



Rapid Pathogen Detection by Metagenomic Next-generation Sequencing of Infected Body Fluids



Stanford
MEDICINE

Department of
Pathology



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Disclosures

- UCSF markets a clinical metagenomic sequencing test
- UCSF filed patent applications

Case 1

- 70s year old man with brain implant presents with fever and encephalopathy
- CSF: WBC 28->700's in 1 month.
Monocyte then neutrophil predominant
- Culture: Negative
- 16S, ITS sequencing: Negative 2X

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Actionable Diagnosis of Neuroleptospirosis by Next-Generation Sequencing

The NEW ENGLAND JOURNAL of MEDICINE

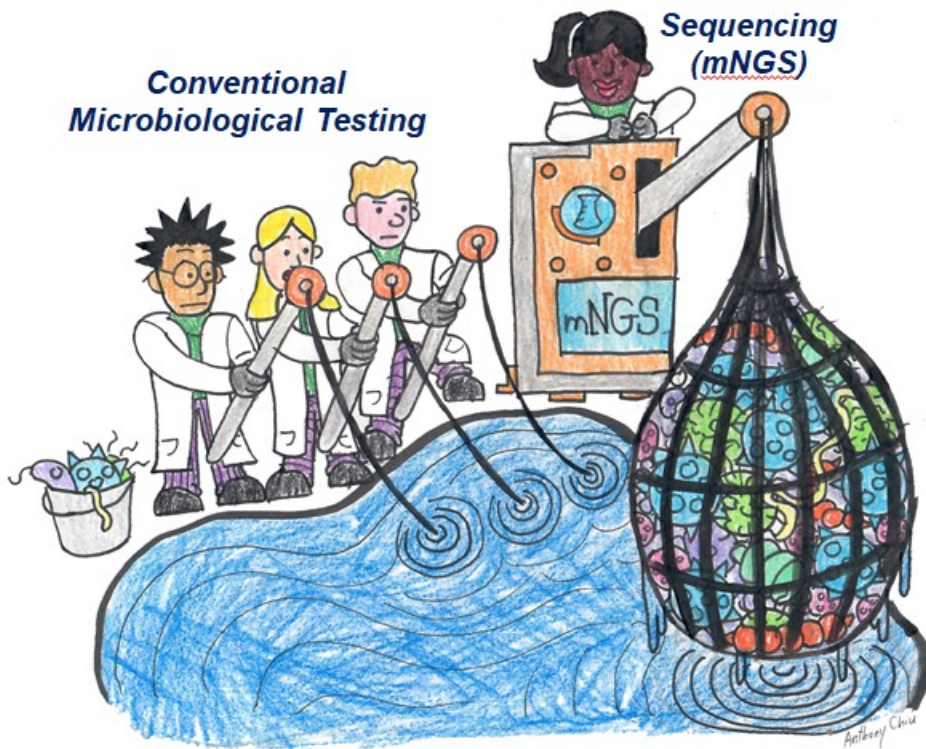
ORIGINAL ARTICLE

Clinical Metagenomic Sequencing for Diagnosis of Meningitis and Encephalitis

Wilson et al. **NEJM** 2014
Wilson et al. **NEJM** 2019

Conventional
Microbiological Testing

Metagenomic
Next-Generation
Sequencing
(mNGS)



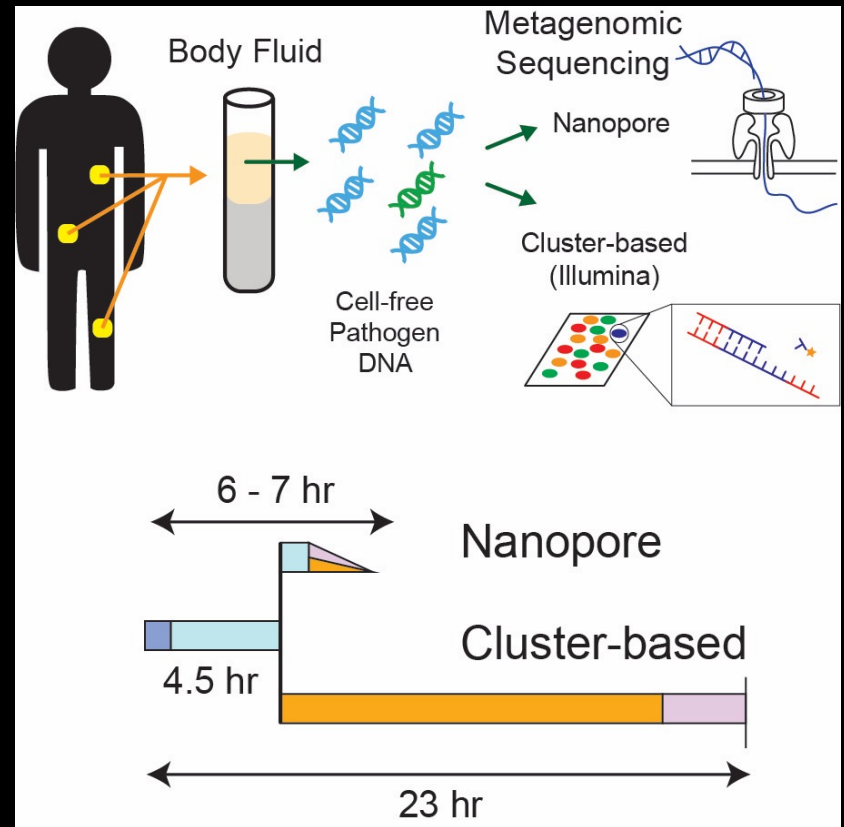
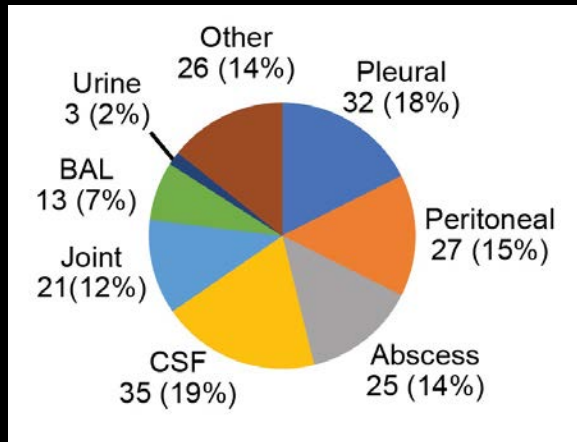
Art courtesy of Dr. Charles Chiu, Anthony Chiu

Pathogen cfDNA in Body Fluids

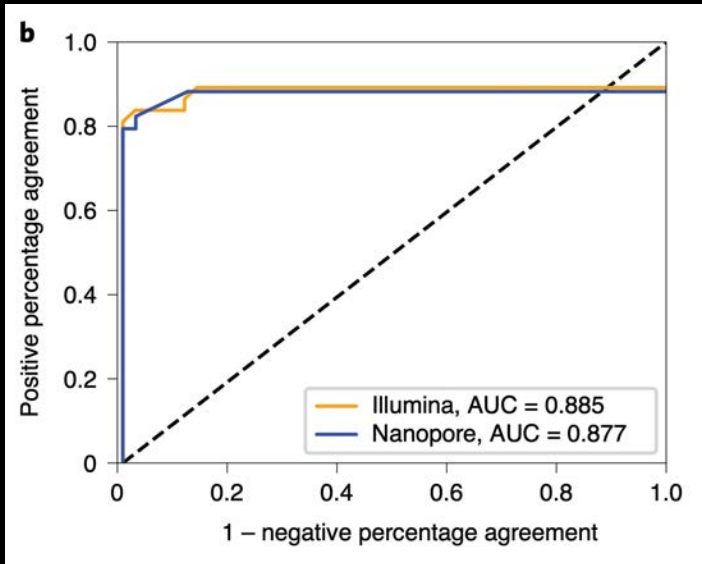
nature medicine **ARTICLES**
<https://doi.org/10.1038/s41591-020-1105-z>

[Check for updates](#)

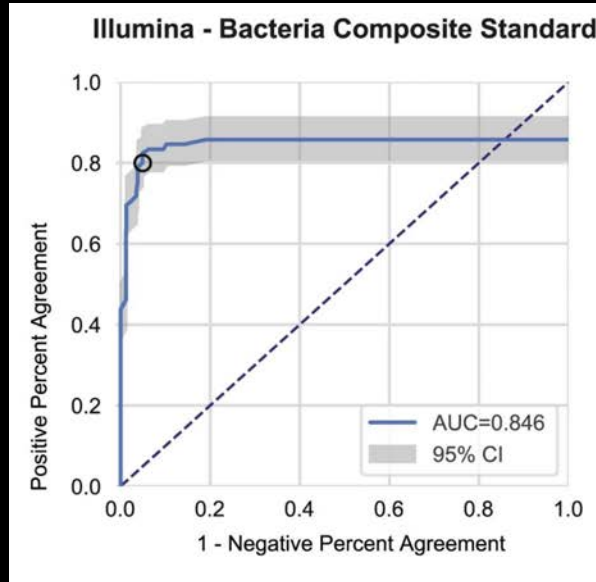
Rapid pathogen detection by metagenomic next-generation sequencing of infected body fluids



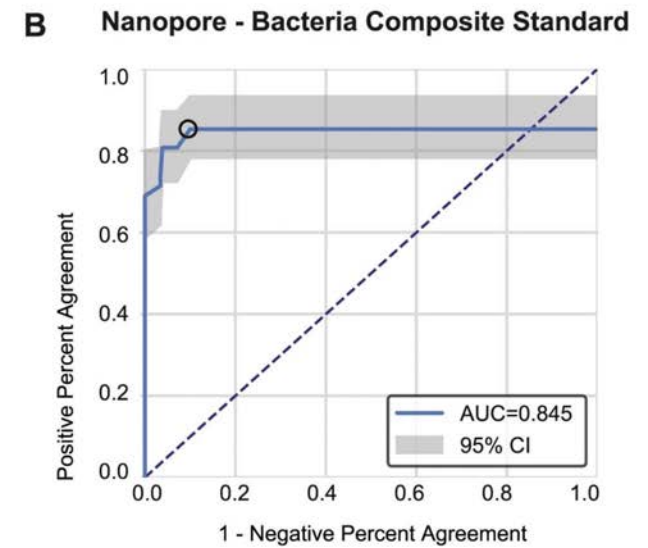
Pathogen cfDNA in Body Fluids



Training



Validation



Composite standard
(culture, 16S PCR, 28S-ITS PCR, other microbiological testing, digital validation PCR, clinical adjudication)

Bacteria: Illumina ($n = 127$)

	+	-
+	68	6
-	17	121

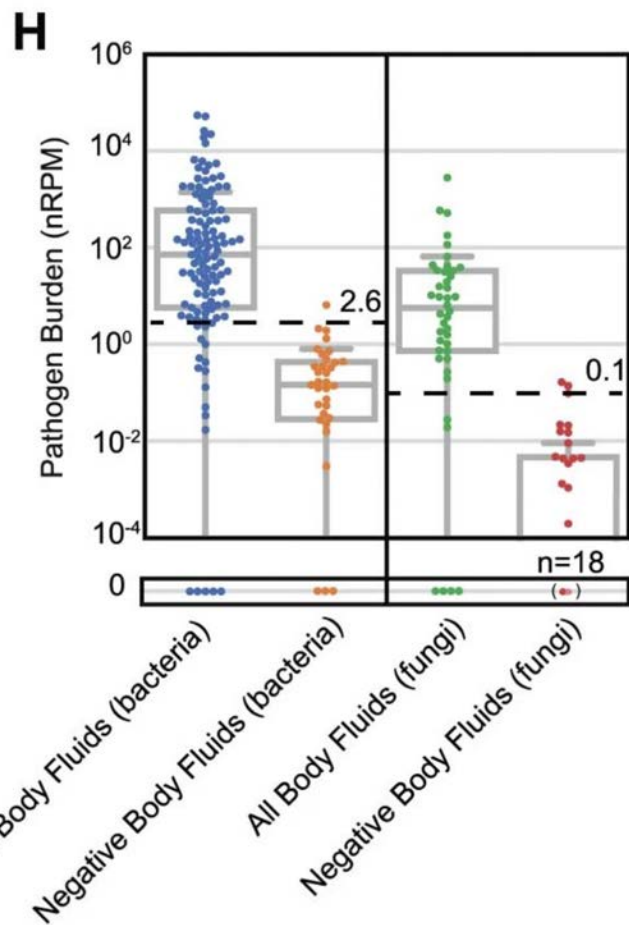
PPA/NPA = 80.0/95.3%

Bacteria: nanopore ($n = 43$)

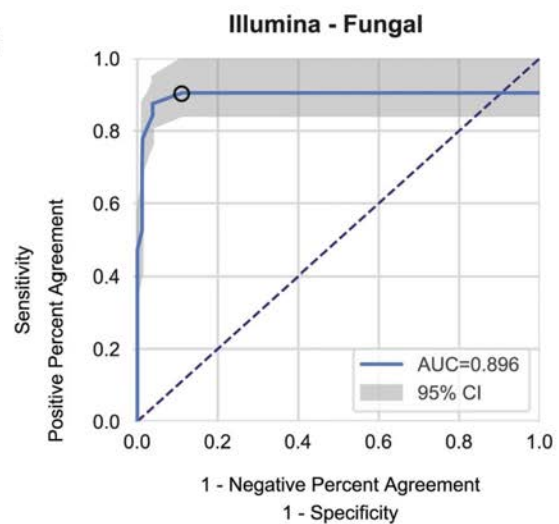
	+	-
mNGS +	34	3
mNGS -	8	40

PPA/NPA = 81.0/93.0%

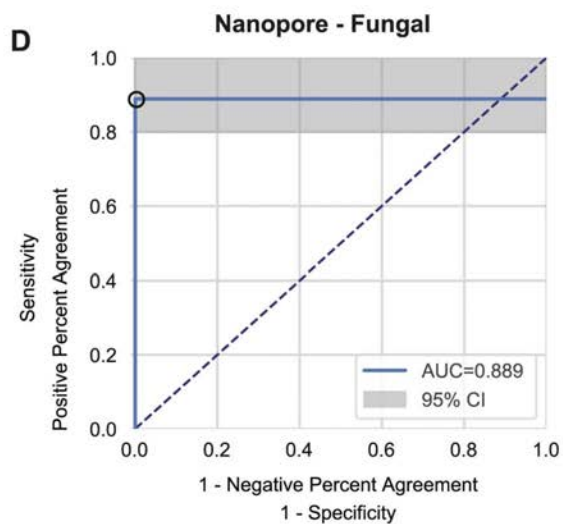
Fungal



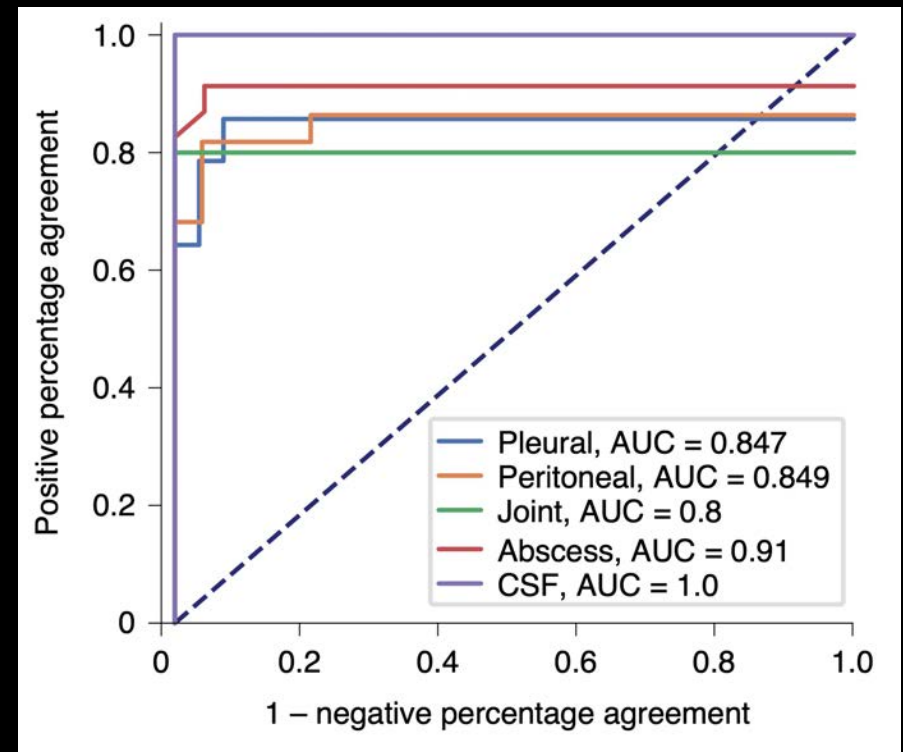
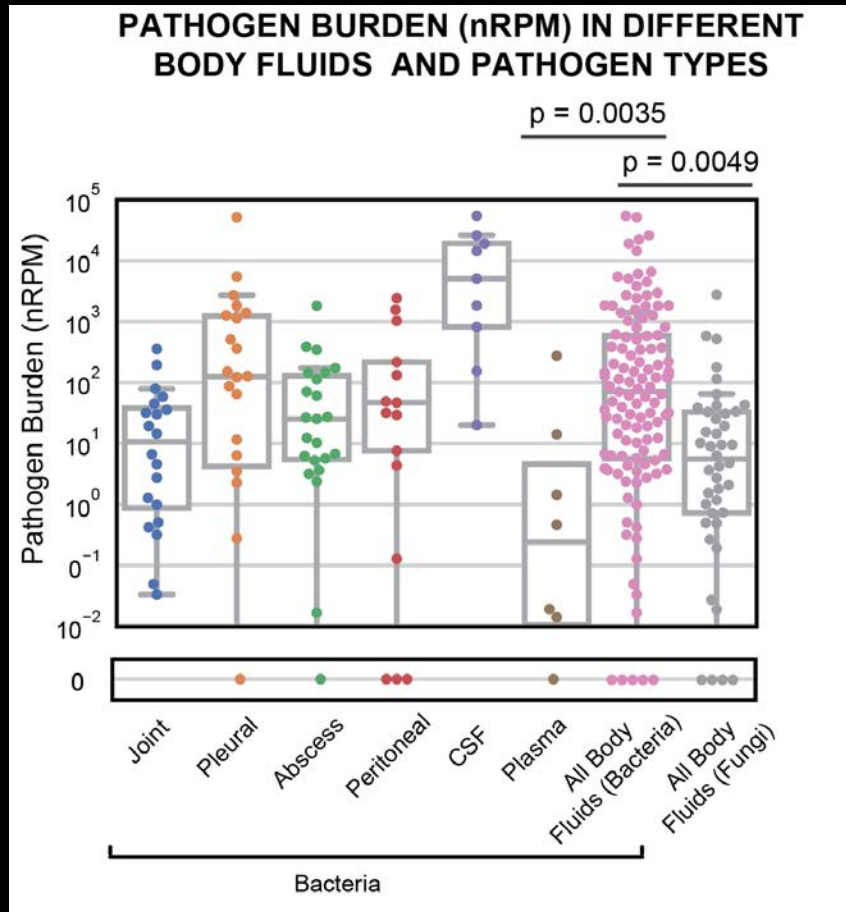
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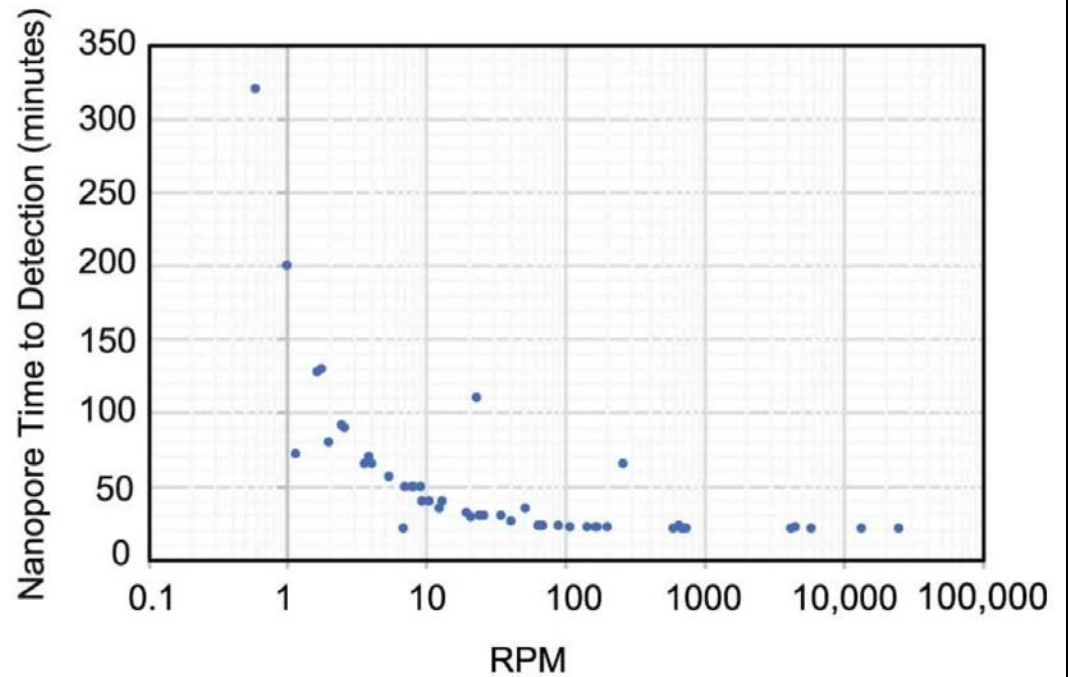
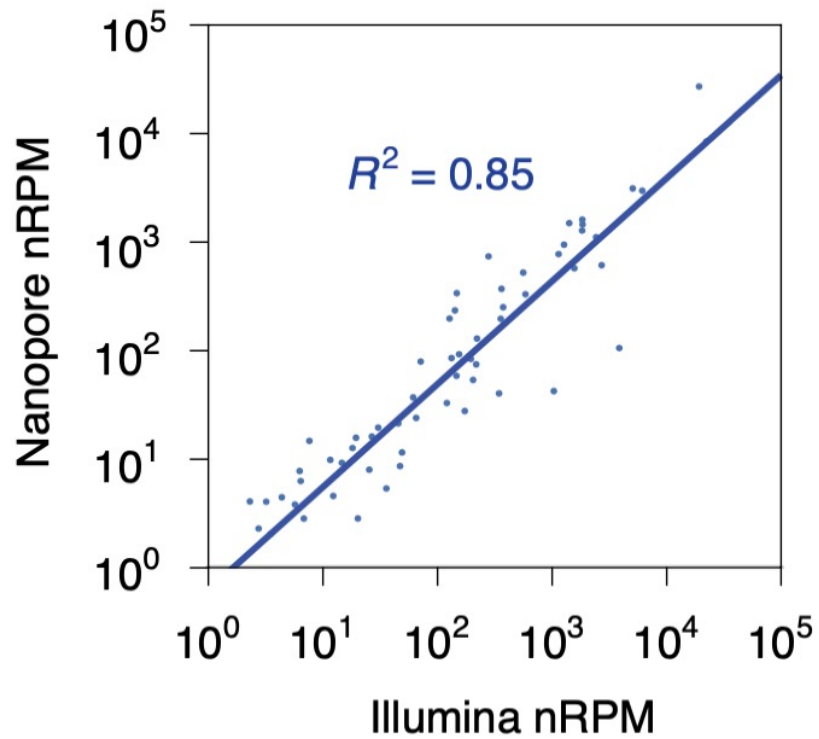
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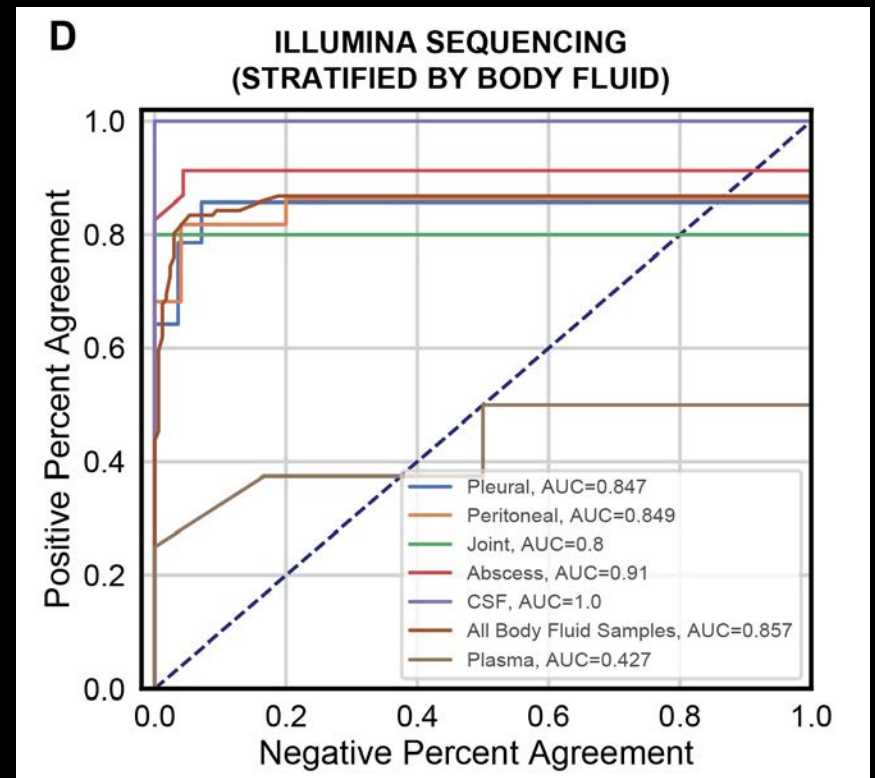
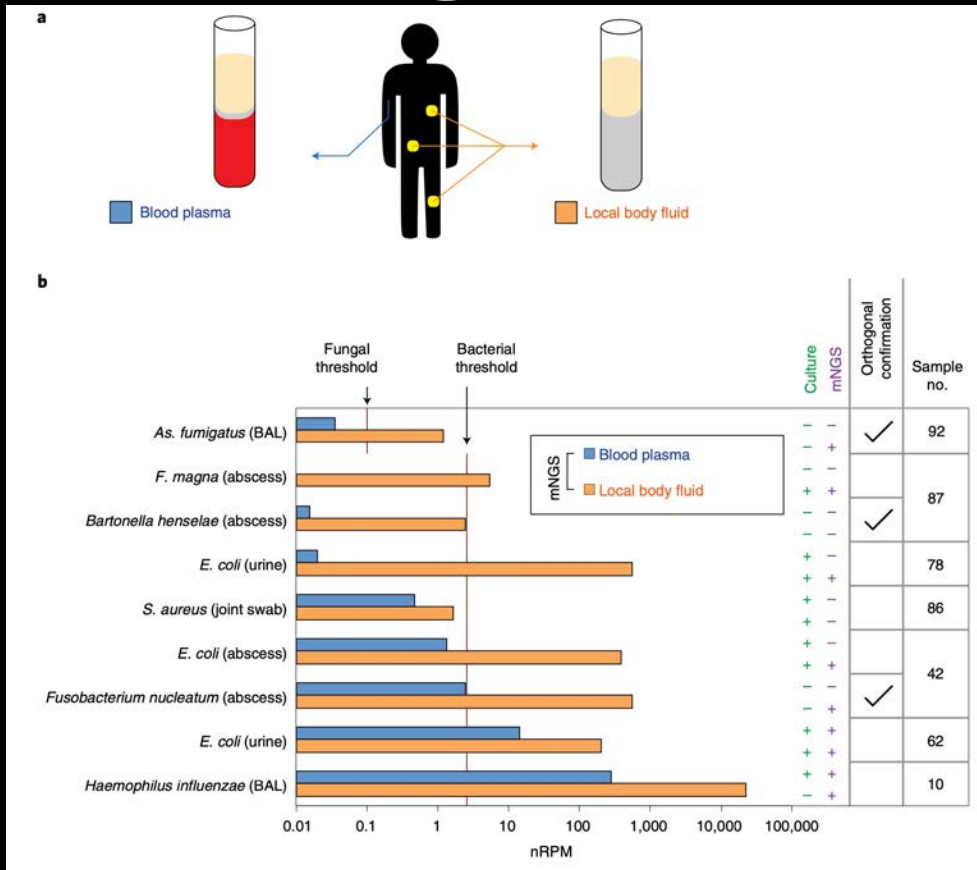
Sample Type



Nanopore: Rapid Sequencing

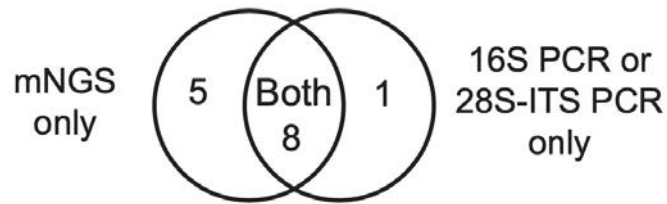


Higher Local Signal

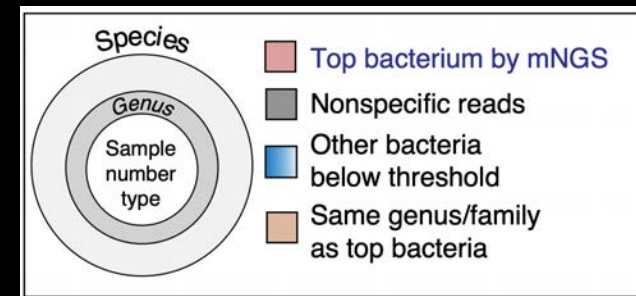


16S Comparison

BODY FLUID SAMPLES WITH
16S/28S-ITS PCR TESTING (n=14)

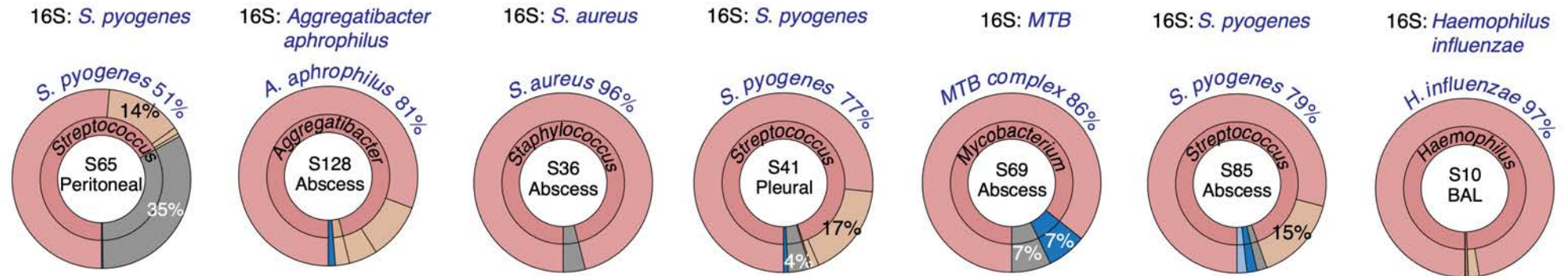


		Composite diagnosis		mNGS	
		+	-	PPA = 86%	NPA = 100%
mNGS	+	8	5	16S/28S-ITS PCR	PPA = 64%
	-	1	0		



b

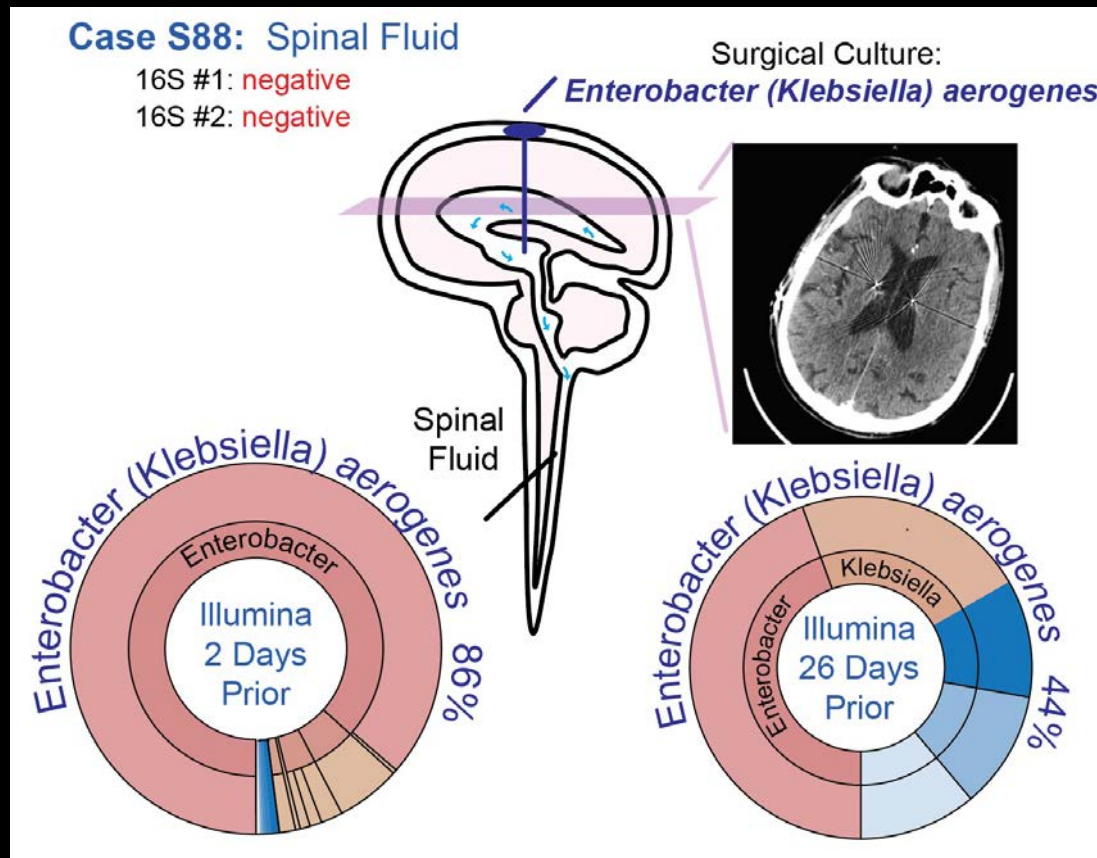
Concordant cases



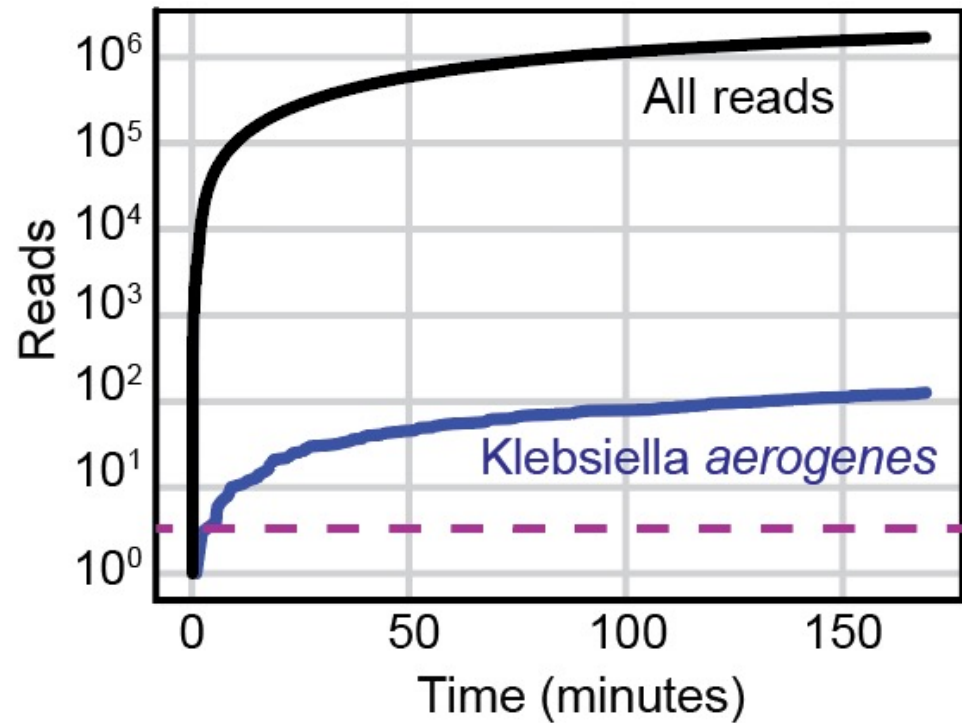
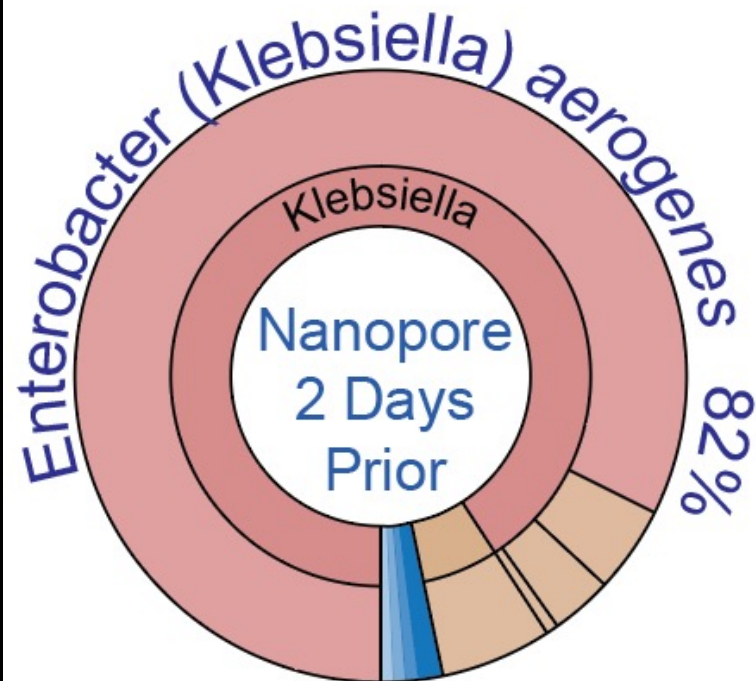
Case 1

- 70s year old man with brain implant presents with fever and encephalopathy
- CSF: WBC 28->700's in 1 month.
Monocyte then neutrophil predominant
- Culture: Negative
- 16S, ITS sequencing: Negative 2X

Case 1: Pathogen Detected



Case 1 - (Nanopore)



Case 2

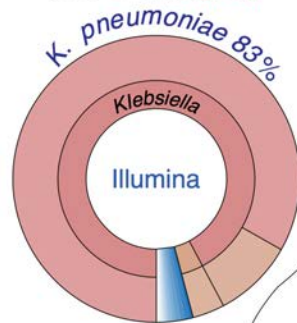
- 3 year old boy with necrotizing pneumonia
- Culture negative
- 16S PCR of Pleural Fluid:
 - Streptococcus *mitis* group

- Are we done?

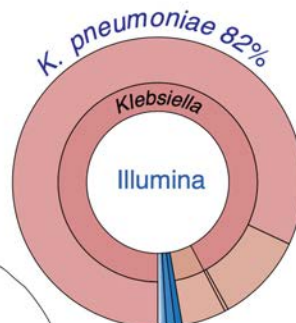
Case 2

Case S31: pleural fluid

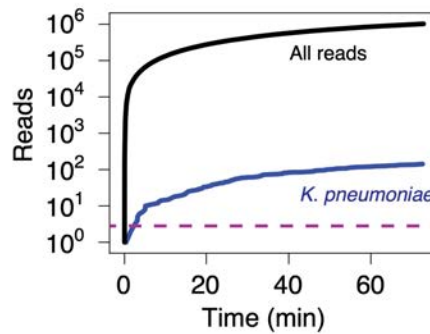
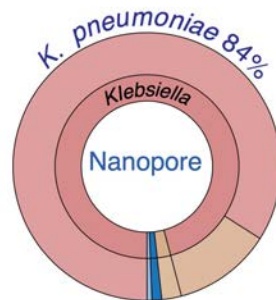
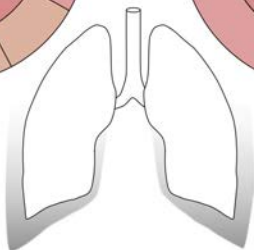
16S: *S. mitis* group



Right pleural fluid

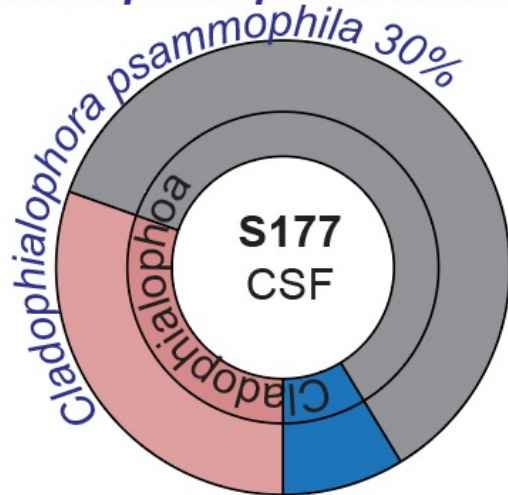


Left pleural fluid

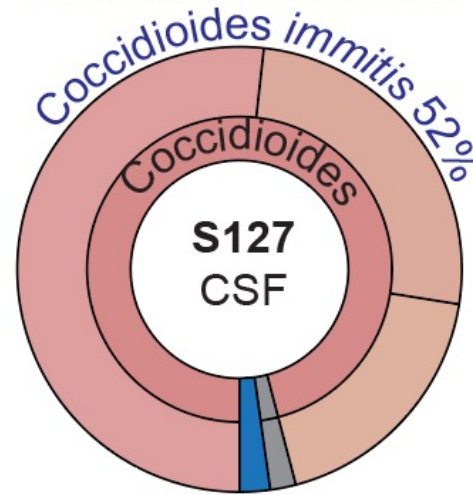


Fungal Discordant Cases

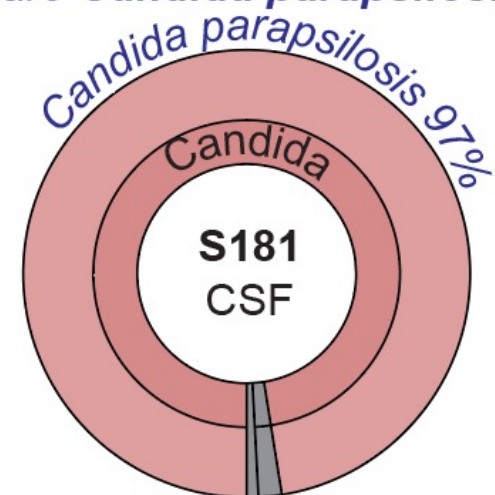
ITS: *negative*
Surgical Culture:
Cladophiala bantiana



ITS: *negative*
Culture: *Rare*
Coccidioides immitis

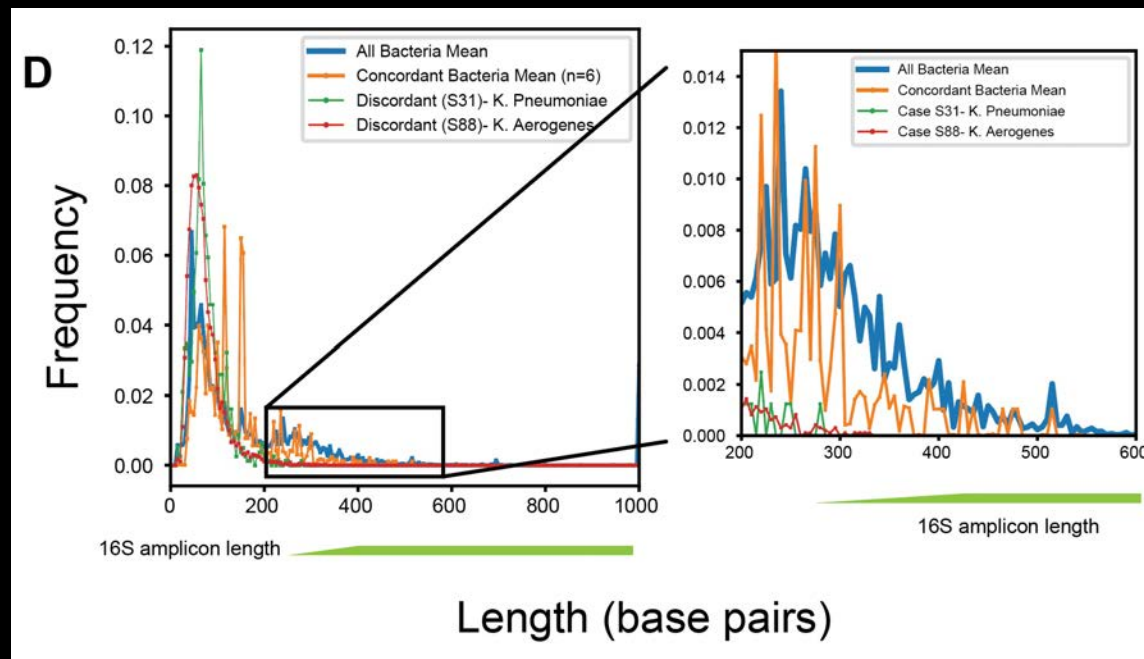
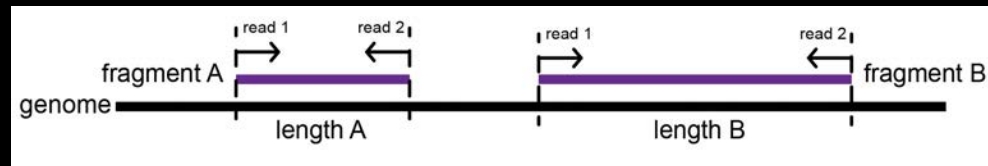


ITS: *negative*
CSF Culture 24 Days Later:
Rare Candida parapsilosis



Why Discordant?

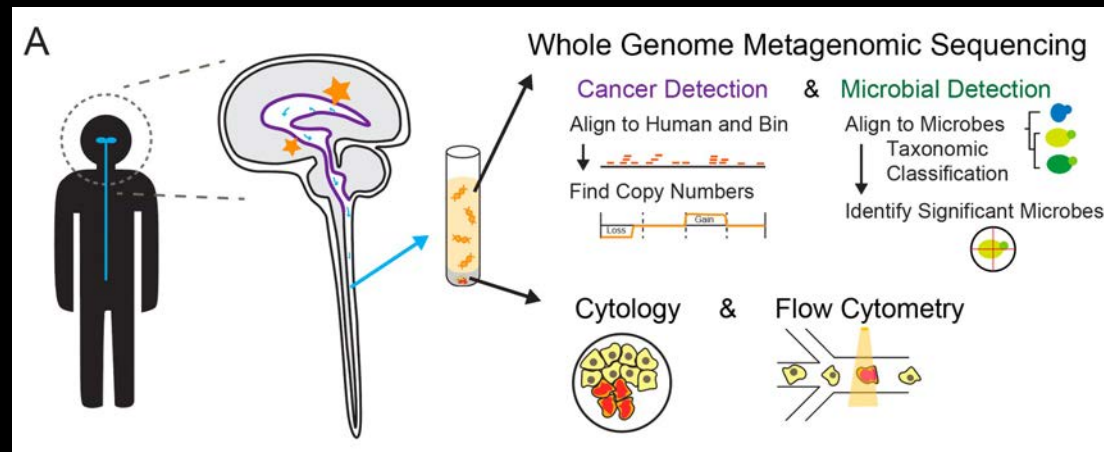
Hypothesis: Invisible DNA



Conclusions

- Universal approach to body fluids
- Detection of 'missed' pathogens
- Max speed nanopore <15 min
- Future directions
 - Larger studies
 - Neoplasm Detection

Same Data → Detect Cancer



- Measure aneuploidy as cancer marker
- 4 studies (3 case-control, 1 cohort)
- 55-68% detection of undetected cancers in primarily in CSF, pleural, peritoneal, BAL fluids

Acknowledgements

UCSF

Gu Lab

Elaine Hsu

Chiu Lab:

Wayne Deng,

Marco Lee,

Yasemin Sucu, Allan Gopez, Scot Federman, Doug Stryke, Kevin Reyes, Candace Wang, Ben Briggs, Charles Chiu

DeRisi Lab: Joe DeRisi,
Hannah Sample

Wilson Lab: Michael
Wilson, Kelsey Zorn

Dept. of Medicine: Amy
Berger

Lab Med: Steve Miller,
Shaun Arevalo

Scientists in Clinical Labs

Funding Acknowledgements

- Charles Chiu, Joe DeRisi, Michael Wilson at UCSF: Marcus Fund, Charles Schwab, Chan-Zuckerberg Biohub, HHMI, CIAPM
- WG: K08 (NCI/NIH), Burroughs Wellcome CAMS Award
- UCSF Laboratory Medicine
- Stanford Pathology

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Thank you Any Questions?



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