

Clinical Metagenomics for the Diagnosis of Acute Infectious Diseases



Meningitis/ Encephalitis:

60-80% unknown cause



Pneumonia: 15-25% unknown cause



Hemorrhagic Fever:

20% unknown cause



Diarrheal Disease: 50% unknown cause



Metagenomic Sequencing – Casting a Wide Net



Accelerating Diagnosis with Next-Gen Testing can Impact Clinical Decision-Making ("Precision Medicine")



Improved patient outcomes

14 y/o male with Meningoencephalitis
3 hospitalizations over 4 months
44 days in the ICU
>100 inconclusive tests
3 empiric treatments with no effect
Brain biopsy and induced coma

(Wilson, et al., 2014, New England Journal of Medicine; photo courtesy of the Osborn family)

14 y/o male with Meningoencephalitis 3 hospitalizations over 4 months 44 days in the ICU >100 inconclusive tests 3 empiric treatments with no effect Brain biopsy and induced coma → Neuroleptospirosis Diagnosed by Metagenomic NGS (mNGS) in 48h Cured 2 weeks after NGS dx with appropriate treatment

(Wilson, et al., 2014, New England Journal of Medicine; photo courtesy of the Osborn family)

Plan:

1 - Translating a Research pipeline to a Clinical assay: The challenges of mNGS ID diagnostics

2 - Prospective case series to study which cases benefit from mNGS ID diagnostics

1 - Translating a Research pipeline to a Clinical assay: The challenges of mNGS ID diagnostics

mNGS pipeline



Challenges of unbiased pathogen sequencing

- 1. Only small % of nucleic acid in clinical samples are from pathogens
- 2. Pathogens have various types of nucleic acids present
- 3. Quick turnaround time
- 4. Controls are needed for validation
- 5. NGS sensitivity leads to potential contamination
- 6. Can be performed by clinical labs

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mNGS pipeline – RNA vs DNA



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SURPI = Sequencing-based Ultra Rapid Pathogen Identification Total NA **Post-DNAse CSF** extraction treatment А 18 COMPREHENSIVE MODE End-to-end processing time (hours) 16:26:34 \triangle 16:00:00 FAST MODE 15 R²=0,4473 15:00:00 End-to-end processing time (hours) 12 \wedge 14:00:00 13:00:00 **Bioanalyzer** 11:39:46 11:32:54 12:00:00 11:00:00 R²=0,9742 10:00:00 9:00:00 8:00:00 100mil 200mi 300mil 400mil 500mil 600mi 7:00:00 6:00:00 5:39:08 # of reads 5:08:41 4:50:16 5:00:00 4:27:46 4:00:00 3:08:48 3:00:00 2:03:43 2:14:46 1:59:54 1:24:27 1:47:24 2:00:00 1:23:12 Sequencing 1:13:39 0:57:32 0:50:50 0:59:49 0:32:37 0:36:27 0:27:26 0:11:40 0:15:44 1:00:00 0:29:57 0:15:25 0:13:08 0:18:21 0:11:07 Rapid Hised #of 508,729,590 158,026,920 143,419,974 28,635,304 12,393,506 10,632,192 6,740,142 100.000.000 47,920,144 28.008.490 17.881.580 14,199,558 13.654.861 11.634.074 reads **100bp SE** F κ в С D Е G н Μ Ν J Pediatric Colon TTVS Hantavirus Encephalitis Hemorrhagic Prostate Prostate Simian Spiked Chronic Hemorrhagic Hemorrhagic Acute hepatitis HIV hepatitis diarrhea cancer fever pulmonary (CSF) fever biopsy cancer pneumonia fever febrile illnes (serum, HiSeq) (serum) (plasma) (serum) (stool) study syndrome (serum, MiSeq) (tissue) (cell line) (lung swab / outbreak Tanzania Rar senartice reads Progracoussy SNAF algement (Inclosed to b) FAST (Inclosed to b) FAST (Inclosed to b) SNAF algement (Inclosed to b) (Inclosed to (biopsy (serum) tissue) DRC, Africa Africa tissue (serum) (serum) Analysis SURPI uses SNAP and

RAPSearch fast aligners Naccache et al., 2014. Genome Research



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mNGS pipeline - Validation





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- performed in CLIA lab
- by CLS personnel
- according to approved LDT protocol

Report

SURPI*viz*

The visual home of SURPI+, a cloud-compatible bioinformatics pipeline for ultrarapid pathogen identification from next-generation sequencing for diagnostics and research.

View results from the SURPI+ runs below:

#	SURPI+ Run	Sequencing	SURPI+ files	Heat maps	Krona plots	Old Coverage
0	Demo Clinical	Sequencing	SURPI+ files	Heat maps	Krona plots	Old Coverage
1	Demo Spike-in	Sequencing	SURPI+ files	Heat maps	Krona plots	Old Coverage
2	1PilotBoardMeeting	Sequencing	SURPI+ files	Heat maps	Krona plots	Old Coverage
3	131025 JLG A1N2DNAseTest	Sequencing	SURPI+ files	Heat maps	Krona plots	Old Coverage

St. Louis encephalitis virus (gi|537790028|, 10936 bp)

St. Louis encephalitis virus strain CbaAr-4005, complete genome

2 - Prospective case series to study which cases benefit from mNGS ID diagnostics

Prospective Case series

Prospective Case series

27 Consecutive patients

22 Months

Will you be the next Dr. House? 3 case reviews

Case Report I: 70 y/o male with fever and pancytopenia

- Evaluated for 2 months of fevers, chills, diarrhea, and fatigue
- Pancytopenic [WBC 2,000 / mm³, HgB 8 g/dL (12.4 14.9 g/dL), platelets 51 / mm³] with elevated liver enzymes (AST = 294 U/L, ALT = 212 U/L)
- On imaging, hepatomegaly, splenomegaly, and liver cysts
- Bone marrow biopsy and lymph node biopsy performed; treated empirically with antibiotics, blood transfusion, G-CSF
- Concern for HLH (hemophagocytic lymphohistocytosis) with negative infectious workup → transferred from community to tertiary care hospital
- 6-week hiking in Spain 10 months prior to admission (500 mile Camino de Santiago)

Case Report I: 70 y/o male with fever and pancytopenia

FFPE bone marrow → SURPI

Case Report I: 70 y/o male with fever and pancytopenia

→ mNGS Dx: Visceral Leishmaniasis

mapping AIS_gy_131202 against Leishmania.361050151.fasta with gi definition gi|361050151|emb|FR799623.2| Leishmania donovani BPK282A1 complete genome, chromosome 36

Reference sequence length = 2713248 bp Coverage in bp = 35161 %Coverage = 1.295900 Average depth of coverage = .033672 Number of reads contributing to assembly = 689

Treated with 2 weeks of IV amphotericin

 \rightarrow clinically improved, now doing well

Case Report II: 15 y/o female with hemorrhagic encephalitis

- 15 year-old girl with type 1 diabetes
- 7 days of headache, vomiting, arm weakness, and confusion
- Elevated WBC count in CSF (pleocytosis); hemorrhagic frontal / occipital lobe brain lesions on MRI
- No international travel, no sick contact, no insect bites
- Contact with alpacas, swimming in freshwater pond 9 months prior
- Extensive diagnostic testing negative
- "Classic" presentation for hemorrhagic form of ADEM → given IV steroids x 5 days

Case Report II: 15 y/o female with hemorrhagic encephalitis

$CSF \rightarrow SURPI$

Case Report II: 15 y/o female with hemorrhagic encephalitis → mNGS Dx: Balamuthia mandrillaris encephalitis

- Brain biopsy pathology from HD 6 → hemorrhagic necrotizing process with numerous amoebae
- Despite combination amoebicidal therapy, she developed intracranial hypertension, cardiac arrest, and died
- Had a complete B.mandrillaris genome been available in Genbank NT, it would have been detected in 1st LP

Case Report III: 55 y/o male with deafness and behavioral change

- 55 y/o male with bone marrow transplant May, 2013. Developed rapidly progressive hearing loss over 2-3 weeks in October.
- CSF unremarkable; MRI negative; PCR for HSV and enterovirus negative; treated empirically with high-dose valacyclovir, antibiotics, IVIg and steroids daily to no effect
- Over next few weeks, developed nausea, fatigue, ataxia, persistent hearing loss, then depressed, irritable mood (unusual for patient per his wife).
- Repeat MRI late December → abnormal signal in thalamus and midbrain bilaterally; frontal lobe biopsy performed (only accessible region)

Case Report III: 55 y/o male with deafness and behavioral change

Brain Biopsy \rightarrow SURPI

Case Report III: 55 y/o male with deafness and behavioral change → Genome Assembly of an Astrovirus

- Origin of virus unknown, but presumably communityacquired
- Patient started on ribavirin and IVIg
- Despite treatment, he continued to deterioriate, and passed away 4 months after NGS diagnosis

Dx

mNGS Dx only

Micro Dx only

Identification of Use Cases for the Precision Medicine Initiative

Prospective metagenomic NGS case series reveals "high-yield" instances:

- 1. Confirmation of ambiguous or suggestive laboratory results
- 2. Epidemiologic or clinical links pointing to infection
- 3. Broad differential diagnosis
- 4. "rule-out" assay in patients remaining undiagnosed despite extensive testing

Clinical Implementation of Metagenomic Next-Generation Sequencing for Precision Diagnosis of Infectious Diseases

Timeline – Clinical CSF mNGS assay in full speed

- High yield cases chosen by PM consult services
- 6 prospective CSF samples sequenced each week
- Results reported in 56h
- Cost-benefit and clinical outcomes analysis after 1 year, 300 samples

University of California San Francisco

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