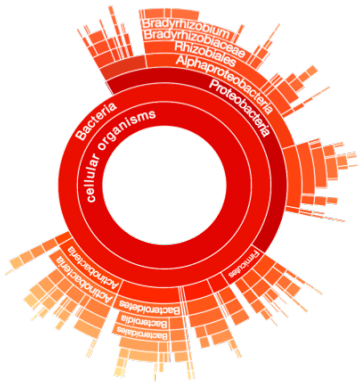




Metagenomics for the Diagnosis of Exotic Infections

Robert Schlager, MD, Dr Med, MPH
Assistant Professor (University of Utah)
Medical Director (ARUP Laboratories)
Chief Medical Officer (IDbyDNA Inc.)



Conventional Infectious Disease Testing

Multimodal

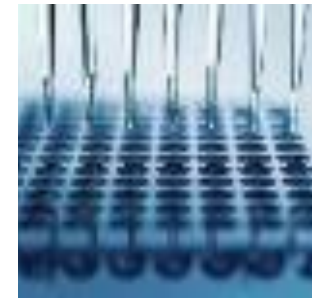
Culture



PCR

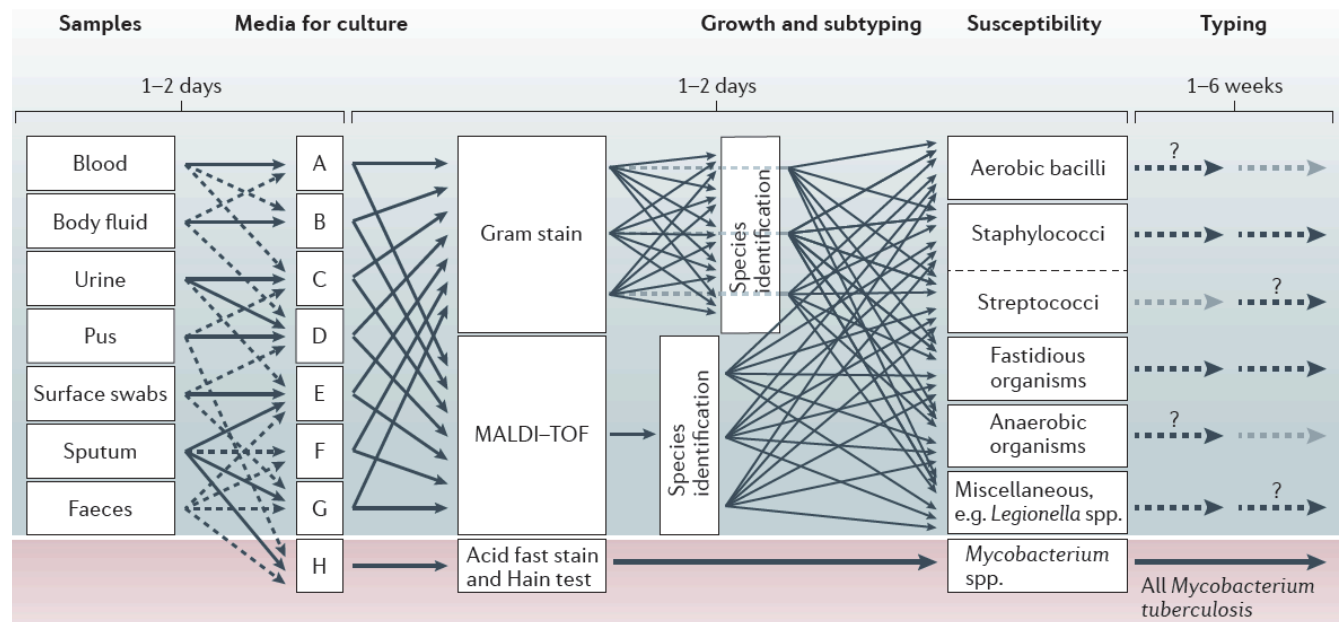


Serology
Ag Detection



Conventional Infectious Disease Testing

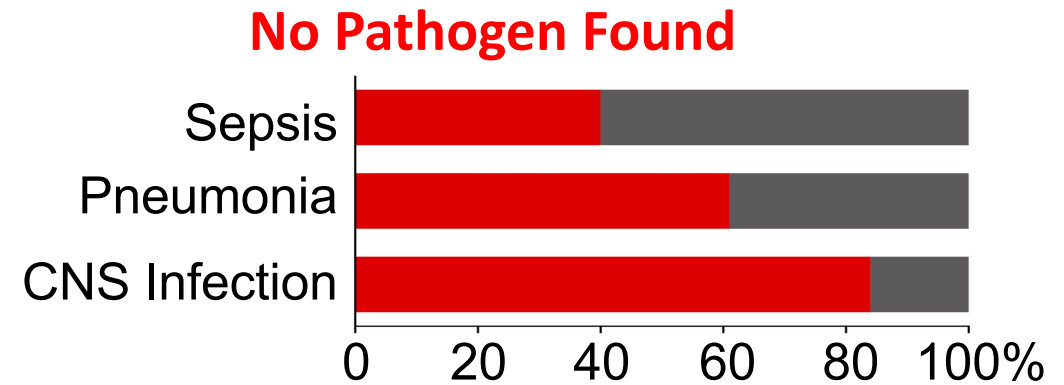
Complex & Sequential Lab Workflow



Limited Success With Many Infectious Syndromes



Test Panels



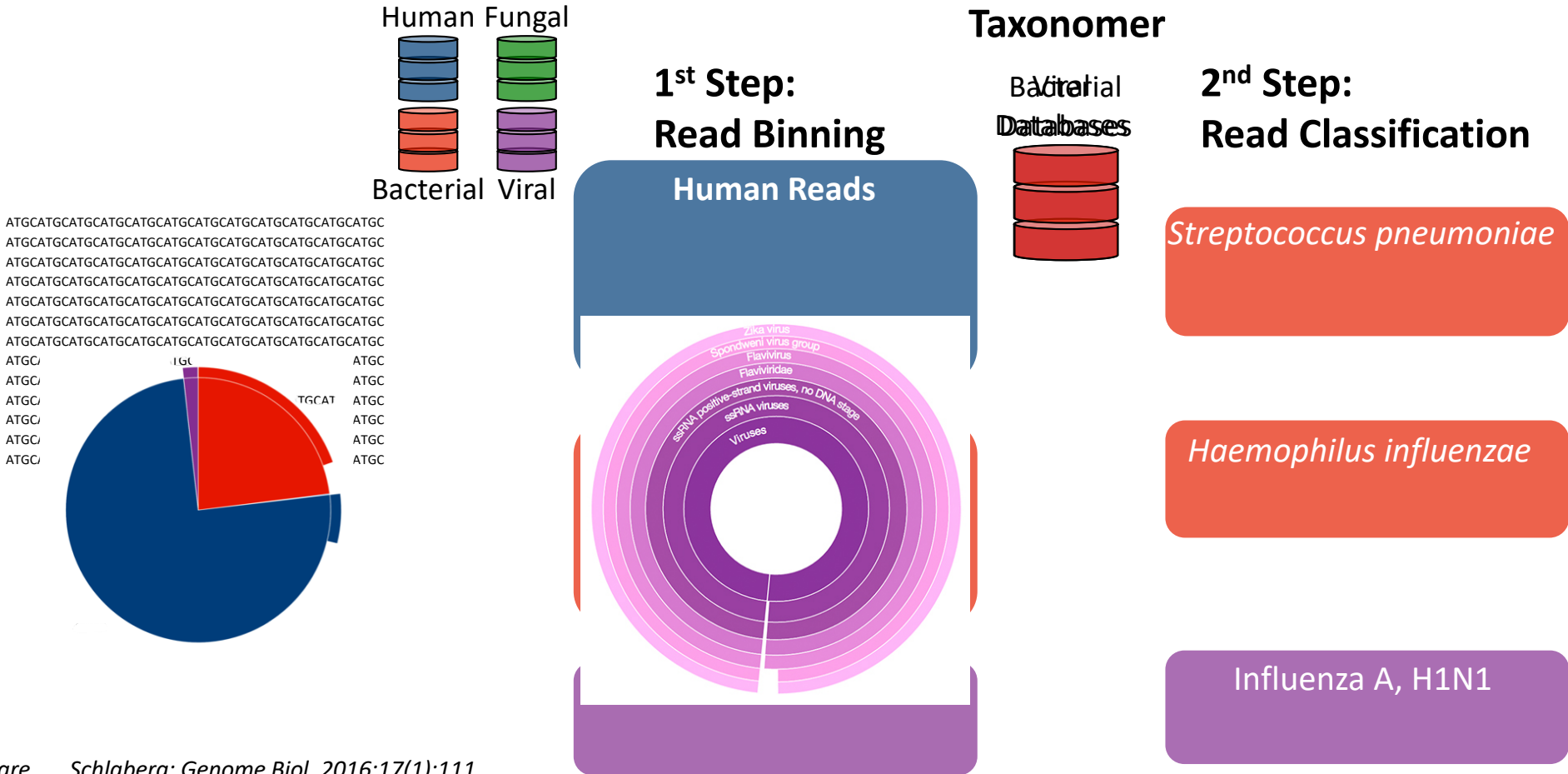
Metagenomics As A One-Stop Testing Option



Single Test



Metagenomics Data Analysis (Research, Taxonomer)



Exotic Infections, Case Examples

1. Patient populations

- Highly susceptible patients
- Unknown epidemiology (e.g. underserved populations)

2. Unexpected, rare pathogens

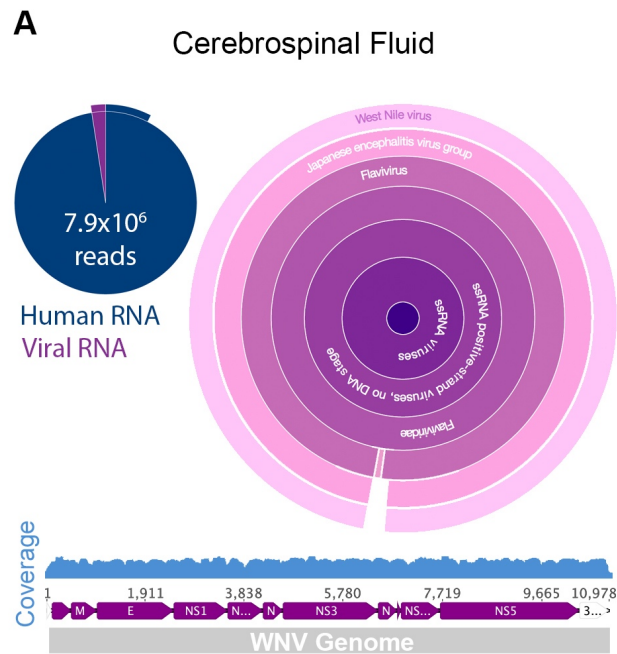
- Unknown exposures

3. Variant strains

- Missed by PCR-based tests (e.g. many viruses)

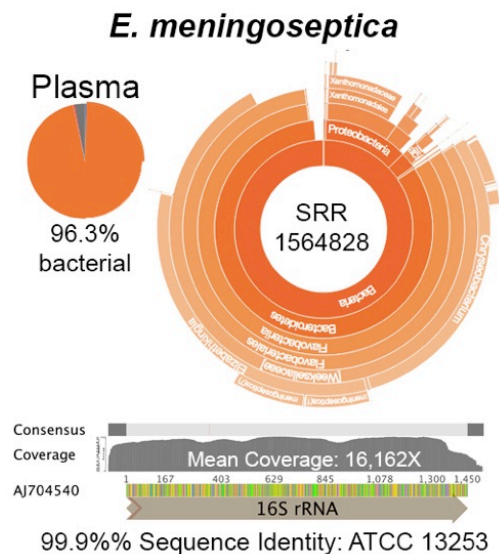
1. Patient Population, Susceptible

Immunosuppressed Patient with Encephalitis



1. Patient Population, Unknown Epidemiology

Suspected EBOV (Sierra Leone)



FUO (Tanzania)

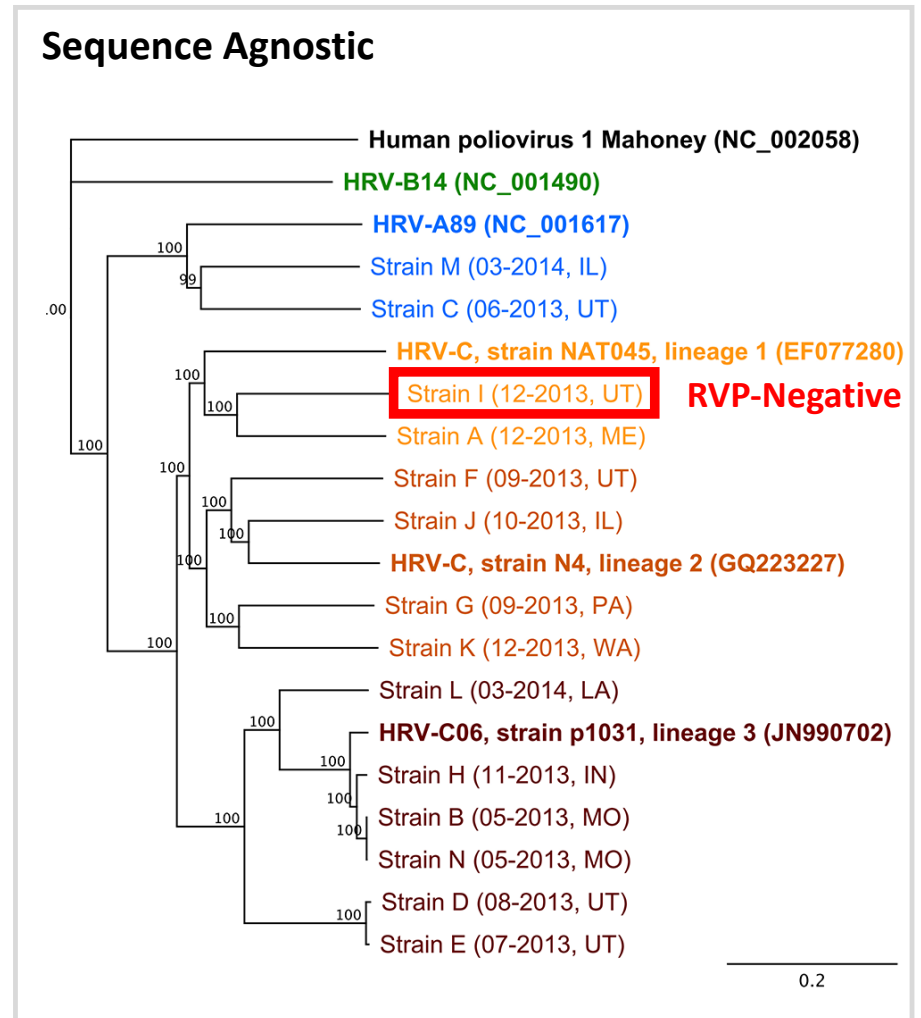
- Parvovirus B19
- Hepatitis E virus
- Enterovirus
- *Enterococcus caecorum*
- *Plasmodium falciparum*
- ...

Data from *Science*, 345,6202:1369-1372

Flygare ... Schlager; *Genome Biol.* 2016 May 26;17(1):111
Rubach, ..., Schlager, manuscript in preparation

3. Variant Strains

- Sequence-independent method
- Less vulnerable to genetic variation
- Protein-based classification can increase tolerance

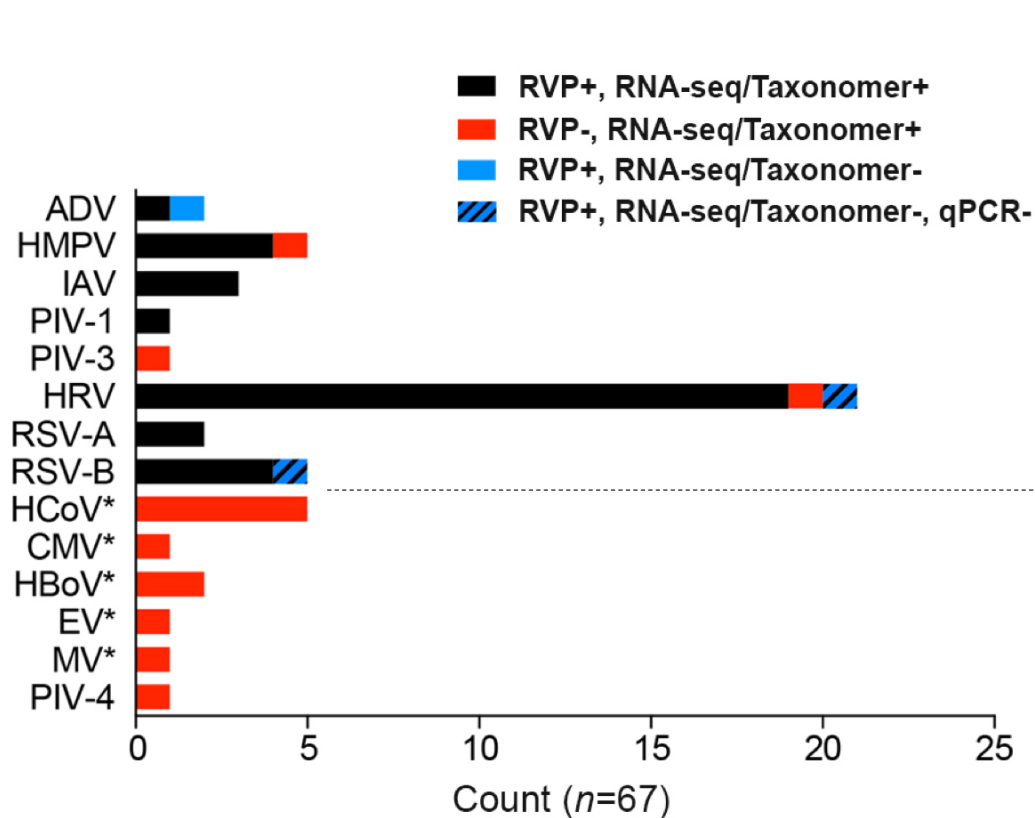


Graf ... Schlager, J Clin Microbiol. 2016 Apr;54(4):1000-7

From Case Reports to Analytical Performance and Clinical Utility

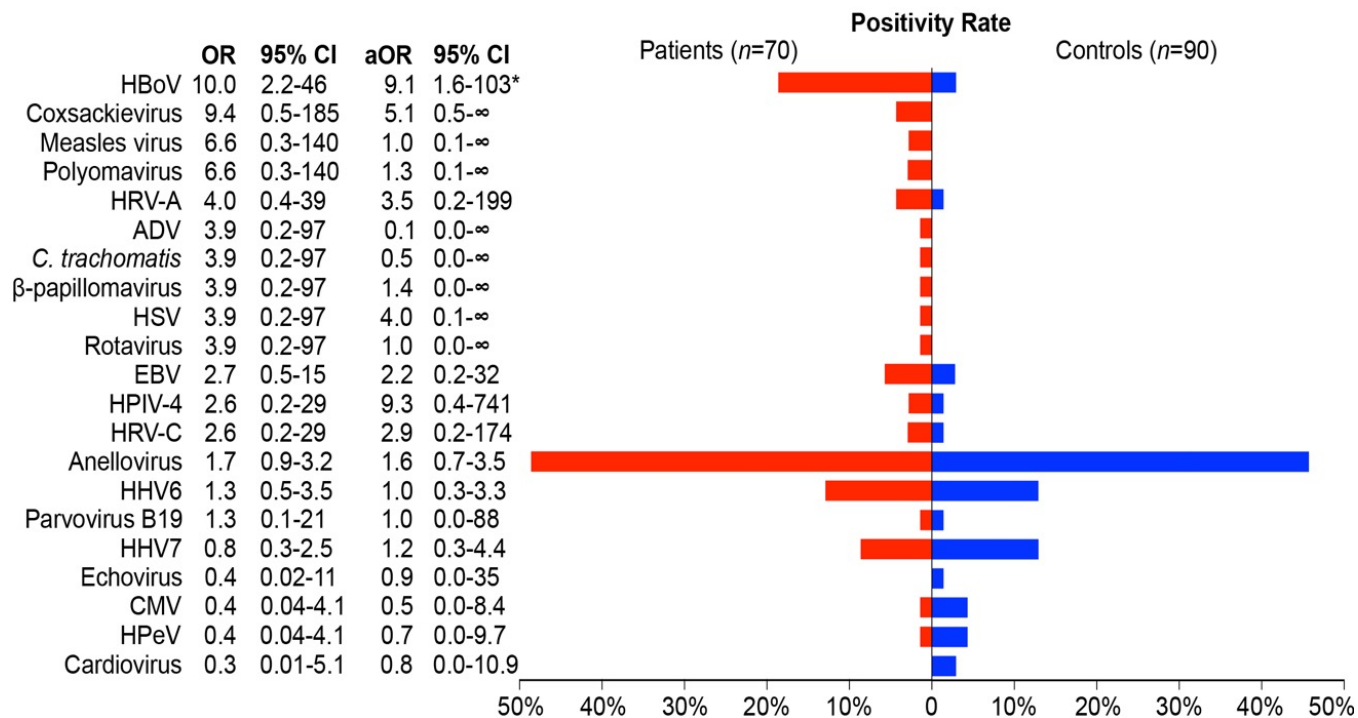
>90% Agreement with PCR Panel, Additional Positives

NP/OP Swabs (n=67)



Pathogens in 30% of Children with Unknown Etiology

NP/OP Swabs (Viral Pathogens)

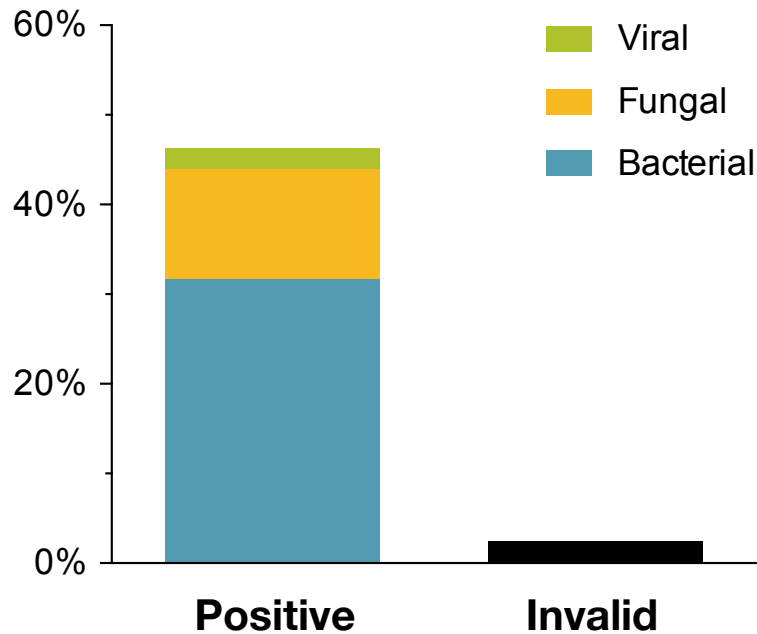


Schlager et al., *J Infect Dis.* 2017 May 1;215(9):1407-1415

Pathogens Detected in ~40% of Patients

A

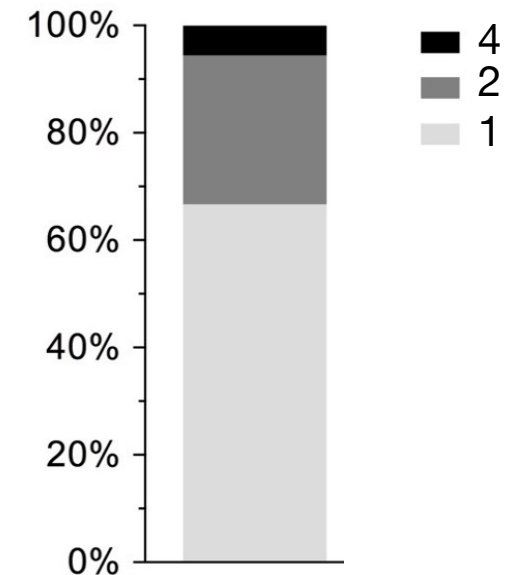
Explify™ Respiratory Results



Viruses	Parainfluenza virus Human Bocavirus Rhinovirus
Fungi	<i>Pneumocystis</i> <i>Mucor</i> <i>Fusarium</i>
Bacteria	<i>Strep. pneumoniae</i> <i>S. maltophilia</i> <i>H. influenzae</i>

B

Number of Pathogens/Case



Main Challenges

Wet-Bench: Efficient Lysis, Host Depletion / Target Enrichment

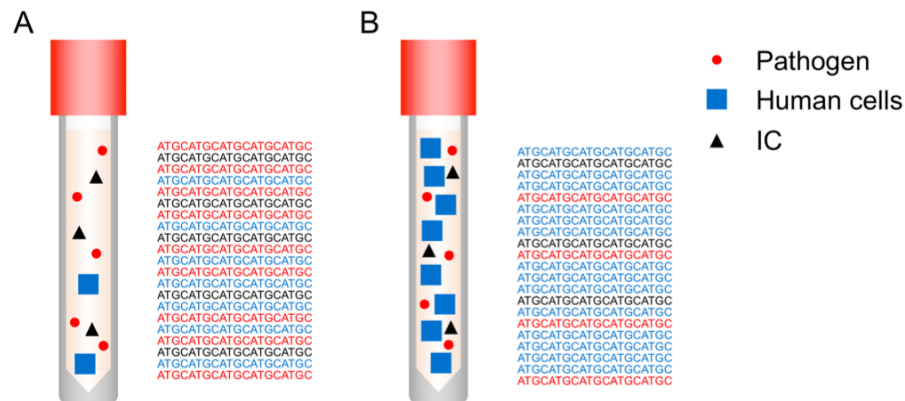
Data Analysis: Databases, Classification Cutoffs, Reporting Criteria

Implementation in Diagnostic Laboratory

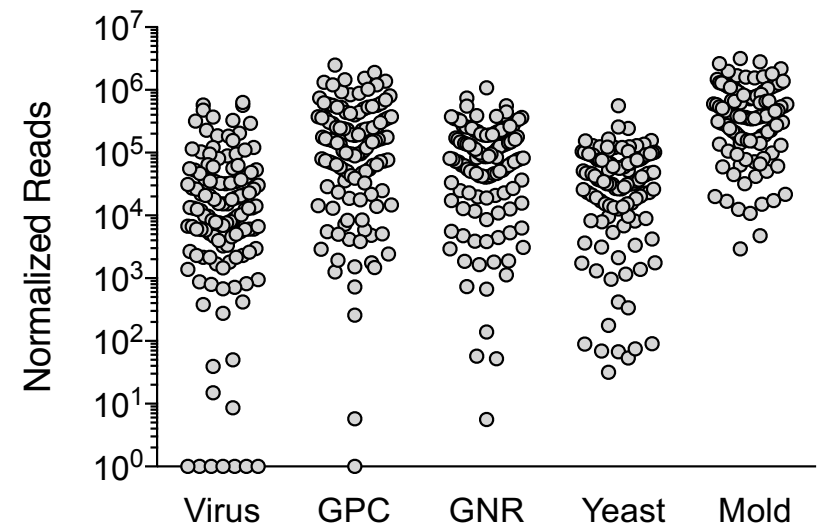
Sample-Dependent Performance, Host Depletion

Analytical sensitivity is variable

- Sequencing depth
- Genome size
- Sample composition/cellularity



Internal controls to flag outliers



Bacterial Contamination, Need for Controls

Reagents contain microbial nucleic acid

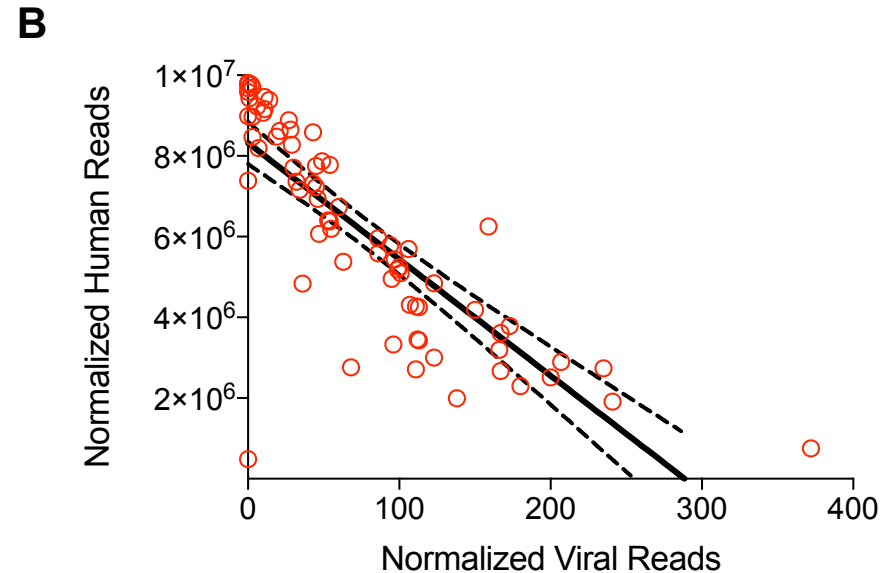
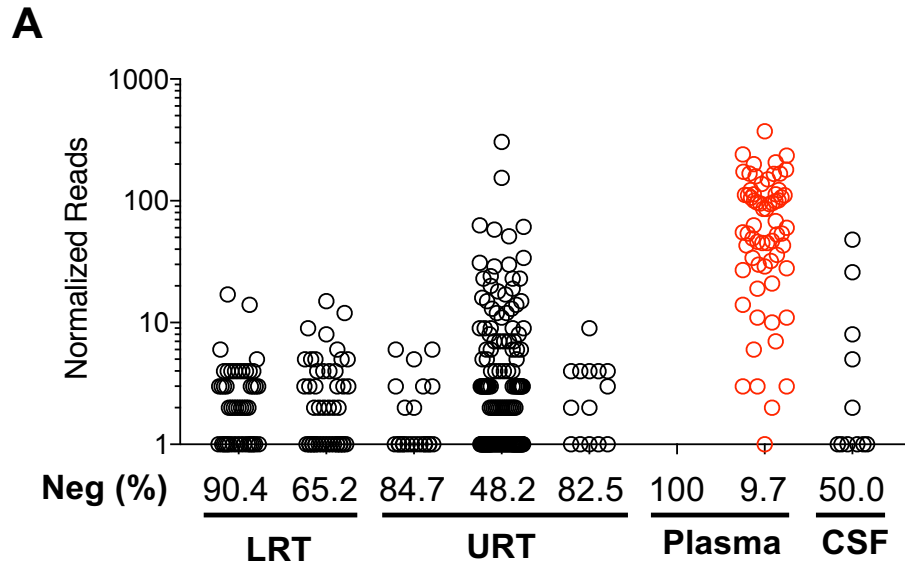
- Low DNA Yield -> High Contamination Risk



- Highly variable between lots/batches (many reagents, frequent lot changes)

Viral Contamination

Reovirus – Kit, NA-Yield Dependent Detection



Missing: Curated, Comprehensive, Balanced Databases

- **Curated**

- Errors: misclassification

- **Comprehensive**

- False-negative results if correct match missing

- False-positive results (near neighbor) if correct match missing

- **Balanced**

- Reduce bias

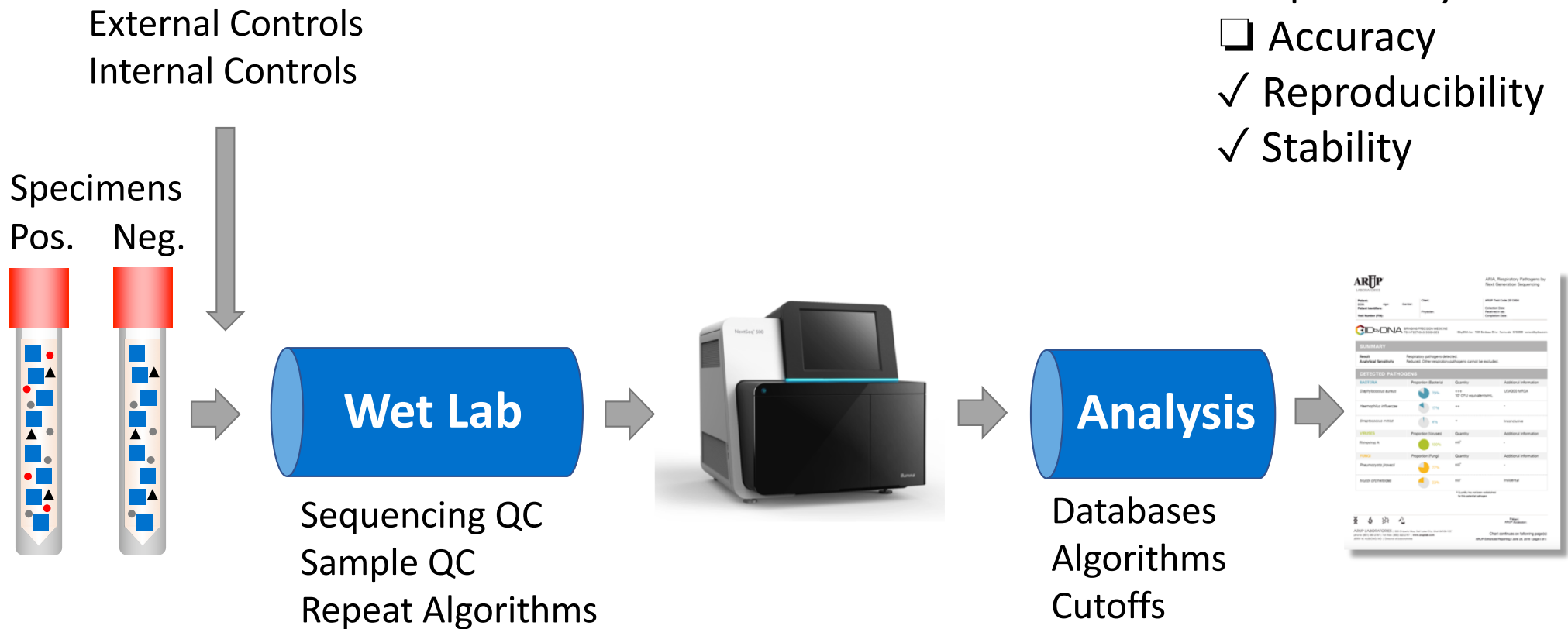
Periodic **updates**, re-validation, version control

Validation

1. Can sequencing reads be generated from sample?
2. Can sequencing reads be accurately classified?

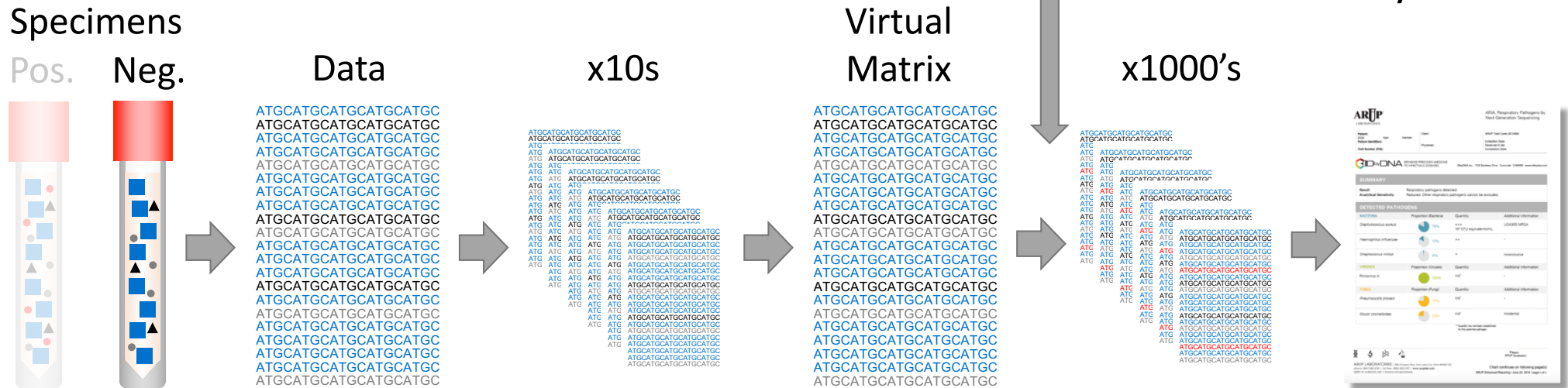
Validation 1: Generating Sequencing Reads

- ✓ Sensitivity
- ☐ Specificity
- ☐ Accuracy
- ✓ Reproducibility
- ✓ Stability



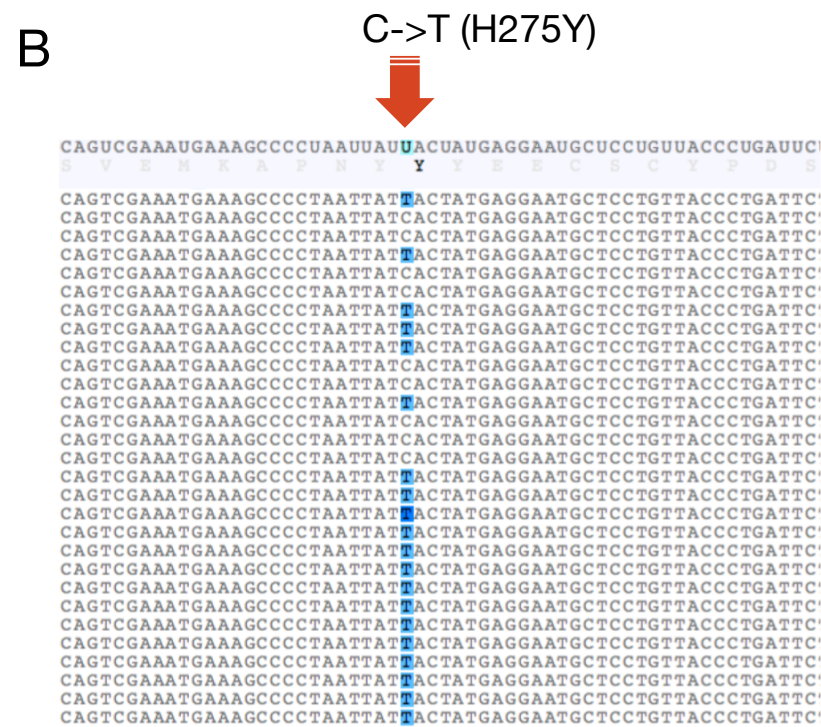
Validation 2: Classification (Virtual Specimens)

Pathogens	✓ Sensitivity
Near neighbors	✓ Specificity
Commensals	✓ Accuracy
Contaminants	✓ Reproducibility
	✓ Stability



Monitoring Oseltamivir Resistance, 3-Year-Old Immunocompromised Child

Monitoring Oseltamivir Resistance, 3-Year-Old Immunocompromised Child



Human Genetics

Brett Kennedy
Mark Yandell

ARUP Laboratories

Keith Tardif
Keith Simmon
Karl Voelkerding
SEQID
Brandy Serrano
Amy Cockerham
Jennie Stanchfield
Diana Mohl

Infectious Diseases

Sankar Swaminathan

Pediatric Infectious Diseases

Chris Stockmann
Carrie Byington
Anne Blaschke
Krow Ampofo
Andy Pavia

Biomedical Informatics

Karen Eilbeck

CHOP

Erin Graf

CDC, Viral Diseases

Seema Jain
Sue Tong
Krista Queen

Pathology

Kim Hanson
Marc Couturier
Maria Pletnikova
Meghan Driscoll

Neurology

Stacey Clardy
Viren Patel

IDbyDNA

Steven Flygare
Heng Xie

BILL & MELINDA
GATES *foundation*

