Harnessing the Host Response for Real Time Clinical Decision Making in Infectious Diseases

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DUKE CENTER for APPLIED GENOMICS & PRECISION MEDICINE

ARLG Antibacterial Resistance Leadership Group

DUKE GLOBAL HEALTH INSTITUTE

Disclosures

- Predigen: Co-Founder

Patents
US 8821876. **Methods of identifying infectious disease and assays for identifying infectious disease.**

- Abbott Molecular/Ibis Diagnostics R
- Biofire/bioMerieux A,R
- Becton Dickinson A,R
- BioMeme R
- Cepheid R
- Elitech R
- GenMark R
- Giner R
- GSK R
- IDbyDNA A
- Luminex/Nanosphere A
- Merck R
- OnSiteGene R
- Qiagen R
- Qvella R
- Tiselia R
- Roche Molecular R
- Sanofi R

A=Advisory, R=Research Contract/Grant

Funding: NIH NIAID, NIA, FOGARTY, DARPA, DTRA

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National Center for Genome Resources

UNC-Chapel Hill

University of Michigan

UVA, Charlottesville

NIH/NIAID

DoD (DARPA, DTRA, USAMRIID)

Coulter Foundation

CDC

Gates Foundation

ACESO/Henry M. Jackson Foundation

Qvella

Brigham and Women's Hospital

University of Utah

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Is It Just a Cold? The Flu? Or More?

- 17-year-old college student
- Develops fevers, mild headache
- Seen at student health, discharged "viral syndrome"
- Becomes confused, roommates take to ED
- T = 38.9, BP 90/70, HR 110

USA TODAY

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Duke student develops meningitis while camping out for UNC game

DURHAM, N.C. (AP) — A Duke University freshman has been diagnosed with meningitis after camping outside for a chance to see the men's basketball game against North Carolina.

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Source: Schoeller T and Schmutzhard E. *N Engl J Med* 2001;344:1372.

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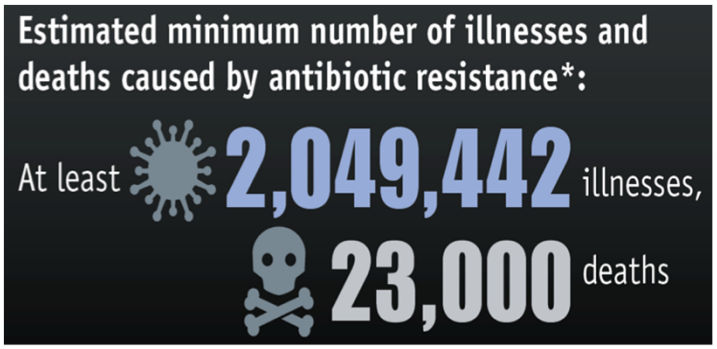
Emergence of Antimicrobial Resistance




The New York Times

SundayReview | EDITORIAL

The Rise of Antibiotic Resistance

By THE EDITORIAL BOARD MAY 10, 2014

- As much as 50% of the time, antibiotics are prescribed when NOT NEEDED
- Antibiotics are frequently misused for **upper respiratory tract infections**, most of which are caused by viruses
  - Need for clinical diagnostic support for providers

Antibiotic resistance threats in the United States, 2013
http://www.cdc.gov/drugresistance/threat-report-2013/

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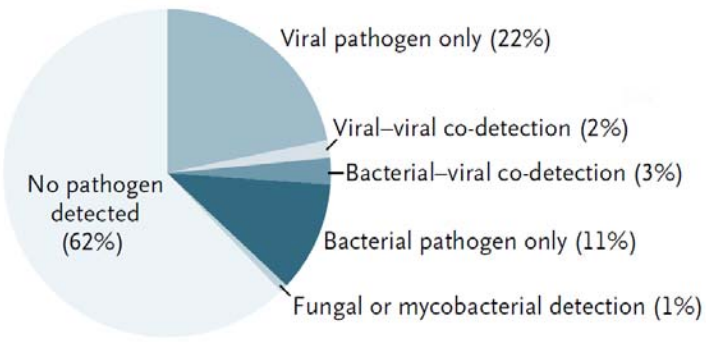
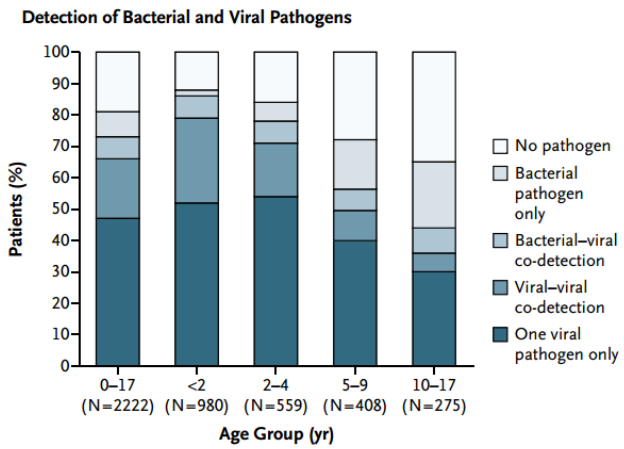
Etiology of Pneumonia in the Community (EPIC)

Adults



Children



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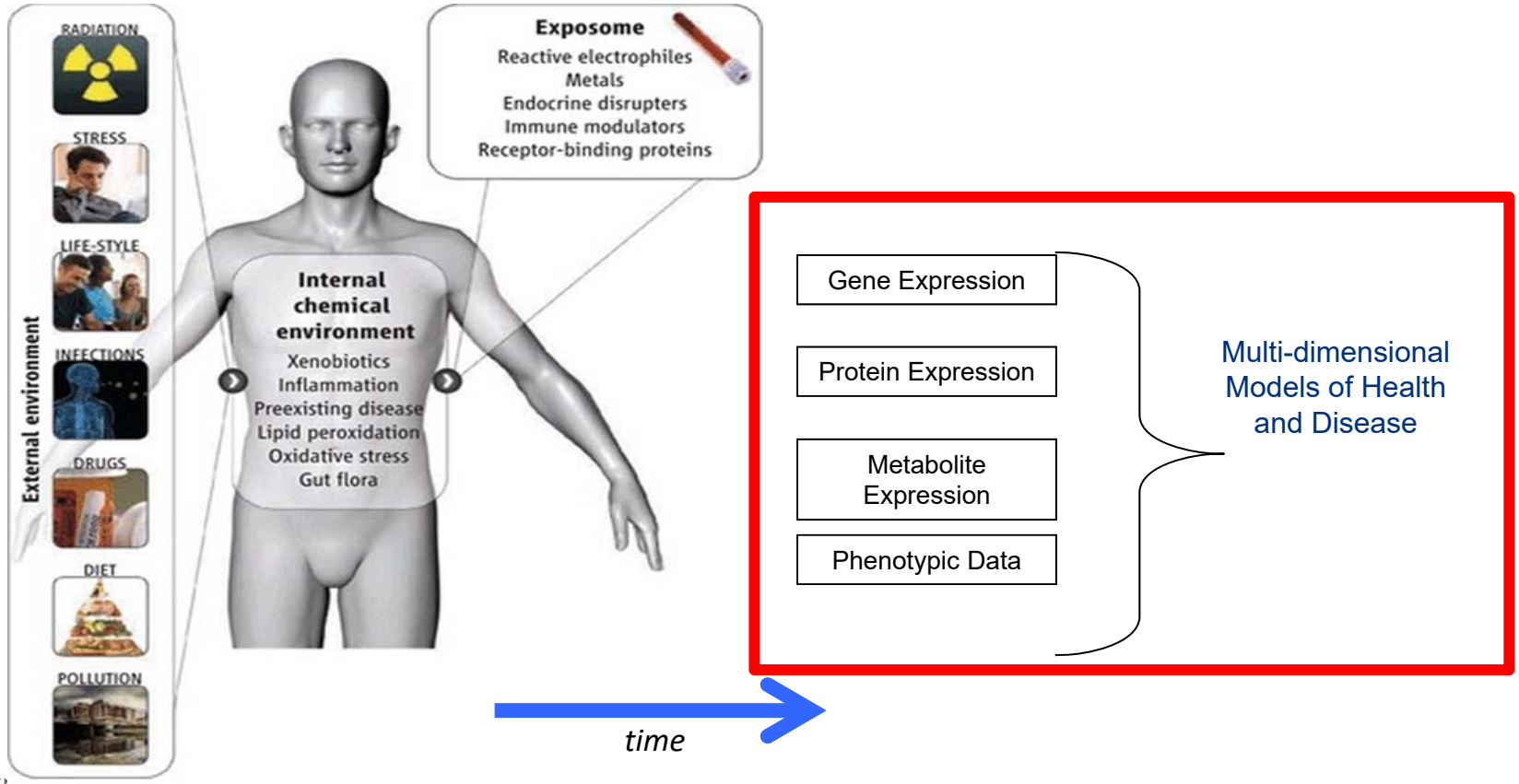
Jain et al. N Engl J Med 2015;373:415-27.
Jain et al. N Engl J Med 2015;372:835-45.

The Human Experimental Model: Host Response to Exposures



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RNA Classifiers in Medical Practice Today

Cancer Diagnosis and Prognosis

- **Oncotoype DX®**: 21 gene RNA signature from breast tumor; 12 gene RNA signature from colon tumor
- **MammaPrint®**: 70 gene RNA signature from breast tumor (FDA approved)
- **BluePrint™**: 80 gene RNA signature that distinguishes basal, luminal and ERBB2 subgroups of breast cancer
- **Pathwork® Tissue of Origin Test**: 2,000 RNAs to classify cancer of unknown primary (FDA approved)

Cardiovascular Disease Diagnosis and Prognosis

- **AlloMap®**: 11 blood gene RNA signature for rejection following cardiac transplant (FDA approved)
- **Corus™ CAD**: 23 gene blood RNA signature for coronary artery disease
- **Triage® Cardiac Panel**: 5 blood protein signature for assessment of chest pain and shortness of breath

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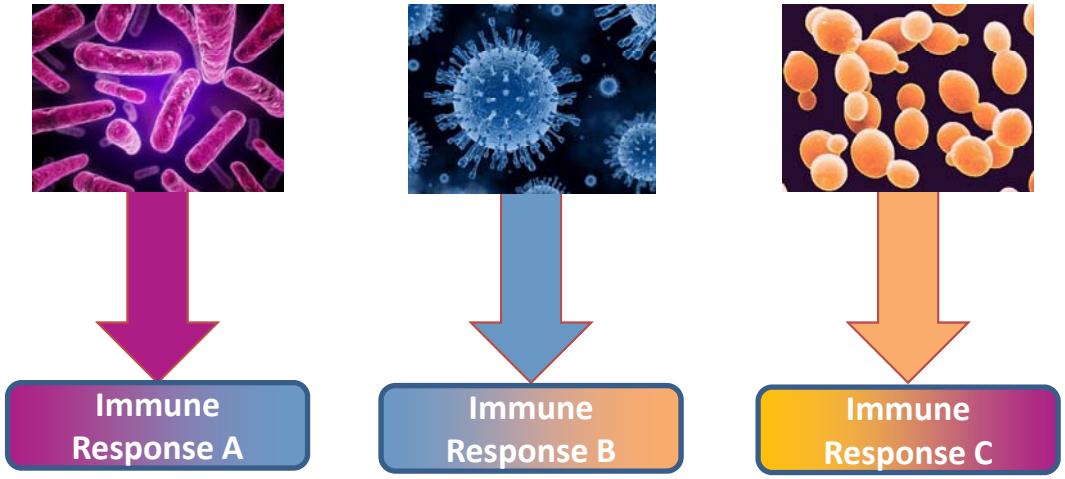
Targeting the Host Response



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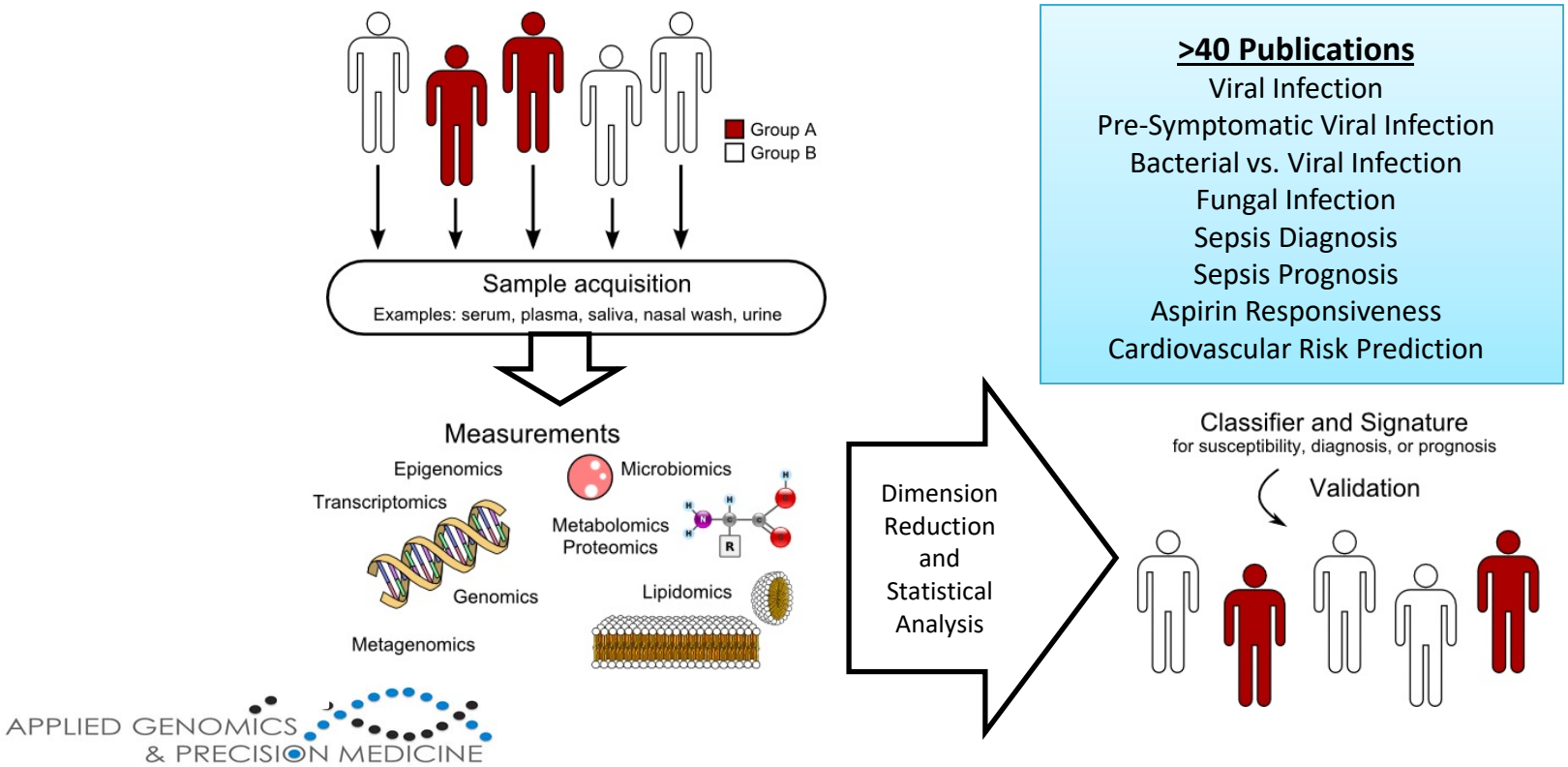
Adapted from Ramilo and Mejias, Cell Host Microbe, 2009. **6**(3): p. 199-200.

Science of Host Response



<u>>40 Publications</u>

Viral Infection
Pre-Symptomatic Viral Infection
Bacterial vs. Viral Infection
Fungal Infection
Sepsis Diagnosis
Sepsis Prognosis
Aspirin Responsiveness
Cardiovascular Risk Prediction

Febrile Illness: Nepal

- Active surveillance for consecutive cases of febrile illness in adult non-surgical patients
  - Axillary temp ≥38.0°C
  - ≥14 years
  - Informed consent
- Wet season: July - August

- Patan Hospital, Kathmandu
  - 251 beds
  - 250,000 outpatients
  - 30,000 ER visits

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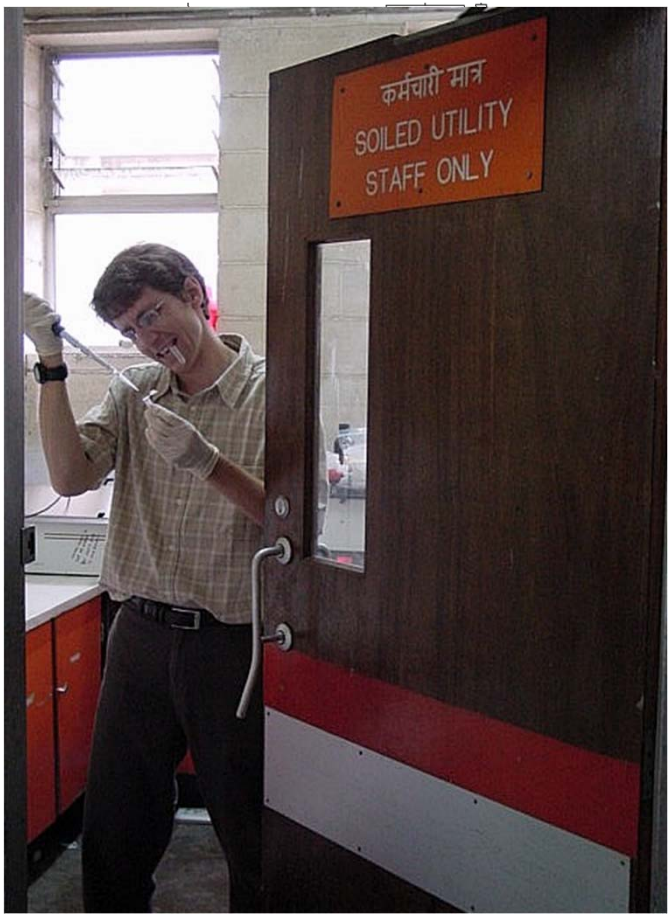
Nepal: Microbiological Diagnoses and Host Response

- Single-agent, well-established Dx, on arrays: n=76
- Also 7 healthy local donors

Etiologic Agent	*n*	Age (Median)	Temp (F) (Median)	WBC (Median)	Sxs (days) (Median)
E. coli	4	30	101.9	12.15	2.5
Leptospira	6	26	100.9	6.5	7.5
O. tsutsugamushi	7	22	102	8.2	7
R. typhi	14	23	101.4	6.8	5.5
S. aureus	3	30	104	11	3
S. pneumoniae	2	26	101.8	10.3	7
*S. enterica**	40	20	102	6	5

*** typhi (22), paratyphi (18)**

Murdoch et al; AJTMH 70(6): 670-675 2004



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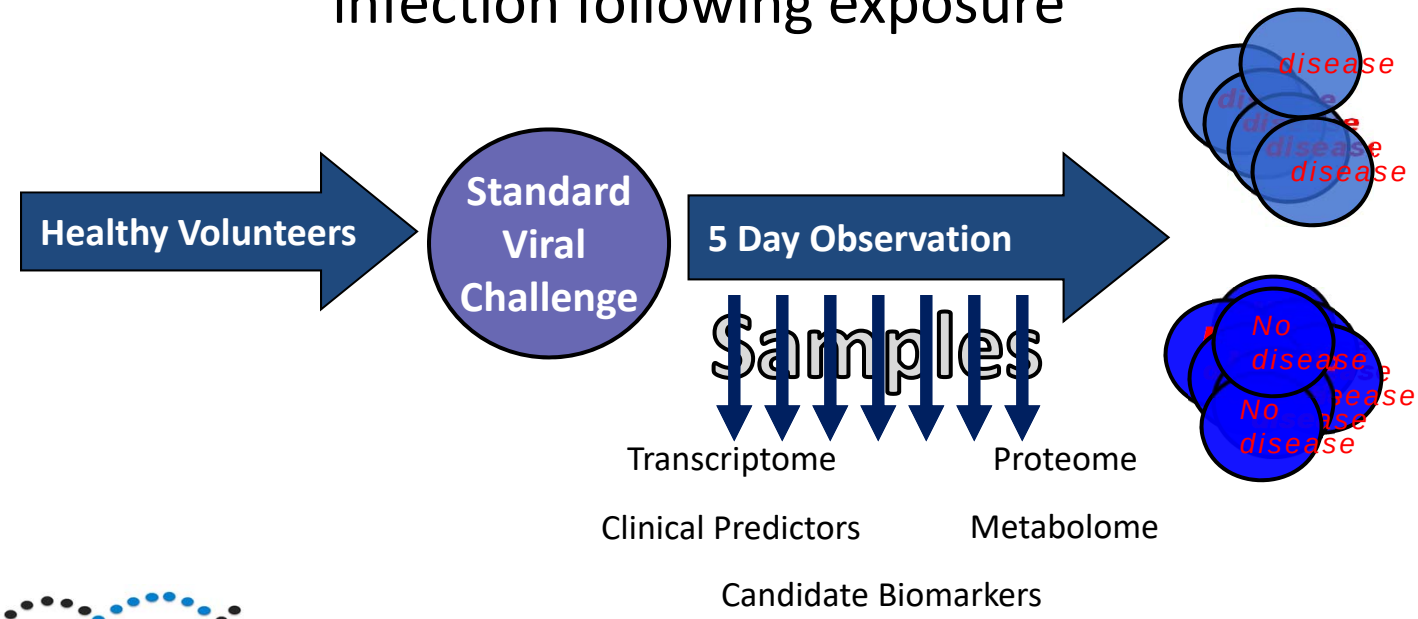
DARPA: Predicting Health and Disease

Develop a predictor of incipient respiratory viral infection following exposure



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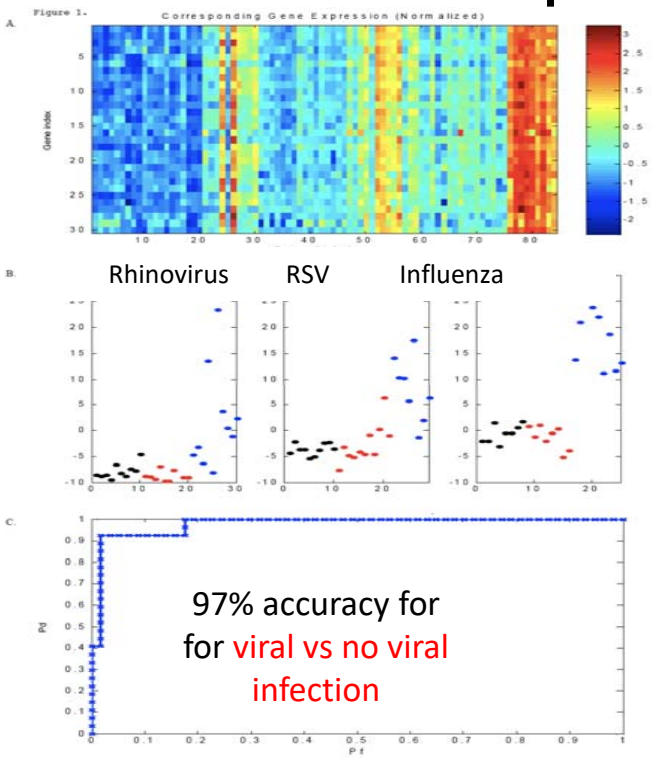
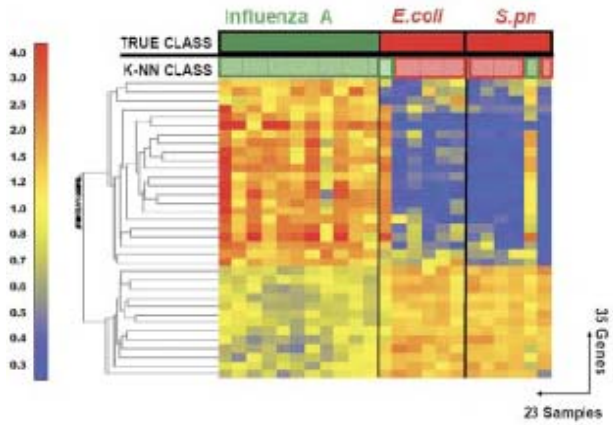
Host Gene Expression Classifier for Acute Respiratory Infection



Viral vs Bacterial Infection



Zaas et al, Cell Host and Microbe, 2009
Woods et al PLoS ONE 2013
Ramilo et al Blood 2007

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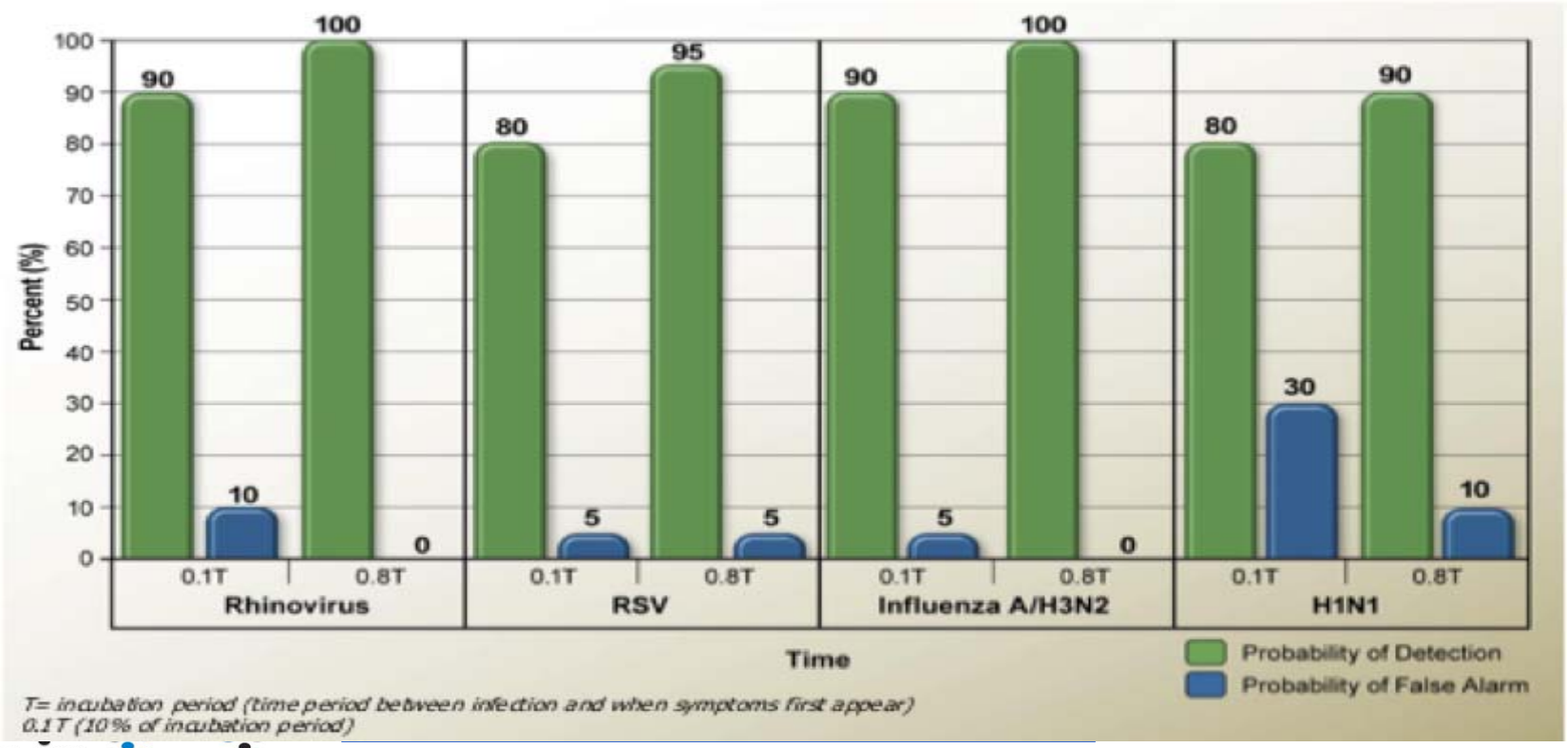
How early does the viral classifier identify those who will become sick?



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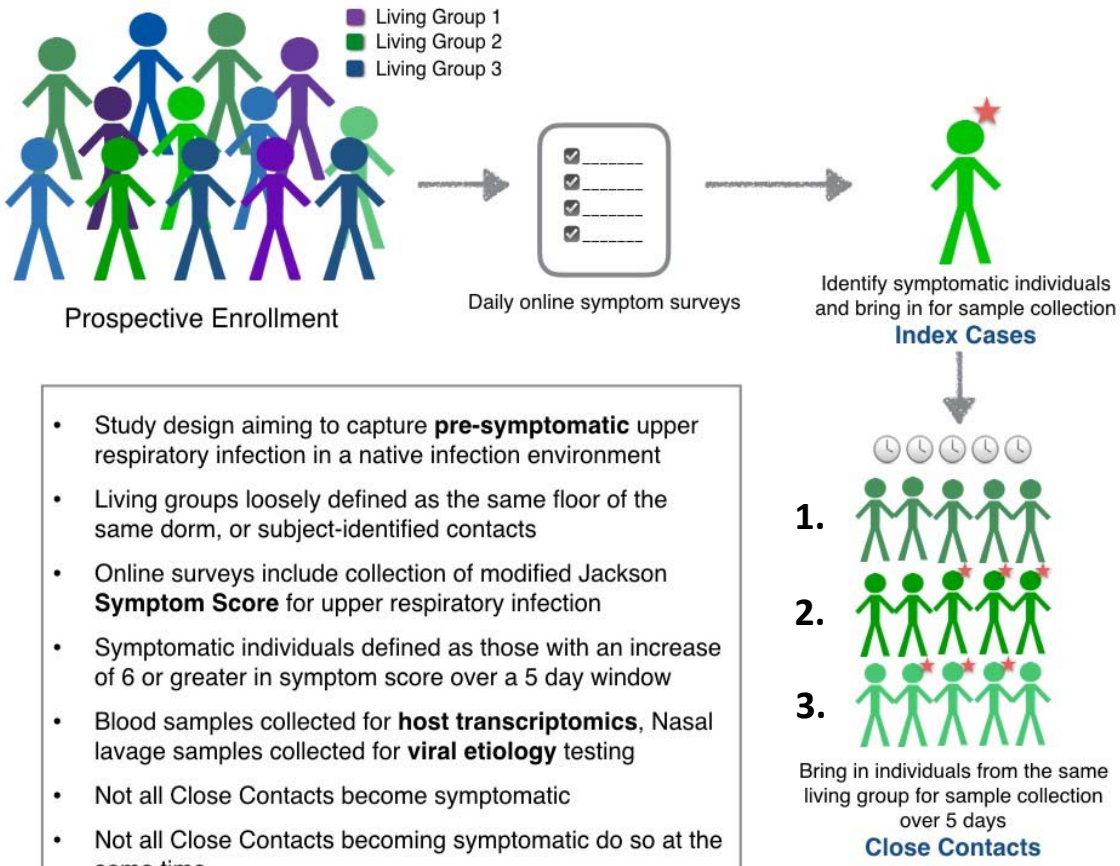
Pre-Symptomatic detection in naturally-acquired infection?



- Study design aiming to capture **pre-symptomatic** upper respiratory infection in a native infection environment
- Living groups loosely defined as the same floor of the same dorm, or subject-identified contacts
- Online surveys include collection of modified Jackson **Symptom Score** for upper respiratory infection
- Symptomatic individuals defined as those with an increase of 6 or greater in symptom score over a 5 day window
- Blood samples collected for **host transcriptomics**, Nasal lavage samples collected for **viral etiology** testing
- Not all Close Contacts become symptomatic
- Not all Close Contacts becoming symptomatic do so at the same time

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Viral etiologies of URI in the Student Cohort

	2009-10	2010-11	2011-12	2013-14	2014-15	Totals
Influenza	8	18	8	4	8	**46**
Parainfluenza	7	22	7	0	5	**41**
Rhinovirus	30	25	15	6	61	**137**
Coronavirus	18	19	8	7	9	**61**
Coxsackie/Echovirus	24	23	10	ND	ND	**57**
Adenovirus	1	1	0	1	6	**9**
Metapneumovirus	6	0	4	4	3	**17**

On Average: 54 % of Index Cases have + Viral Etiology
17% have multiple viruses

15% of Close Contacts have + Viral Etiology
57% of these match their Index Case

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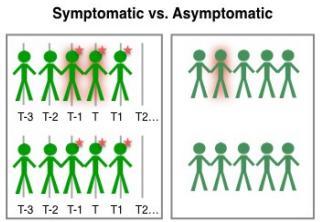
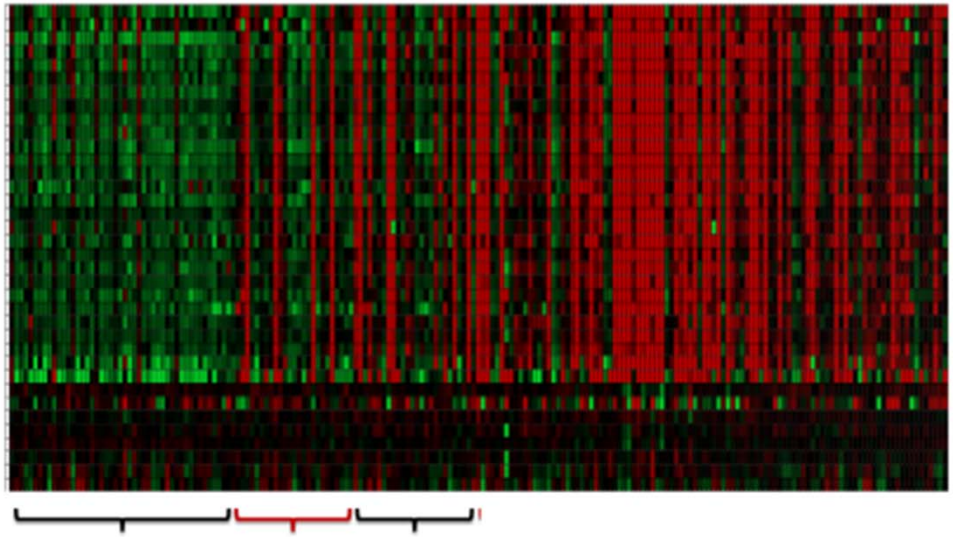
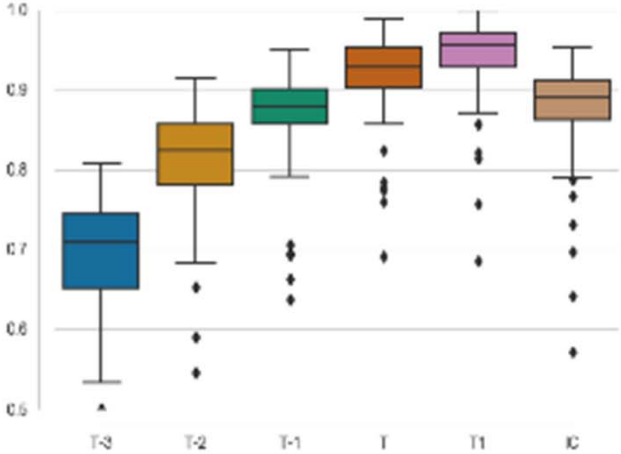
Performance accuracy of a gene signature of viral infection across time







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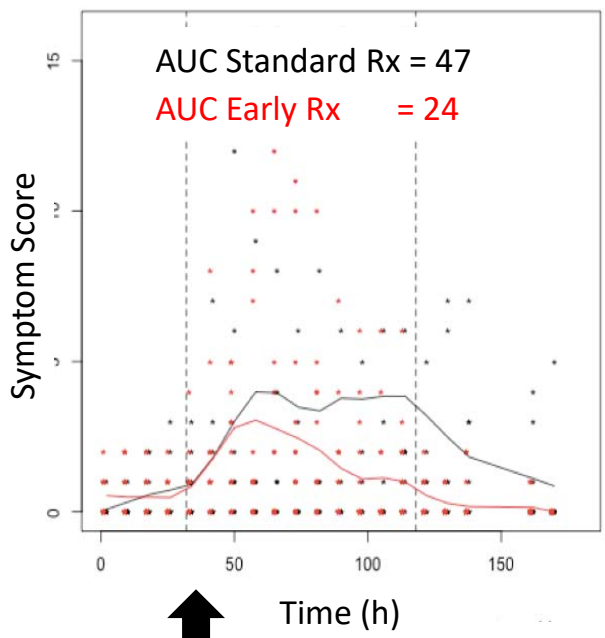
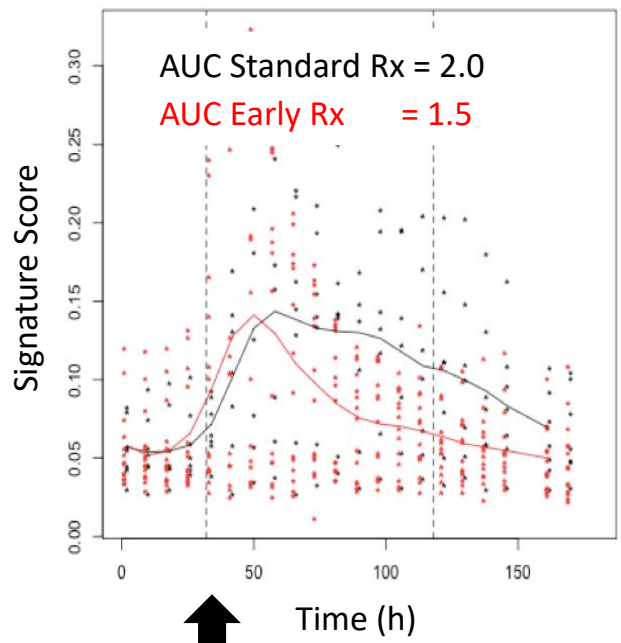
Early Detection, Early Cure:
Early Oseltamivir Reduces Symptom and Classifier Intensity





Oseltamivir

Oseltamivir

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McClain et al, Open Forum of Infectious Diseases, 2016

Science Translational Medicine AAAS

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Science Science Translational Medicine Science Signaling Science Advances

Host gene expression classifiers diagnose acute respiratory illness etiology

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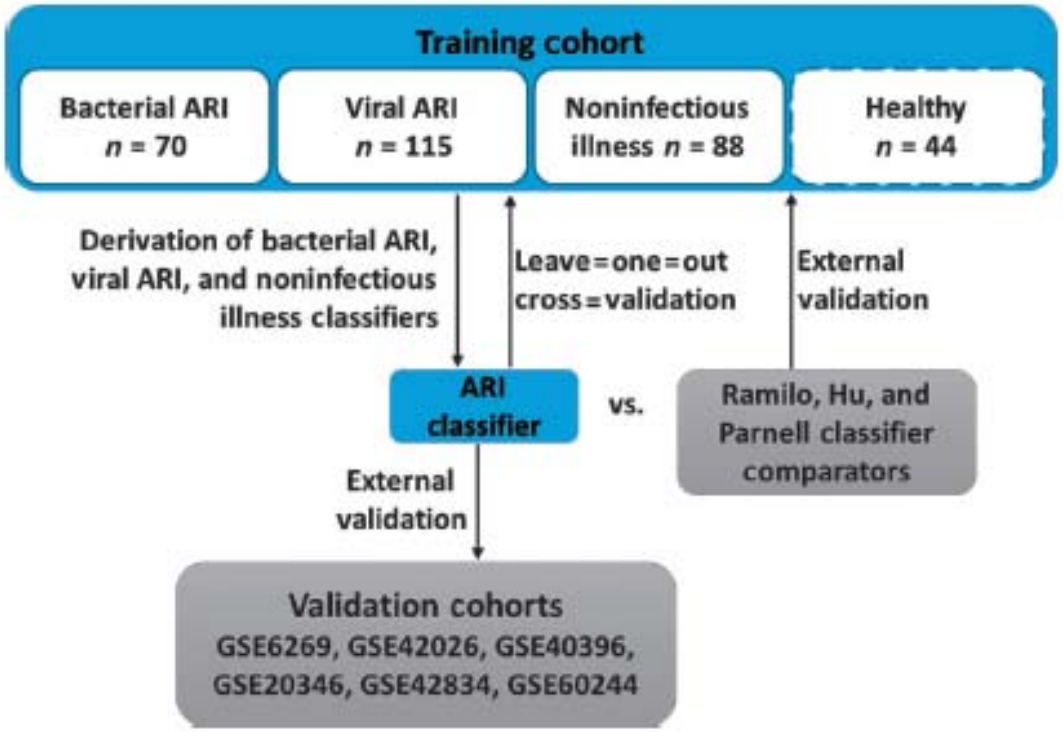
Deriving a Host Response ARI Classifier



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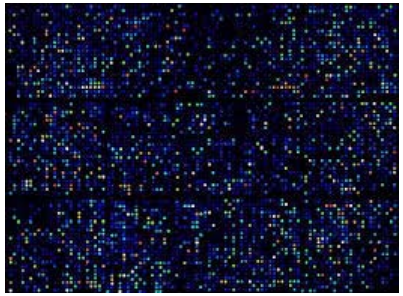
Deriving a host response ARI Classifier

- Training cohort of host gene expression measured by microarray



- Generate binary classifiers
  - Bacterial vs. Non-Bacterial (71 genes)
  - Viral vs. Non-Viral (33 genes)
  - Non-Infection vs. Infection (26 genes)
- Apply all three classifiers to each individual
- Generate three discrete probabilities for each condition
- Highest probability wins

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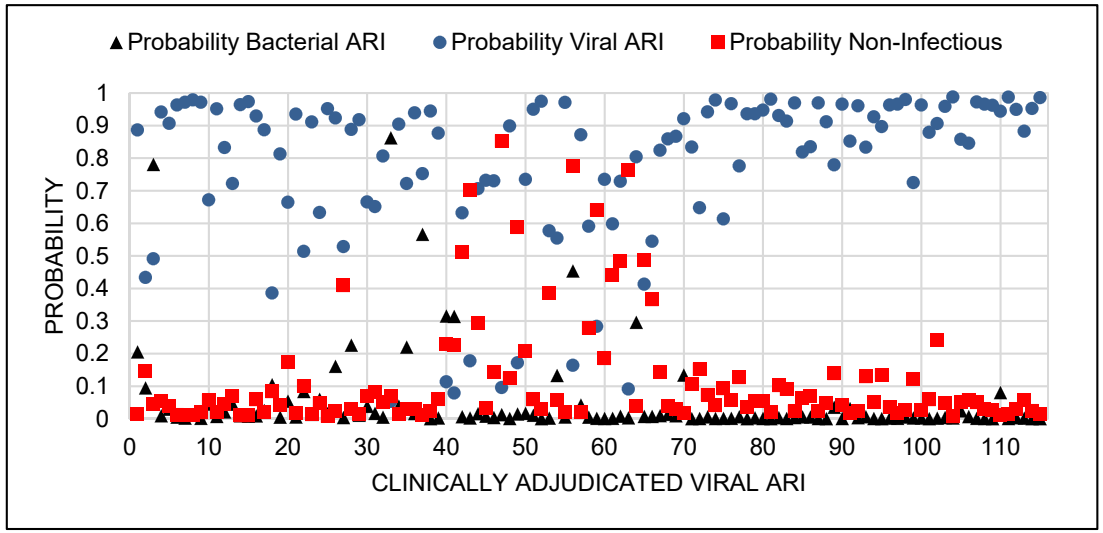
Viral ARI Classification



104/115 correctly identified (90%)
145/158 correctly excluded (92%)

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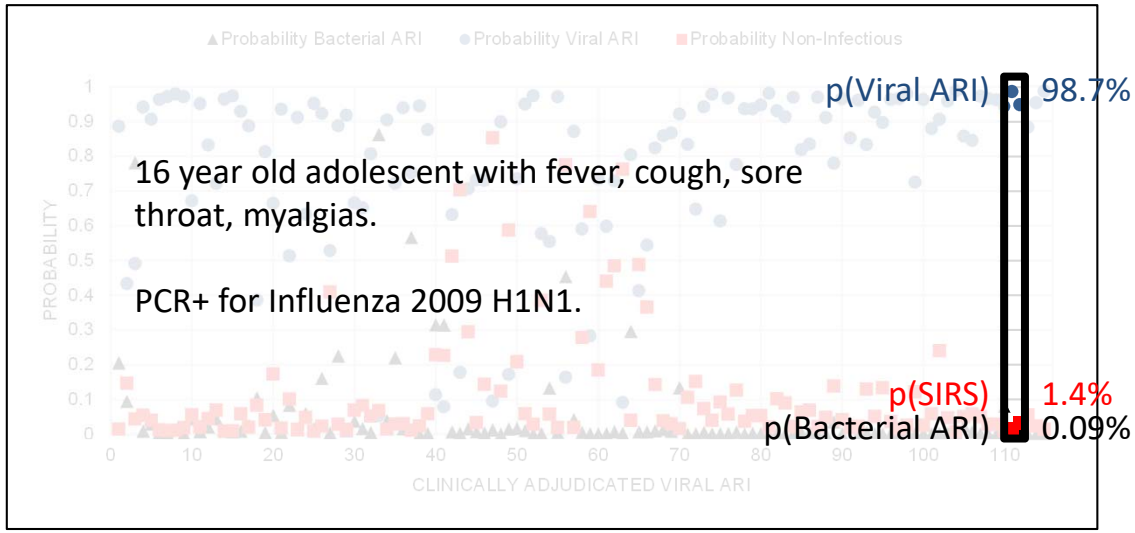
Viral ARI Classification



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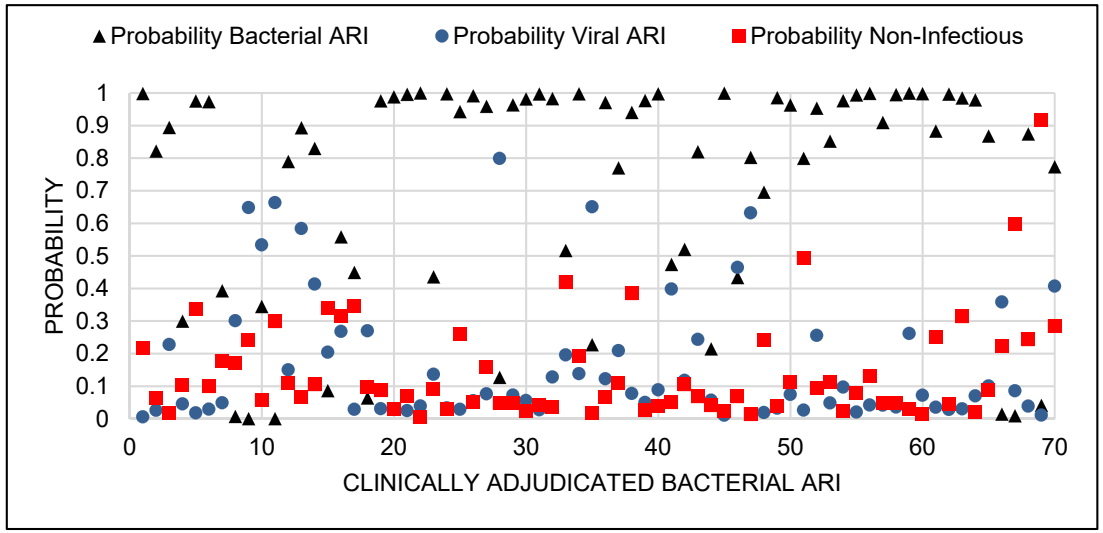
Bacterial ARI Classification



58/70 correctly identified (83%)
179/191 correctly excluded (94%)

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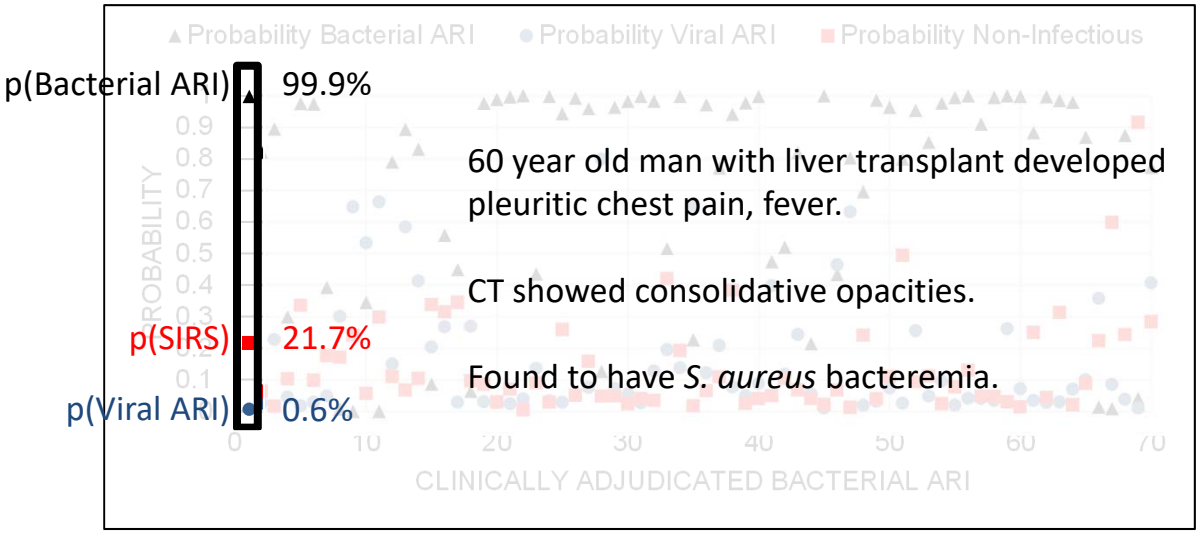
Bacterial ARI Classification



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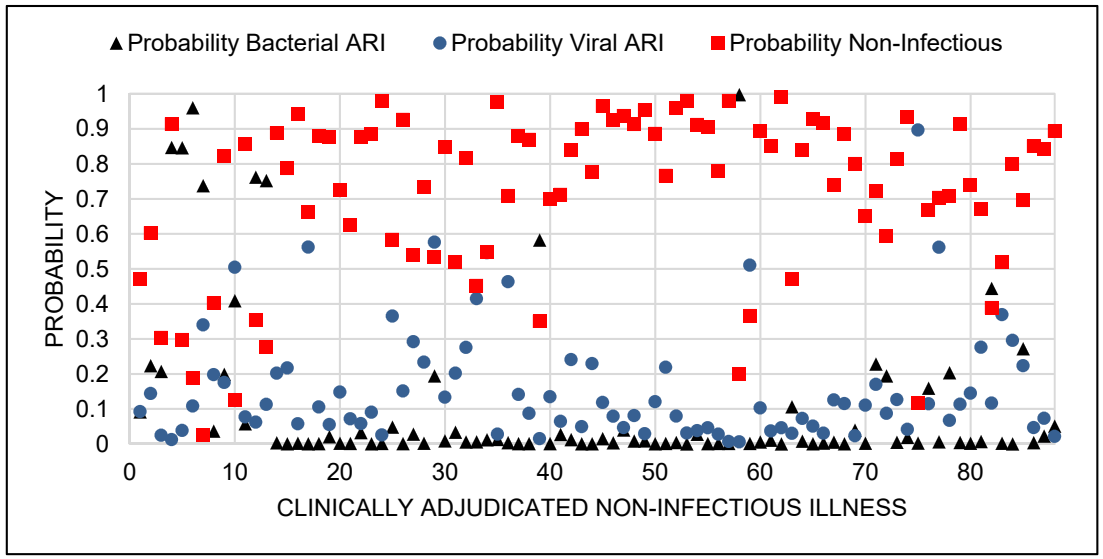
Non-Infectious SIRS Classification



Infection excluded in 76/88 cases (86%)

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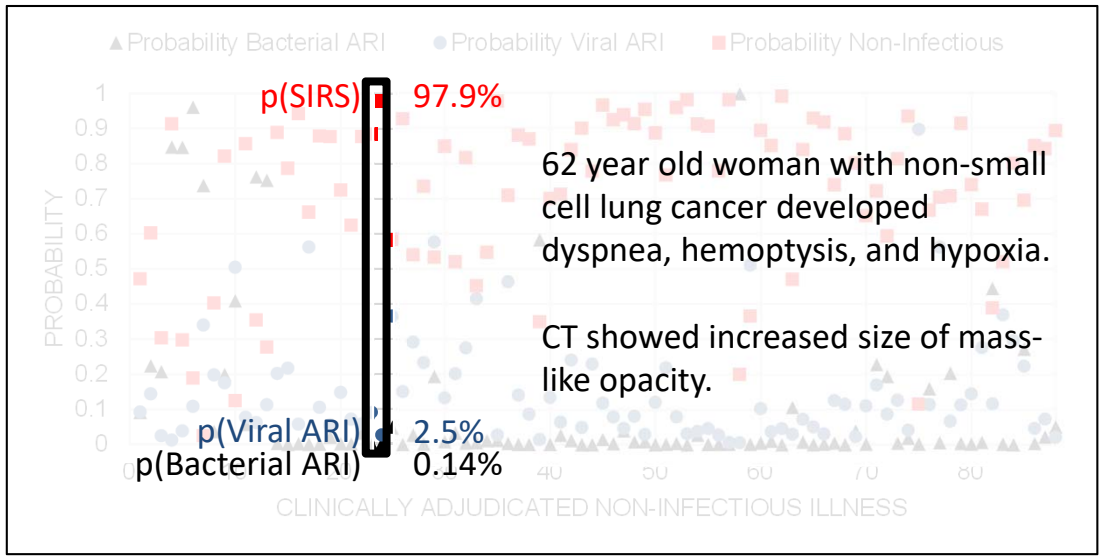
SIRS Classification



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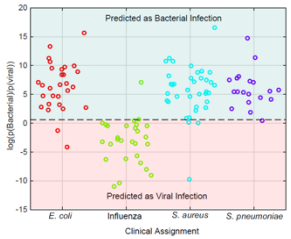
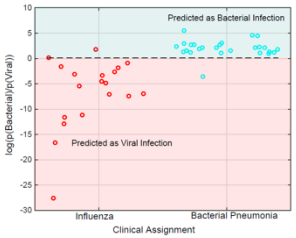
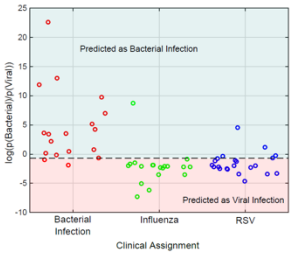
External Validation Studies



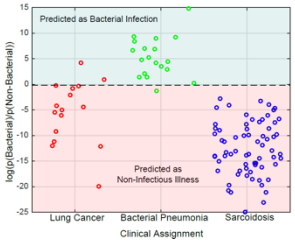
- **AUC 0.95**



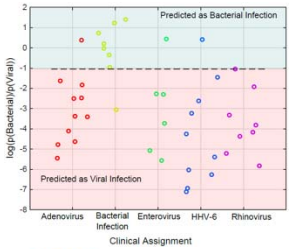
AUC 0.99



AUC 0.90



AUC 0.99



AUC 0.93

Includes adults and children; mild infection and severe infection; inpatients and outpatients; US and international

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Migration of Microarray Results to RT-PCR

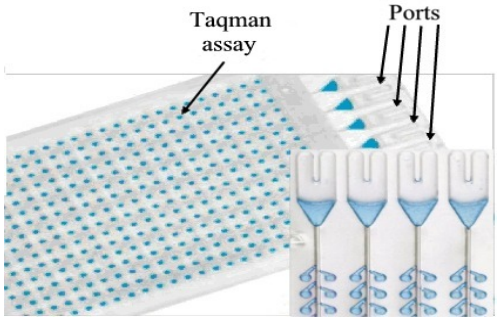
RESEARCH ARTICLE

INFECTIOUS DISEASE

A Host-Based RT-PCR Gene Expression Signature to Identify Acute Respiratory Viral Infection

Aimee K. Zaas,[1,2] Thomas Burke,[1] Minhua Chen,[3] Micah McClain,[1,2] Bradly Nicholson,[4] Timothy Veldman,[1] Ephraim L. Tsalik,[1,2,4] Vance Fowler,[2] Emanuel P. Rivers,[5] Ronny Otero,[5] Stephen F. Kingsmore,[6] Deepak Voora,[1,2] Joseph Lucas,[1] Alfred O. Hero,[7] Lawrence Carin,[8] Christopher W. Woods,[1,2,4]* Geoffrey S. Ginsburg[1,2]*

- RT-PCR
  - 29 genes in the "Pan-Viral" Factor
  - Interferon pathway
  - Oligo adenylate synthase pathway
  - MX1
  - RSAD2
  - Control genes



Taqman RT-PCR Low Density Array (TLDA) Card

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Zaas et al, Sci Trans Med (2013)

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Antimicrobial Resistance Rapid, Point-of-Need Diagnostic Test' Challenge

Overview | Participation Requirements | View FAQ's | Resources | Forum | Letter of Intent | Submission Form | Executive Summary

Notice

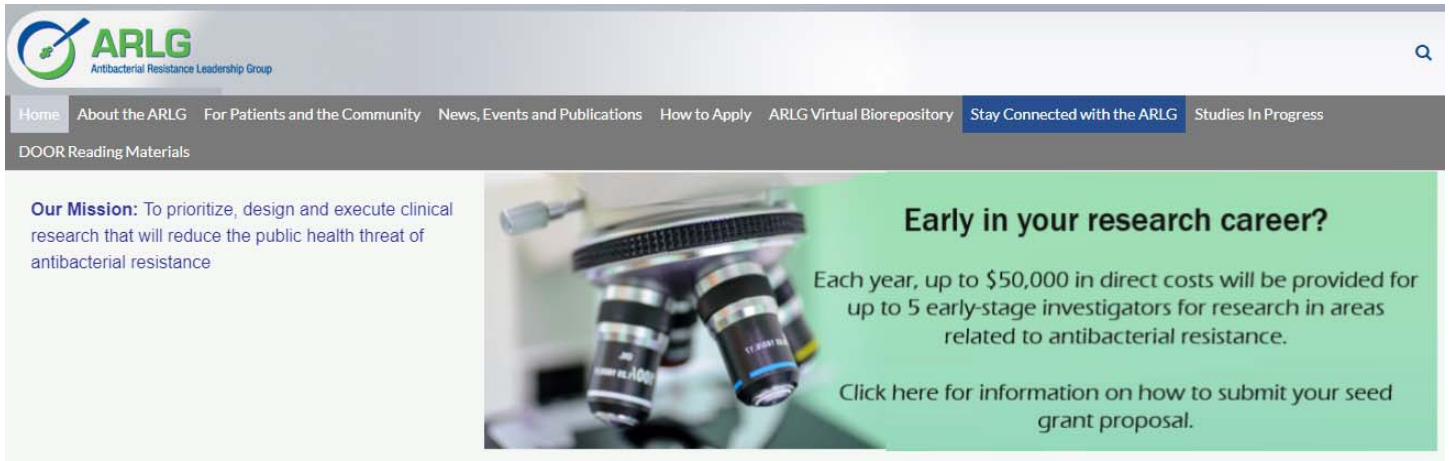
Antimicrobial Resistance Diagnostic Challenge selects 10 semifinalists in first phase of competition

Each will receive $50,000 to develop prototypes of diagnostics to improve detection of drug resistant bacteria

Ten semifinalists have been selected in the first phase of the Antimicrobial Resistance Diagnostic Challenge, a federal prize competition that will award up to a total of $20 million in prizes, subject to the availability of funds, for innovative rapid, point-of-need diagnostic tests to combat the emergence and spread of drug resistant bacteria. The semifinalists were selected for their concepts for a diagnostic based on a technical and programmatic evaluation from among 74 submissions. While semifinalists will each receive $50,000 to develop their concepts into prototypes, anyone can submit a prototype to compete in the second phase of the challenge to win up to $100,000.

Read the Full Press Release

Antibacterial Resistance Leadership Group



<u>Four Priority Areas</u>

Gram-negative bacteria

Gram-positive bacteria

Infection Control/Stewardship

Diagnostics

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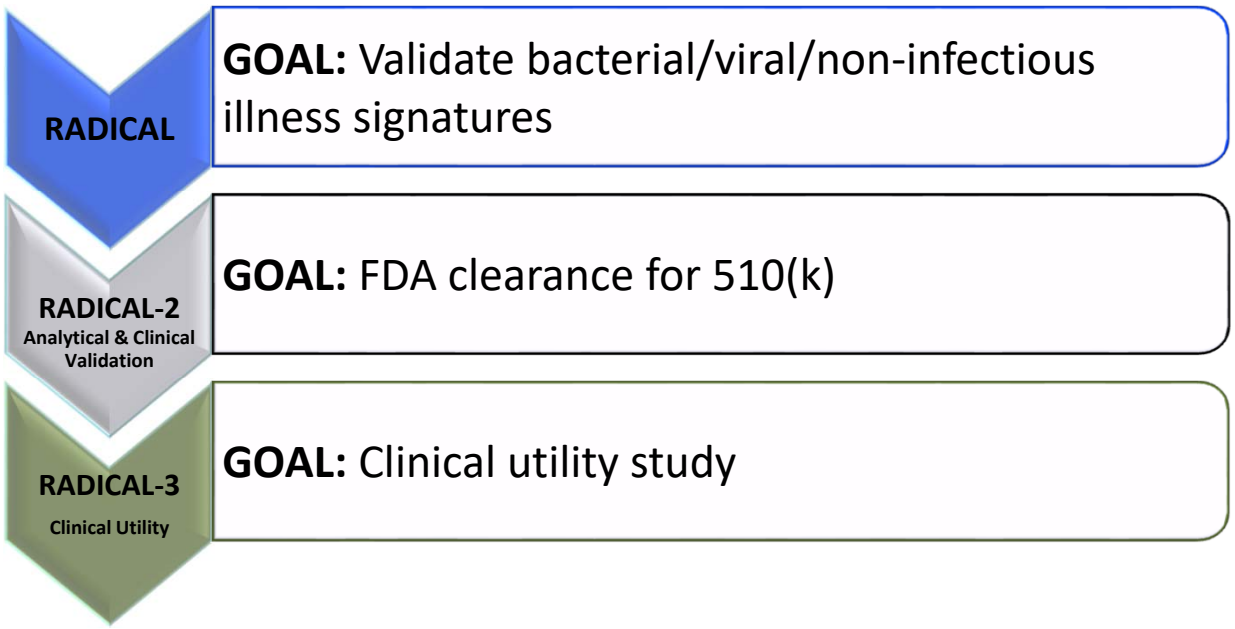
Rapid Diagnostics in Categorizing Acute Lung Infections (RADICAL)



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Antibacterial Resistance Leadership Group

Developing a Host Gene Expression Test

- Rapid
  - <5 minutes Field/Home
  - <20 minutes Outpatient
  - <1hr ER
  - <4 hours hospital
- Low complexity
- Inexpensive
- Non-invasive/Minimally invasive
- High degree of multiplexing
- Integrated sample processing

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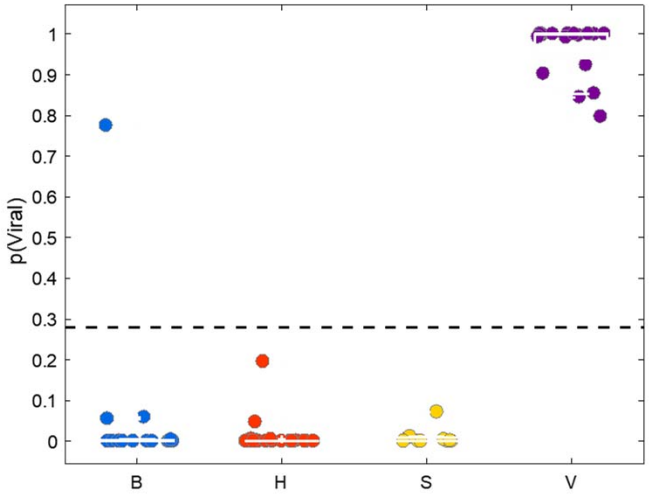
Qvella



68 Whole blood samples	24 healthy controls
	19 viral infections
	15 bacterial infections
	10 SIRS (non-infectious)

Data Analysis		
True positive rate (sensitivity)	19/19	100%
True negative rate (specificity)	48/49	98%
Accuracy	67/68	98.5%

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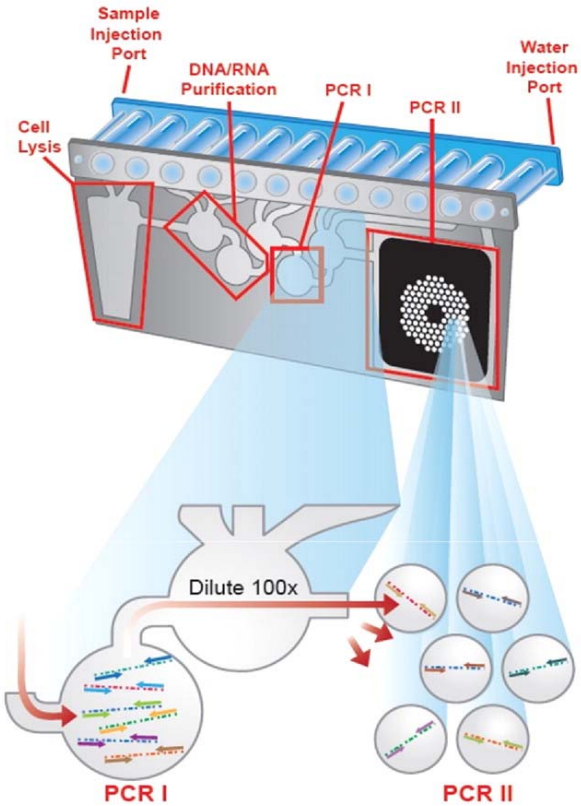
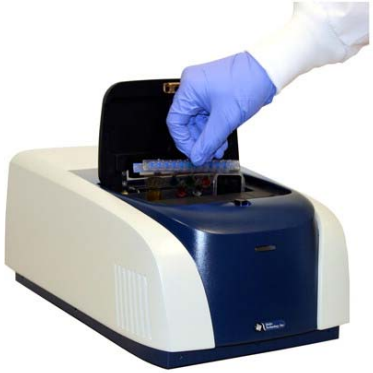
The FilmArray Pouch





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BIOFIRE
A BIOMÉRIEUX COMPANY

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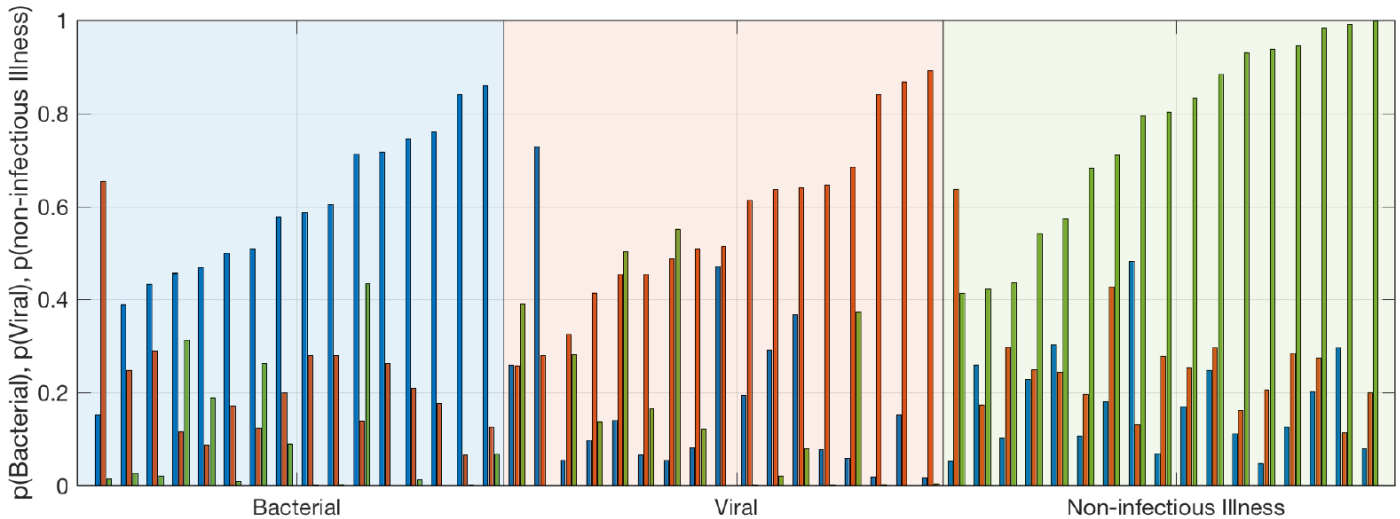
Antibacterial Resistance Leadership Group

BioFire Pilot Data

50 subjects, 88% overall accuracy



Proportions

True vs. Pred	Bacterial	Viral	SIRS
Bacterial	0.937	0.062	0.000
Viral	0.058	0.764	0.176
SIRS	0.000	0.058	0.941

Classifier	AUC	TPR	TNR
Bacterial	0.936	0.938	0.912
Viral	0.923	0.882	0.909
SIRS	0.984	1.000	0.909

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BioFire – Host Response B/V Test

- FilmArray pouch with host response targets for viral infection and bacterial infection
- Can identify viral infection, bacterial infection, co-infection, or non-infectious illness
- 325 subjects tested
  - 117 Bacterial
  - 165 Viral
  - 103 Non-infectious illness

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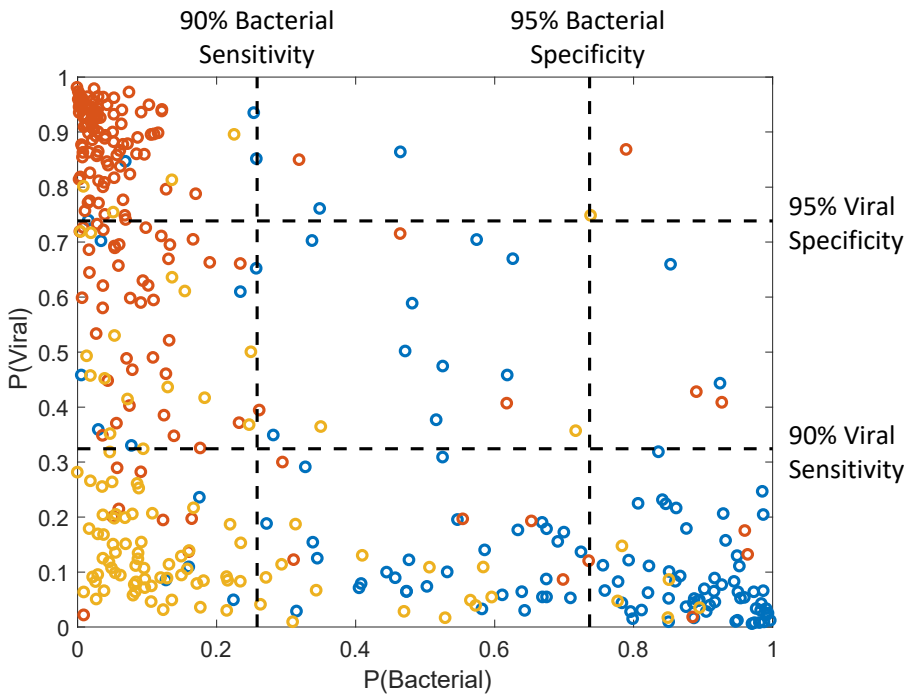
BioFire – Host Response B/V Test



Clinical Adjudication

- Bacterial
- Viral
- Non-Infectious Illness

Area under the curve (AUC)

Bacterial – 0.92

Viral – 0.92

Non-Infectious Illness – 0.89

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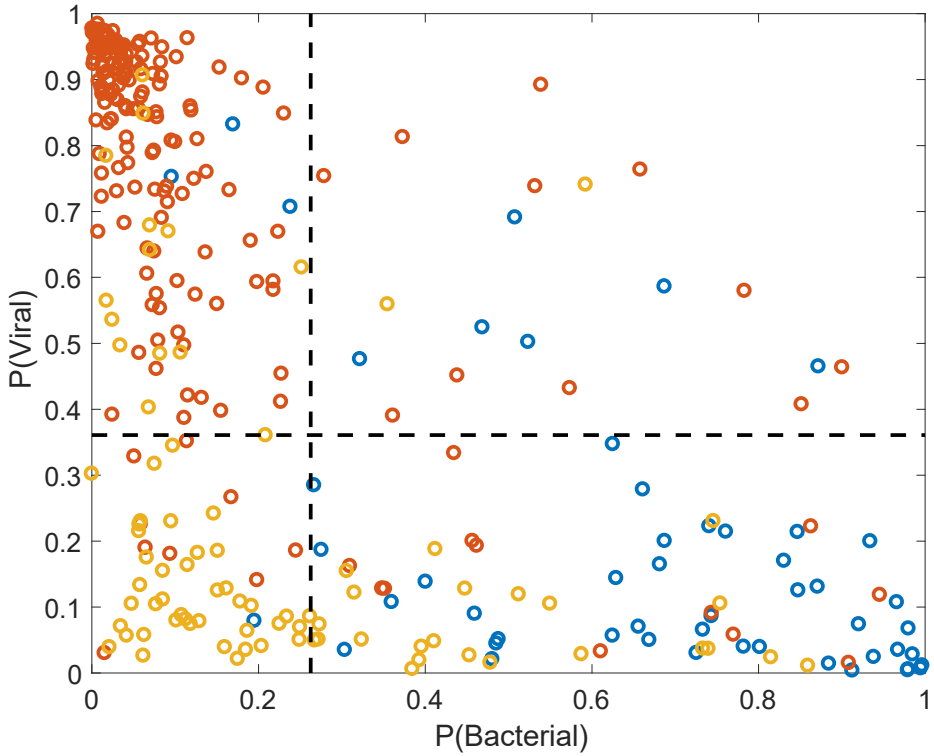

Average Weight Accuracy

Bacterial – 87%

Viral – 85%

Probability of viral infection vs. probability of bacterial infection. The vertical dashed line is the threshold (.263) which maximizes the bacterial test AWA (87%). The horizontal line is the threshold (.361) which maximizes the viral test AWA (85%). Blue = adjudicated bacterial infection; red = viral infection; gold = non-infectious illness. Each circle represents one subject.

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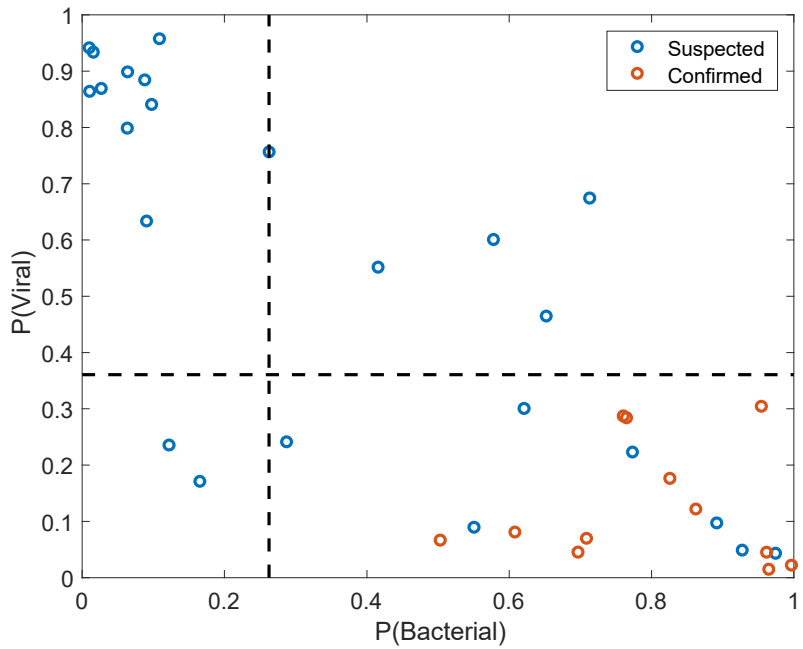
Co-Infection



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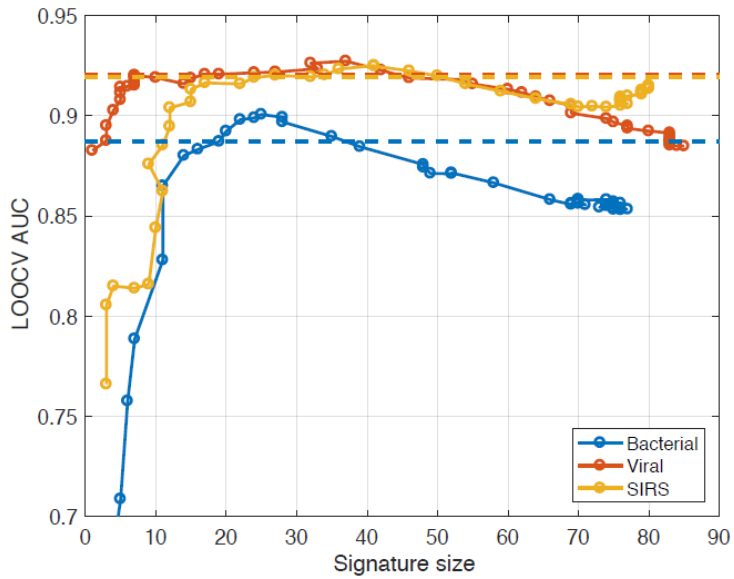
Achieving Sparsity



Modeling the impact of signature size on predicated accuracy as measured by leave one out cross validated (LOOCV) area under the curve (AUC).

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ANNOUNCEMENTS

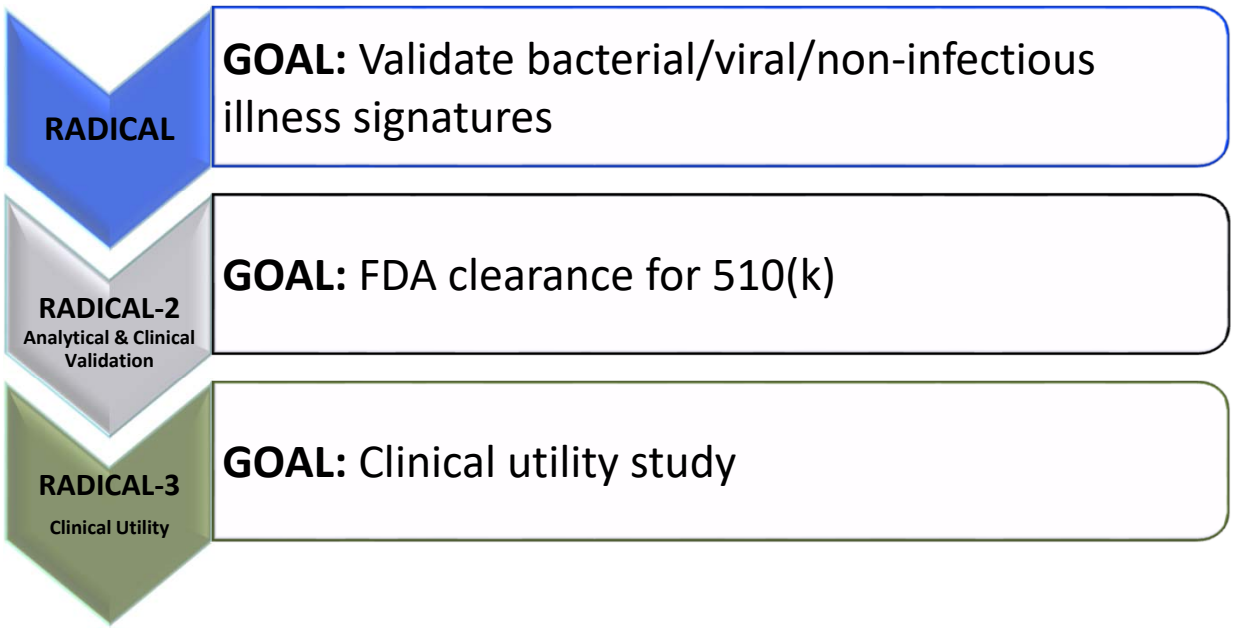
December 3, 2018

[Antimicrobial Resistance Diagnostic Challenge names five finalists]

Host gene expression to classify viral and bacterial infection using multiplex PCR

Ephraim Tsalik, Duke University, Durham, North Carolina

Rapid Diagnostics in Categorizing Acute Lung Infections (RADICAL)



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RADICAL-2

- 1200 subject validation
- 8 sites: Duke, UNC, Henry Ford, U. Utah, BWH, Newton-Wellesley, UC Davis, U. Texas-Houston
- Enrollment 2017 – 2019
- Results will be used to validate available platforms (e.g., BioFire)
- Support FDA application

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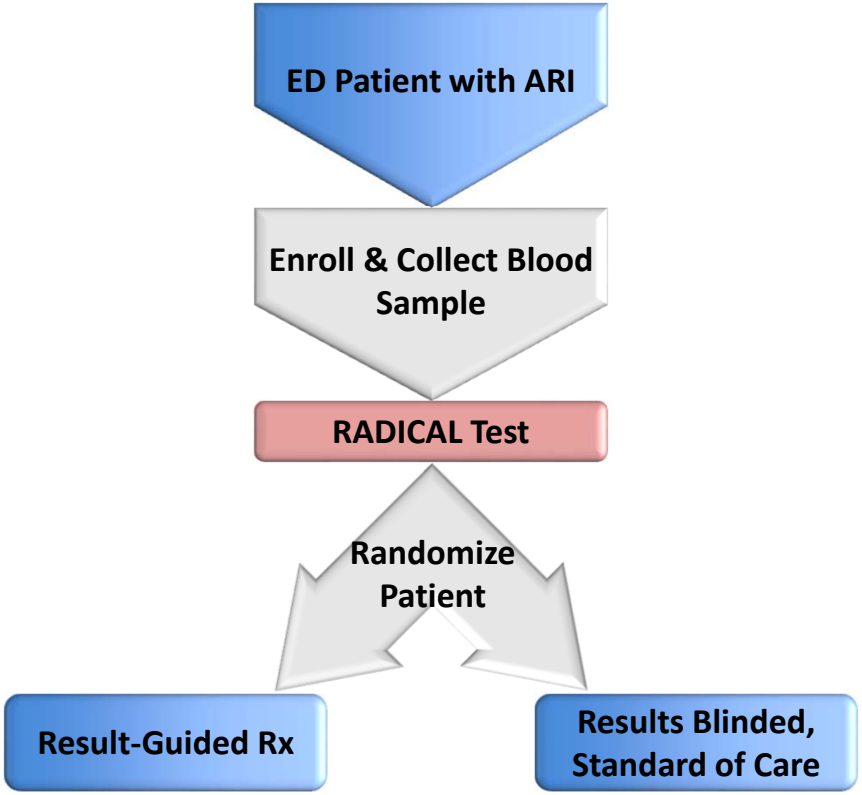
RADICAL 3 Design (~2020)



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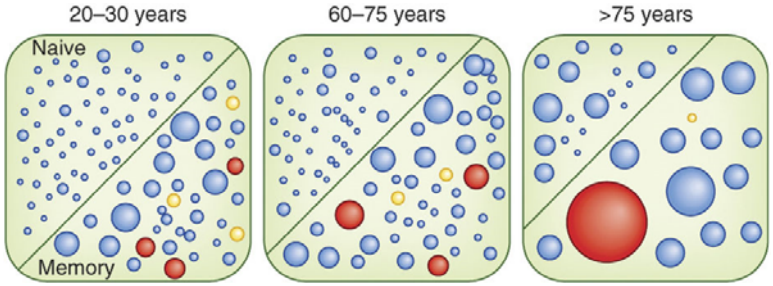
How do host response-based diagnostics perform in hosts with altered immune responsiveness?

1. **Host biomarker signatures for diagnosis of acute respiratory syndromes in CF patients following lung transplant.** Cystic Fibrosis Foundation
   - Biobanked samples of viral and bacterial infection in normal hosts as comparators
   - Banking of CF / Lung transplant samples for future applications

2. **Performance of biomarker diagnostics in an aging population** (VA)
   - Biobanked samples of younger adults as comparator arm.

3. **Resilience in Older Adults** (UH2/UH3, NIA - Whitson, Colon-Emeric)
   - Biobanked samples of older adults with respiratory infections – biomarker discovery for resilience to infection



Nature Immunology **14**, 428–436

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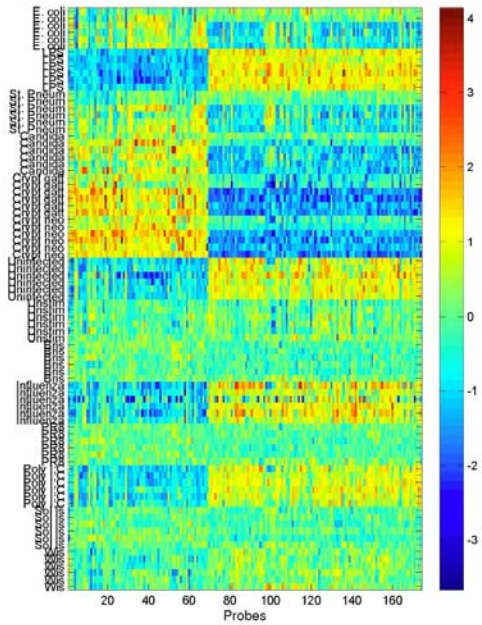
Diagnostics– in vitro PBMC Modeling

Data Binary comparisons Probe lists comparison todo

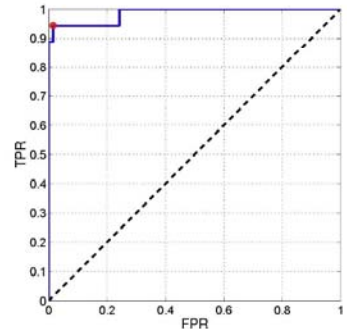
Fungal

- Bonferroni correction
- 174 significant with threshold 0.01

Candida and Crypto



- Sparse linear classifier
- Leave-one-out cross-validation



max AUC 0.9856
threshold: 0.1308
TPR: 0.9444
TNR: 0.9848
probes: 25

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Diagnostic host genomic signatures can be stable across multiple forms of host immunosuppression

Experimental Condition	Classifier-Predicted Condition: Aspergillus	Aspergillus + prednisone	Aspergillus + cyclophosphamide	Control	Control + prednisone	Control + cyclophosphamide
Aspergillus	**11**	0	0	0	0	0
Aspergillus + prednisone	0	**9**	0	0	2	0
Aspergillus + cyclophosphamide	0	0	**6**	0	0	1
Control	2	0	0	**55**	0	0
Control + prednisone	0	2	0	0	**8**	0
Control + cyclophosphamide	0	0	1	0	0	**9**

Murine aspergillus infection model. Numbers in the diagonal dark blue cells indicate correct classifications.

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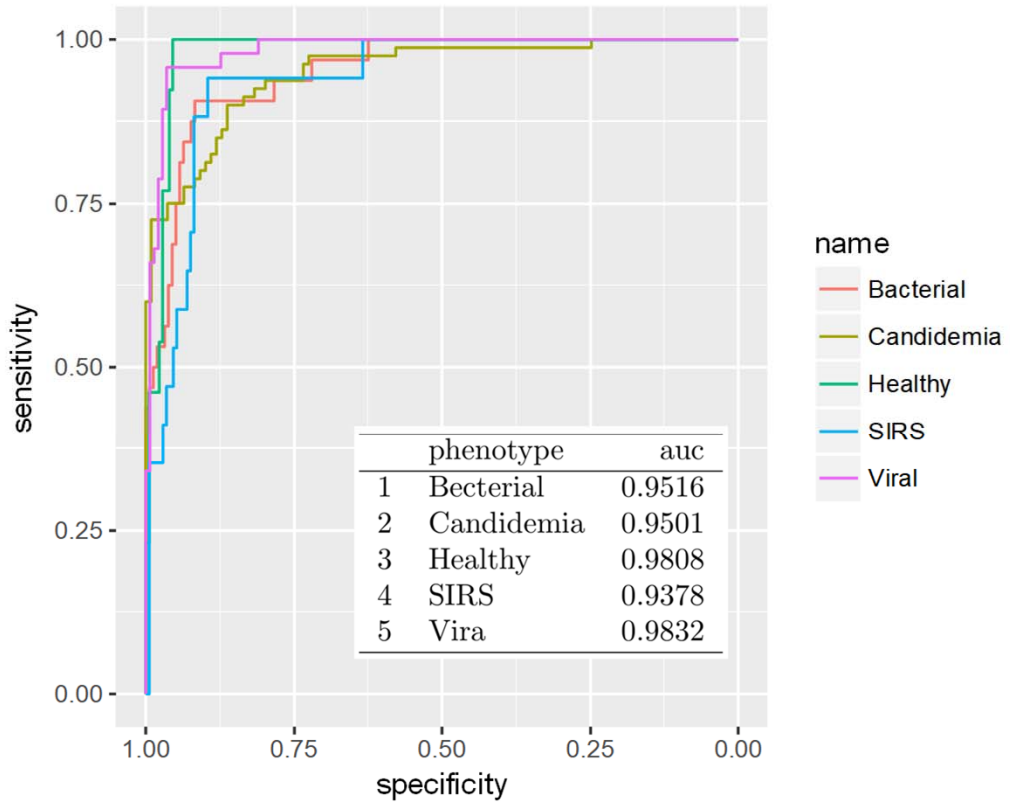
Host-derived genomic signature of human candidemia
NIAID R21



	phenotype	auc
1	Becterial	0.9516
2	Candidemia	0.9501
3	Healthy	0.9808
4	SIRS	0.9378
5	Vira	0.9832

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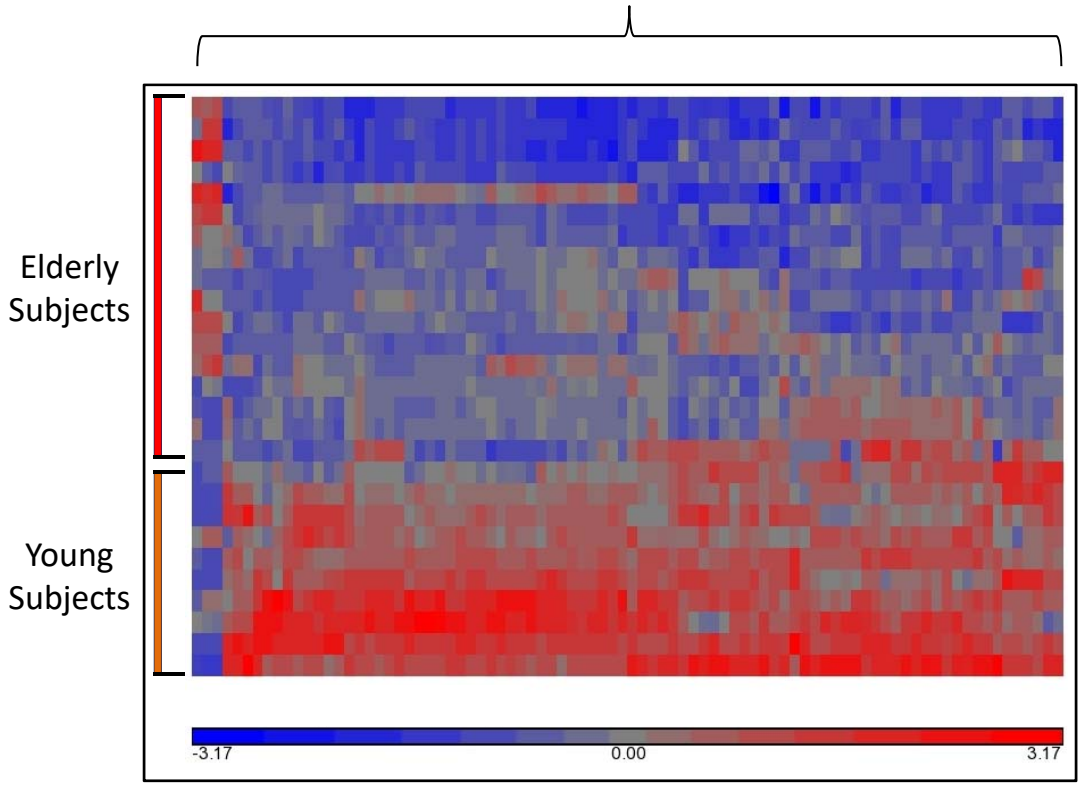
Elderly vs Young Veterans with viral URI: Differentially expressed genes



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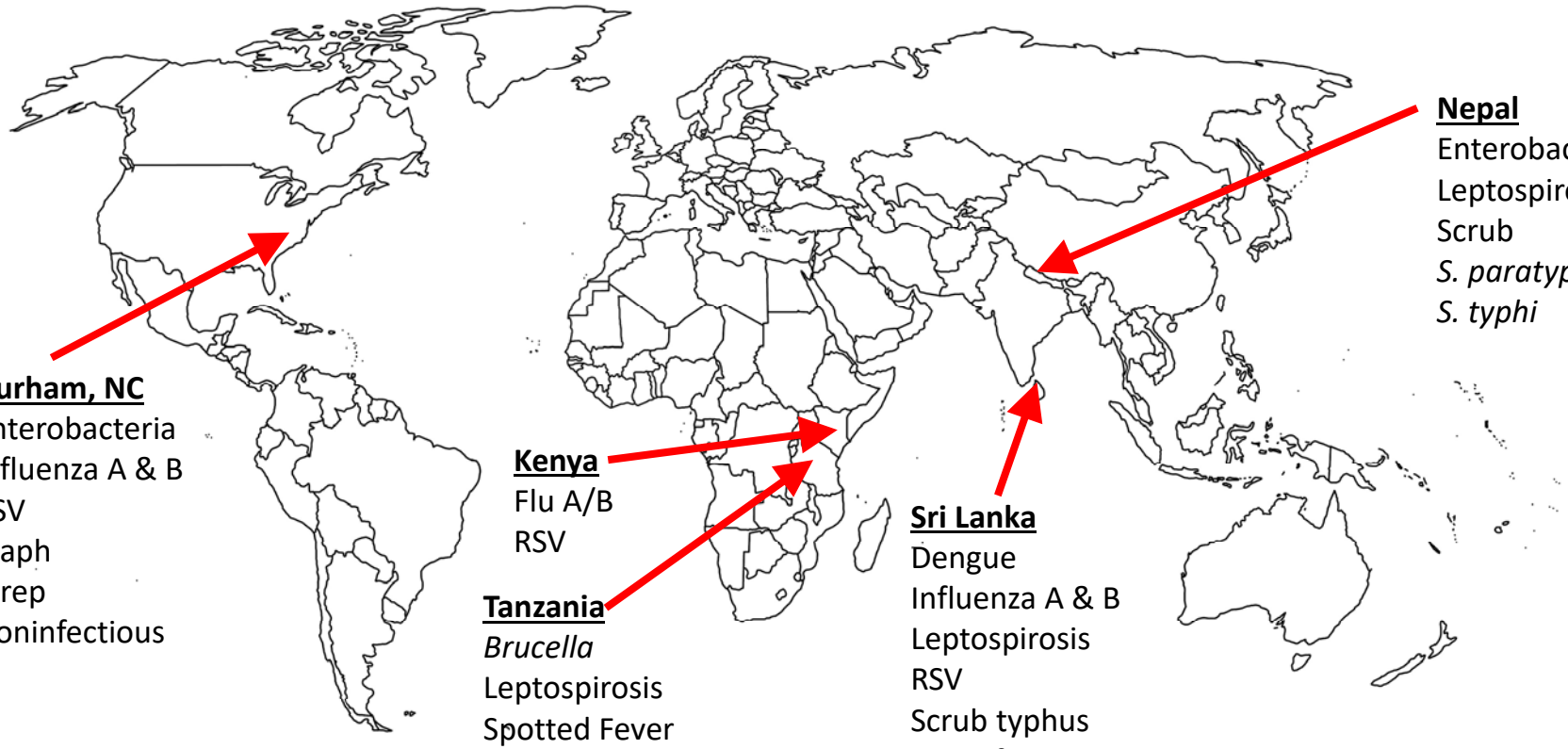
Global Febrile Illness



Durham, NC
Enterobacteria
Influenza A & B
RSV
Staph
Strep
Noninfectious

Kenya
Flu A/B
RSV

Tanzania
Brucella
Leptospirosis
Spotted Fever

Sri Lanka
Dengue
Influenza A & B
Leptospirosis
RSV
Scrub typhus
Noninfectious

Nepal
Enterobacteria
Leptospirosis
Scrub
S. paratyphi
S. typhi

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Global Fever mRNA classifiers: Discovery

Class: 338	Pathogen	Duke	Sri Lanka
Bacterial: 128	*Staphylococcus* spp.	11	
	Streptococcus spp.	13	
	Gram Negative Rods (*E.coli* & *K. pneumoniae*)	17	
	Leptospira spp.		24
	Coxiella burnetii		17
	Scrub Typhus		22
	Spotted Fever Group		
Viruses: 138	Respiratory viruses: Flu A/B RSV	 26 13	 44
	Dengue		40
NI-SIRS: 72	NI-SIRS	69	3

Global Fever mRNA classifier: Validation and Exploratory

Bacterial/Viral Co-Infection: Duke/Sri Lanka (7)
QC checks (possible mismatch): Duke/Sri Lanka (7)
Updated etiology: Duke/Sri Lanka (21)
Bacterial/Parasitic Co-Infection: Tanzania (2)

Confounded Sites:

	Tanzania	Nepal	Emory	Nicaragua
Bacterial: *E.coli*	5	3		
Salmonella	1	23		
Lepto	12	9		12
Coxiella	5			7
Brucella	13			
Scrub		3		
Rickettsial	8			5
Parasitic: Malaria	3			
Viral: Zika			4	
Dengue				15

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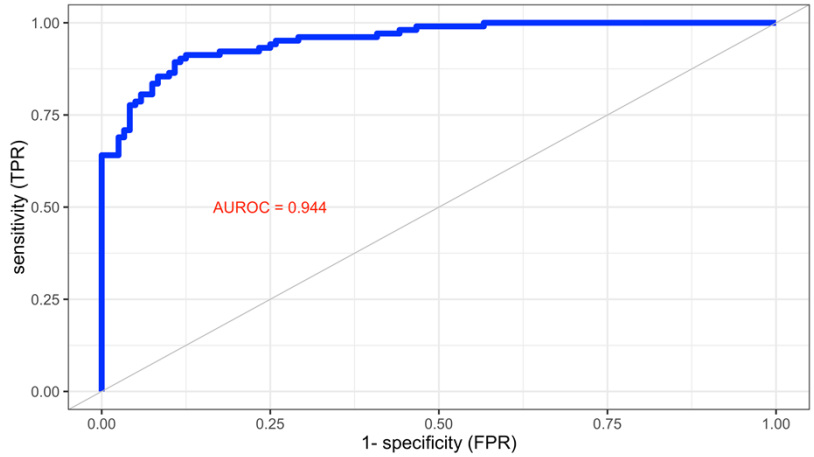
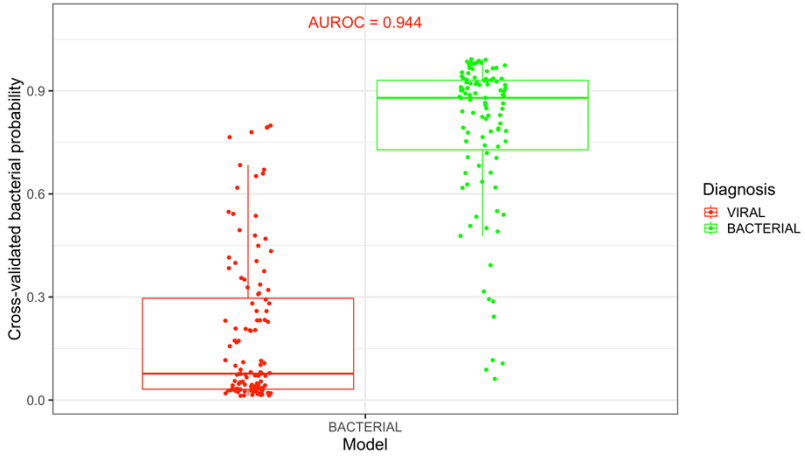
Novel Signature Bacterial/Viral Classifier: Performance in Global Fever Cohort





Performance characteristics for predicting bacterial infection (n=338):
Sensitivity: 0.90; Specificity: 0.88

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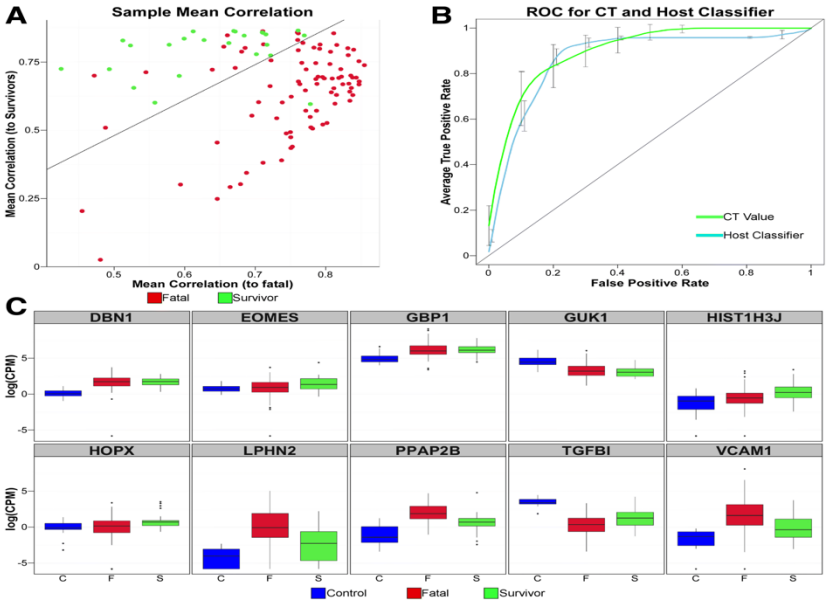
Transcriptomic Signatures Classify Ebola Deaths vs. Survivors



Liu et al. Transcriptomic Signatures Differentiate Survival from Fatal Outcomes in Humans Infected with Ebola Virus. Genome Biology August 2017

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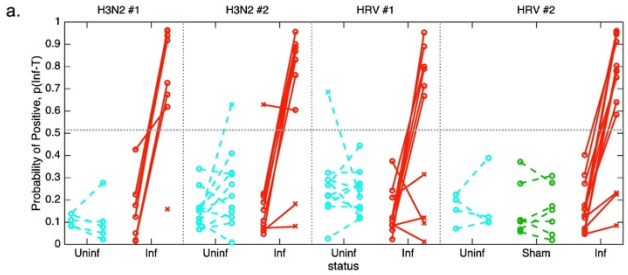
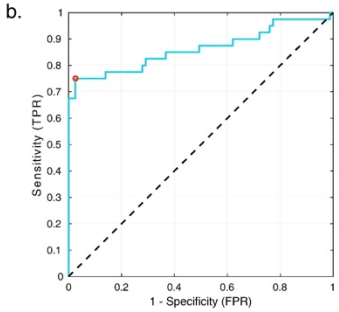
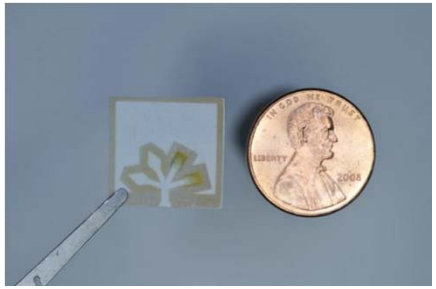
Nasal Lavage Proteomics:
Peptides Detect Viral Response in Human Challenge Model

Classifier Based on 8 Targeted Peptides
(leave one out cross-validation, p-value 2e-5)





Burke et al, EmBio 2017



http://gmwgroup.harvard.edu/research/index.php?page=24

Smart Tissues?

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Current and Future Directions

- Developing a model for the 'healthy state'
- Biomarkers for
  - Durable vaccine response
  - Hospital-Acquired Infections
  - Prosthetic joint infections
  - Rickettsial infections
  - Bacterial and viral gastroenteritis
  - Pathogens of global importance (e.g., Dengue, Malaria)
- Translate biomarkers into useable diagnostic platforms
- Utility in aged, and immunosuppressed populations
- Statistical methods for complex temporal data analyses

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Ongoing Programs

- NIH/NIAID/ARLG RADICAL
- DARPA Sigma Plus
- DARPA Prometheus
- DARPA Biochronicity
- DTRA Global Fever
- VA Tick Borne Illness
- VA/NIA Resilience in the Elderly
- NIH/VTEU Valley Fever Program
- NIH Candidemia
- Biomarkers in Cystic Fibrosis
- DARPA ECHO

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