Valley Fever: Technology to increase sensitivity of diagnostics

Amanda Burnham-Marusich, PhD

DxDiscovery

University of Nevada, Reno
School of Medicine

Unmet need: rapid diagnosis soon after symptom onset

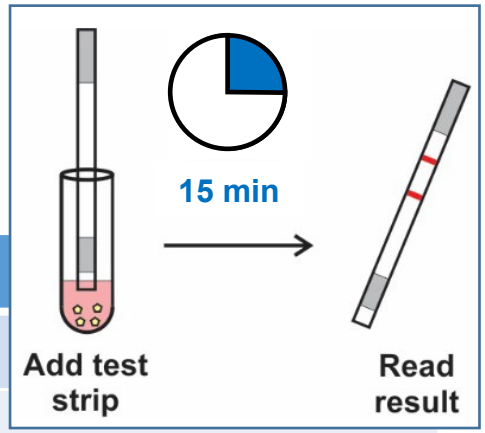
- **What delays diagnosis with current tests?**
  - Turnaround time (not point-of-care)
  - Serial testing to achieve needed clinical sensitivity

- **Consequences of delayed diagnosis:**
  - No diagnosis (patients lost to follow-up)
  - Unnecessary antibiotics (patient and community impact)
  - Additional blood work, scans, *etc.* that could be pre-empted by a prompt positive VF diagnosis
  - Increased morbidity/mortality risk

Immunoassays: serology *vs.* antigen-detection

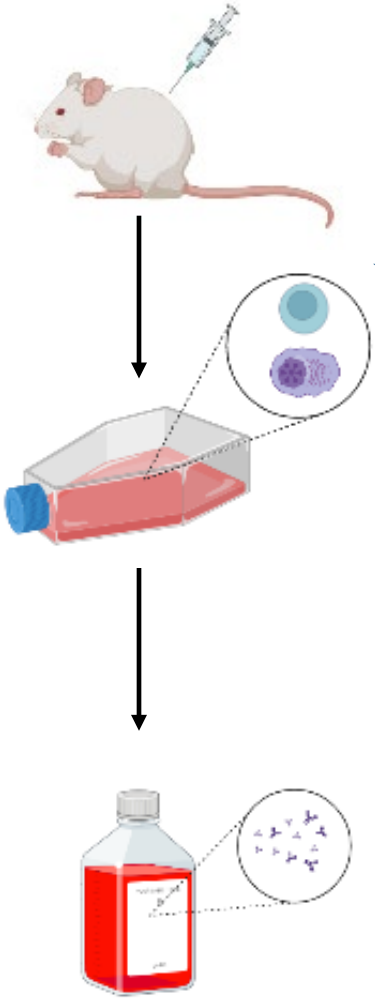
- Serology: **detects patient antibodies** to *Coccidioides* antigens
  - Con: Must wait for patient to generate antibody response to infection; positive tests can be due to antibodies from past infection
  - Pro: Once generated, patient antibodies are typically abundant, so analytical sensitivity is theoretically not an issue
- Antigen-detection: **detects *Coccidioides* antigens** via lab-made antibodies
  - Con: Microbial antigens are typically present at low concentrations, so exquisite (pg/mL) analytical sensitivity is ideal
  - Pro: Can be used early during infection (at time of symptom onset); reflect current patient status
- Both are suitable for lateral flow immunoassay platform

Proposed solution: antigen-detection LFIA



Key performance parameters	
Indication	Diagnosis of Valley Fever in symptomatic patients
Specimen	Urine or serum
Biomarker	*Coccidioides* spp. mannan (precedent for mannan as biomarker of many other invasive fungal diseases)
Readout	Ideal: Visual detection (colloidal gold) Acceptable: reader-based fluorescence detection
Time	< 15min sample-to-answer
Complexity	Minimal (seek CLIA-waiver for POC use); similar to COVID-19 rapid tests

Develop library of mAbs to *Coccidioides* mannan



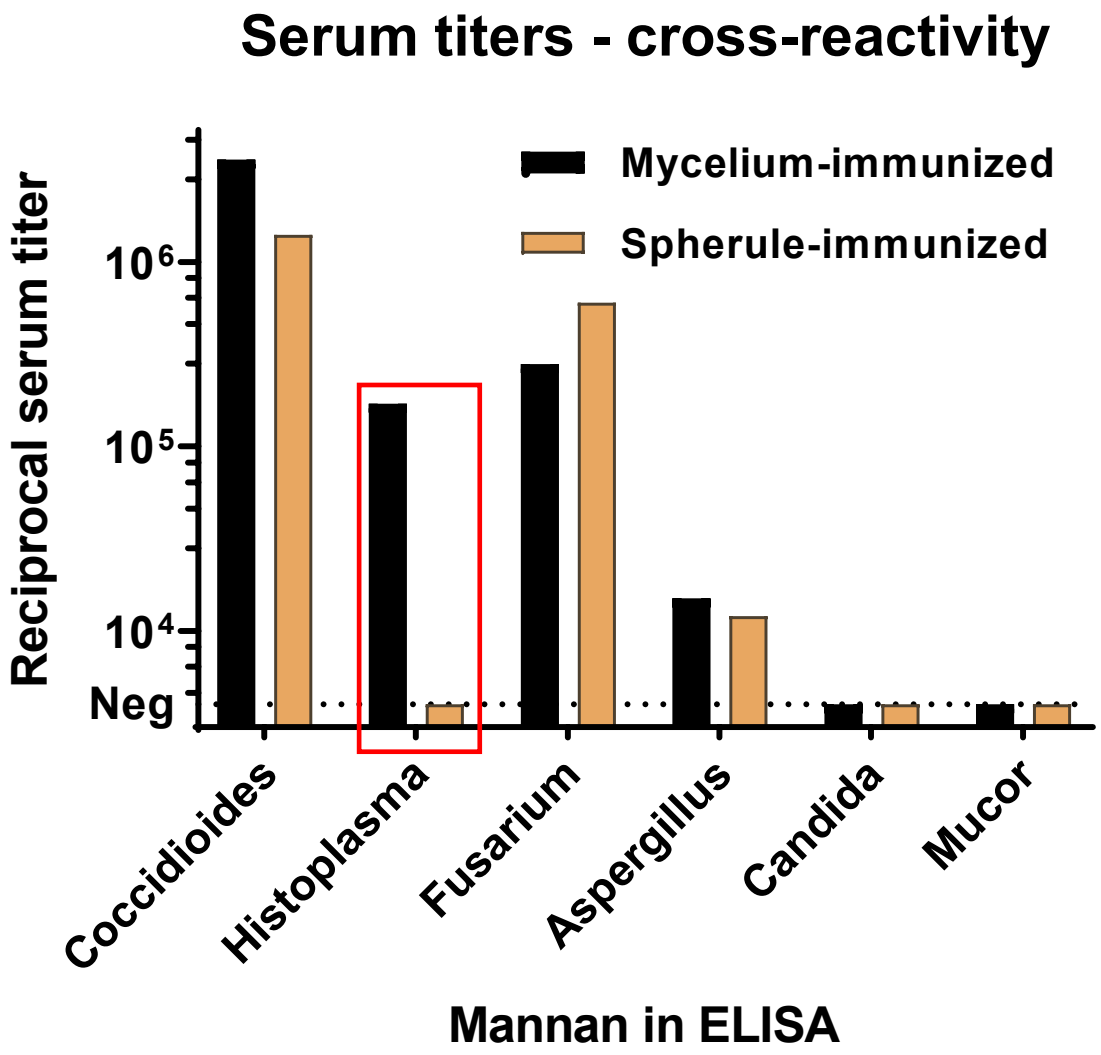
1. Immunize for high titers

2. Screen: Indirect ELISA

- ✓ Cell line stability & monoclonality
- ✓ Specificity to *Coccidioides*
- ✓ Inclusivity across *Coccidioides* spp.
- ✓ Affinity

3. Prototype development: antigen-capture ELISA & LFIA

Spherule-immunized splenocytes could be rich source of *Cocci*-specific mAbs



mAb reactivity patterns reflect terminal bleed sera

	Purified fungal mannan coated onto ELISA plate									
	Coccidioides posadasii		*Coccidioides immitis*		*Histoplasma*	*Fusarium*	*Aspergillus*	*Candida*	*Mucor*	*Rhizopus*
mAb	Mycelium	Spherules	Mycelium	Spherules						
7B12	++++	++++	++++	++++	–	–	–	–	–	–
11C9	++++	++	+++	+	+	+	–	–	–	–
4E3	++++	++++	++++	++++	+++	+++	–	–	+	+
6C12	++++	++++	++++	++++	+++	+++	–	–	–	–

- Ideal mAb has high inclusivity across *Coccidioides* spp. and high specificity to *Coccidioides* genus

Antigen-detection ELISA with mAb 7B12

Inclusivity	
Mannan	LOD (ng/ml)
C. immitis (RS) - mycelia	1
C. immitis (RS) - spherules	1
C. posadasii (Silveira) - mycelia	2
C. posadasii - spherules	2

Specificity	
Mannan	LOD (ng/ml)
Histoplasma capsulatum (Hc17)	>200
Histoplasma capsulatum (G217B)	>200
Candida albicans	>200
Candida auris (CAU-07)	>200
Fusarium solani	>200
Aspergillus fumigatus	>200
Mucor circinelloides	>200

- ✓ Inclusivity across *Coccidioides*
- ✓ Analytical specificity to *Coccidioides*

Next: move to LFIA platform and enhance sensitivity (analytical sensitivity drives clinical sensitivity)

Strategies to increase sensitivity

- Detector mAb labeling options
  - colloidal gold < gold nanoshells < Europium (best sensitivity)
- Alternative conjugation chemistries
  - Passive adsorption *vs.* covalent (NHS-ester or site-directed)
- Enrich analyte concentration in specimen
  - Concentration (urine); magnetic immunoprecipitation (urine/serum)
- Trade-offs for better sensitivity:
  - Higher cost
  - Electronic reader required (cost, electricity)
  - Test complexity (need to be careful to maintain POC & CLIA-waiver)

LFIA readers for europium now reaching POC

- Original options were expensive and research-grade (or cheap but unregulated UV pen)

- Now small footprint, lower cost, simplified user interface and data interpretation (+ electronic records and reporting)

  - Quidel Sofia

  - C2Sense HALO

Summary

- Antigen-detection LFIA: technology to enhance VF diagnosis (especially POC diagnosis soon after symptom onset)
- Multiple strategies for increasing LFIA clinical sensitivity (balance enhanced sensitivity *vs.* POC & accessibility)
- Clinical needs determine which strategies are feasible
- Considerations for future discussion:
  - Use of reader acceptable? (cost + electricity)
  - How big a window of clinical utility? (from symptom onset to when?)
  - Screening or rule-out usage? (high specificity + moderate sensitivity *vs.* extremely high sensitivity)

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burnham-marusich@DxDiscovery.com