Real-Time Metagenomic Next-Generation Analysis for Diagnosis of Infectious Diseases

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Targeting Acute Infectious Diseases in Hospitalized Patients

Up to 60% of hospitalized patients with pneumonia, sepsis, and encephalitis / meningitis are managed and treated without a laboratory-confirmed cause of their disease, resulting in:

*delayed and ineffective therapy, increased mortality, and excess healthcare costs*
Conventional Testing

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

Bacteria

Viruses

Fungi

Parasites
Precision Diagnosis of a Mysterious Infection

3 hospitalizations over 4 months
44 days in the ICU
>100 inconclusive tests

Brain biopsy and induced coma (Wilson, et al., 2014, *New England Journal of Medicine*; photos courtesy of the Osborn family)
Leptospira santarosai
Leptospira borgpetersenii
unclassified
Leptospira interrogans
Propionibacterium acnes
mNGS Clinical Workflow (48-72 hr TAT)

Naccache, et al. (manuscript in preparation)
Precision Diagnosis of Acute Infectious Diseases (PDAID)

Meningitis / Encephalitis
40-60% unknown cause

7 hospitals in CA and nationwide
Enroll/consent patients
151 to date
CSF collected
Clinical chart review

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

Clinical microbial sequencing board
Clinical report in patient EMR

Diagnosis of neurologic infection in 23.2% of cases, one-third of which were not identified by conventional testing
88% sensitivity, 97% specificity (excluding cases dx’ed by serology)
Precision Diagnosis of Acute Infectious Diseases (PDAID) Study

Neurological Infections Missed by Conventional Testing

- St. Louis encephalitis virus (first case in California since 1986)
- Human coronavirus 229E
- Hepatitis E virus
- *Enterobacter aerogenes*
- *Candida tropicalis*
- *Neisseria meningitidis*
- *Streptococcus agalactiae*
Nanopore Sequencing for Real-Time Metagenomic Pathogen Detection in Febrile Illness

MinION (Oxford Nanopore Technologies)

(Greninger, et al., 2015, *Genome Medicine* 7:99)
Viral Reads Detected <8 Min into Sequencing Run

- **Chik1 (9.1x10⁷ copies/mL)**
  - All reads (n=19,452)
  - CHIKV reads (n=556)
  - 6 min

- **Ebola1 (1x10⁷ copies/mL)**
  - All reads (n=13,090)
  - EBOV reads (n=41)
  - 8 min
Low Serum Titers in Acutely Infected ZIKV Patients

(Quick, et al., 2016, Nature 530:228-232)
Nanopore Metagenomic Sequencing Protocol (<3 hr turnaround time)

RNA extraction + DNase (0.5 hr)

Reverse transcription with random and targeted priming of RNA (0.5 hr)

PCR amplification (1.5 hr)

First read detection (3 - 30 min)

Library preparation (15 min - 2 hr)
Targeted Primers Increase Sensitivity But Do Not Impact Off-Target Metagenomic Detection
Nanopore Sequencing in Space
SURPlr Analysis of Nanopore Data Collected on the ISS

E. coli de novo assembly (Canu)
- raw 1D and 2D reads (n=84,502)
  - (4 mapped contigs, 99.8% complete, 98.5% pairwise identity)

Circle chart:
- Bacteria (6,279, 42.3%)
- Non-host Eukaryote (0.40%)
- Other (1.9%)
- Mus musculus (6,220, 41.8%)
- Low quality / complexity (14)
- Viruses (520, 3.5%)
- Unidentified (1,482, 10.0%)
Increasing Read Throughput of Nanopore Sequencing

Detection of Zika virus @ $10^2$ copies/mL

- 5,000 reads / min
- 200,000 reads / hr
- 7.4 million reads total for run
- Detection of Zika virus @ 15 min
Diagnosis of Lyme Disease using Human Host Biomarkers

59 human gene transcripts → 96% sensitivity / 100% specificity in distinguishing Lyme patients from controls
Machine Learning-Based Prediction of Causes of Infection from Human Gene Expression Data (RNA-Seq)

74% accuracy (preliminary analysis)
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