Point of Care Testing in Microbiology and Laboratory Diagnosis of Valley Fever

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DISCLOSURES:

None
Objectives

• Be able to discuss important considerations of POC testing for microbiology

• Be able to explain why POC molecular testing has unique costs and benefits

• Be able to explain available diagnostic tests for valley fever

• Be able to discuss the role of laboratory test results in diagnosis and prognosis of Valley Fever
Point of Care Molecular Testing: 
*the Dawn of a New Age. Maybe.*
Definitions

• POC: Point of Care (does not mean waived)
• POI: Point of Impact (when POC is done right)
• Near-patient: in-room, ED, urgent care, etc.
• Waived Status: FDA cleared to be run by non-laboratory staff
• Moderate Complexity: FDA cleared to be run by non-certified laboratory technologists.
Influenza Background

• RNA virus—highly variable
• Transmission: large droplets
• Fever, muscle aches, headache, fatigue, cough, sore throat
• Yearly epidemics
  • >200,000 hospitalized/year
  • >36,000 deaths/year
• Keys to reduce transmission:
  • Hand hygiene
  • Vaccination
• Antiviral treatments available

• See also: 2015 Hot Topic Influenza Update by Dr. Matt Binnicker.
Vaccines for 2016/2017

- World Health Organization organizes a group who recommends vaccine composition
- In USA, FDA chooses final vaccine composition and approves vaccine products
- USA: 2016/2017 Vaccines contain 3 or 4 strains
  - Influenza A strains: H1N1, H3N2
  - Influenza B strain(s)
- FDA Approved vaccine formulations:
  - IIV: Inactivated Influenza Vaccine (injection)
  - RIV: Recombinant Influenza Vaccine (injection)
  - For >65 years (high dose or adjuvant)
  - Egg-free (grown in cell culture)
Vaccination Updates for 2016/2017

• Advisory Committee on Immunization Practices (ACIP)
  • Appointed by the Secretary of US Department of Health and Human Services (DHHS)
  • Decides how to use vaccines

• The nasal spray vaccine (Live Attenuated Influenza Vaccine—LIAV) is not recommended for 2016/2017 season

• Egg allergies:
  • Mild egg allergies (hives), any licensed vaccine
  • More severe symptoms:
    • Any licensed vaccine
    • Given in a medical setting with health care supervision to recognize and manage any allergic reaction.
  • No longer 30 minute wait
Traditional Methods of Detection

- **Culture**
  - Patient: slow and moderate sensitivity
  - Laboratory: high level of space and effort required, viral strains helpful for public health

- **Rapid antigen testing**
  - Patient: quick and variable sensitivity
  - Laboratory: varied impact
    - Point of Care—less laboratory interaction
    - Laboratory performed—simple but effort intensive
    - Negative results should be confirmed by alternative methods
  - Prevalence affects performance
    - Positive result in low prevalence more likely false-positive
    - Negative result during high prevalence more likely false-negative
POC ≠ POC

• Chemistry usually measures things that “should be there”

• Microbiology testing usually has a reference range of “negative.”

• POC for Micro
  • Downside: POC for antigens are not sensitive (mediocre performance)
  • Upside: POC for antigens are not sensitive (low risk for contamination)
Clinical Factors to consider

- Actionable information?
- Impact of TAT on outcome & cost?
- Surrogate measure or information available to approximate same answer?
- Specimen integrity (labeling, contamination, temperature, timing, storage).
- Risk/benefit of empiric treatment
- Seasonality of testing
- Potential for contamination within collection & testing environment
Operational Factors

• Cost of POC test vs. alternatives
• Cost vs. Charge
• Opportunity cost of space & staff, menu of platform?
• Training of staff collecting/performing testing
• Logistics of training, competency, maintenance, quality control, proficiency, procedures, etc.
• Comparison to existing and available gold standards
• Seasonality of testing
• Logistics/daily routine of testing needs
• Waste, “green” supplies
Psychology of testing

• Why do we test?
  • To treat provider? Patient? Parent?
  • Interesting? Available?

• Sensitivity vs. Specificity

• PPV vs. NPV (Positive & Negative Predictive Value)
  • Affected by age, season, geography, etc.

• The power of objective, black & white print

• Permanence: cannot be undone, ignored, or discounted
Case 1

• January 2014: 3 year old girl with 2 day history of runny nose, malaise, irritability

• Taken to CVS Minute Clinic

• Rapid Influenza test negative

• What do we do next?
## Pilot POC Micro Lab

- 23 tests
- TAT < 4 hr
- 2 years
- 51,179 tests
- 6244 Dx
- 8% of tests influenced management of ED patients

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Test result</th>
<th>n*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation for contagiousness</td>
<td>Positive influenza detection (A/H1N1)</td>
<td>545</td>
</tr>
<tr>
<td></td>
<td>Positive RSV detection</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>Positive <em>B. pertussis</em> detection</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Positive rotavirus/adenovirus detection</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>Positive <em>C. difficile</em> detection</td>
<td>7</td>
</tr>
<tr>
<td>Avoid unnecessary hospitalization</td>
<td>Positive enterovirus detection</td>
<td>117</td>
</tr>
<tr>
<td>Avoid unnecessary treatment</td>
<td>Positive RSV detection</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>Negative procalcitonin detection</td>
<td>294</td>
</tr>
<tr>
<td></td>
<td>Negative <em>S. pyogenes</em> detection</td>
<td>1,827</td>
</tr>
<tr>
<td></td>
<td>Infectious mononucleosis diagnosis</td>
<td>17</td>
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<tr>
<td></td>
<td>Positive enterovirus detection</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>Negative <em>S. agalactiae</em> detection</td>
<td>763</td>
</tr>
<tr>
<td></td>
<td>Dengue diagnosis</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td><em>C. tetani</em> antibodies</td>
<td>8</td>
</tr>
<tr>
<td>Replace empiric with documented treatment</td>
<td>Positive A/H1N1 influenza detection</td>
<td>335</td>
</tr>
<tr>
<td></td>
<td>Presence of urinary pneumococcal antigens</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Presence of urinary <em>L. pneumophila</em> antigens</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Positive <em>M. pneumoniae</em> detection</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Bacterial meningitis</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>HSV meningitis</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Malaria</td>
<td>149</td>
</tr>
</tbody>
</table>
Influenza: case example for POC issues

• Seasonal
• Treatable (sort of)
• Treatment success is time-dependent
• Large strain on ED/urgent care
• Age differences
• Many tests available
Influenza POC - Antigen

• Manual card tests
  • Many assays available

• Automated card reader tests
  • BD Veritor
  • 3M Sophia
Table 2. 95% Confidence Intervals: Data from two tests cleared during the past few years.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Influenza type</th>
<th>Population a</th>
<th>Sensitivity (95% CI) c</th>
<th>% Specificity (95% CI) c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throat swab</td>
<td>Influenza A</td>
<td>Pediatric b</td>
<td>65 to 90</td>
<td>81 to 91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adult</td>
<td>24 to 91</td>
<td>69 to 94</td>
</tr>
<tr>
<td>Throat swab</td>
<td>Both Influenza A &amp; B</td>
<td>Not specified</td>
<td>59 to 82</td>
<td>81 to 93</td>
</tr>
<tr>
<td>Nasopharyngeal wash/aspirate</td>
<td>Influenza A</td>
<td>Pediatric b</td>
<td>82 to 95</td>
<td>98 to 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adult</td>
<td>53 to 87</td>
<td>90 to 100</td>
</tr>
<tr>
<td>Nasal wash</td>
<td>Influenza A</td>
<td>Pediatric b</td>
<td>36 to 88</td>
<td>92 to 99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adult</td>
<td>9 to 99</td>
<td>59 to 100</td>
</tr>
<tr>
<td>Nasal wash and aspirate</td>
<td>Influenza A</td>
<td>Not specified</td>
<td>65 to 84</td>
<td>95 to 99</td>
</tr>
<tr>
<td>Nasal swab</td>
<td>Both Influenza A &amp; B</td>
<td>Not specified</td>
<td>65 to 87</td>
<td>87 to 97</td>
</tr>
</tbody>
</table>

a From the U.S., Australia, or New Zealand during seasons where A/H3 and A/H1 were predominant circulating influenza A viruses (derived from WHO Flunet, http://gamapserver.who.int/GlobalAtlas/home.asp)
b Age range not specified; majority are <10 years  
c 95% Confidence Interval
### Quidel Sophia

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal Swabs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All Ages</td>
<td>90% (124/138)</td>
<td>95% (500/527)</td>
</tr>
<tr>
<td></td>
<td>(95%CI=84%-94%)</td>
<td>(95%CI=93%-96%)</td>
</tr>
<tr>
<td>&lt;6 years</td>
<td>95% (62/65)</td>
<td>95% (210/221)</td>
</tr>
<tr>
<td></td>
<td>(95%CI=87%-99%)</td>
<td>(95%CI=91%-97%)</td>
</tr>
<tr>
<td>6 to 21 years</td>
<td>87% (46/53)</td>
<td>95% (193/204)</td>
</tr>
<tr>
<td></td>
<td>(95%CI=75%-94%)</td>
<td>(95%CI=91%-97%)</td>
</tr>
<tr>
<td>22 to 59 years</td>
<td>78% (14/18)</td>
<td>96% (82/85)</td>
</tr>
<tr>
<td></td>
<td>(95%CI=54%-92%)</td>
<td>(95%CI=90%-99%)</td>
</tr>
<tr>
<td>60 Years and up</td>
<td>100% (2/2)</td>
<td>88% (15/17)</td>
</tr>
<tr>
<td></td>
<td>(95%CI=29%-100%)</td>
<td>(95%CI=64%-98%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nasopharyngeal Swabs</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Ages</td>
<td>97% (100/103)</td>
<td>95% (596/630)</td>
</tr>
<tr>
<td></td>
<td>(95%CI=91%-99%)</td>
<td>(95%CI=93%-96%)</td>
</tr>
<tr>
<td>&lt;6 years</td>
<td>97% (61/63)</td>
<td>94% (444/470)</td>
</tr>
<tr>
<td></td>
<td>(95%CI=89%-100%)</td>
<td>(95%CI=92%-96%)</td>
</tr>
<tr>
<td>6 to 21 years</td>
<td>97% (35/36)</td>
<td>94% (136/144)</td>
</tr>
<tr>
<td></td>
<td>(95%CI=85%-100%)</td>
<td>(95%CI=89%-97%)</td>
</tr>
<tr>
<td>22 to 59 years</td>
<td>100% (4/4)</td>
<td>100% (15/15)</td>
</tr>
<tr>
<td></td>
<td>(95%CI=45%-100%)</td>
<td>(95%CI=76%-100%)</td>
</tr>
<tr>
<td>60 Years and up</td>
<td>N/A (0/0)</td>
<td>100% (1/1)</td>
</tr>
<tr>
<td></td>
<td>(95%CI=17%-100%)</td>
<td>(95%CI=68%-100%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nasopharyngeal Aspirate/Wash</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Ages</td>
<td>99% (68/69)</td>
<td>96% (554/580)</td>
</tr>
<tr>
<td></td>
<td>(95%CI=91%-100%)</td>
<td>(95%CI=93%-97%)</td>
</tr>
<tr>
<td>&lt;6 years</td>
<td>99% (68/69)</td>
<td>95% (544/570)</td>
</tr>
<tr>
<td></td>
<td>(95%CI=91%-100%)</td>
<td>(95%CI=93%-97%)</td>
</tr>
<tr>
<td>6 to 21 years</td>
<td>N/A (0/0)</td>
<td>100% (10/10)</td>
</tr>
<tr>
<td></td>
<td>(95%CI=68%-100%)</td>
<td>(95%CI=68%-100%)</td>
</tr>
<tr>
<td>22 to 59 years</td>
<td>N/A (0/0)</td>
<td>N/A (0/0)</td>
</tr>
<tr>
<td>60 Years and up</td>
<td>N/A (0/0)</td>
<td>N/A (0/0)</td>
</tr>
</tbody>
</table>
**BD Veritor**

Table 1: Summary of the Performance of the BD Veritor System for Rapid Detection of Flu A+B Test Compared to PCR for All Swabs - U.S. Sites

<table>
<thead>
<tr>
<th></th>
<th>Reference PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>POC: BD Flu A</strong></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>122</td>
</tr>
<tr>
<td>N</td>
<td>33*</td>
</tr>
<tr>
<td>Total</td>
<td>155</td>
</tr>
<tr>
<td><strong>POC: BD Flu B</strong></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>75</td>
</tr>
<tr>
<td>N</td>
<td>26**</td>
</tr>
<tr>
<td>Total</td>
<td>101</td>
</tr>
</tbody>
</table>

- **PPA: 78.7% (95% C.I. 71.6%-84.4%)**
- **NPA: 97.8% (95% C.I. 95.7%-98.9%)**

* Of the 33 PCR positive, **BD Veritor** negative Influenza A specimens, eight were positive in the **BD Veritor** assay using a second swab specimen (reference method specimen) collected from the same patient.

** Of the 26 PCR positive, **BD Veritor** negative Influenza B specimens, six were positive in the **BD Veritor** assay using a second swab specimen (reference method specimen) collected from the same patient.
Value of results depends on: Sensitivity, Specificity, **Prevalence**

Consider a test: Sensitivity = 80%
Specificity = 95%

*Prevalence affects Value in this way:*

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>False Pos in 1000 tests</th>
<th>True Pos in 1000 tests</th>
<th>Negative Predictive Value (%)</th>
<th>Positive Predictive Value (%)</th>
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</thead>
<tbody>
<tr>
<td>0.1</td>
<td>50</td>
<td>0.8</td>
<td>100</td>
<td>1.6</td>
</tr>
<tr>
<td>2.5</td>
<td>49</td>
<td>20</td>
<td>99.5</td>
<td>29.1</td>
</tr>
<tr>
<td>25</td>
<td>37.5</td>
<td>200</td>
<td>93.4</td>
<td>84.2</td>
</tr>
<tr>
<td>90</td>
<td>5</td>
<td>720</td>
<td>34.5</td>
<td>99.3</td>
</tr>
</tbody>
</table>
Categories of molecular testing

• Regulatory status
  • LDT (Lab developed tests)
  • FDA cleared

• Operators
  • Waived (non-laboratory personnel)
  • Moderate complexity
  • High complexity

• Analytes
  • Single
  • Small panel
  • Multiplex/syndromic
Molecular Detection: nucleic acid amplification tests

• Paradigm 1: single analyte tests
• Paradigm 2: syndromic testing panels
• Paradigm 3: rapid point of care tests
Paradigm 1: Single analyte tests

• Examples
  • Laboratory Developed Tests (LDTs)
  • FDA cleared assays

• Laboratory impact
  • New instruments, new methods
  • New laboratory skills
  • High cost, high effort, often batched

• Patient impact
  • Higher charge
  • Better sensitivity/specificity
  • Longer time to result than rapid antigen tests
  • Care decisions made on reliable information
Paradigm 2: Syndromic Testing

- **Examples:**
  - Influenza A/B and RSV
  - 3-20 pathogens including non-virus targets

- **Laboratory Impact:**
  - New instruments, often lower complexity
  - New Quality control challenges
  - High cost, logistics of ordering & reporting

- **Patient impact:**
  - High or very high charge
  - Often faster than single analyte testing
  - Care decisions may not change for some positive results on outpatients
Paradigm 3: Point of Care

- Examples:
  - Several FDA cleared assays with results <30 minutes
  - Waived status: can be performed outside laboratory

- Laboratory Impact:
  - High effort if performed in lab
  - Loss of control if performed in clinics
  - Revenue? Reporting/documentation?

- Patient Impact:
  - Care decision can be made before patient leaves
  - May be best tool for antimicrobial stewardship
  - Testing may occur when inappropriate
  - Positive test may not lead to change in care
    - Antivirals can be expensive
    - Antibacterials may be warranted for secondary bacterial infections
Copernican Revolution

• 10-15 years ago:
  • Culture was common and default gold standard
  • Antigen testing was widespread
  • Molecular testing was emerging

• Now
  • Culture use is less common
  • Antigen tests: use carefully and with ancillary testing
  • Wide array of options for molecular
Test Selection: Step 1  
Define Current State and Resources

- Evaluate current testing practices and identify any rapid antigen testing that may be occurring
- Consider standalone Influenza testing vs. small panels vs. syndromic panels
- Consider space and skills to support point of care and/or rapid testing in the laboratory
Test Selection: Step 2

Engage the Practice to Support Needs

- Start with the patient and provider
  - What decisions are made? What actions taken?
  - If treatment is not indicated, testing may not be needed
  - Define range of opportunity
    - Time to result
    - Local resources for testing or post-visit support

- Engage institutional leadership
  - Influenza testing/treatment recommendations
  - Evaluate opportunities to support the recommendations
Test Selection: Step 3  
**Synthesize needs and resources**

- Evaluate intersection of available resources and opportunities to impact care decisions
- Educate practice on orders, methods, expectations
- Communicate
  - Email, departmental visits, internal web sites
- Evaluate
  - Test utilization
  - Cost, reimbursement, FTE impact
POC Molecular testing

• Questions?
Laboratory Diagnosis of Valley Fever.

You say “Valley Fever,” I say Coccidioidomycosis
**Coccidioides** biology

- **Order: Onygenales**
  - (also includes *Histoplasma*, *Blastomyces* and *Paracoccidioides*)

- **Family: Onygenaceae**
  - Only dimorphic pathogen within this family

- **Two species, one disease**
  - *C. immitis*: California
  - *C. posadasii*: Arizona and everywhere else
Coccidioides epidemiology

- 20,000 reported infections each year
- >120,000+ unreported cases
- 10-30% of community acquired pneumonia (CAP) in endemic areas

<table>
<thead>
<tr>
<th>Organism</th>
<th>Incidence per 100,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coccidioides sp</td>
<td>42.6 (reported)</td>
</tr>
<tr>
<td></td>
<td>~200 (inc. unreported)</td>
</tr>
<tr>
<td>Blastomyces</td>
<td>6.1</td>
</tr>
<tr>
<td>Histoplasma</td>
<td>2</td>
</tr>
<tr>
<td>Borrelia burgdorferi</td>
<td>50-100</td>
</tr>
</tbody>
</table>
Diagnostic modalities for Valley Fever

- Culture
- PCR
- Serology
- Antigen testing
- Skin testing
- Radiology
Diagnostic modalities for Valley Fever

- Culture
- PCR
- Serology
- Antigen testing
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- Radiology
Diagnostic modalities for Valley Fever

• Culture
• PCR
• Serology
• Antigen testing
• Skin testing
• Radiology
Complement Fixation

**Reactive**
- Serum with antibodies
- Specific antigen added to bind antibodies
- Complement added to bind antigen-antibody complexes
- Sensitized red cells added but no surplus complement
- Intact red cells settle in pellet

**Nonreactive**
- Serum but no antibodies
- Free antigen
- Added complement remains unbound
- Free complement binds sensitised red cells
- Red cells lyse

http://micrognome.priobe.net/2013/08/how-serology-works/

tji 2013. Creative Commons
Immunodiffusion

- Use different antigens to test
  - IgG (IDCF) chitinase
  - IgM (IDTP) beta-glucosidase
Enzyme Immunoassay

- FDA cleared (Meridian, Immuno-Mycologics [Immy])
- Can be automated

![Diagram of Enzyme Immunoassay process]

- Specimen is added to solid matrix coated with capture antigen and incubated
- Enzyme labeled, detector antibody is added and incubated
- Enzyme substrate is added, incubated and washed to remove unbound excess substrate
- General interpretation: Reactivity = Antibody Present, No reactivity = Antibody Absent

- Target antibody from specimen
- Enzyme labeled detector antibody
- Adhered, captured antigen
- Enzyme substrate, inactivated
- Enzyme substrate, activated

MiraVista antigen test

- Antibodies against *Coccidioides* galactomannan
- Sensitivity is moderate (50-73%, serum & urine)
- Specificity concerns with cross reactivity to *Histoplasma* and/or *Blastomyces*
- Recently shown useful for CNS infections by testing CSF specimens (93% sensitive, vs. 85% for EIA IgG and 85% for ID & CF combined).

References:
Diagnostic modalities for Valley Fever

- Culture
- PCR
- Serology
- Antigen testing
- Skin testing
- Radiology
Skin testing

- 1930s, C.E. Smith and colleagues developed Coccidioidin (mycelial extract). Used for many epidemiological studies in form of a skin test.
- Similar to TB skin test, measures Delayed-Type Hypersensitivity (DTH)
- 1950s: spherules propagated in culture enabled development of spherulin (late 1970s)
- 1987: more concentrated form of spherulin introduced.
- 1999: spherulin no longer available.
Diagnostic modalities for Valley Fever

• Culture
• PCR
• Serology
• Antigen testing
• Skin testing
• Radiology
Spherusol™

• FDA cleared (2011) for determining cell-mediated immunity to *Coccidioides* in patients with established history of pulmonary coccidioidomycosis, ages 18-64.
  
  • Solution and placement:
    
    • Preservative is phenol
    • Performance comparable to earlier literature using spherulin
    • 0.1 mL of solution placed intradermally and read at 48 hours.
    • Induration of ≥5 mm is positive (redness/discholoration not used)
    • Immediate reaction within 15-60 minutes does not count (true positive reaction does not occur until 6 hours)

  • Interpretation:
    
    • Patients develop positivity in 3 days to 3 weeks of symptom onset
    • Does not affect serologic testing
    • Positive result suggests good prognosis and development of protective immunity.
    • Serial testing may be helpful

Spherusol™

• Cautions
  • May be negative in anergic individuals
  • May be false-negative in severe infections
  • Avoid in patients with erythema nodosum
  • Future immunosuppression may put skin test positive patients at risk

Diagnostic modalities for Valley Fever

- Culture
- PCR
- Serology
- Antigen testing
- Skin testing
- Radiology
Radiology

- Cavity (can mimic things like TB or other fungal infections)
- Nodule (can mimic cancer)
- Miliary (suggests dissemination)
Cavitary lesion (CT scan)

- Cavitary pneumonia

4 years later: nodule

Cavity of coccidioidomycosis

- Thin walled, typically not calcified
Immunocompromised patient: miliary pattern

## Diagnostic modalities for coccidioidomycosis

<table>
<thead>
<tr>
<th>Test</th>
<th>Turnaround Time</th>
<th>Evidence</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>Several Days</td>
<td>Direct</td>
<td>Requires good specimen</td>
</tr>
<tr>
<td>PCR</td>
<td>~48 hours</td>
<td>Direct</td>
<td>Cost, and requires good specimen, not clearly better than culture</td>
</tr>
<tr>
<td>EIA (IgG/IgM)</td>
<td>24 hours</td>
<td>Indirect</td>
<td>Serologic response can be slow and unreliable.</td>
</tr>
<tr>
<td>Complement Fixation (CF)</td>
<td>Several Days</td>
<td>Indirect</td>
<td>Quantitative. IgG only (chitinase) Serologic response can be slow and unreliable.</td>
</tr>
<tr>
<td>Immunodiffusion (IDTP = IgM)</td>
<td>Several Days</td>
<td>Indirect</td>
<td>Good specificity. IgG and IgM Serologic response can be slow and unreliable.</td>
</tr>
<tr>
<td>Histology</td>
<td>24 hours</td>
<td>Direct</td>
<td>Requires procedure for specimen collection</td>
</tr>
<tr>
<td>Spherusol</td>
<td>48 hours</td>
<td>Indirect</td>
<td>Skin test FDA cleared for verifying cellular immunity. Serologic response can be variable. Logistics</td>
</tr>
<tr>
<td>Imaging</td>
<td>24 hours</td>
<td>Indirect</td>
<td>Non-specific</td>
</tr>
<tr>
<td>Antigen Testing (MiraVista)</td>
<td>Several Days</td>
<td>Direct</td>
<td>50-70% sensitive in urine &amp; serum, cross reactive to other dimorphic fungi</td>
</tr>
</tbody>
</table>
## A staged criteria (expanded from EORTC)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Spherules visualized or culture growth</th>
<th>Symptoms compatible with coccidioidomycosis</th>
<th>Radiographic abnormalities</th>
<th>Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed</td>
<td>+</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Highly Probable</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>IgG or IgG and IgM positive</td>
</tr>
<tr>
<td>Probable</td>
<td>-</td>
<td>Presence of symptoms or radiographic abnormalities</td>
<td>IgG or IgG and IgM positive</td>
<td></td>
</tr>
<tr>
<td>Possible</td>
<td>-</td>
<td>Presence of symptoms or radiographic abnormalities</td>
<td>IgM only reactivity</td>
<td></td>
</tr>
<tr>
<td>Unconfirmed</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>IgM only reactivity</td>
</tr>
</tbody>
</table>
Case 1: 24 year old woman

- 1 week history of coughing up blood, Mild pain in chest (4/10 intensity)
- After 3 days admitted to outside hospital for evaluation
- L upper lobe cavity 21 x 18 mm, R lower lobe nodule 5 x 4 mm.
- AFB smears negative x3, Cocci negative IgG, IgM. Strep pneumo urine test was negative. Mycoplasma IgG was positive. Couldn’t produce sputum in hospital, only when in shower.
- Sent home on oral Levaquin.
- Later that day presents to Mayo Clinic ED.
- Temperature of 36.3
- Heart rate 93, BP 120/79,
- Respirations 16, Sat 99%
- Hemoglobin of 12.9
- Hematocrit 38
- WBCs 8.1
- Platelets 216
Upon further questioning

- Blood streaked sputum 6 months prior
- Returned from Germany and Italy 2 weeks ago, with extensive European travel over past 2 years
- Occasionally coughing over past few weeks
- Also spent time in Wisconsin recently, where cough seemed to be better
**Coccidioides testing**

- Blood draw from ED sent for serology

<table>
<thead>
<tr>
<th>Test</th>
<th>Initial draw</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIA IgM</td>
<td>Neg</td>
</tr>
<tr>
<td>EIA IgG</td>
<td>Neg</td>
</tr>
<tr>
<td>ID IgM</td>
<td>Neg</td>
</tr>
<tr>
<td>ID IgG</td>
<td>Pos</td>
</tr>
<tr>
<td>CF</td>
<td>Neg</td>
</tr>
</tbody>
</table>

- Could this be *Coccidioides*?
- Other causes for cavity in a traveler…
• Following day: bronchoscopy performed
  • Negative for Respiratory Pathogen Panel
  • 2+ respiratory flora
  • Grew 1 colony *Coccidioides*

• 2 weeks later, still hemoptysis on 800 mg Fluconazole, another bronchoscopy
  • 3+ respiratory flora
  • Grew 1 colony *Coccidioides*
• Wedge resection
  • Necrotizing granulomatous inflammation and organisms consistent with *Coccidioides*
  • Lung tissue grew 2+ *Coccidioides*
• 1 month after resection, feeling well.
• Serology:

<table>
<thead>
<tr>
<th>Test</th>
<th>6 weeks after ED, 4 weeks post resection</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIA IgM</td>
<td>Neg</td>
</tr>
<tr>
<td>EIA IgG</td>
<td>Neg</td>
</tr>
<tr>
<td>ID IgM</td>
<td>Neg</td>
</tr>
<tr>
<td>ID IgG</td>
<td>Neg</td>
</tr>
<tr>
<td>CF</td>
<td>Neg</td>
</tr>
</tbody>
</table>
Case 1 pearls

• Sputum can be difficult to obtain
• Serology showed IgG by ID only
  • Any positive serology in appropriate clinical context must trigger an investigation for coccidioidomycosis
• Patient received a course of unnecessary antibiotics
• Infection refractory to treatment, required resection
Serology: it’s great, except when it isn’t

- EIA (results vary based on kit and prevalence)
  - 50% of patients positive at 2 weeks\(^1\), 90% positive by 1 month\(^1\)
  - Some studies question specificity\(^2\)
  - Evaluation data vs. ID/CF or combined standard?

- ID
  - Best specificity
  - Least sensitive (75% overall)\(^1\)

- CF
  - Great for following titer
  - Not as sensitive in early infection\(^1\)

- Early treatment may blunt serologic response\(^3\)

## Serology in immunocompromised hosts

(concept likely true for everyone)

### Table 2. Seropositivity among 62 immunocompromised hosts with serologic confirmation of coccidioidomycosis detected by various serologic tests

<table>
<thead>
<tr>
<th>Category of immunosuppression</th>
<th>Type of serologic testing, no. (%)</th>
<th>Any test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EIA (IgM and IgG)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tested</td>
<td>Positive</td>
</tr>
<tr>
<td>Hematologic malignancy (N = 14)</td>
<td>12</td>
<td>4 (33)</td>
</tr>
<tr>
<td>Cancer and chemotherapy, nonhematologic (N = 19)</td>
<td>18</td>
<td>13 (72)</td>
</tr>
<tr>
<td>HIV infection (N = 4)</td>
<td>4</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Organ transplantation (N = 7)</td>
<td>7</td>
<td>5 (71)</td>
</tr>
<tr>
<td>Rheumatologic illness (N = 13)</td>
<td>11</td>
<td>9 (82)</td>
</tr>
<tr>
<td>Other ICH illness* (N = 11)</td>
<td>10</td>
<td>9 (90)</td>
</tr>
<tr>
<td>All patients†</td>
<td>57</td>
<td>38 (67)</td>
</tr>
<tr>
<td>Healthy patients tested ≤ 1 y after symptom onset (N = 261)</td>
<td>244</td>
<td>212 (87)</td>
</tr>
</tbody>
</table>

CF, complement fixation; EIA, enzyme immunoassay; ICH, immunocompromised; ID, immunodiffusion; HIV, human immunodeficiency virus.

*Patients with other causes of immunocompromise include 3 inflammatory bowel disease (1 taking infliximab), 2 autoimmune blood dyscrasias (hemolytic anemia and idiopathic thrombocytopenic purpura) taking prednisone, 1 autoimmune polyneuropathy, and 5 taking corticosteroids long-term for sarcoid, cough, other pulmonary diseases (chronic obstructive pulmonary disease, interstitial pulmonary fibrosis, or normal interstitial pneumonia).

†Six patients have 2 immunosuppressive illnesses and are represented in each category.

Summary

• Many useful modalities available, none are perfect
• If suspicious of coccidioidomycosis, considering ordering all serologic tests: EIA, CF, and ID
• Follow up on any positive serology in context of clinical symptoms
• New tools available: skin testing, antigen testing
• Immunocompromised hosts are complicated
Questions?