Multiplexed Diagnostics:

Valley Fever assessment using Immunosignatures

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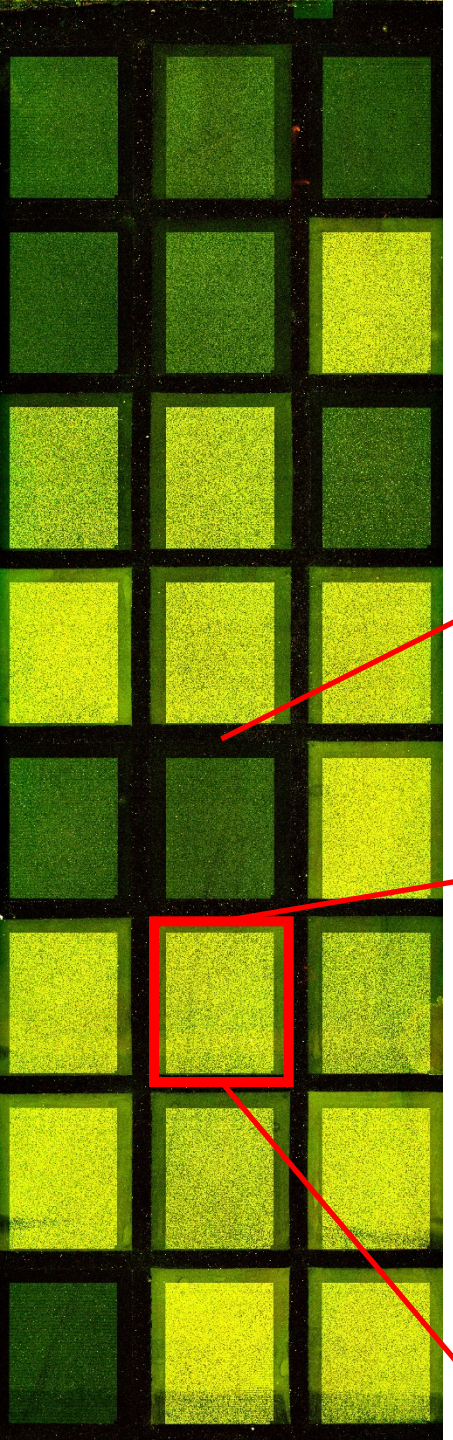
Multiplexed Diagnostics: Specificity, Sensitivity, Modularity, Performance

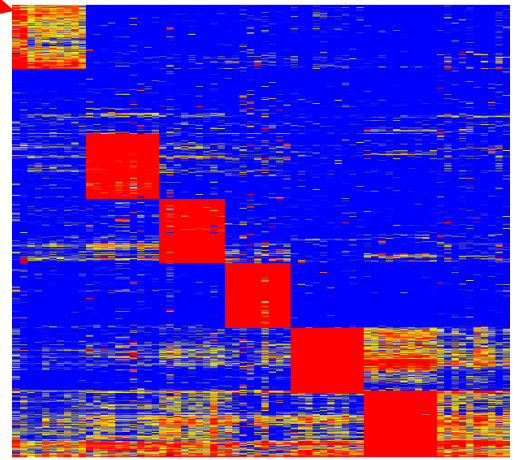
- What is multiplexing?
- Why do we need multiple tests?
- Pros and cons?
- Commercial applications?
- How does Valley Fever make the case for multiplexing?

Introduction to Immunosignatures

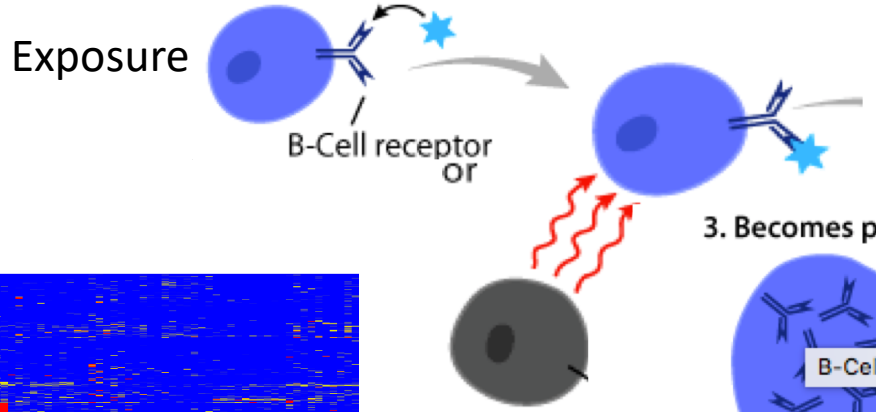
- Immunosignatures are a serological test for the presence of antibodies to a pathogen
- Exposure can be a recent as 3 days (acute) or convalescent
- Immunosignatures can resolve multiple disease using the same platform (multiplexing)
- Immunosignatures use many biomarkers rather than narrowing down to a few

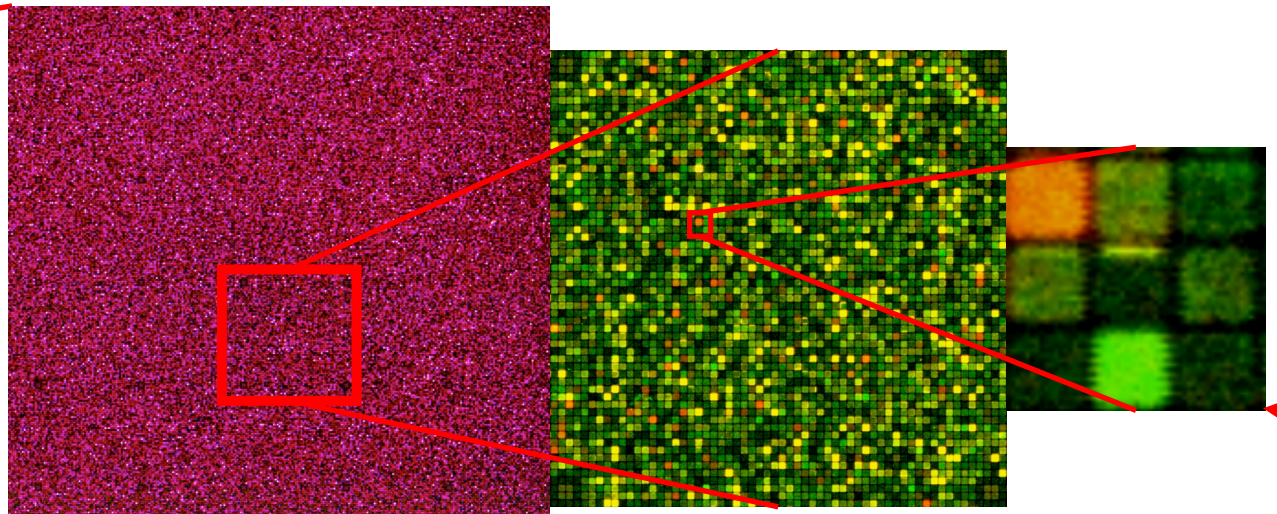


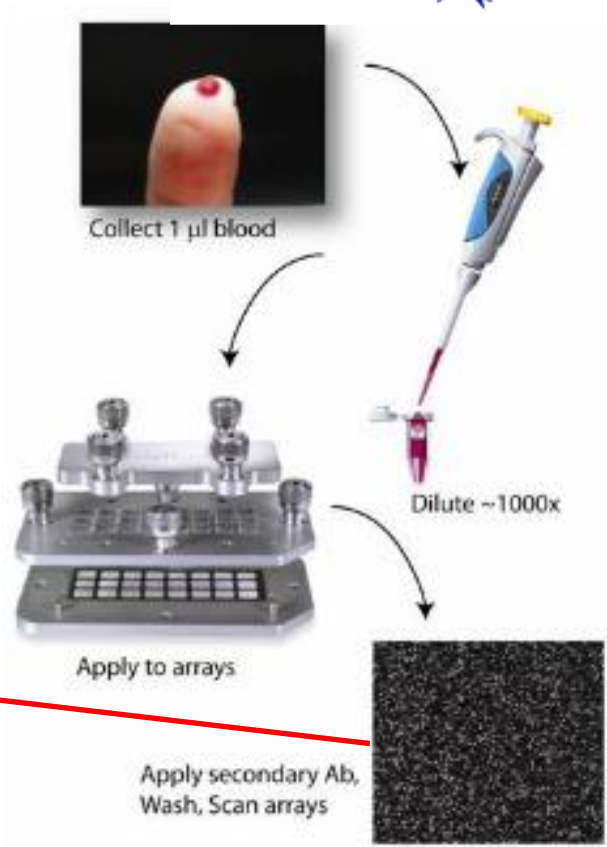

Biomek FX^P Laboratory Automation Workstation



Diagnosis


Exposure
B-Cell receptor
or
3. Becomes plasma cell
B-Cell maturation
4. Releases
antibodies

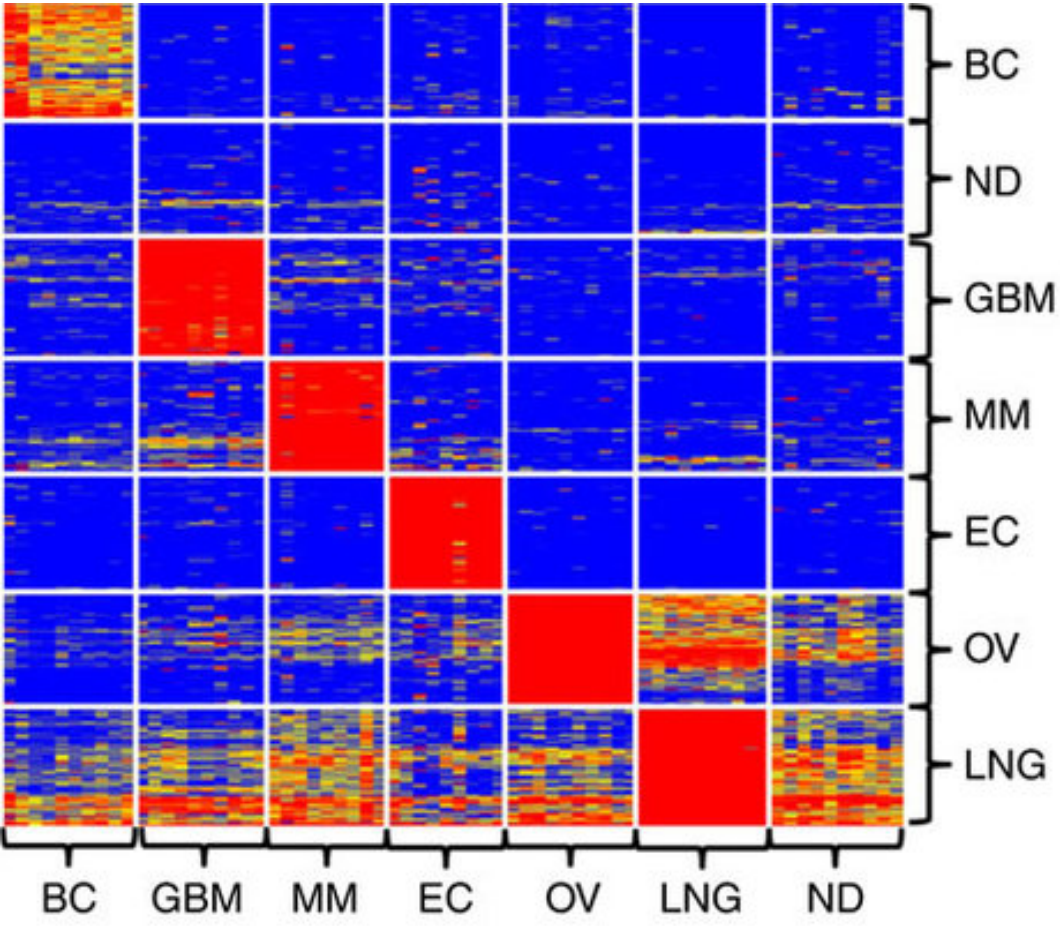



Collect 1 µl blood
Dilute ~1000x
Apply to arrays
Apply secondary Ab,
Wash, Scan arrays

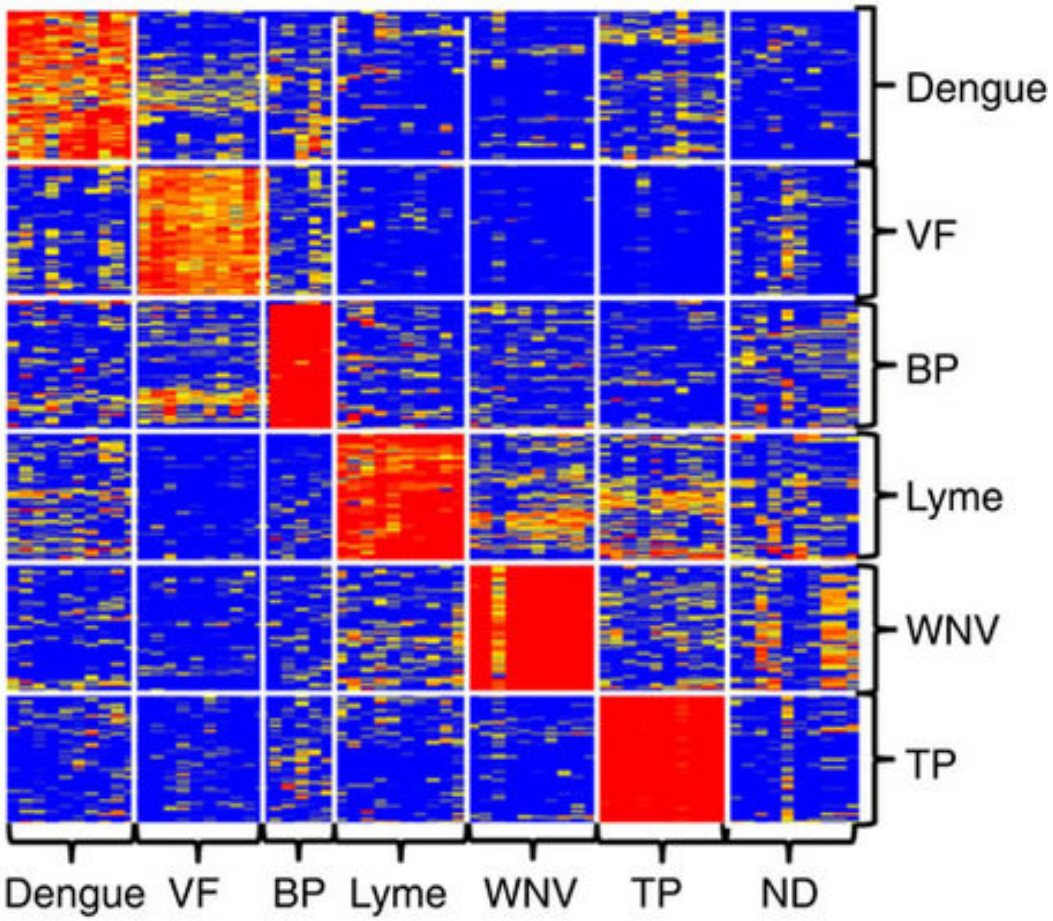
Introduction to immunosignatures

- With 125,000 random peptide markers, we can identify disease-specific patterns of reactivity
- The pattern for disease 1 is likely different from disease 2

The Case for multiplexing



Leave-10%-out 100-fold cross-validation
100% specificity
100% sensitivity
100% accuracy



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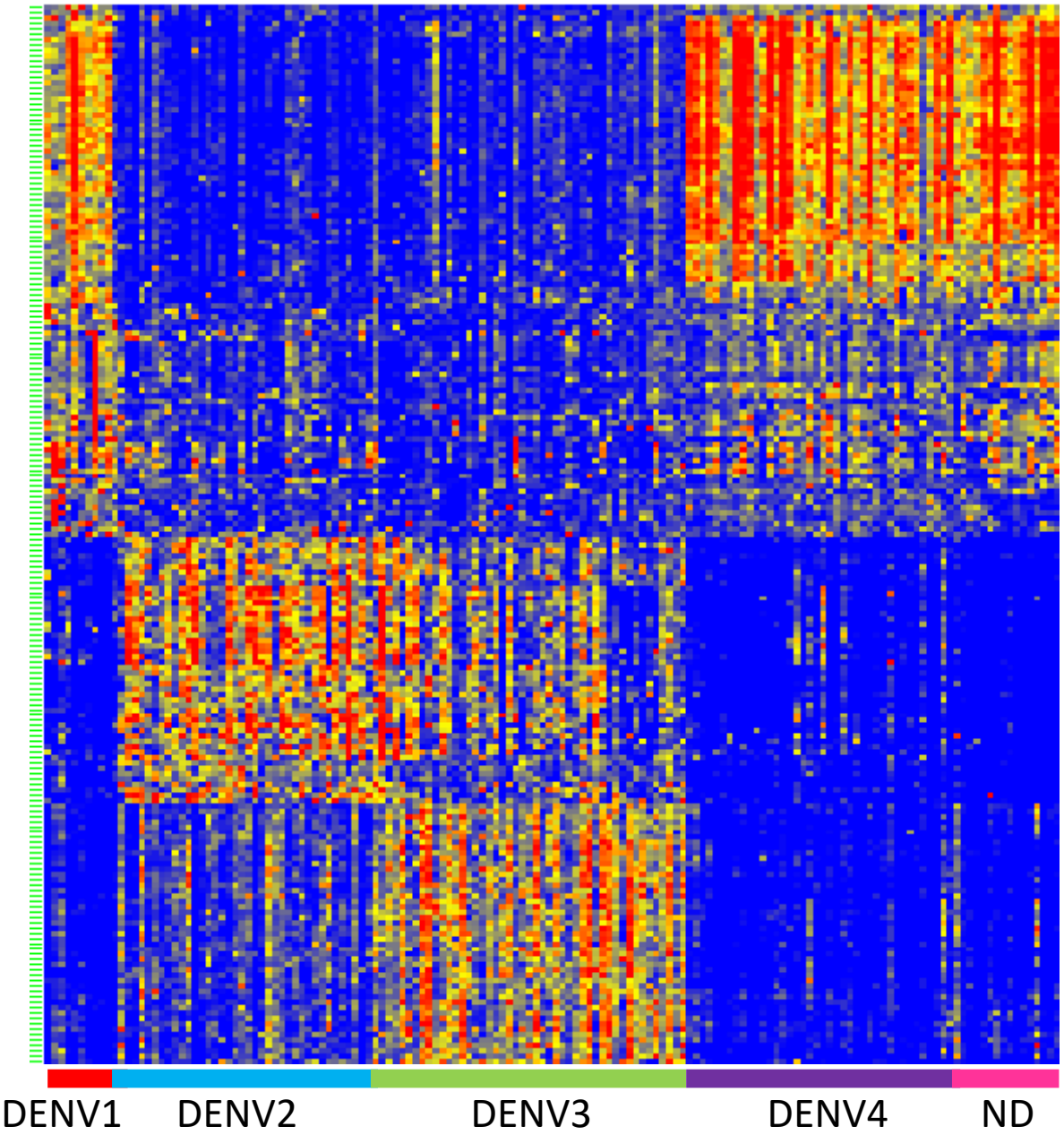
The Case for multiplexing

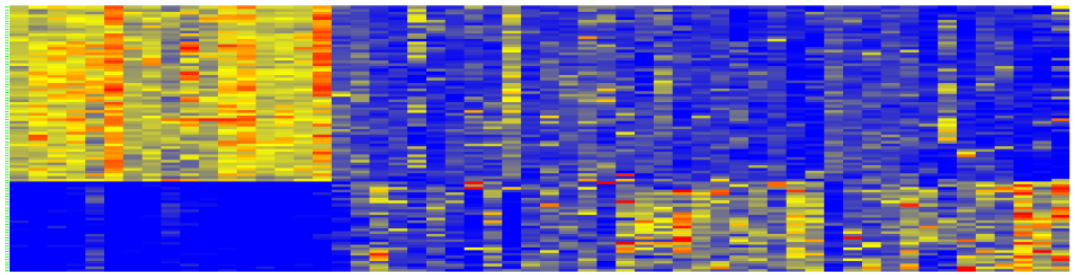


Dengue serotype test

Crossvalidation results (convalescent patients):
Leave 10% out, 100-fold crossvalidation
83.8% overall accuracy

Dengue vs. Zika

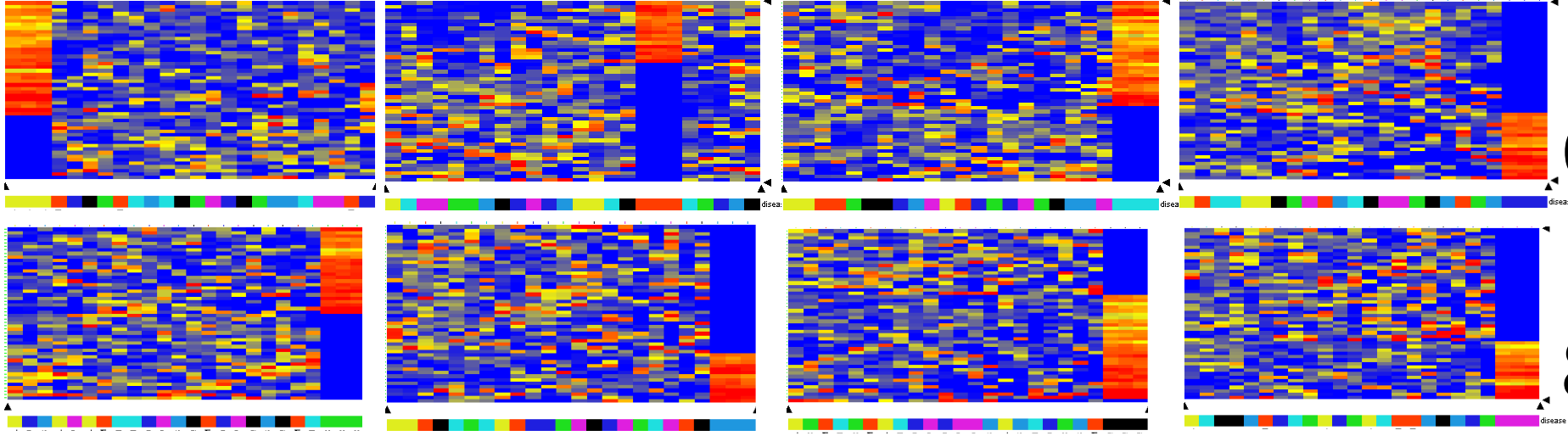
Crossvalidation results (convalescent patients):
Leave 10% out, 100-fold crossvalidation
83.8% overall accuracy



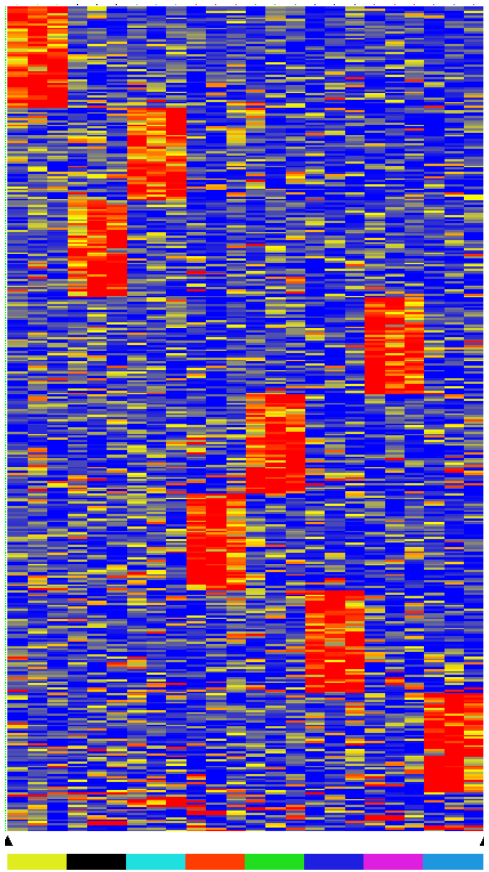
DENV positive ZIKV positive

Multiplexed Diagnostics: Specificity, Sensitivity, Modularity, Performance

- **Modularity**
  - Multiplexing advantages include modularity – adding a new disease to an existing platform
  - A high-sales diagnostic could detect common diseases (CAP, viral vs. bacterial infections, nosocomial infections) – this would promote sales
  - The same diagnostic could be trained to detect rarer diseases like Valley Fever
  - Same platform can be used for:
    - Autoimmune and rare diseases
    - Pediatric diagnostic
    - Infectious disease diagnostic
    - Cancer and chronic disease diagnostic
  - Once added, an over-the-air software update will upgrade existing diagnostic



One vs. All
100% sensitivity
88% specificity



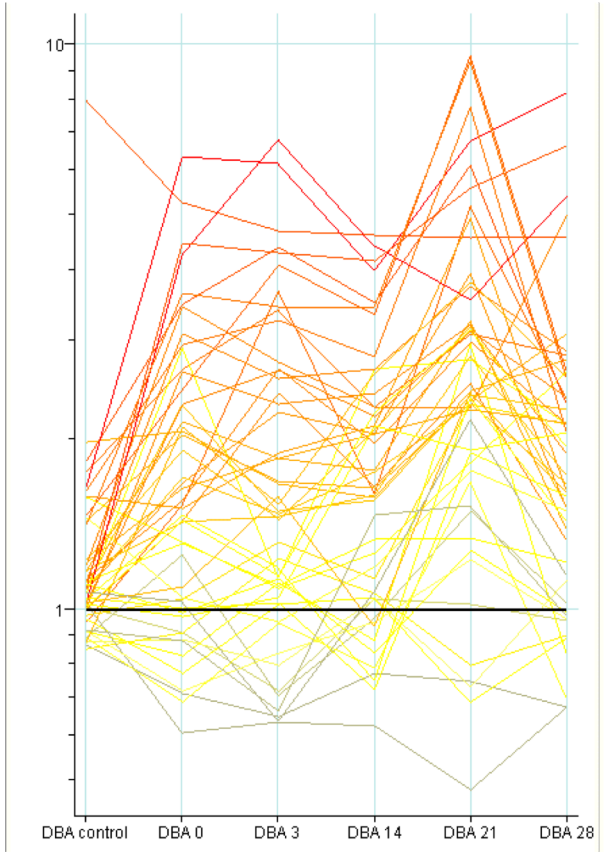
Multiclass
100% sensitivity
100% specificity

The case for Valley Fever Diagnosis

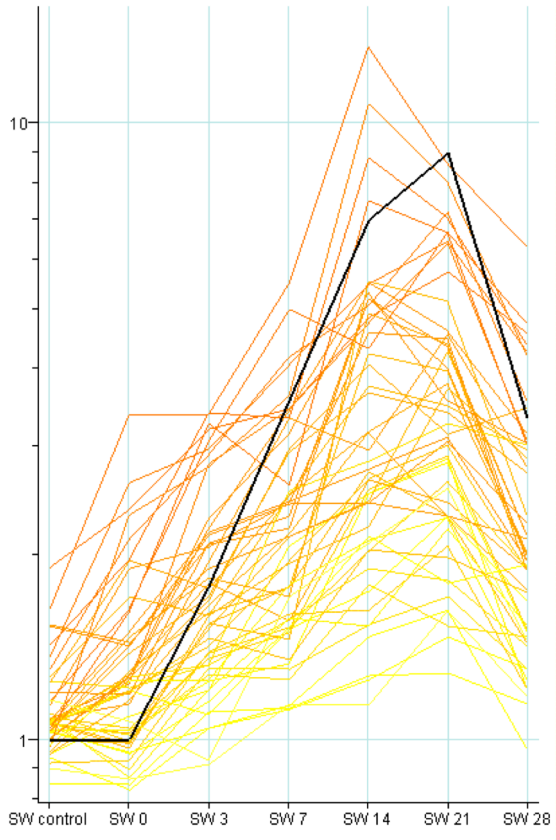
Valley Fever has different characteristics when infecting

In three different mouse strains, the immunosignature is the same but timing varies

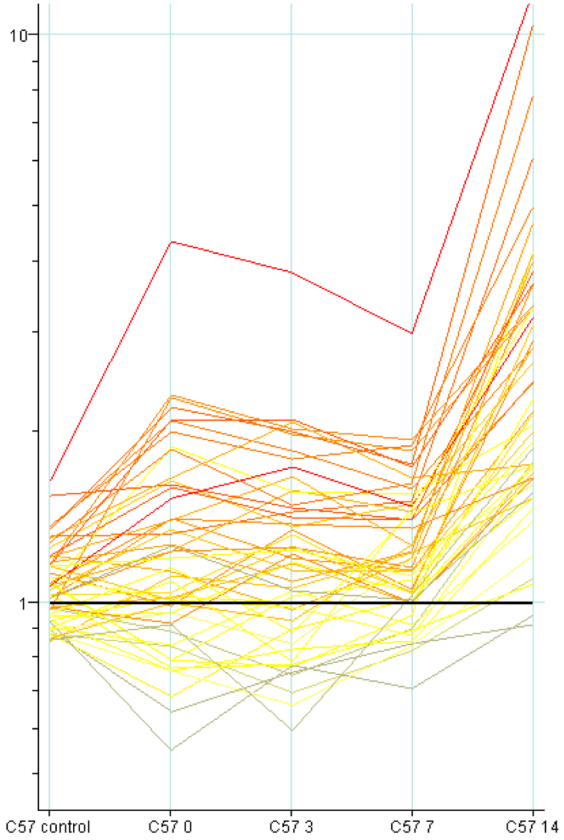
DBA/2N – resistant
Lethal dose = 10^5 arthroconidia



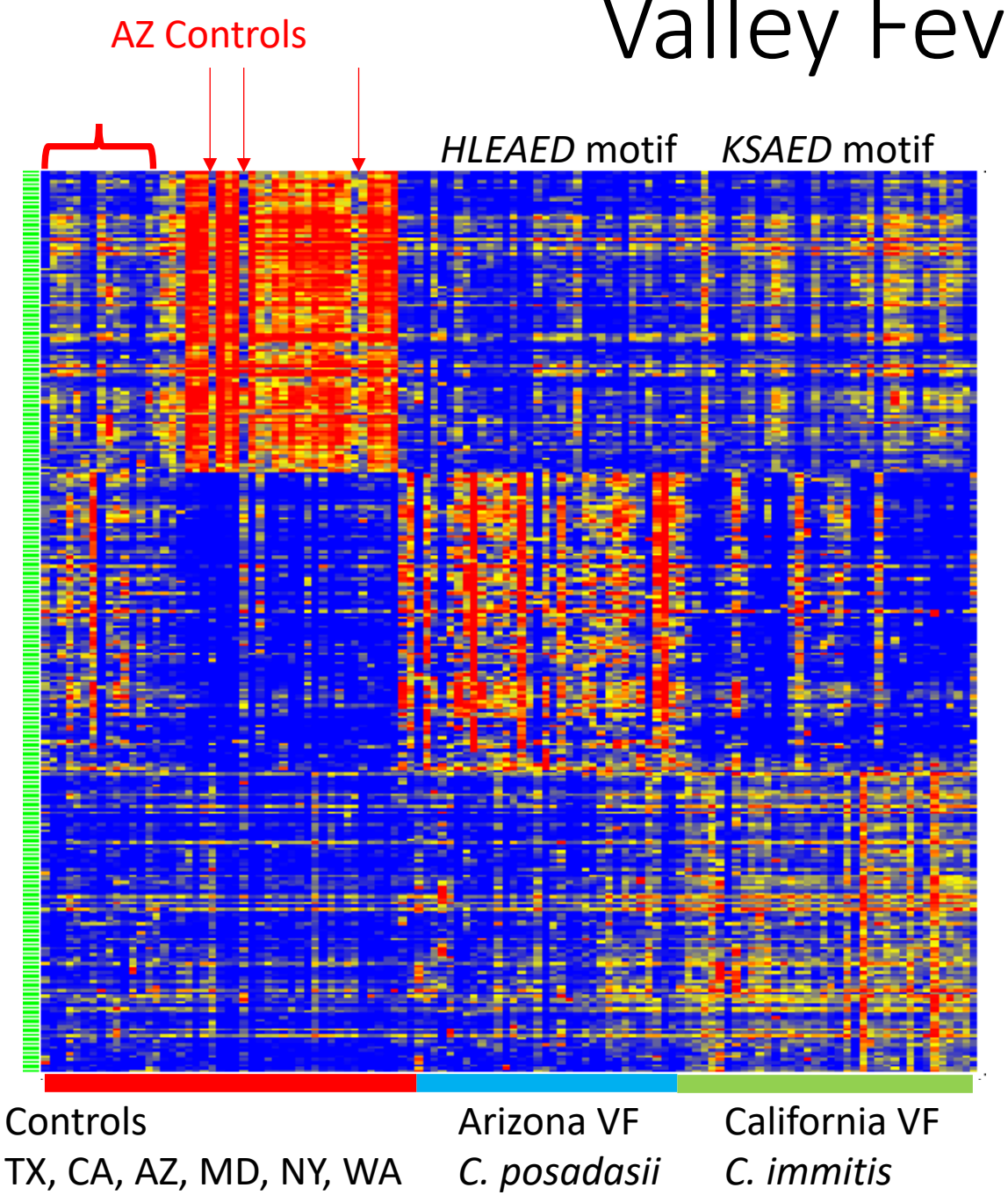
SW – moderate resistance
Lethal dose = 10^4 arthroconidia



C57 – susceptible
Lethal dose = 10^3 arthroconidia



Valley Fever Diagnosis - by strain



Valley Fever species test

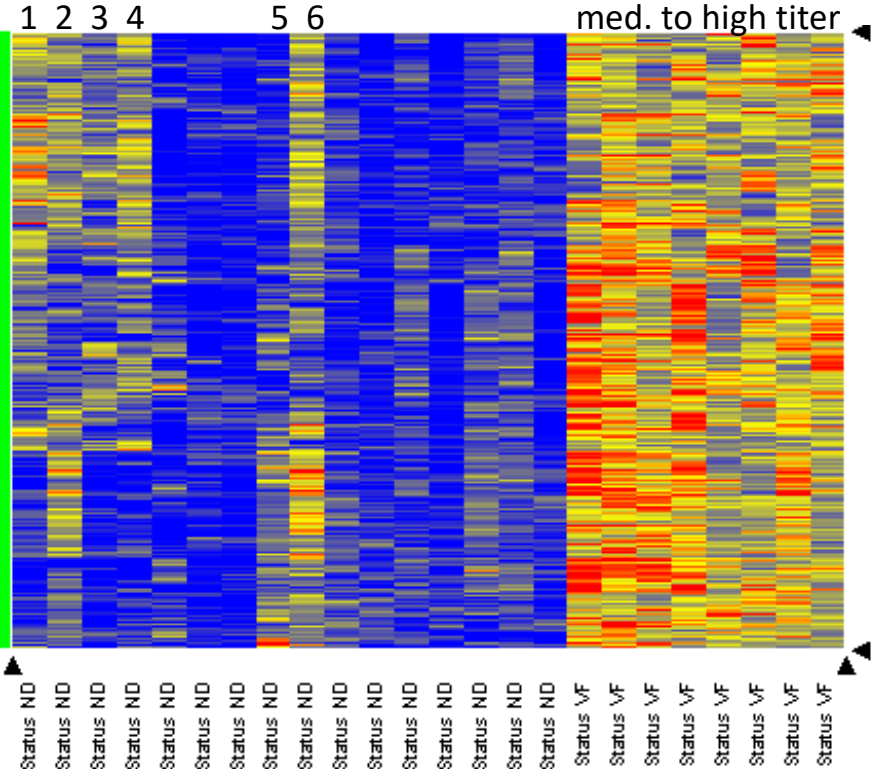
Immunosignatures are sensitive. In many cases Cocci signatures appear similar to endemic AZ residents. Here, 'AZ controls' are those living in Phoenix for 10 years. These controls look similar to real infections.

Immunosignatures can distinguish *posadasii* from *immmitis*. Leave 10% out, 100-fold crossvalidation 98.1% overall accuracy (2 AZ controls called VF)

Controls are from TX, CA, AZ, MD, NY and WA

Valley Fever Diagnosis – early chronic infection

It is possible that early diagnosis of Valley Fever is difficult with standard serology, false negatives are common.
IMS shows a pattern intermediate between diagnosed VF patients and negatives and endemic AZ residents.
This intermediate pattern may be a way to increase sensitivity without overwhelming false positives.



1, 2, 3, 4, 5, and 6 are patients who felt sick, had long-term coughing, were AZ residents for 6-10 years, but were negative by standard diagnosis.

Eventually 3, 5 and 6 were diagnosed correctly.

Thanks to:

- Stephen Johnston, CIM PI
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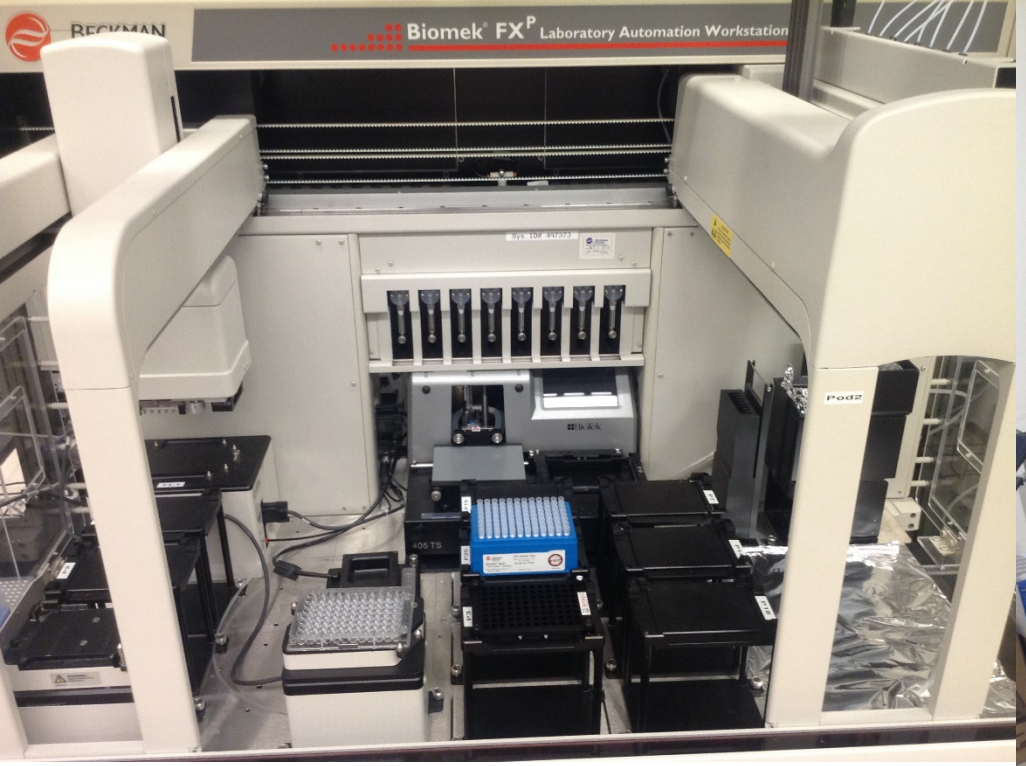
- Zhan-Gong Zhao, Geneva Nguyen, Doug Daniel, Peptide synthesis
- Neal Woodbury, Algorithms
- SAJ, NW, HealthTell Founders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Microarray Scanner
InnoScan 910 AL
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