Overview of Syphilis Diagnostics: Serologic Assays and Algorithms
Outline

1. Non-Treponemal Assays
2. Treponemal Assays
3. CLIA-waived Rapid Testing
4. Testing Algorithms
Non-Treponemal Assays

• Detects “reagin”, a non-specific antibody-like substance released upon *T. pallidum* infection
• Qualitative and Quantitative
• Traditionally used for screening
  – Rapid
  – Inexpensive
Venereal Disease Research Laboratory Assay (VDRL)

- Original non-treponemal testing method
- Qualitative and Quantitative
- Microscopic slide flocculation
- Only FDA-approved non-treponemal method for CSF specimens
Rapid plasma reagin (RPR)

- The reagin binds to the test antigen which is bound to charcoal, causing **macroscopic** flocculation
- Qualitative and Quantitative

Reactive
(Clumping/agglutination appears).

Minimally Reactive
(Slight clumping/agglutination).

Non-reactive
(Smooth gray pattern).

Images courtesy of CDC
# Comparison of Non-Treponemal Assays

<table>
<thead>
<tr>
<th></th>
<th>RPR</th>
<th>VDRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient samples per card/slide</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Sensitivity- Primary Syphilis</td>
<td>86 (77-99)</td>
<td>78 (74-87)</td>
</tr>
<tr>
<td>Specificity</td>
<td>98 (93-99)</td>
<td>98 (96-99)</td>
</tr>
<tr>
<td>Requires Microscope</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Approved for CSF</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Adapted from Larsen, S.A., et. Al. (1995) Table 2, pg 7
Non-Treponemal Assays

**Advantages**

- High Sensitivity
- Low cost
- Does not detect past infections
- Requires little equipment for testing
- Usually requires only one reflex test
- Useful for treatment monitoring (Change in titer)

**Disadvantages**

- Lower specificity
- Labor intensive
- Subjective results
- Manual data manipulations
- Detection of late latent syphilis
Treponemal Assays

- Detect specific antibody against *T. pallidum*
- Qualitative
- Reactivity can persist over lifetime
- Traditionally utilized for confirmation
Types of Treponemal Tests

- Treponemal Pallidum Particle Agglutination Assay (TP-PA)
- Fluorescent Treponemal Antibody Absorption (FTA-ABS)
- Immunoassays
  - Enzyme Immunoassay (EIA)
  - Chemiluminescent Immunoassay (CIA)
  - Microbead Immunoassay (MIA)
**TP-PA**

- Qualitative particle agglutination assay
- Subjective interpretation

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>Test Cell</th>
<th>Control Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive</td>
<td><img src="image1" alt="Test Cell Image" /></td>
<td><img src="image2" alt="Control Cell Image" /></td>
</tr>
<tr>
<td>Non-reactive</td>
<td><img src="image3" alt="Test Cell Image" /></td>
<td><img src="image4" alt="Control Cell Image" /></td>
</tr>
</tbody>
</table>

Images courtesy of CDC
FTA-ABS

• Alternative to TP-PA as confirmatory assay
• Requires experienced microscopist
• Subjective interpretation
Immunoassays (EIA, CIA, MIA)

- Qualitative assays that measure IgG, IgM, or both
- Commonly used as screening assays for reverse algorithm
- Can be automated
- Objective interpretation based on cutoff values of specific assay
  - Reactive, Non-reactive, Equivocal
Comparison of Treponemal Assays

<table>
<thead>
<tr>
<th></th>
<th>TP-PA</th>
<th>FTA-ABS</th>
<th>IA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potential for high-throughput</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Interpretation</td>
<td>Subjective</td>
<td>Subjective</td>
<td>Objective</td>
</tr>
<tr>
<td>Maximum patient specimens per plate/slide</td>
<td>24</td>
<td>5</td>
<td>12-180*</td>
</tr>
<tr>
<td>Common use</td>
<td>Confirmation</td>
<td>Confirmation</td>
<td>Screening/Confirmation</td>
</tr>
</tbody>
</table>

* Maximum number of specimens that can be run depends on the platform of IA that is selected
Treponemal Assays

Advantages

• Detection of specific treponemal antibody
• Objective results (EIA, CIA, and MIA)
• Less labor intensive (if automated)
• Ability to detect early primary infection

Disadvantages

• Cannot differentiate between active and past infection
• Subjective results (TPPA, FTA)
• More expensive than non-treponemal
• If IgG-only assay utilized, can miss early primary infections
CLIA-Waived Rapid Testing

• One FDA-cleared, CLIA-waived test

Reactive  Non-reactive  Invalid

Per package insert
CLIA-Waived Rapid Testing

Advantages
- May be utilized in nontraditional laboratory settings
- Results obtained while client present and can link to care
- Samples do not need to be batched and can be run when needed
- Could be utilized as the treponemal test in a syphilis testing algorithm to expedite results

Disadvantages
- More expensive than other treponemal tests
- Subjective results
- Nontraditional settings still require training and quality assurance
- Only one assay currently FDA-cleared
- Performance characteristics not fully evaluated for all settings as compared to laboratory testing
Traditional Syphilis Algorithm

* If titer <1:4 consider these values associated with possible serofast condition
Reverse Syphilis Algorithm

* The second treponemal test should utilize a unique platform and or antigen, different than the first treponemal test
Considerations for Algorithm Selection

Factors to consider when evaluating the best algorithm for the laboratory or jurisdiction

- Testing volume
- Laboratory workflow and staffing
- Prevalence and incidence of syphilis in your jurisdiction
- Cost and reimbursement
- Programmatic and clinical input
References

Resources

Images

- [http://phil.cdc.gov/phil/home.asp](http://phil.cdc.gov/phil/home.asp)

General

- [CDC Sexually Transmitted Disease Surveillance 2013](https://www.cdc.gov/std/surveillance/
- [STD Treatment Guidelines 2015](https://www.cdc.gov/std/tg2015/
- [Syphilis Fact Sheets](https://www.cdc.gov/std/factsheets/
- [CDC Division of STD Prevention (DSTD)](https://www.cdc.gov/std/
- [APHL STD Homepage](https://www.cdc.gov/std/hiv/

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