Introduction

Coccidioidomycosis (coccidioides), also known as Valley Fever (VF), is a respiratory fungal infection that is prevalent in the southwestern US and Central and South America. Approximately 60% of all reported US cases occur in Arizona. Because Arizona does not require clinical criteria to confirm a case, public health surveillance data is based predominantly on laboratory tests.

Serological testing comprises the vast majority of reports:
- Immunofluorescence (ID)
- Enzyme Immunoassay (EIA)

Due to cost and availability, most laboratories utilize Enzyme Immunoassay (EIA), which is readily available in a commercial kit to diagnose Coccidioides.

These EIA kits are currently available from two manufacturers:
- Immunodiagnostic, Inc – Premier Coccidioides EIA (Immy)
- Meridian Biosciences, Inc – Coccidioides Antibody EIA (Meridian)

However, some concerns exist about the reproducibility of EIA results among laboratories and the specificity of EIA IgM and IgG false positive results.

Objectives

Primary objective: to evaluate the laboratory reproducibility of Coccidioides EIA IgM and IgG results by comparing the percent agreement among laboratories using sera from known cases and controls.

Secondary objective: to assess EIA sensitivity and specificity

Methods

EIA IgM and IgG results from two different manufacturers (Immy and Meridian) were compared using sera from the same patients divided among three laboratories, two in Arizona & one in California.

150 sera from confirmed coccidioidomycosis cases were selected retrospectively and frozen. All specimens were

- Laboratory confirmed with ID and/or Complement Fixation (CF)
- Independently reviewed for clinical evidence of coccidioides by an infectious disease physician

50 remnant sera from CDC employees were used from 150 sera from confirmed coccidioidomycosis cases.

All 200 specimens were divided into aliquots, blinded, and distributed by Kern County Public Health Laboratory.

All specimens were divided among three laboratories, two in Arizona & one in California.

Results

Percent agreement was calculated:

- Numerator: Number of times all three laboratories obtained the same result (all negative or all positive) for a particular test (IgG or IgM) using a particular test kit (Immy or Meridian) on a particular specimen
- Denominator: Total number of specimens with indeterminate results counted as “negative”

IgG and IgM combined: If either the IgG or IgM EIA result is positive, the “test set” is considered “positive.” If both are negative, the test set is considered “negative.” IgG and IgM test sets from a particular test kit (Immy or Meridian) have to be in agreement among all 3 laboratories.

Percent agreement for EIA IgM and IgG combined among the three labs:

- 85.5% for Immy (90% for IgM and 89% for IgG alone)
- 70.5% for Meridian (67% for IgM and 81% for IgG alone)

Sensitivity for EIA IgM and IgG combined:

- 68.5% for Immy
- 72.4% for Meridian

Specificity for EIA IgM and IgG combined:

- 99.3% for Immy
- 91.3% for Meridian

Table 1: EIA Sensitivity and Specificity

<table>
<thead>
<tr>
<th>Laboratory Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIA Immy Kit, Lab A</td>
<td>Combined IgM &amp; IgG 65.8</td>
<td>Combined IgM &amp; IgG 100.0</td>
</tr>
<tr>
<td>EIA Immy Kit, Lab B</td>
<td>62.7</td>
<td>100.0</td>
</tr>
<tr>
<td>EIA Immy Kit, Lab C</td>
<td>77.0</td>
<td>98.0</td>
</tr>
<tr>
<td>EIA Meridian Kit, Lab A</td>
<td>87.3</td>
<td>74.0</td>
</tr>
<tr>
<td>EIA Meridian Kit, Lab B</td>
<td>57.3</td>
<td>100.0</td>
</tr>
<tr>
<td>EIA Meridian Kit, Lab C</td>
<td>72.7</td>
<td>100.0</td>
</tr>
<tr>
<td>EIA Immy Kit, All Labs</td>
<td>68.5</td>
<td>99.3</td>
</tr>
<tr>
<td>EIA Meridian Kit, All Labs</td>
<td>72.4</td>
<td>91.3</td>
</tr>
<tr>
<td>EIA Both Kits, All Labs</td>
<td>70.5</td>
<td>95.3</td>
</tr>
</tbody>
</table>

Table 2: Specificity among 50 controls

<table>
<thead>
<tr>
<th>Combined IgM/IgG</th>
<th>True Negative (%)</th>
<th>False Positive (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIA Immy Lab A</td>
<td>50</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>EIA Immy Lab B</td>
<td>50</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>EIA Immy Lab C</td>
<td>49</td>
<td>1</td>
<td>98</td>
</tr>
<tr>
<td>EIA Meridian Lab A</td>
<td>37</td>
<td>13</td>
<td>74</td>
</tr>
<tr>
<td>EIA Meridian Lab B</td>
<td>50</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>EIA Meridian Lab C</td>
<td>50</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Conclusions

- Variability between laboratories exists when performing serology using the same brand of EIA test kit on the same specimens.
- Percent agreement is greater for the Immy test kit for IgG, IgM and both combined compared with the Meridian test kit.
- Lab A yielded an increased number of false positive EIA IgM results (13 of 50 or 26%).
- Excluding this outlier, EIA IgG and IgM are very specific in our investigation ranging from 98% – 100%.
- Both Immy and Meridian EIA IgG and IgM appear to be less sensitive than predicted ranging from 57% – 87%.

Limitations

Sensitivity and specificity results may not be generalizable due to the relatively small number of specimens included in this investigation.

Discussion

The Meridian EIA test kit requires a “wash step” that is often automated by commercial laboratories with large specimen volumes. Laboratory A uses an automated wash process whereas Laboratories B and C do the wash step by hand, which might explain the increase in false positive Meridian EIA results from Laboratory A. The Immy EIA test kit does not require a wash step.

It is unclear why the sensitivities of both EIA test kits were lower than those previously reported. The timing of specimen collection may contribute to these findings.

Recommendations

- Compare the automated vs the “hand” wash step using the Meridian EIA test kit in a single laboratory with the same specimens to determine if the wash step methods could explain the false positives.
- Determine day of laboratory specimen collection in relation to symptom onset to evaluate low sensitivity.

Disclaimer

The findings and conclusions in this presentation are those of the author(s) and do not necessarily represent the views of the Centers for Disease Control and Prevention.