antibiotics, with infections not identified in conventional testing

https://nextgendiagnostics.ucsf.edu

Unknown Case

- 40 y/o male with AML s/p allogeneic stem-cell transplant in October of 2019 admitted with cough, fever, and found to have relapse of AML
- Developed acute respiratory decompensation requiring intubation and progression with new nodular consolidation in the upper lobes that was concerning for invasive mold infection
- Repeat chest CT → evolution of upper lobe opacities, less nodular and more diffuse, with tree-in-bud abnormalities
- Workup for viral etiologies (RVP, adenovirus, CMV, HHV6) and PCP (beta-D-glucan, cytology) unrevealing, tracheal aspirates/BAL cultures negative
- On pip-tazo and ambisome but with increasing oxygen requirement
- Discussion to transition to comfort care


R
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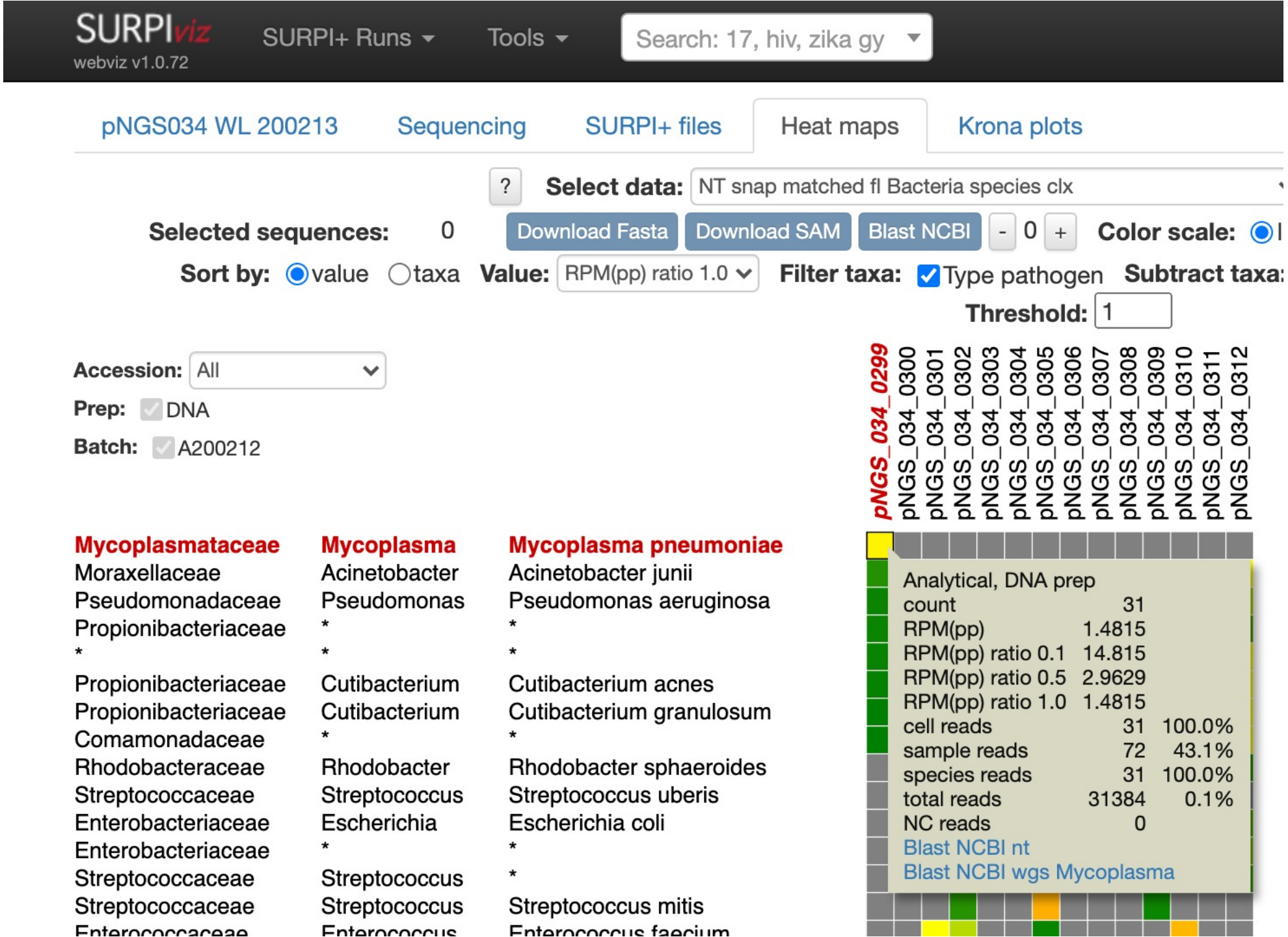
P


SURPIviz
webviz v1.0.72
SURPI+ Runs
Tools
Search: 17, hiv, zika gy
pNGS034 WL 200213
Sequencing
SURPI+ files
Heat maps
Krona plots
?
Select data:
NT snap matched fl Bacteria species clx
Selected sequences: 0
Download Fasta
Download SAM
Blast NCBI
- 0 +
Color scale:
Sort by: value taxa
Value: RPM(pp) ratio 1.0
Filter taxa: Type pathogen
Subtract taxa:
Threshold: 1
Accession: All
Prep: DNA
Batch: A200212
pNGS_034_0299
pNGS_034_0300
pNGS_034_0301
pNGS_034_0302
pNGS_034_0303
pNGS_034_0304
pNGS_034_0305
pNGS_034_0306
pNGS_034_0307
pNGS_034_0308
pNGS_034_0309
pNGS_034_0310
pNGS_034_0311
pNGS_034_0312
Mycoplasmataceae
Mycoplasma
Mycoplasma pneumoniae
Moraxellaceae
Acinetobacter
Acinetobacter junii
Pseudomonadaceae
Pseudomonas
Pseudomonas aeruginosa
Propionibacteriaceae
*
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Propionibacteriaceae
Cutibacterium
Cutibacterium acnes
Propionibacteriaceae
Cutibacterium
Cutibacterium granulosum
Comamonadaceae
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Rhodobacteraceae
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Rhodobacter sphaeroides
Streptococcaceae
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Streptococcus uberis
Enterobacteriaceae
Escherichia
Escherichia coli
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Streptococcaceae
Streptococcus
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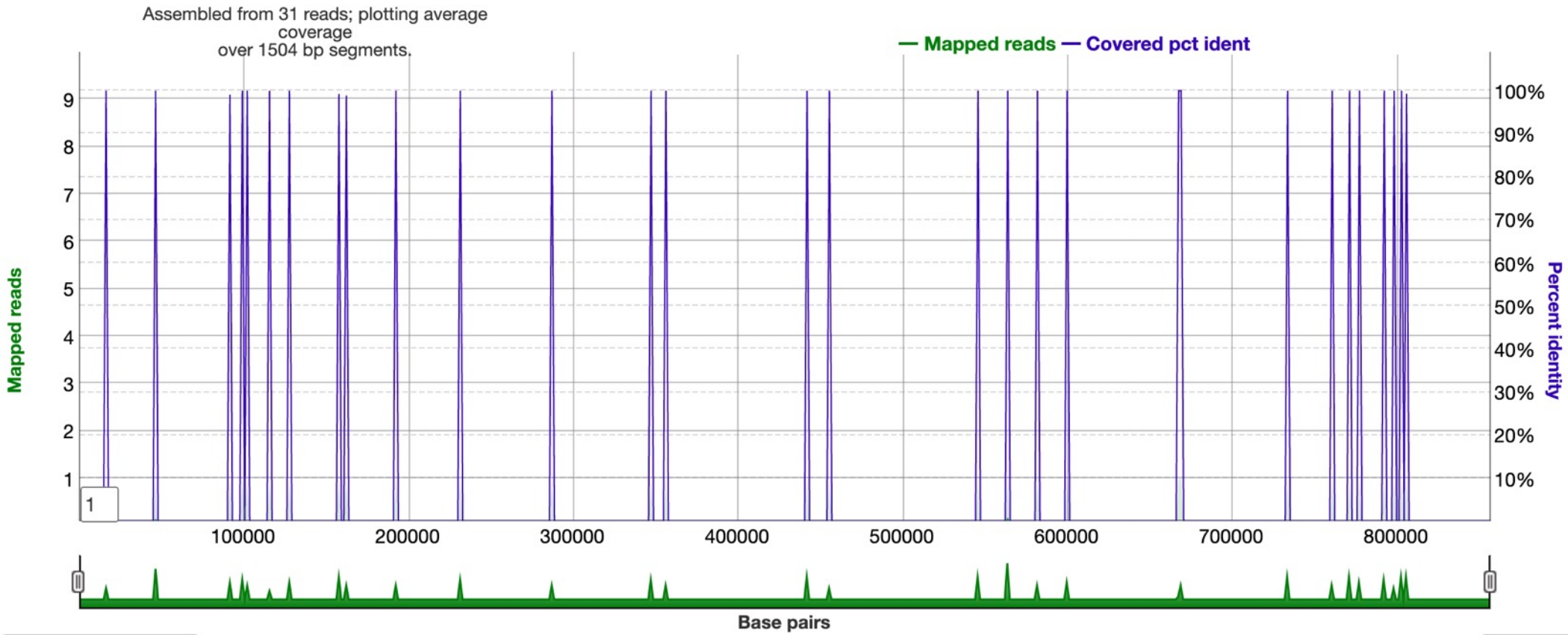
Streptococcaceae
Streptococcus
Streptococcus mitis
Enterococcaceae
Enterococcus
Enterococcus faecium
Analytical, DNA prep
count 31
RPM(pp) 1.4815
RPM(pp) ratio 0.1 14.815
RPM(pp) ratio 0.5 2.9629
RPM(pp) ratio 1.0 1.4815
cell reads 31 100.0%
sample reads 72 43.1%
species reads 31 100.0%
total reads 31384 0.1%
NC reads 0
Blast NCBI nt
Blast NCBI wgs Mycoplasma

? **Select file:** Mycoplasma Mycoplasma_pneumoniae CP008895.1 genus **Series:** Coverage Percent identity **Shading:** min/max

Mycoplasma pneumoniae M29 (CP008895.1, 857799 bp)

Mycoplasma pneumoniae M29, complete genome



Toggle Log/Linear Y-axis

Download Consensus

Reset Zoom

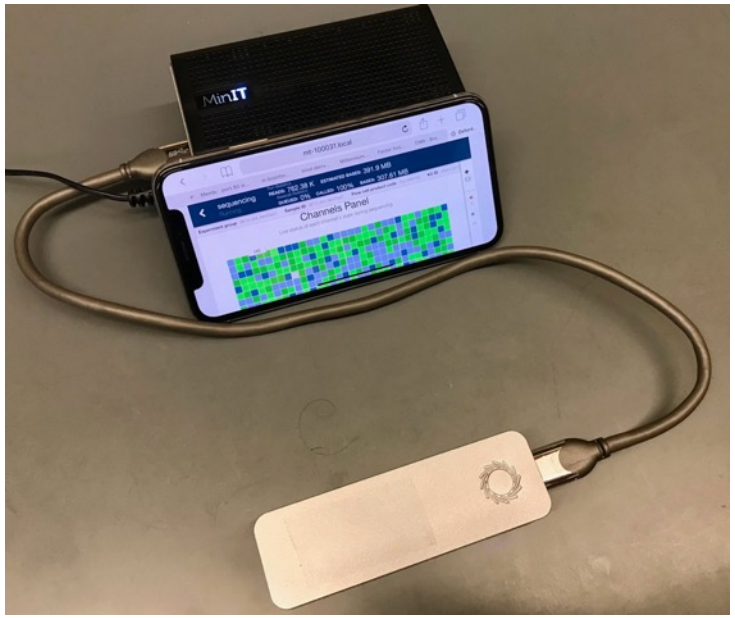
Save PDF

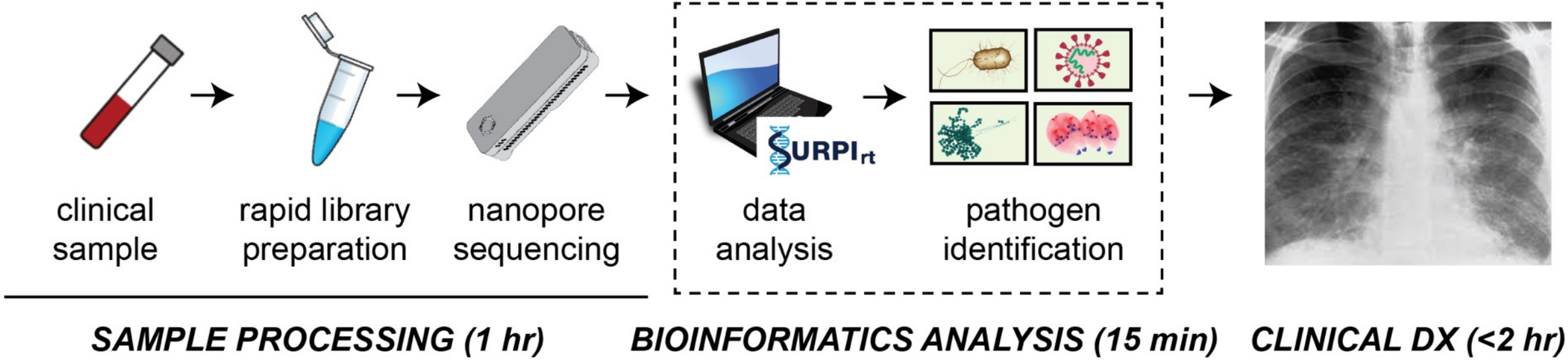
	Reference length bp	*Coverage in bp*	*Percent coverage*	*Avg coverage depth*	*Covered pct ident*
Overall	857799	3301	0.38	0.00	99.88
Displayed	857799	3301	0.38	0.00	99.88

Unknown Case

- Patient started on azithromycin
- Improved rapidly and was extubated, on room air, and working with physical therapy
- Also had received steroids for possible pulmonary graft-versus-host-disease that was continued
- Notably, he had received a five-day course of azithromycin as an outpatient, but this was thought insufficient to treat his fulminant disease given his degree of immunocompromise

Clinical Nanopore Metagenomic Sequencing

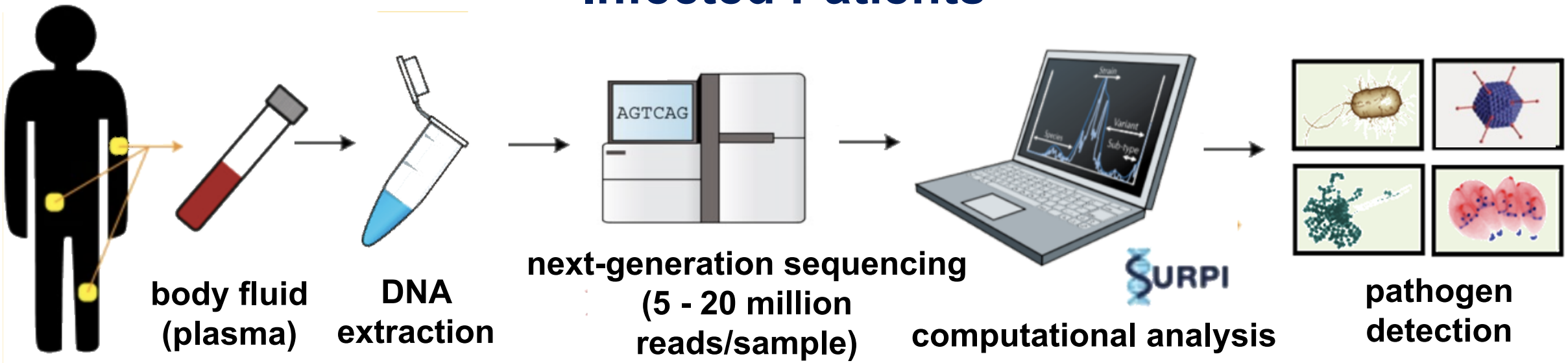




Gu, et al., 2021, *Nature Medicine*, 25:115-124.
Zhang, et al., 2021, manuscript in preparation


FIRST AID KIT

Metagenomic Sequencing of Body Fluids from Infected Patients



body fluid (plasma) → DNA extraction → next-generation sequencing (5 - 20 million reads/sample) → computational analysis → pathogen detection

Article | Published: 09 November 2020

Rapid pathogen detection by metagenomic next-generation sequencing of infected body fluids

Wei Gu, Xianding Deng, Marco Lee, Yasemin D. Sucu, Shaun Arevalo, Doug Stryke, Scot Federman, Allan Gopez, Kevin Reyes, Kelsey Zorn, Hannah Sample, Guixia Yu, Gurpreet Ishpuniani, Benjamin Briggs, Eric D. Chow, Amy Berger, Michael R. Wilson, Candace Wang, Elaine Hsu, Steve Miller, Joseph L. DeRisi & Charles Y. Chiu ✉

Nature Medicine **27**, 115–124 (2021) | Cite this article

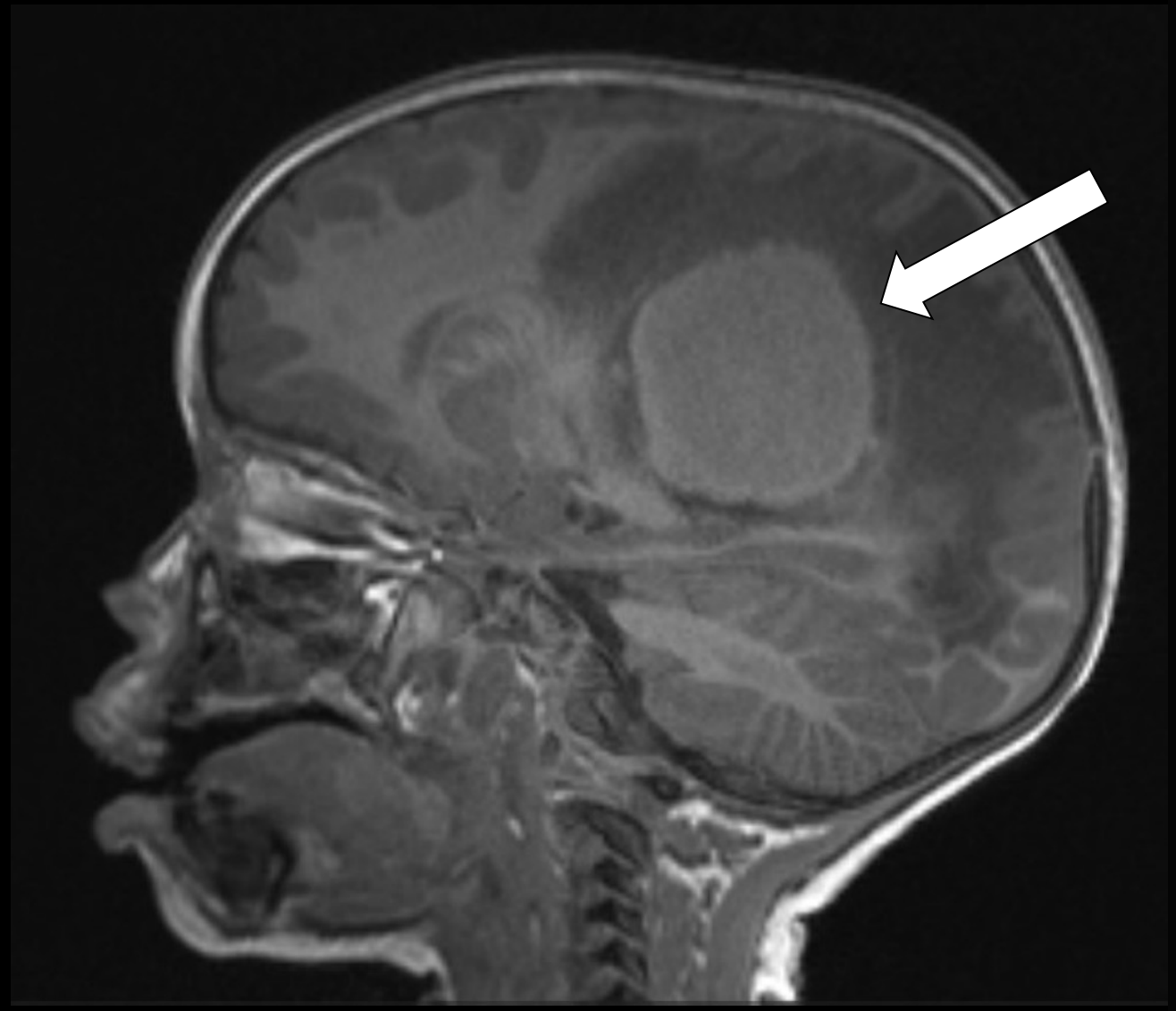
8963 Accesses | **13** Citations | **174** Altmetric | Metrics

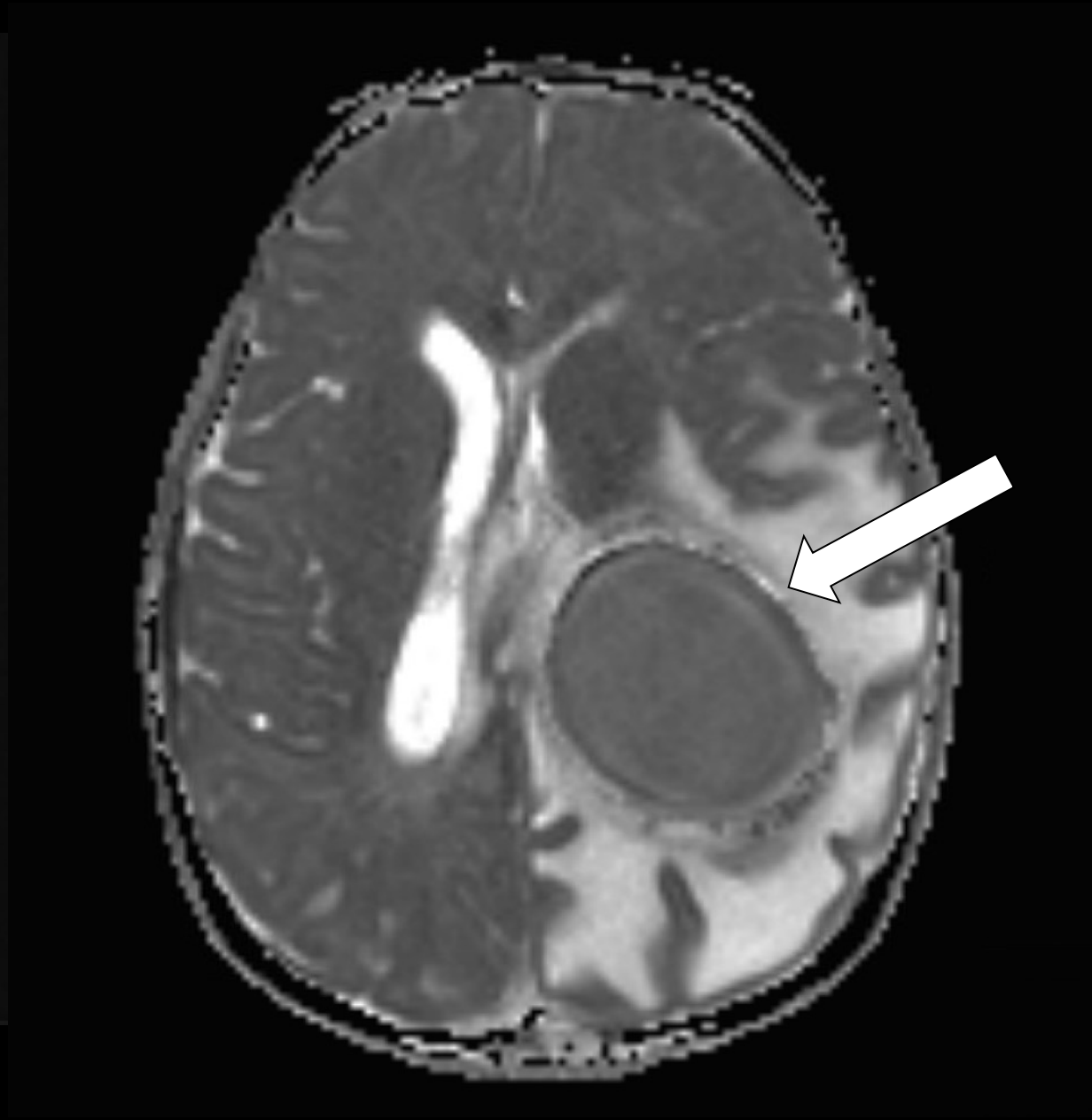
Wei Gu, MD/PhD

Gu, et al., 2021, *Nature Medicine*, 25:115-124.

Unknown Case – 2 year old with new-onset seizures and large left brain abscess

- History of multiple ear infections and cellulitis
- treated with ceftriaxone and azithromycin
- Presented with seizures, wobbly gait, decreased use of right hand, hearing difficulties (associated with otitis media)
- No significant exposure history
- Admitted for workup



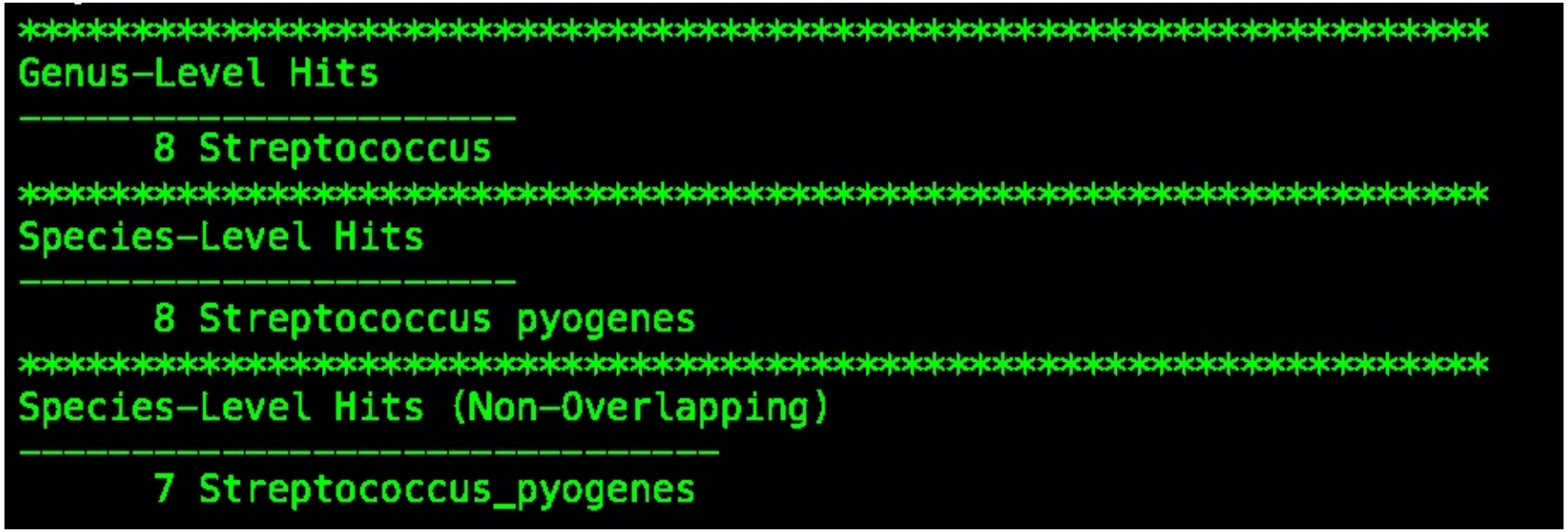


2-year with new-onset seizures and large left brain abscess

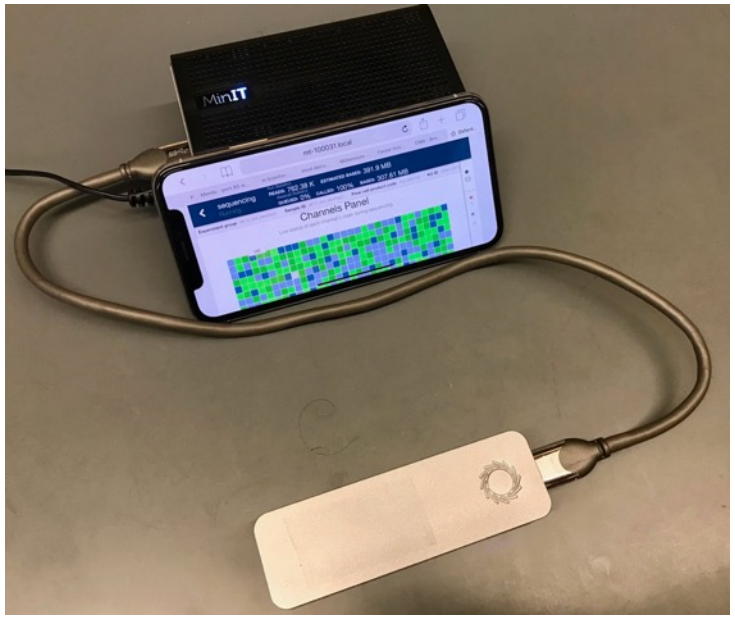
- Workup for immunodeficiency (lymphocyte subsets, HIV testing)
- Extraction of 40 cc of purulent fluid from abscess
- Differential diagnosis:
  - Anaerobic infection
  - *Balamuthia mandrillaris*
  - Neurocysticercosis
  - *Nocardia spp.*
  - *Mycobacterium tuberculosis*
  - Toxoplasmosis
  - *Listeria monocytogenes*
  - Non-infectious etiology (malignancy)

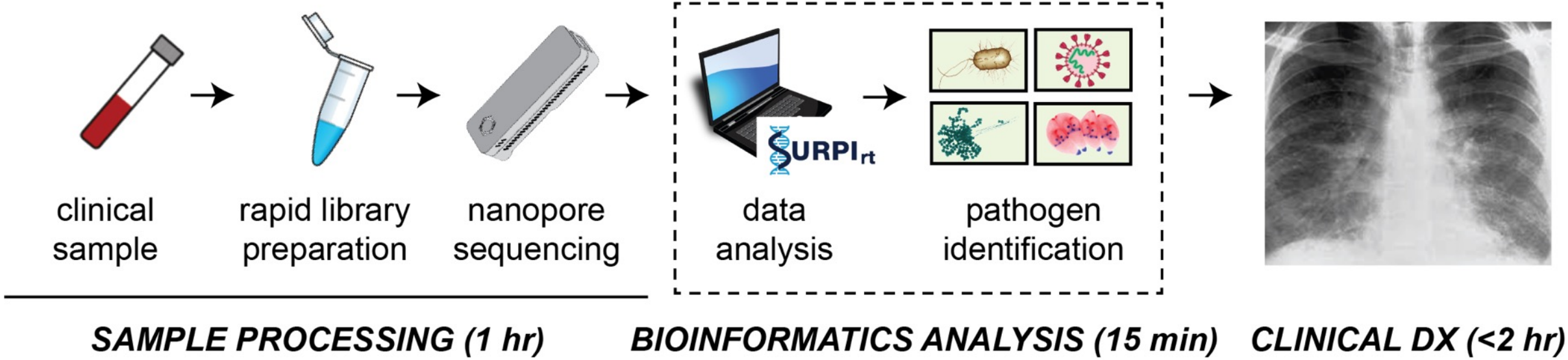
Streptococcus pyogenes abscess

- Direct DNA extraction → nanopore sequencing
- First read detected in 40 minutes
- Overall turnaround time from sample to answer: 3 hours
- 16S universal PCR also positive for *S. pyogenes*, cultures negative



Clinical Nanopore Metagenomic Sequencing



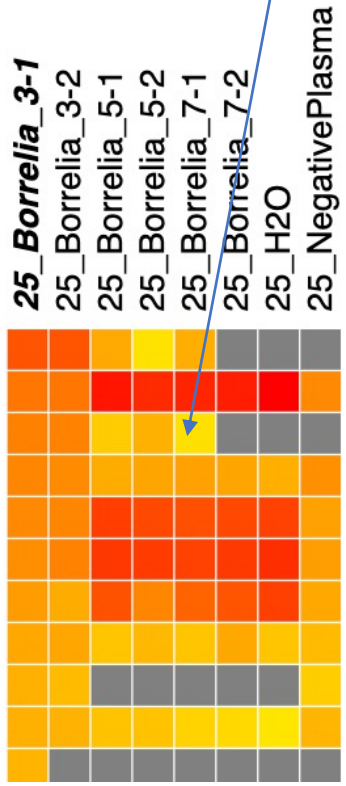


Gu, et al., 2021, *Nature Medicine*, 25:115-124.
Zhang, et al., 2021, manuscript in preparation

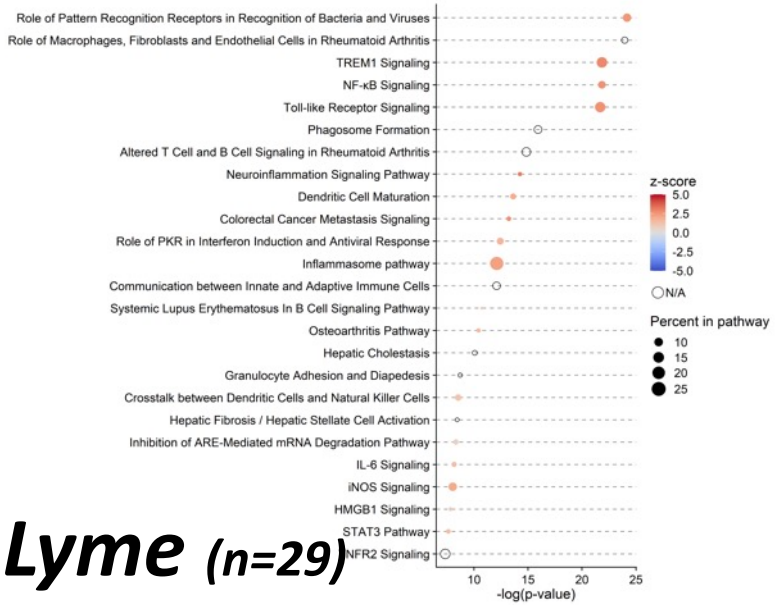
Nanopore Sequencing of *Borrelia burgdorferi*

Spirochaetaceae	Borrelia	*
*	*	*
Spirochaetaceae	**Borrelia**	**Borrelia burgdorferi**
Pseudomonadaceae	Pseudomonas	Pseudomonas pseudoalcaligenes
Enterobacteriaceae	Escherichia	Escherichia coli
Enterobacteriaceae	*	*
Propionibacteriaceae	Propionibacterium	Propionibacterium acnes
Comamonadaceae	Acidovorax	Acidovorax sp. KKS102
Methylophilaceae	*	*
Pseudomonadaceae	Pseudomonas	*
Pseudomonadaceae	Pseudomonas	Pseudomonas sp. TKP

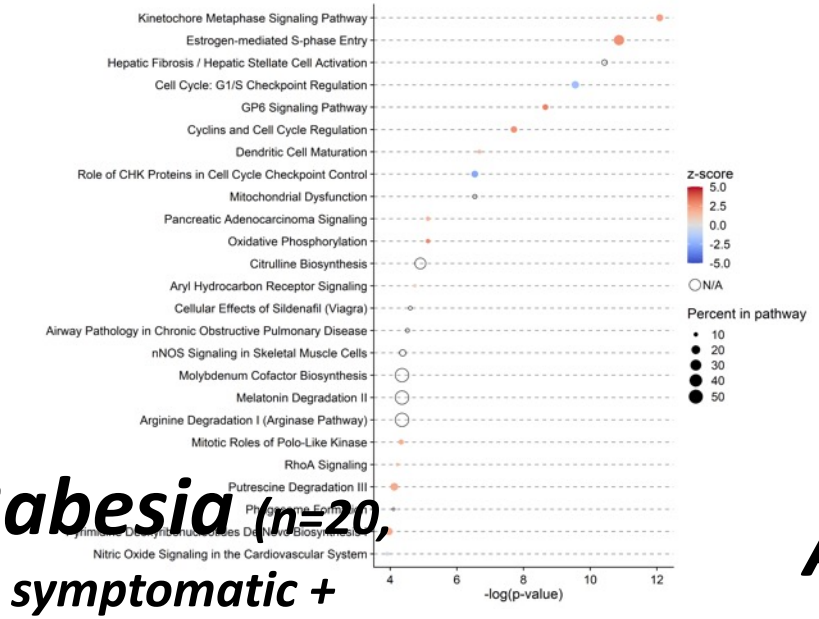
1 copy/mL



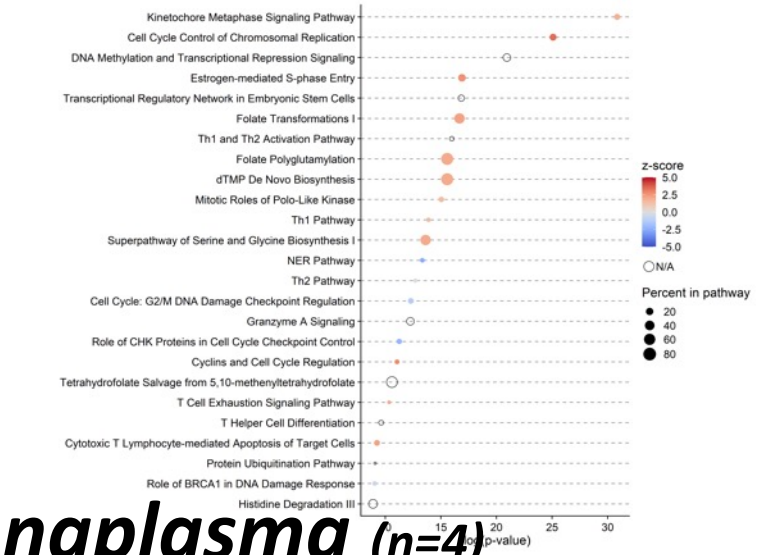
RNA Host Response Profiles of Febrile Infection



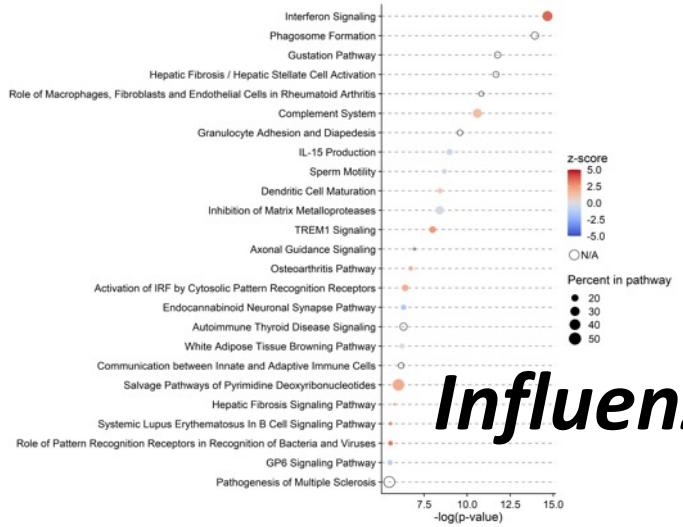
Lyme *(n=29)*



Babesia *(n=20, symptomatic + asymptomatic)*

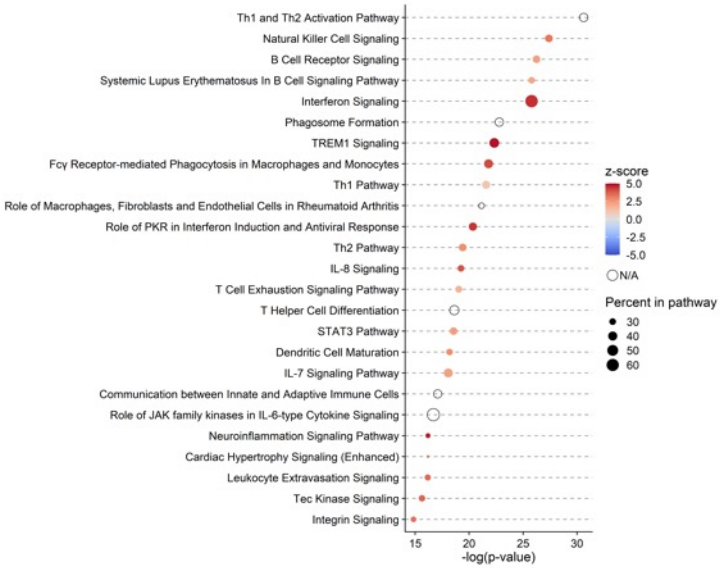


Anaplasma *(n=4)*



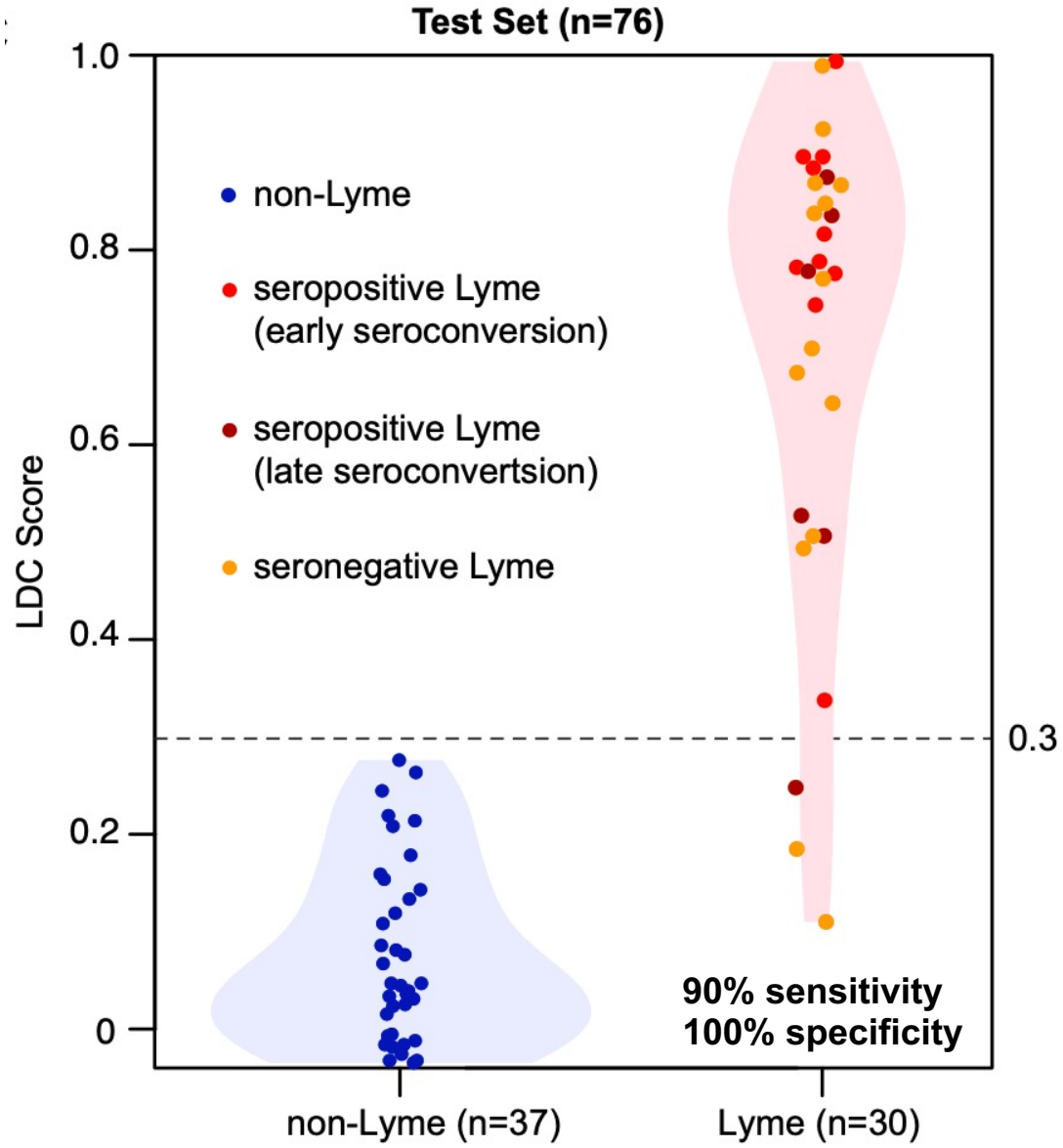
Influenza *(n=18)*

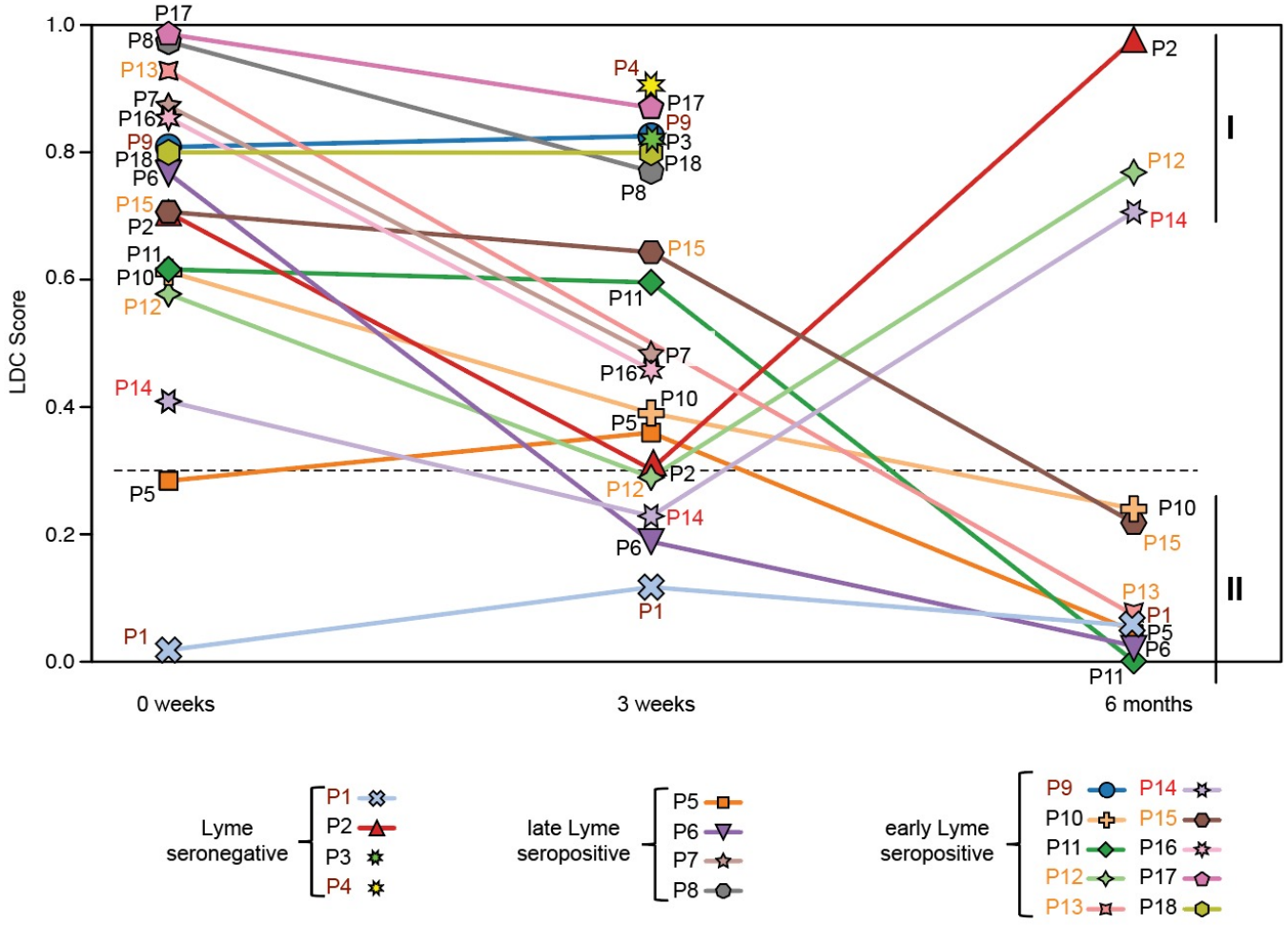
Bacterial sepsis *(n=5, symptomatic + asymptomatic)*



Ng, et al., 2020, manuscript under review

Lyme Disease Gene Classifier (LDC) for Diagnosis of Early Lyme Disease





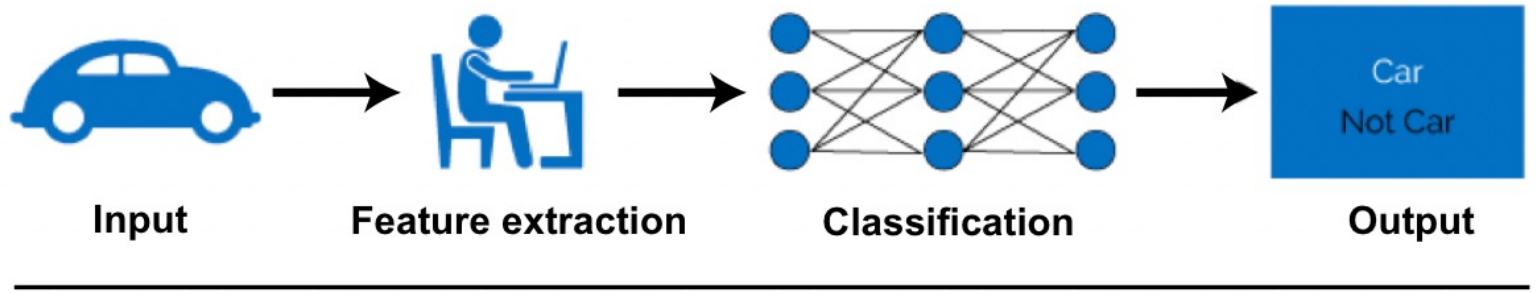
Pn return to usual state of health at 6 months and 1 year

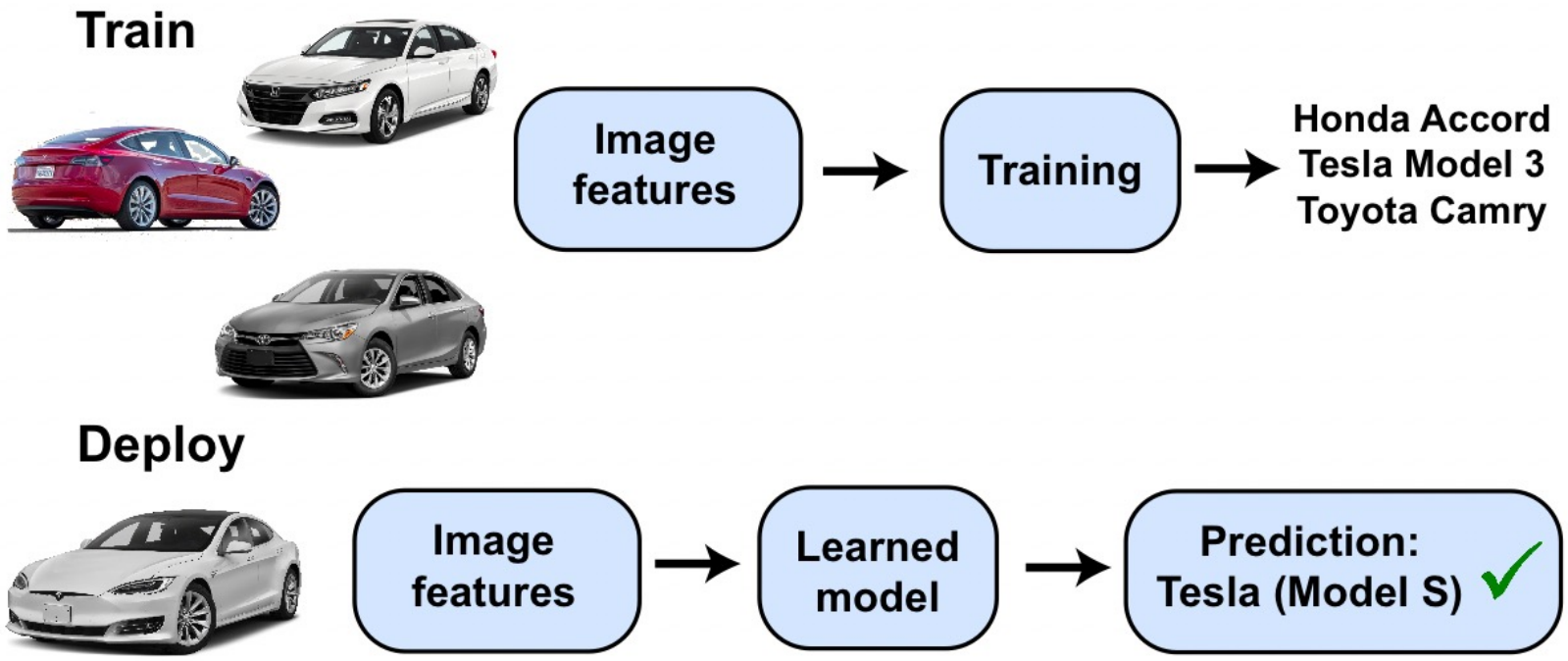
Pn symptomatic at 6 months, return to usual state of health at 1 year

Pn symptomatic at 12 months (but not 6 months)

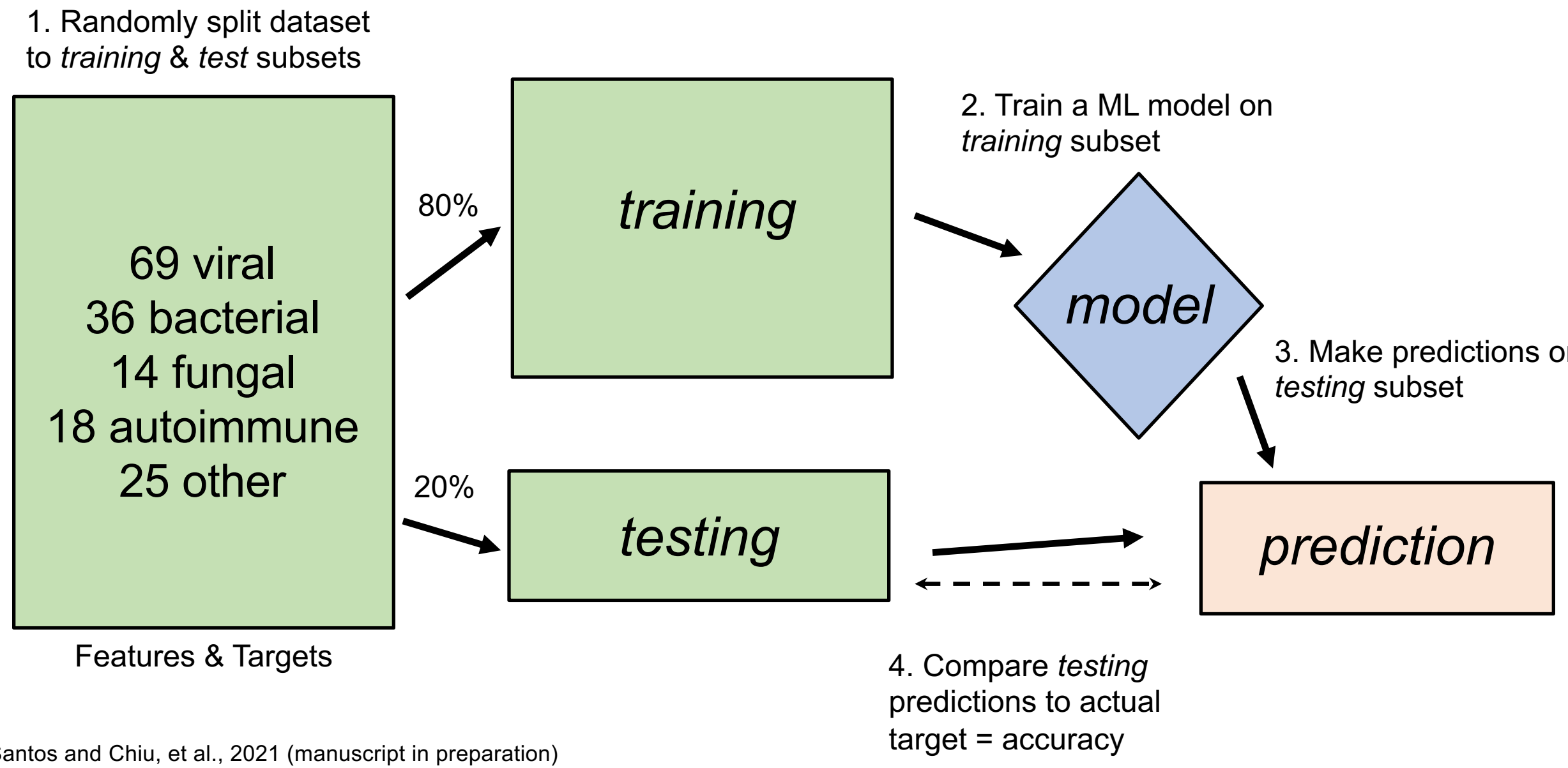
Pn PTLDS diagnosis at 6 months or 12 months

Transcriptome Profiling of Human (Patient) Host Responses using Machine Learning Analyses of RNA-Seq Data



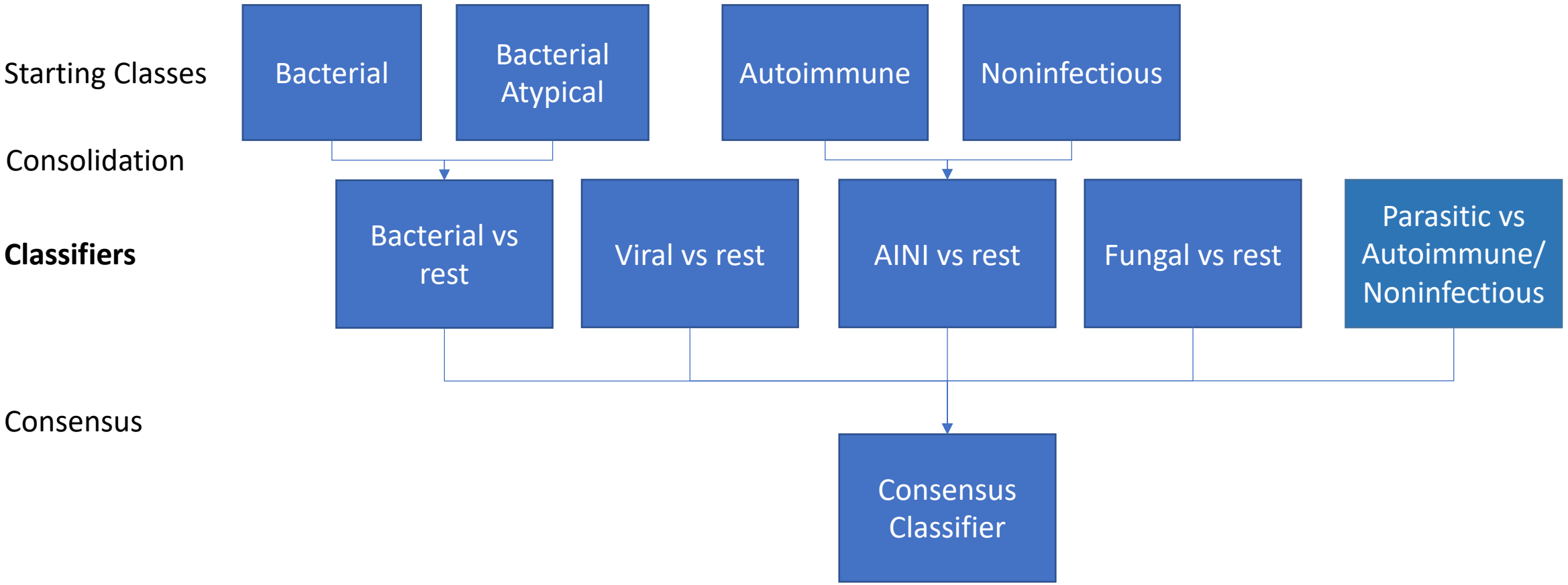


Predictive Model from CSF RNA-Seq (Transcriptome) Data



Santos and Chiu, et al., 2021 (manuscript in preparation)

CSF Host Response Classifier Algorithm



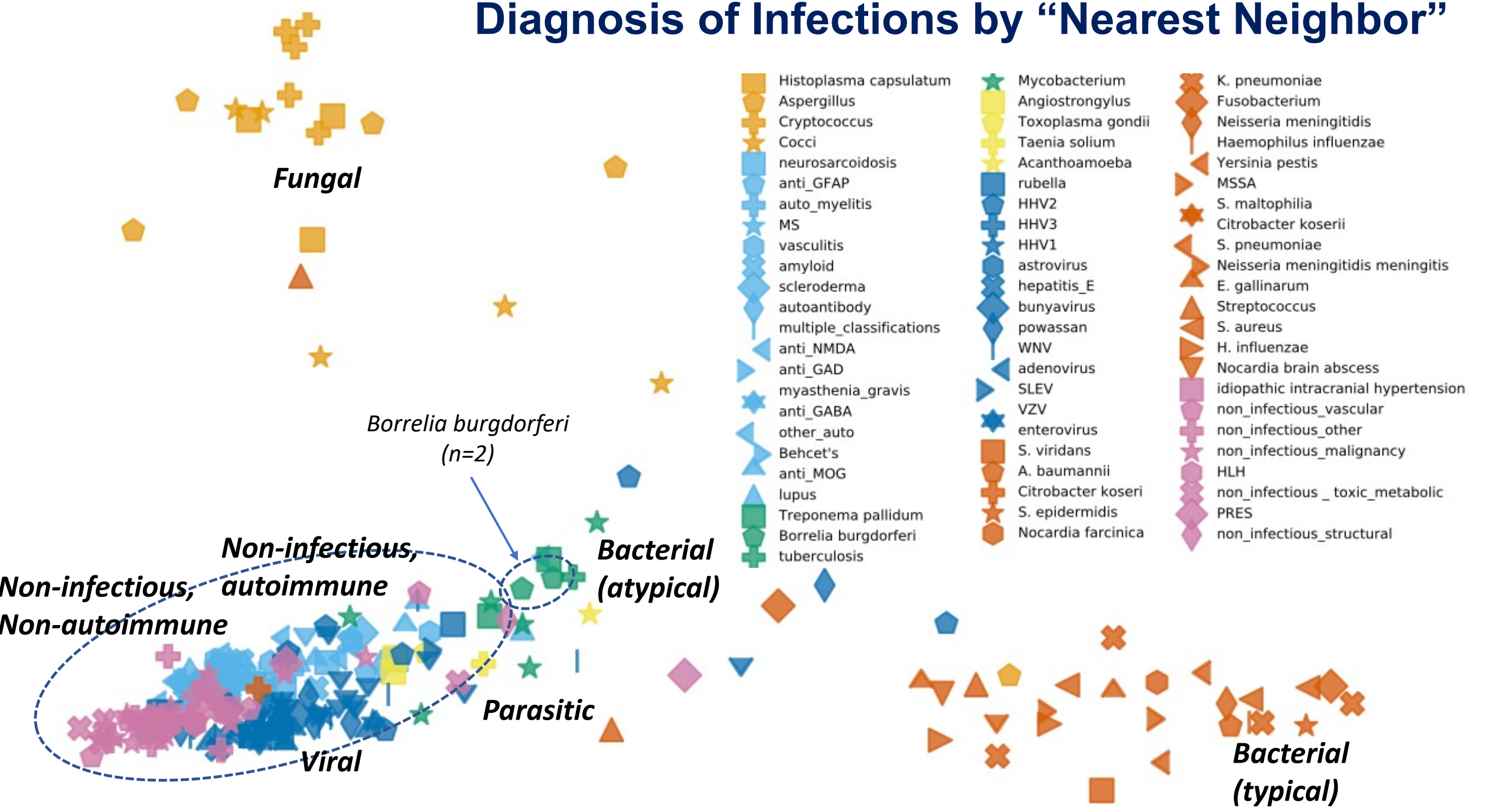
Performance of the Spinal Fluid Classifier

- ***Bacterial infection*** *(86% sensitivity, 93% specificity)*
- ***Viral infection*** *(81% sensitivity, 93% specificity)*
- ***Fungal infection*** *(75% sensitivity, 95% specificity)*
- ***Parasitic infection*** *(60% sensitivity, 100% specificity)*
- ***Autoimmune / non-infectious etiology*** *(83% sensitivity, 96% specificity)*

Host Response Classification of Metagenomic RNA-Seq Data for Infection Diagnosis

Patient Dx	mNGS result	AINI	Bacterial	Fungal	Parasitic	Viral	Classifier Result
subthreshold *Mycobacterium tuberculosis*	negative	0.61%	64.07%	21.18%	1.30%	12.84%	Bacterial
GABA-receptor mediated encephalitis	negative	80.92%	8.10%	1.36%	2.34%	7.29%	AINI
unknown, likely viral / autoimmune	multiple bacterial taxa, including P. acnes, Micrococcus luteus, and Corynebacterium sp.	35.20%	11.80%	8.74%	6.93%	37.33%	Viral
subthreshold Aspergillus	negative	7.32%	17.68%	65.71%	0.86%	8.43%	Fungal
Zika virus	Zika virus	16.24%	4.93%	0.91%	2.13%	75.78%	Viral

Diagnosis of Infections by “Nearest Neighbor”



Unknown Case

FINAL REPORT mNGS for pathogen detection in CSF

STUDY ID	n/a
PATIENT NAME	
MRN	
INSTITUTION	
FAX (Lab Result)	
SENDER MRN	
SENDER SAMPLE ID	
SAMPLE TYPE	CSF
SAMPLE ACCESSION #	
COD	9/30/21 12:15

Library Type	Analytical
Description	Clinical
mNGS ACCESSION #	NGS_368_5359

Run prepared by	
Date: D/M/YYYY	
CLS comments	

Supervisor Review	CC
Date	10/15/21

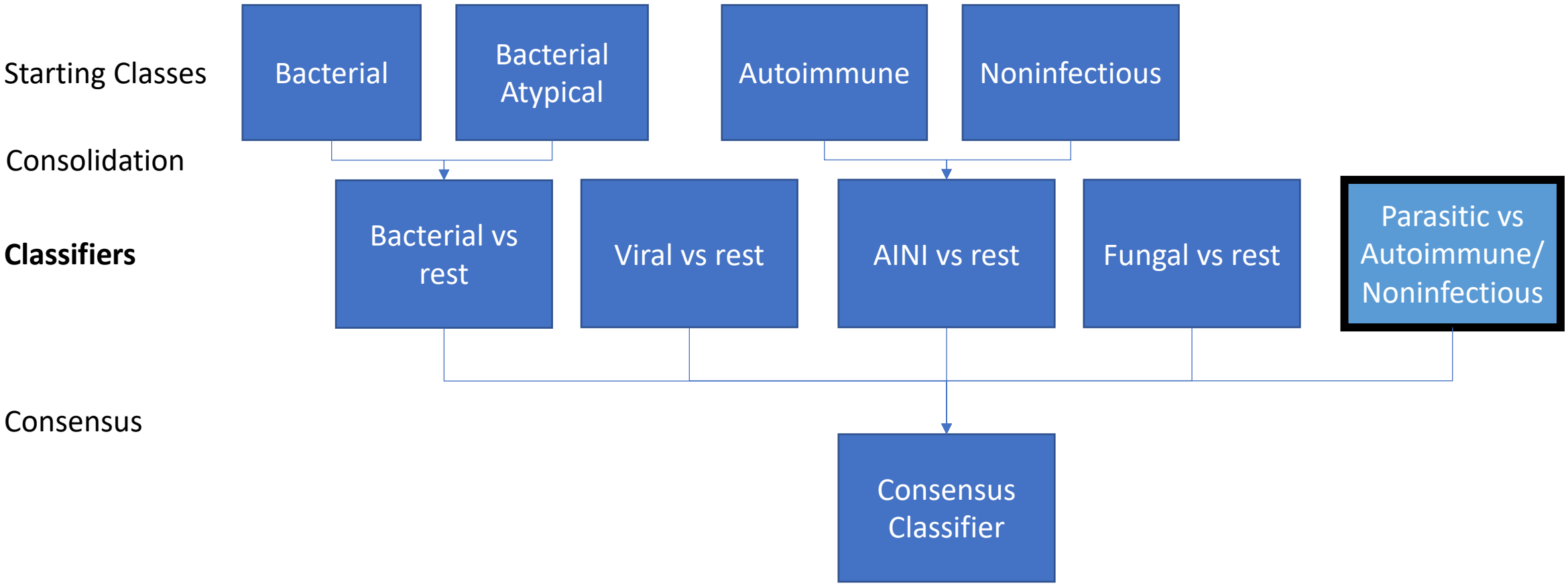
Organism Type	Taxonomic Name / Not Detected / Pending	Synonym	Interpretive Note
DNA VIRUSES	Not Detected		
RNA VIRUSES	Not Detected		
BACTERIA	Not Detected		
FUNGI	Not Detected		
PARASITES	Not Detected		

- Elderly male presenting with acute encephalopathy with lymphocytic pleocytosis in CSF
- Microbiological workup negative
- Differential Dx: parasitic infection (positive *Toxocara* Ab) versus autoimmune disease

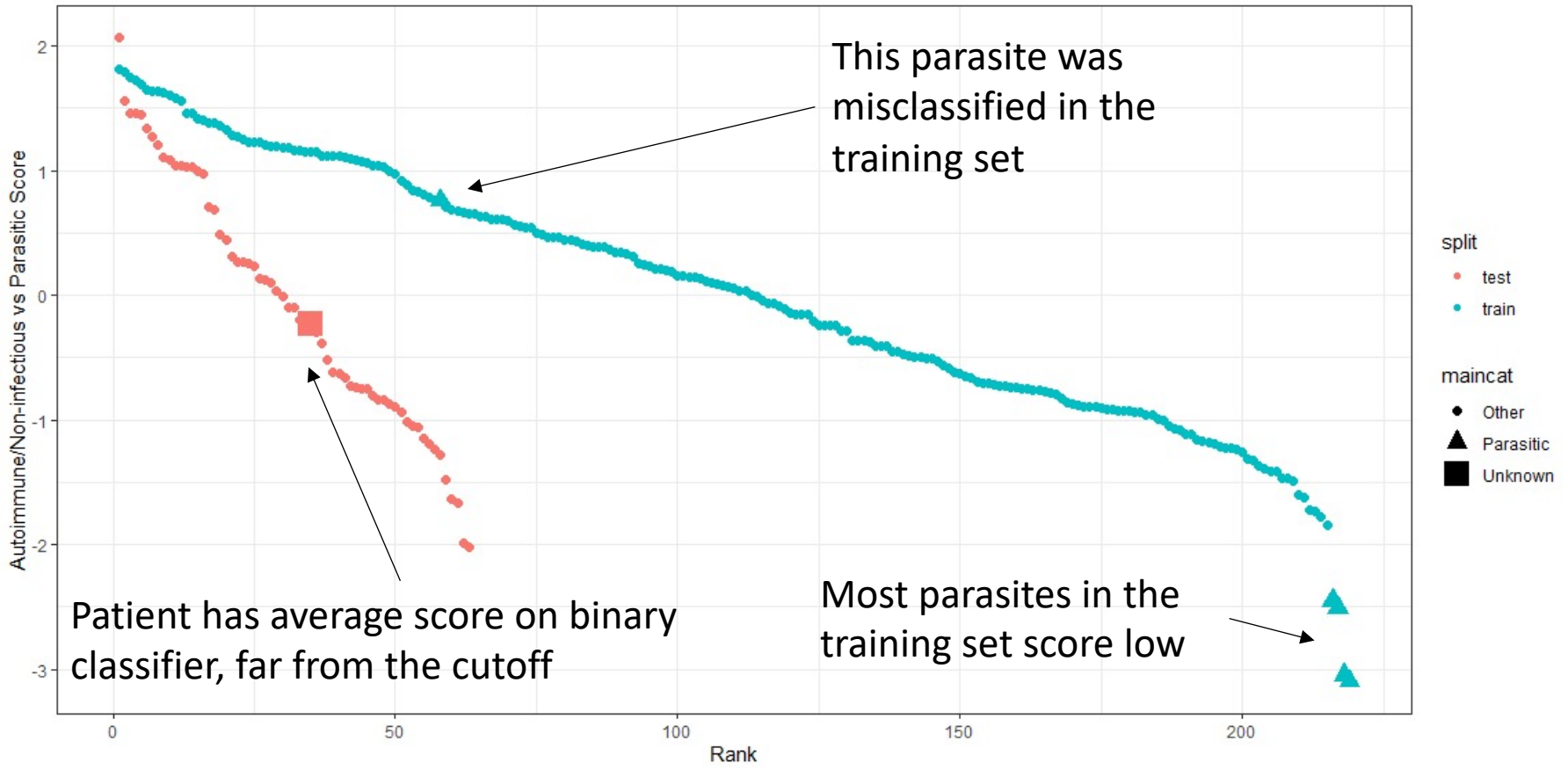
Host Response Classifier Analysis

AINI	81%
Bacterial	8%
Fungal	1%
Parasitic	2%
Vir	7%
Consensus	AINI

CSF Host Response Classifier Algorithm



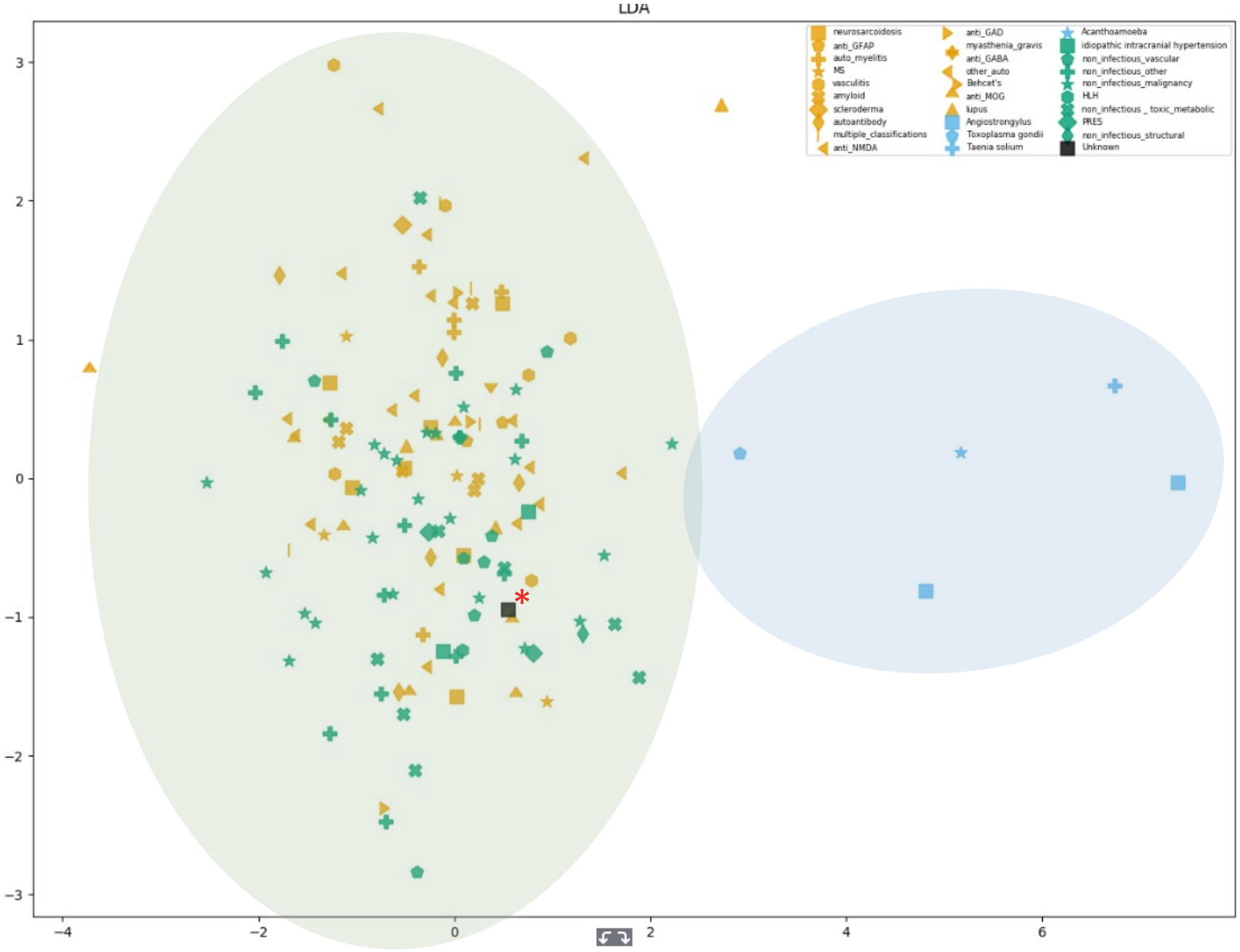
Results from the Binary AINI / Parasite Classifier



- Scores on the y-axis (high = more like AINI, low = more like Parasitic
- 4 of the 5 parasites have reasonable scores
- The 5th parasite indicates that it is possible that a non-represented species could have a higher score
- Overall, however, this binary classifier suggests that this is not a parasitic sample

Linear Discriminant Analysis Plot

(top 27 genes in AINI/parasitic binary classifier)



Tickborne Infections in the United States

- Anaplasmosis
- Babesiosis
- Bourbon virus disease
- Colorado tick fever
- Ehrlichiosis
- Heartland virus disease
- Powassan disease
- **Lyme disease**
- Tickborne relapsing fever
- *Rickettsia parkeri* rickettsiosis
- Rocky Mountain spotted fever
- *Borrelia miyamotoi* infection
- Tularemia
- STARI
- 364D rickettsiosis

CDC website

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