Host Gene Expression for Diagnosis of Infectious Diseases

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Outline

- Background
- Peripheral Blood Gene Expression for Diagnosis of Acute Respiratory Viral Illness
  - Hypotheses
  - Study Design
  - Statistical Analysis
  - Results
  - Conclusions
- *Peripheral Blood Gene Expression for Diagnosis of Candidemia*
  - *Study Design*
  - *Results*
  - *Future Directions*
- Conclusions

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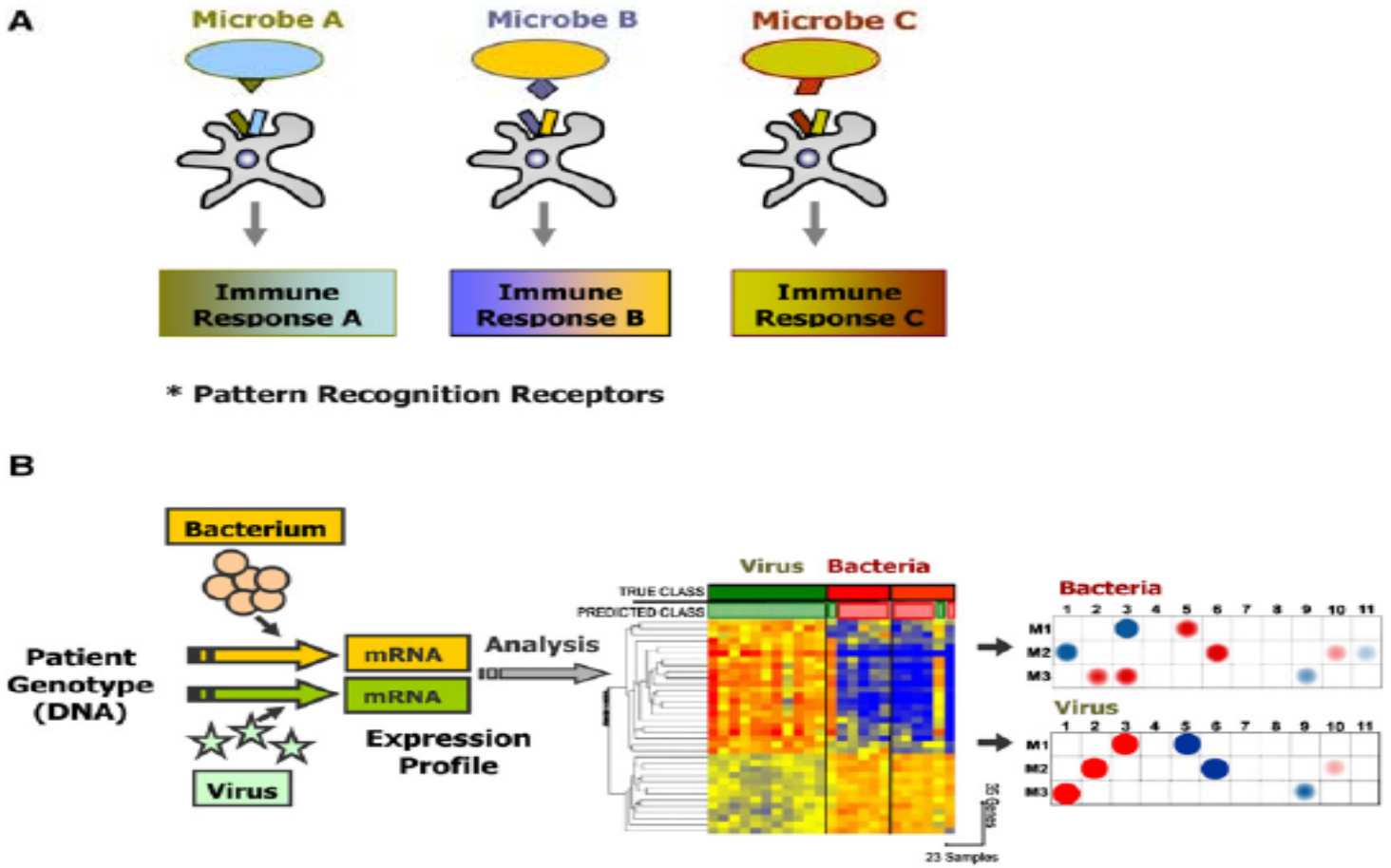
Gene Expression Can Discriminate Between Pathogens



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Can We Classify Acute Respiratory Viral Illness?

- Hypotheses:

  Peripheral blood gene expression at time of peak symptoms in experimentally infected cohorts can differentiate between symptomatic and asymptomatic subjects

  The above derived peripheral blood gene expression signatures can accurately classify other subjects with viral respiratory infection and differentiate viral from bacterial infection

- Methodology:

  Serial sampling and symptom scores of experimentally infected individuals

  Unsupervised analysis of peripheral blood gene expression data at time of peak symptoms

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Human Viral Challenge Sites: HRV, RSV, Influenza A

HRV Challenge: Charlottesville, VA 11/2007

Influenza Challenge (Cambridge, UK 10/2008)

RSV Challenge (Brentwood, UK 7/2008)

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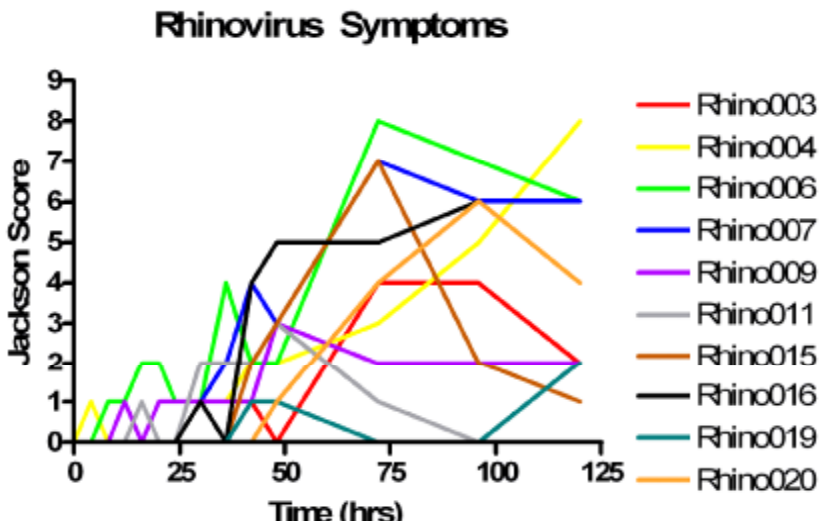
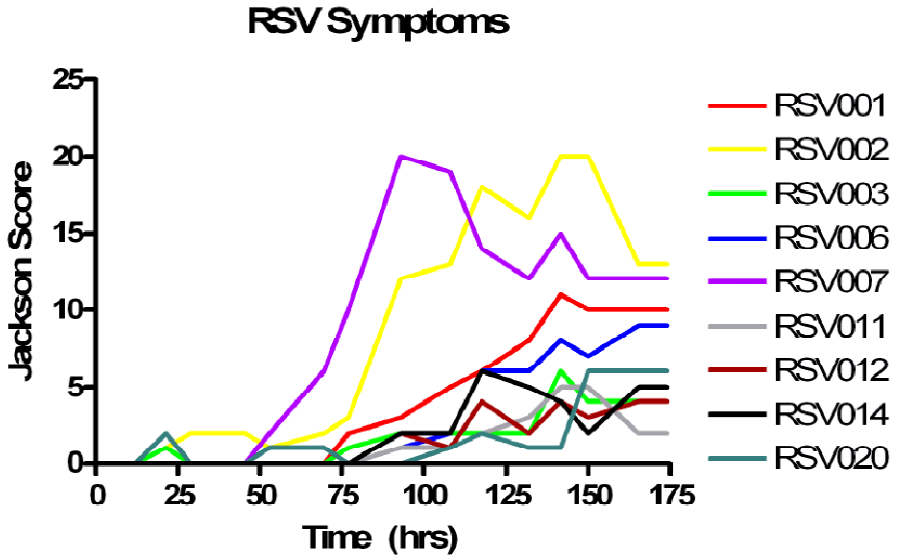
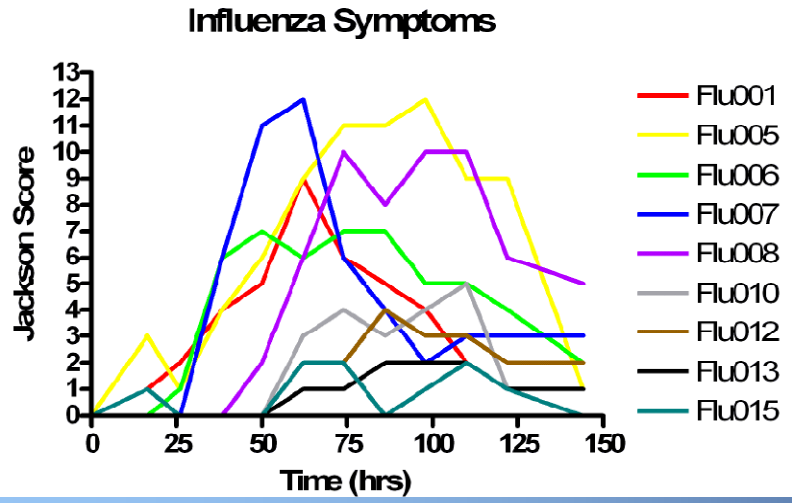
Human Viral Challenges: Symptom Scores

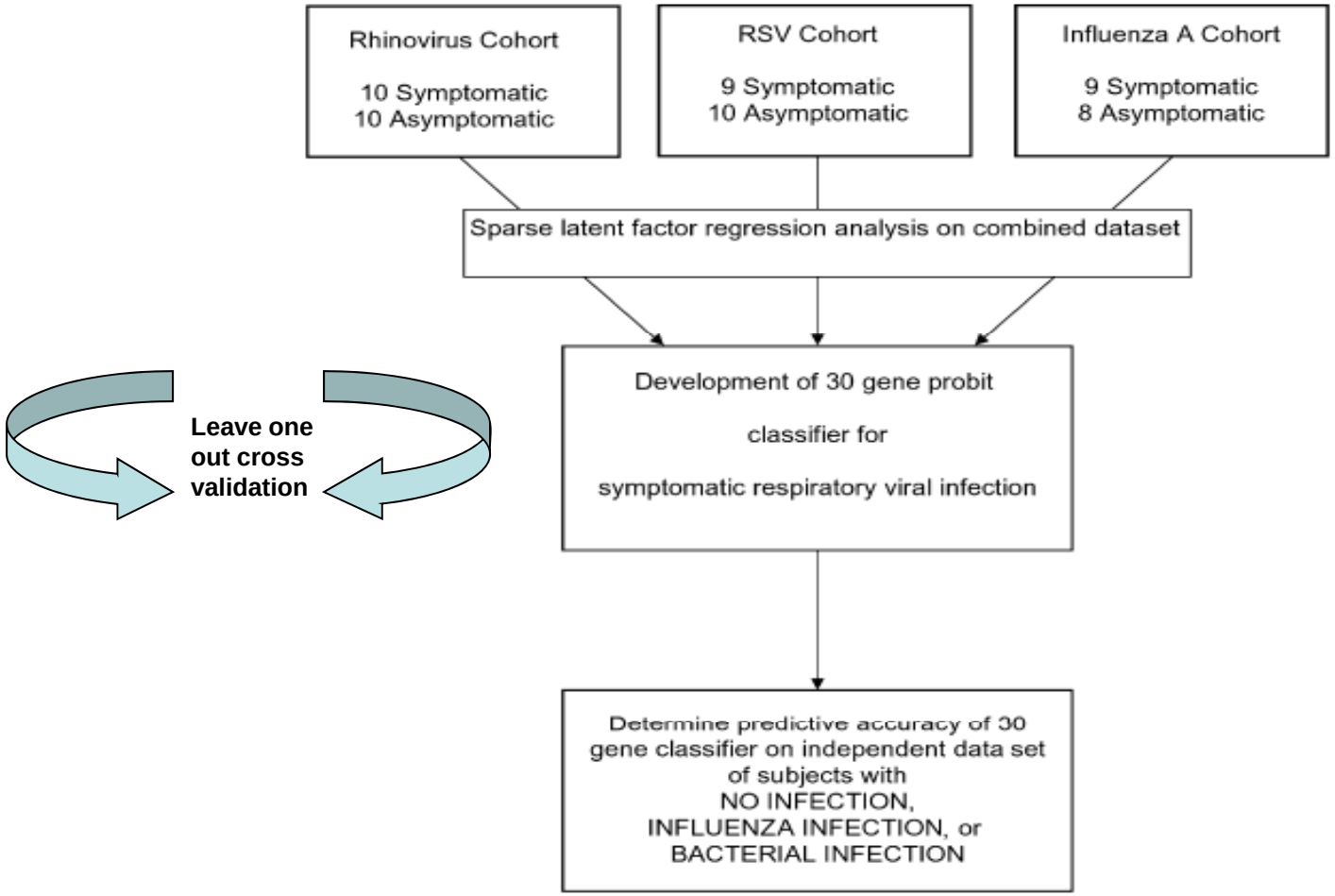






Cohort	Number Exposed	Number Symptomatic	Median Time "T": Time to Peak Symptoms
Rhinovirus	20	10	72 hours
RSV	20	8	141.5 hours
Influenza	17	9	80 hours

Study Design



Zaas AK, *et al*. Cell Host Microbe 2009.

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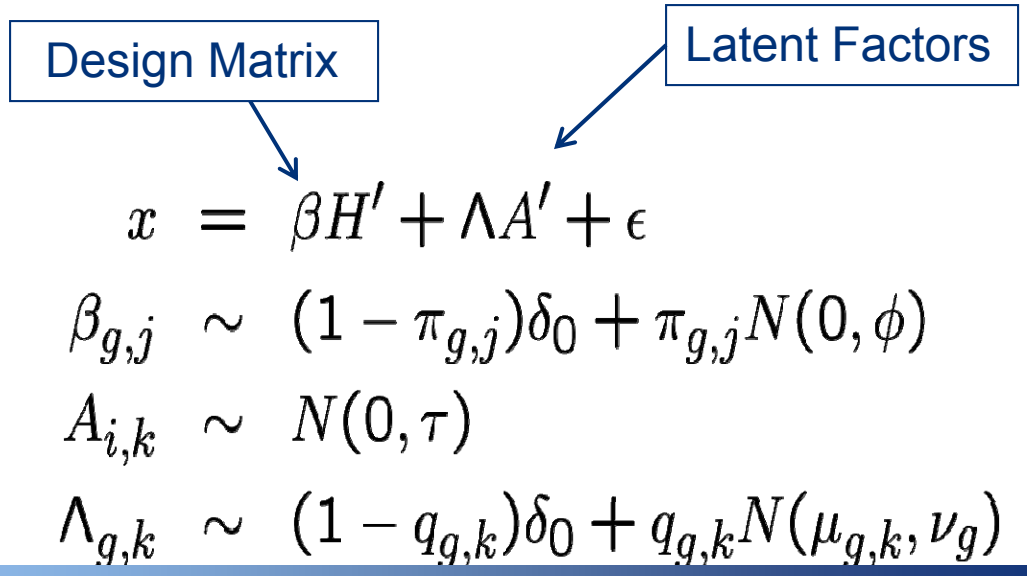
Sparse Latent Factor Regression Analysis

- Latent Factor = Co-expressing genes = Signature
- Assumes MOST genes on array do not have differential expression between varied conditions (“sparseness”)
- “Unsupervised”: does not use class information to derive factors
- Signature can be used to classify new samples as they become available



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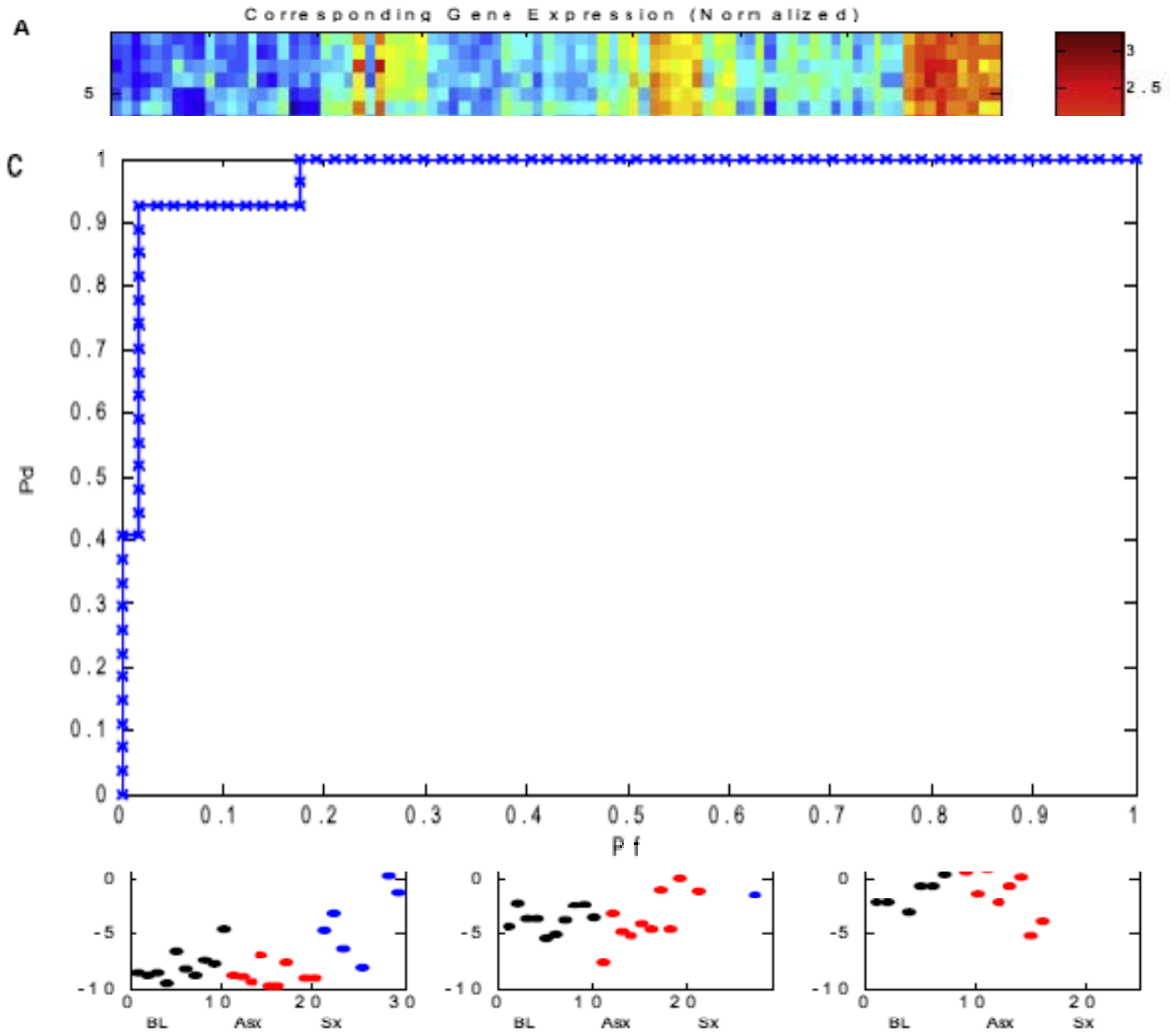

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An "Acute Respiratory Viral" Signature Dominates at Time T

Table 2. Intra-Data Set Probit Classification Cross-Validation Results

	Test: HRV	Test: RSV	Test: Influenza
Train: HRV	1/30 (*RSAD2*)	2/29 (*RTP4*)	0/25 (*ISG15*)
Train: RSV	1/30 (*RSAD2*)	2/29 (*RTP4*)	0/25 (*ISG15*)
Train: Influenza	1/30 (*RSAD2*)	2/29 (*RTP4*)	0/25 (*ISG15*)

The error rate is shown based on the top gene (noted in parentheses) selected from the training set probit classifier. For this model, the top 40 genes from the training set discriminative factor were used to build the probit classifier for testing in the validation data set.

Zaas AK, *et al*. Cell Host Microbe 2009.

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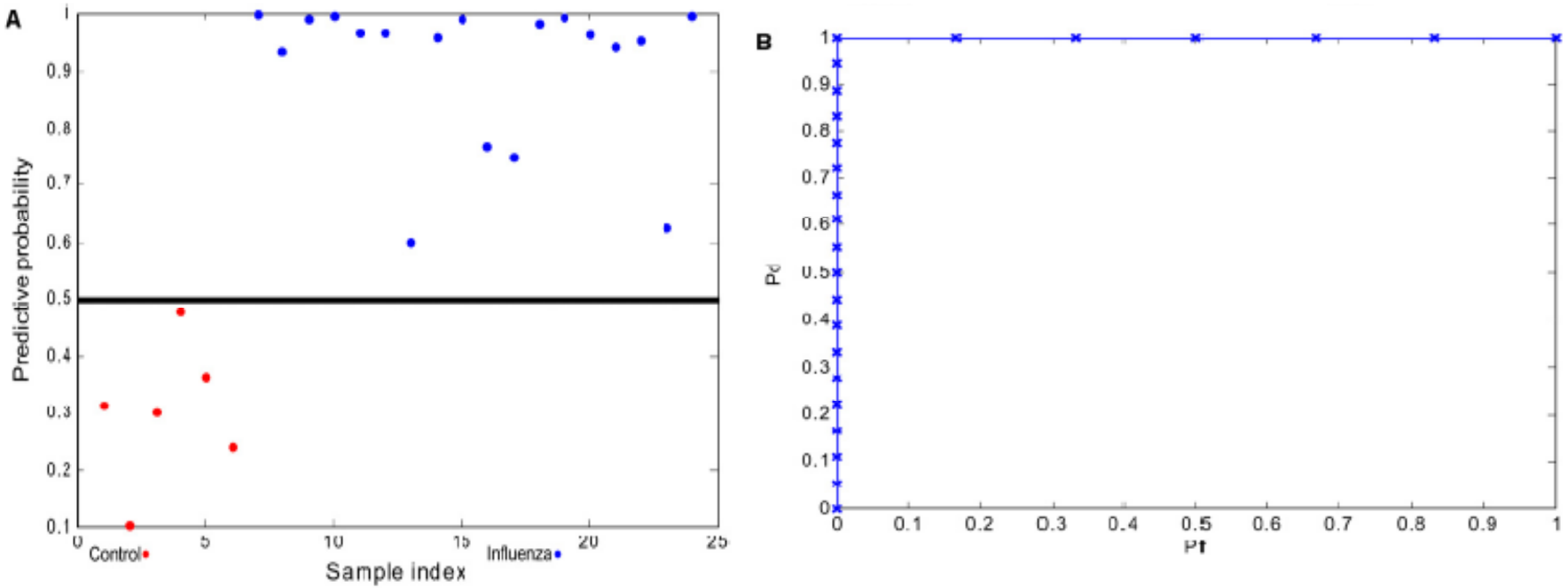
The Acute Respiratory Viral Signature Validates in a Historical Cohort



Perfect classification of pediatric subjects with Influenza A (blue) versus hospitalized controls (red)

Zaas AK, *et al*. Cell Host Microbe 2009.

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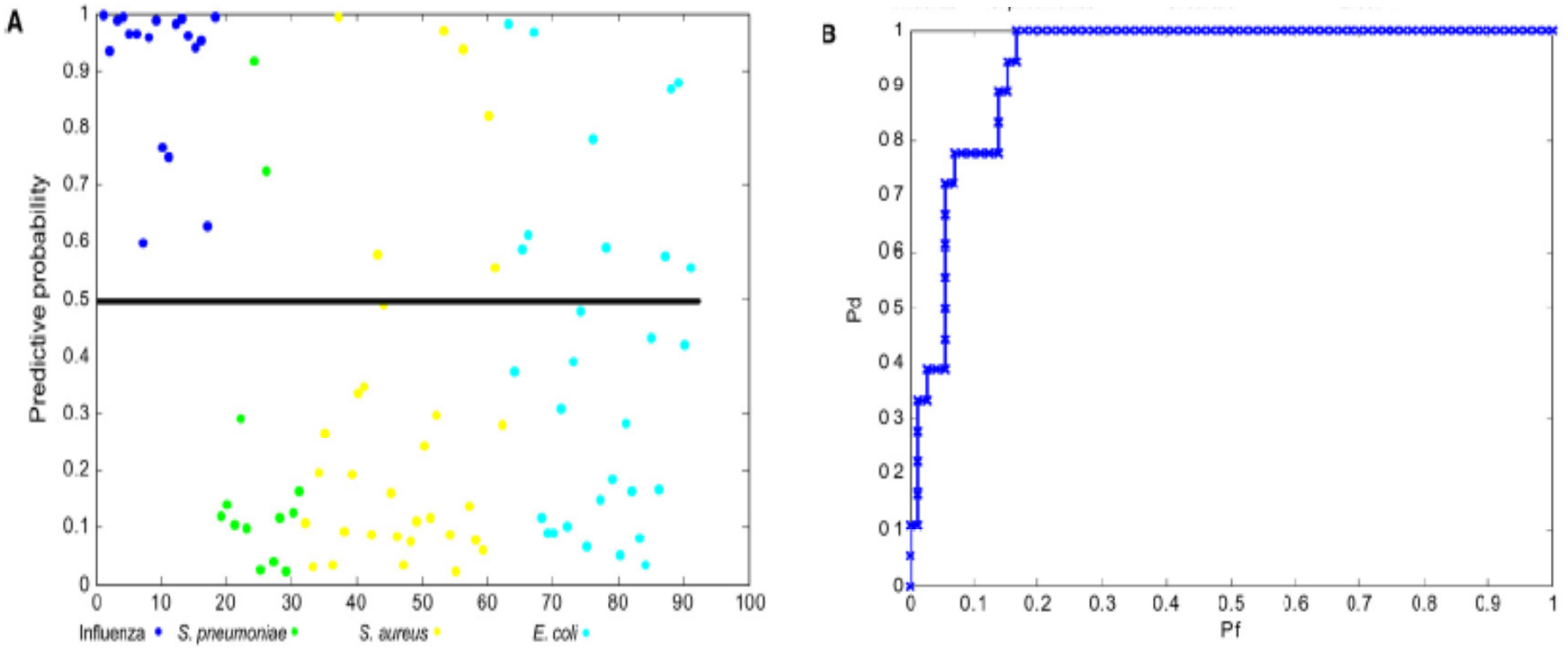
The Acute Respiratory Viral Signature Validates in a Historical Cohort



73/91 [80%] subjects accurately classified for Influenza A versus Bacterial Infection (*S. pneumonia, S. aureus* or *E. coli*)

Zaas AK, *et al*. Cell Host Microbe 2009.

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Conclusions: Classification is Highly Accurate at Maximal Symptoms

- Sparse latent regression analysis identifies a gene expression signature that accurately classifies experimentally infected individuals with symptomatic viral respiratory infection at time of maximal symptoms
- Genes contained in this signature have direct relationship to known viral response pathways
- At time of maximal symptoms, a “pan-viral” signature is dominant
- This methodology, and other methodologies, can be used to develop classifiers that function at earlier timepoints

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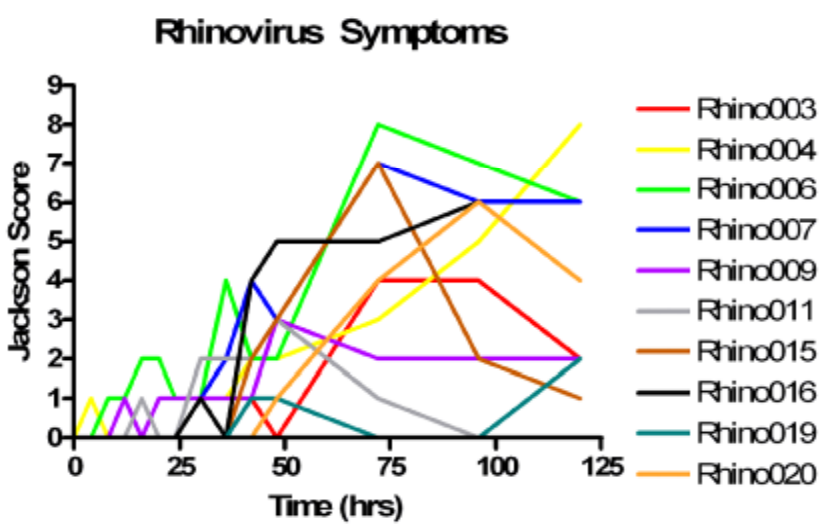
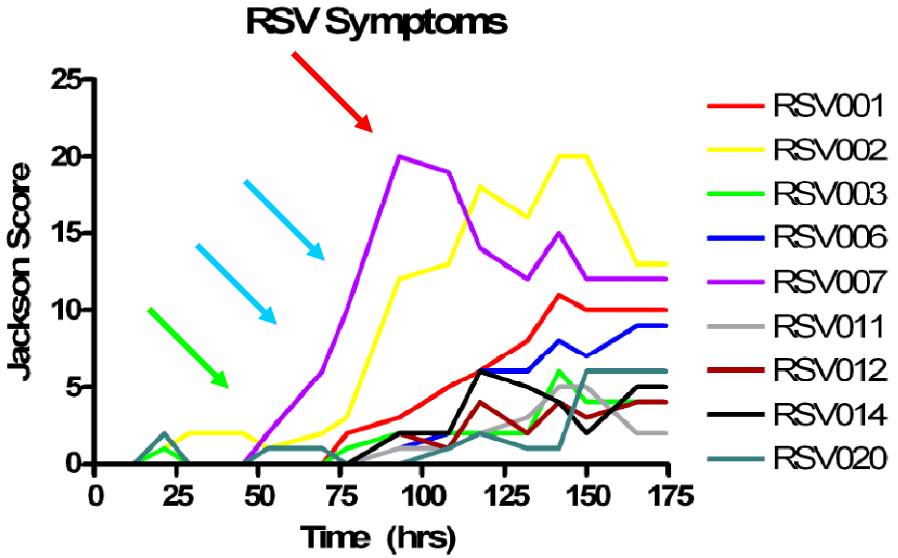
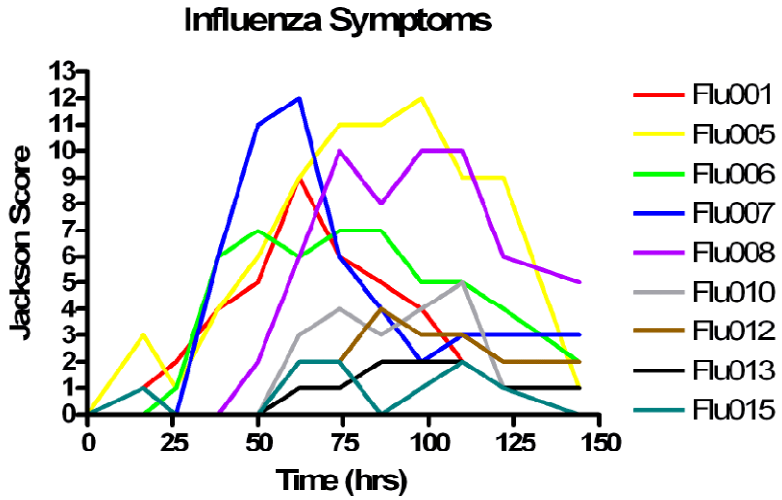
What About Earlier Than Maximal Symptoms?







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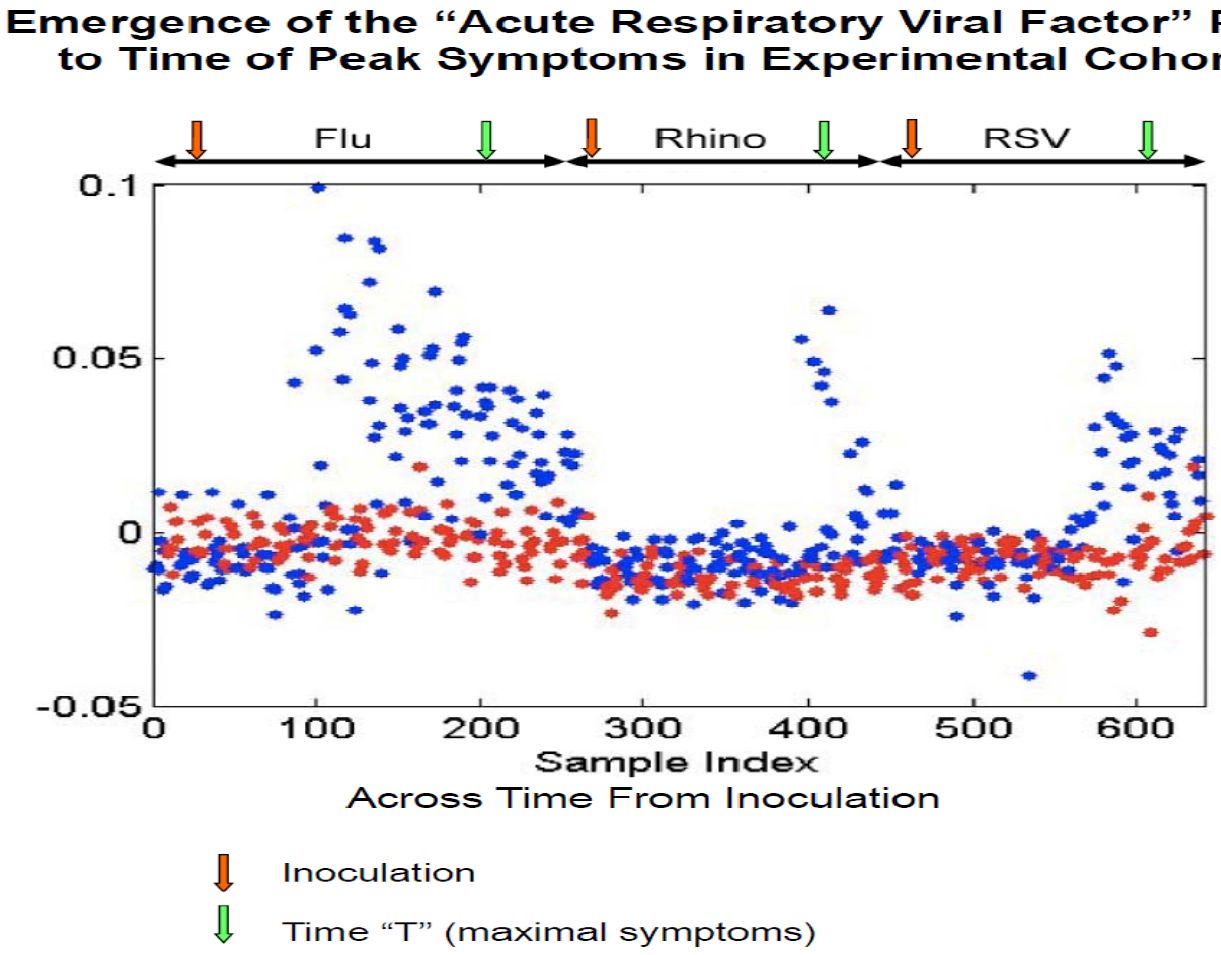
Early Emergence of the "Acute Respiratory Viral" Factor



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Can We Move Detection Earlier?

- 1) Improve on the sensitivity of detection
  - RT-PCR
    - Dynamic range of gene expression greater than Affymetrix array
    - Potential to build classifier on basis of degree of gene expression
    - Potential to reduce the number of genes in the classifier
- 2) Use additional statistical methods to achieve earlier classification
    - Bayesian Elastic Net
- 3) Use combination of clinical (i.e. symptoms/physical signs) and molecular (i.e. gene expression) data to achieve earlier classification

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From Healthy Adults to Immunocompromised?

- Paradigm increases in complexity as host increases in complexity
- Perhaps can extrapolate healthy young adults to healthy kids
- Important parameters to consider
  - Effect of immunosuppressive regimens on “baseline” gene expression
  - Difficulties with specimen procurement in neutropenia/leukopenia (adequate cells for RNA extraction)
  - Co-infection, effect of herpesvirus reactivation

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The DARPA Team

Infectious disease

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Bayesian machine learning

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bioinformatics

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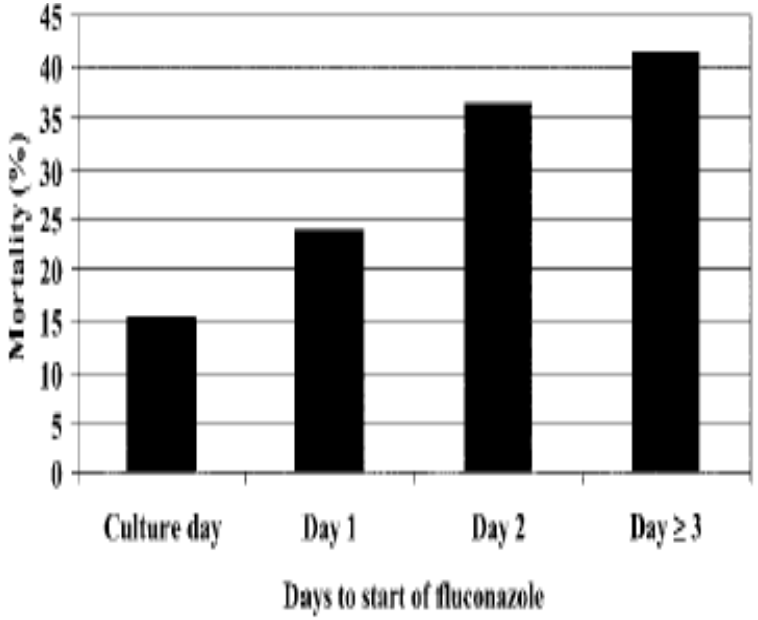
Invasive Candidiasis – An Important Medical Problem

Candidemia is common and life-threatening

- 4th most common nosocomial BSI[1]
- Excess mortality rates: 10% – 49%
- Average total cost of candidemia: $44,536[2]

Current diagnostic paradigms inadequate

- Variable and nonspecific presentation
- Gold standard for diagnosis: blood culture
  - Sensitivity approximately 50%
- Delay in appropriate therapy increases mortality[3]



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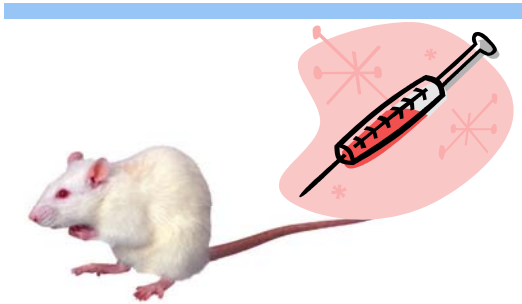
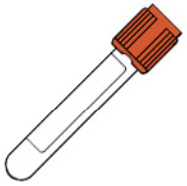
Hypothesis

Predictive models based on global changes in gene expression of peripheral blood immune cells can distinguish between infectious causes of illness, particularly candidemia vs. bacteremia.

Study Design

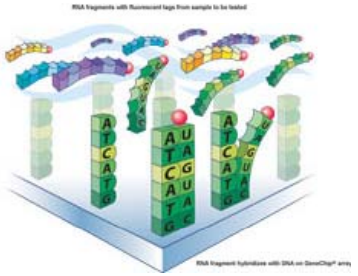


C. albicans:
discovery (n = 28)
validation (n=12)
PBS (n=12;5)
S. aureus (n = 12)

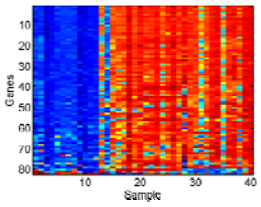


Collections at
24, 48, 72, and
96 hrs

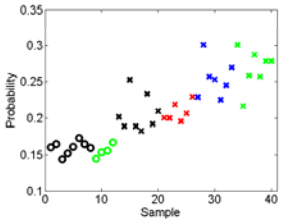
RNA Extraction &
Globin Reduction



Is there a Signature?



Validation



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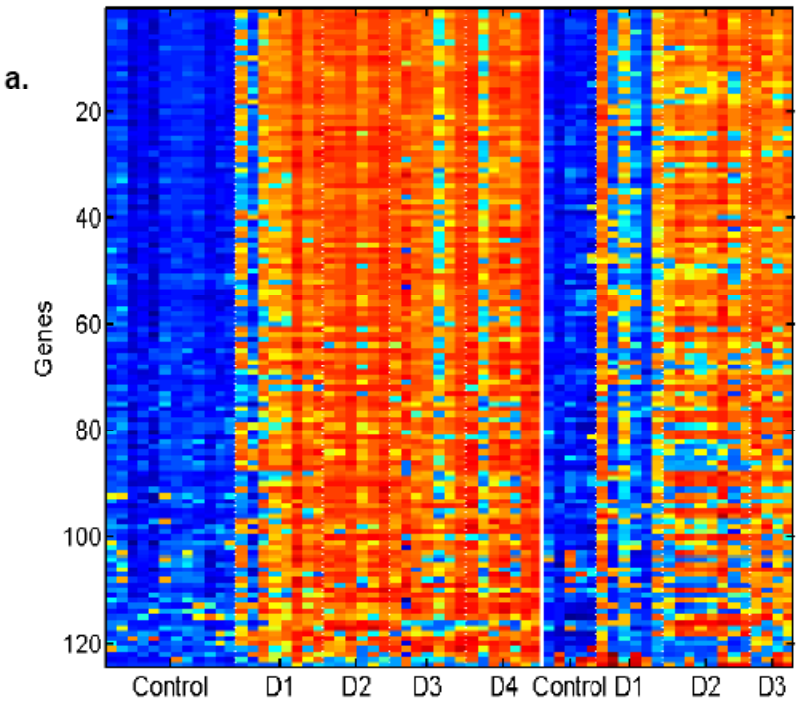
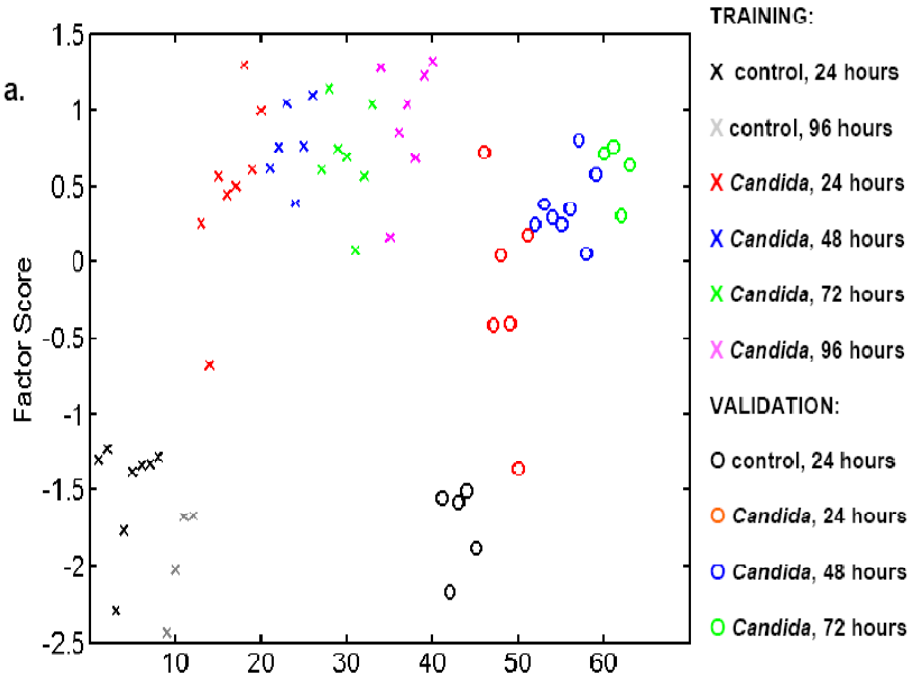
A Disease-Defining Factor





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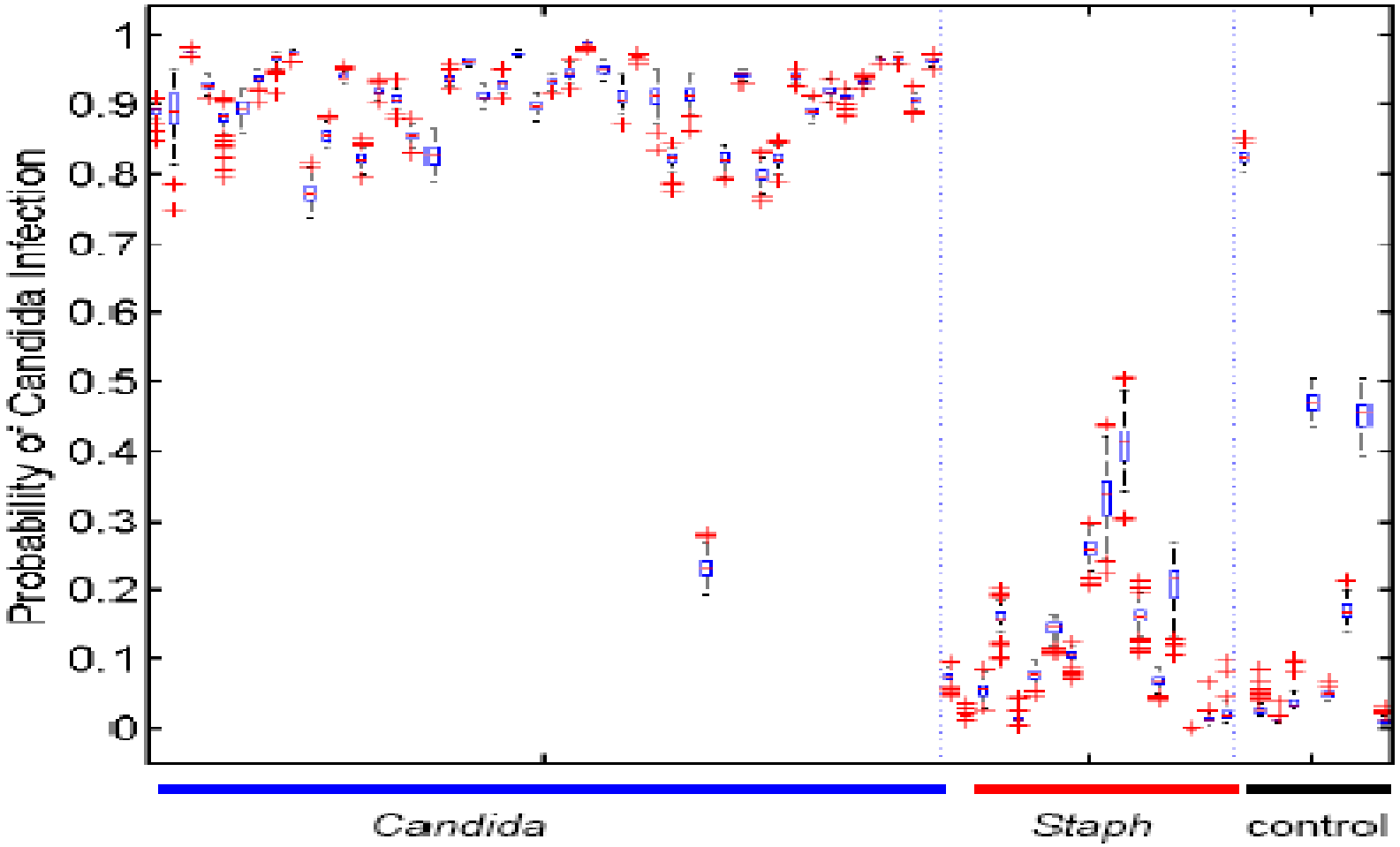
Gene Expression Can Distinguish Between Candidemia and *S. aureus* Bacteremia



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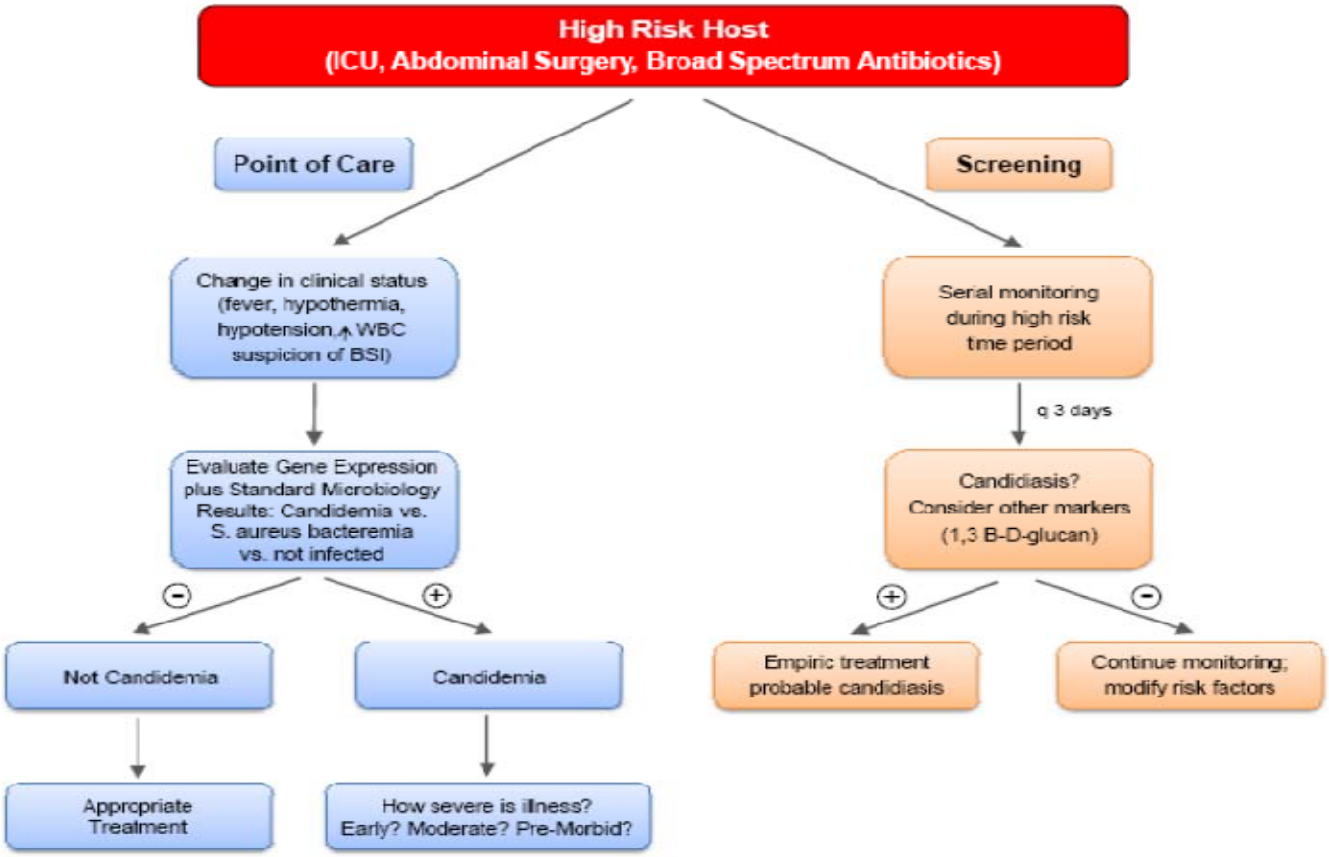
Proposed Clinical Application



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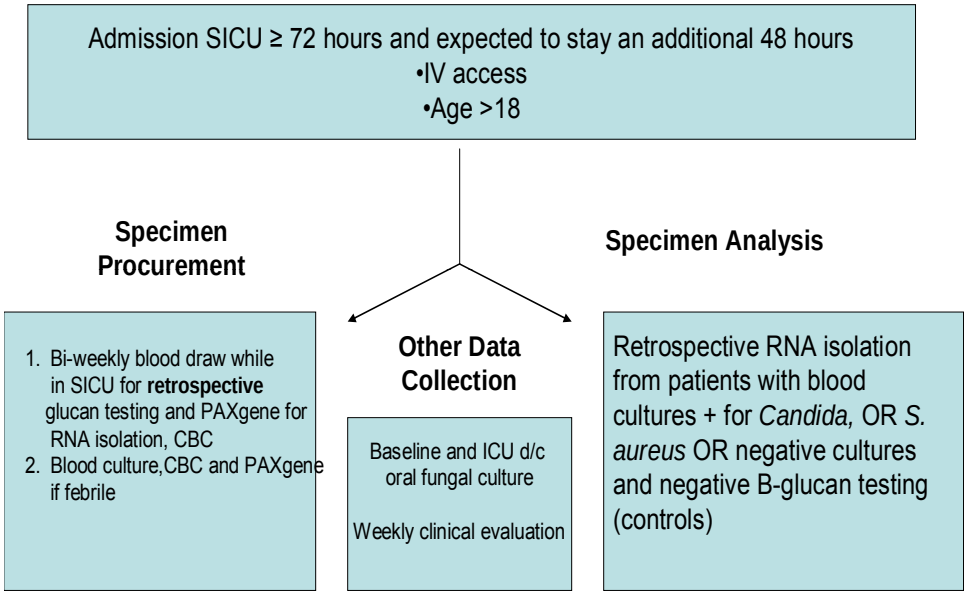
Candida: Conclusions

- Distinct gene expression signatures can identify murine candidemia
- Gene expression signatures change with disease severity
- Genes contained in signature ("factor") are involved in host-pathogen response
- Validation vs. bacteremia AND in human cohort needed



CLINICAL DATA:
Age, gender, underlying illness, medications, Immune suppression, surgery, central line, TPN, Microbiologic culture data

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Conclusions

- Diagnosis of infectious diseases can be enhanced by "breaking tradition" from pathogen-based diagnostics
- Combining host and pathogen findings may provide optimal means of classifying infected individuals
- Future directions: Prediction of therapeutic successes or failures

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The *Candida* Team

- Hamza Aziz, MSIII
- Joseph Lucas, PhD
- John Perfect, MD
- Holly Dressman, PhD
- Geoff Ginsburg, MD, PhD

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