Syphilis
Disease & Testing Algorithms

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Senior Clinical Specialist
Disease State Marketing

History of Syphilis
First Reported Cases
- Syphilis was first reported in Europe in 1494 among soldiers (and their camp followers) involved in a war between France and Naples.
- The disease was striking in two ways: for its unpleasantness and for its status as a new disease, unknown to the ancient medical authorities.
- Syphilis would remain a significant social and medical problem through the mid-20th century.

The “French Disease”
- Until the 18th century, syphilis was known by many different names, but the most common was the “French Disease.” (The French called it the “Neopolitan disease.” In a pattern that would repeat itself elsewhere, Russians, for instance, sometimes called it the “Polish disease.”)

Origins in the New World
- Syphilis is generally believed to have come originally from the New World, imported into Europe by Christopher Columbus’s sailors after their famous voyage of 1492.

Epidemiology
- 12 million new cases worldwide per year
- Greatest numbers in adolescents and young adults
- Greatest numbers of new cases in South and Southeast Asia, followed by sub-Saharan Africa

Primary and Secondary Syphilis by Sex, 1990-2010

Syphilis in Wartime

The Tuskegee Syphilis Experiment
- A clinical study conducted between 1932 and 1972 in Tuskegee, Alabama, by the U.S. Public Health Service.
- 399 African-American sharecroppers with syphilis recruited for research related to the natural progression of the untreated disease.
- By 1947 penicillin had become the standard treatment for syphilis.
- Tuskegee scientists continued the study, withholding penicillin treatment and information about it from the patients.
- After the study and its consequences became front-page news, it was ended in a day.
Syphilis: United States

Syphilis elimination campaign in the US, circa 1940

The Syphilis Elimination Effort (SEE)

CDC Syphilis Campaign, 2009
The Syphilis Elimination Effort (SEE) is a national initiative that brings together health care providers, policy makers, community leaders and state and local public health agencies, to reduce syphilis rates in the United States.

SEE goal: less than 2.2 cases of syphilis per 100,000 in the general population

Primary and Secondary Syphilis—Rates by State, United States and Outlying Areas, 2010

Primary and Secondary Syphilis—Rates by County, United States, 2010

Note: In 2010, a total of 2,194 (69.9%) of 3,141 counties in the United States reported no cases of primary and secondary syphilis.
May become chronic without treatment

Etiologic agent:

Siemens Healthcare Diagnostics

Mississippi

Louisiana

New York

Nevada

Texas

Humans are the only natural host, unable to survive outside host

Baby born to mother with untreated syphilis

Secondary lesions occur 3 to 6 weeks after the primary lesion appears; may persist for weeks to months

Primary and secondary stages may overlap

Characterized by the formation of gummas which are soft, tumor-like balls of inflammation known as granulomas

Typical painless but highly infectious and can spontaneously infect multiple times!

Humans are the only natural host, unable to survive outside host

Transmission: Groups at Risk

Primary

Secondary

Latent

Tertiary
Disease Course of Untreated Syphilis

- **Chancre**
  - Infection with *T. pallidum*
  - Primary Syphilis
  - Secondary Syphilis
  - Latent Syphilis

- Rash

No Further Complications 72%

Tertiary syphilis 28%

Damaged Causes by Syphilis

- **Tertiary syphilis**
  - a late stage of the disease which can follow the initial infection, primary syphilis, by several years
  - Pockets of damage accumulate in various tissues such as the bones, skin, nervous tissue, heart, and arteries.
  - These lesions are called gummas and are very destructive

Untreated Syphilis - Outcome

1/3 of untreated patients will proceed to tertiary syphilis

Treatment

- Treatment is available, effective and relatively inexpensive
- Penicillin is the drug of choice (84-97% effective)
- (Tetracycline or Doxycycline if allergic to penicillin)
- Follow-up includes clinical evaluation at 1 to 2 weeks followed by clinical and serologic evaluation at 3, 6, 9, 12, and 24 months after treatment
- Drug therapy can eliminate vertical transmission
- No vaccine is available

Who should be tested?

- Screen High-Risk Individuals
  - Those who engage in high-risk sexual behavior
  - Persons with other sexually transmitted disease
  - Men who have sex with men
  - Commercial sex workers
  - Those who exchange sex for drugs
  - Incarcerated adults

Who Should be Tested for Syphilis?

- Screen pregnant women
  - All pregnant women should be tested at their first prenatal visit, with repeat serologic testing in the third trimester in high-risk groups.
- Screen high-risk individuals
  - Populations at increased risk include those who engage in high-risk sexual behavior, persons diagnosed with other sexually transmitted disease, men who have sex with men, commercial sex workers, those who exchange sex for drugs, and incarcerated adults.
Timing of Testing

For Pregnant Women
• First visit (ideally first trimester)
• Third trimester
• At delivery

Neonates and all Others
• As soon as suspected

Laboratory Evaluation for Syphilis: Serology

Laboratory Evaluation: Dark Field Microscopy

T. pallidum is a spirochete that is difficult to culture
One way of diagnosing syphilis is through direct microscopic detection of the organism
When unstained, T. pallidum cannot be seen with standard bright-field microscopy due to its small cell diameter
The spirochete is best seen with dark-field microscopy

Laboratory Evaluation: Molecular

PCR has been used to detect T. pallidum
PCR has been used for the identification of congenital and early syphilis
It has also been used for the diagnosis of neurosyphilis

Laboratory Evaluation for Syphilis: Serology

Two Types of Serology Tests

Treponemal Tests
Direct detection of T. pallidum antigens

Non-Treponemal Tests
Detection of antiphospholipid antibodies (IgG and IgM) formed by the host in response to infection
Nontreponemal and Treponemal Tests

<table>
<thead>
<tr>
<th>Nontreponemal</th>
<th>Treponemal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venereal Research Disease Laboratory (VDRL)</td>
<td>Enzyme Immunoassay (EIA)</td>
</tr>
<tr>
<td>Rapid Plasma Reagent (RPR)</td>
<td>Fluorescent Treponemal Antibody Absorption (FTA-ABS)</td>
</tr>
<tr>
<td>Unheated Serum Reagent (USR)</td>
<td>Treponema pallidium Particle Agglutination (TP-PA)</td>
</tr>
<tr>
<td>Toluidine Red Unheated Serum Test (TRUST)</td>
<td>Western Blot</td>
</tr>
</tbody>
</table>

Laboratory Testing: Nontreponemal Tests

**ADVANTAGES**
- Detects antilipoidal IgG and IgM (released by damaged cells/treponemes)
- Useful for general screening (qualitative)
- Indicates active infection
- Useful for monitoring response to therapy (quantitative)
- Has the ability to detect re-infection

**DISADVANTAGES**
- False positive rate of about 1-2% in the general population
- Potential false negatives due to hook-effect seen in early primary and latent syphilis
- Low PPV in low prevalence populations
- Temperature sensitive (false positives)
- Manual procedure (time, cost, errors)

Laboratory Testing: Treponemal Tests

**ADVIA Centaur Syphilis Assay**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Syphilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection</td>
<td>Qualitative</td>
</tr>
<tr>
<td>Peak Size (tests)</td>
<td>200T</td>
</tr>
<tr>
<td>Design</td>
<td>1 step</td>
</tr>
<tr>
<td>Sample Types</td>
<td>Serum/Plasma</td>
</tr>
<tr>
<td>Sample Size (µL)</td>
<td>100 µL</td>
</tr>
<tr>
<td>Turn-Around Time</td>
<td>29 min</td>
</tr>
<tr>
<td>Calibration</td>
<td>2 point</td>
</tr>
<tr>
<td>On-Board Shelf-life</td>
<td>60 days</td>
</tr>
<tr>
<td>Calibration Interval</td>
<td>28 days</td>
</tr>
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</table>
ADVIA Centaur Syphilis Assay
Performance – Total Study Population

<table>
<thead>
<tr>
<th>ADVA Centaur</th>
<th>Comparative Method 1</th>
<th>Non-Reactive</th>
<th>Reactive</th>
<th>Equivocal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive</td>
<td>703</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>707</td>
</tr>
<tr>
<td>Equivocal</td>
<td>2</td>
<td></td>
<td></td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Non-Reactive</td>
<td>1434</td>
<td></td>
<td>1</td>
<td>0</td>
<td>1437</td>
</tr>
<tr>
<td>Total</td>
<td>2107</td>
<td></td>
<td>1</td>
<td>0</td>
<td>2108</td>
</tr>
</tbody>
</table>

Sensitivity = 99.7% and Specificity = 99.5%

<table>
<thead>
<tr>
<th>ADVA Centaur</th>
<th>Comparative Method 2</th>
<th>Non-Reactive</th>
<th>Reactive</th>
<th>Equivocal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive</td>
<td>266</td>
<td>9</td>
<td></td>
<td>0</td>
<td>275</td>
</tr>
<tr>
<td>Equivocal</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Non-Reactive</td>
<td>572</td>
<td></td>
<td>1</td>
<td>0</td>
<td>573</td>
</tr>
<tr>
<td>Total</td>
<td>581</td>
<td></td>
<td>1</td>
<td>0</td>
<td>583</td>
</tr>
</tbody>
</table>

Sensitivity = 99.6% and Specificity = 99.5%

ADVIA Centaur Syphilis Assay
Performance – Apparently Healthy Population

1. Pregnant

<table>
<thead>
<tr>
<th>Population</th>
<th>Reactive</th>
<th>Equivocal</th>
<th>Non-Reactive</th>
<th>Total</th>
<th>Resolved</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone</td>
<td>330</td>
<td>0</td>
<td>8</td>
<td>349</td>
<td>100%</td>
<td>99.9%</td>
</tr>
<tr>
<td>Pediatric</td>
<td>77</td>
<td>0</td>
<td>66</td>
<td>143</td>
<td>100%</td>
<td>99.5%</td>
</tr>
<tr>
<td>A.B.B. not Pregnant</td>
<td>396</td>
<td>0</td>
<td>6</td>
<td>408</td>
<td>100%</td>
<td>99.4%</td>
</tr>
<tr>
<td>Total</td>
<td>476</td>
<td>0</td>
<td>81</td>
<td>557</td>
<td>100%</td>
<td>99.4%</td>
</tr>
</tbody>
</table>

2. Medically Diagnosed

<table>
<thead>
<tr>
<th>Population</th>
<th>Reactive</th>
<th>Equivocal</th>
<th>Non-Reactive</th>
<th>Total</th>
<th>Resolved</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult not Pregnant</td>
<td>234</td>
<td>0</td>
<td>10</td>
<td>244</td>
<td>100%</td>
<td>96.2%</td>
</tr>
<tr>
<td>Total</td>
<td>236</td>
<td>0</td>
<td>102</td>
<td>248</td>
<td>100%</td>
<td>96.2%</td>
</tr>
</tbody>
</table>

Sensitivity = 99.6% and Specificity = 99.5%

ADVA Centaur Syphilis Assay
Performance – Expected Positive Population

1. TPPA Reactive

<table>
<thead>
<tr>
<th>Population</th>
<th>Reactive</th>
<th>Equivocal</th>
<th>Non-Reactive</th>
<th>Total</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone</td>
<td>221</td>
<td>1</td>
<td>0</td>
<td>222</td>
<td>99.5%</td>
</tr>
<tr>
<td>Pediatric</td>
<td>217</td>
<td>0</td>
<td>5</td>
<td>222</td>
<td>99.3%</td>
</tr>
<tr>
<td>A.B.B. not Pregnant</td>
<td>261</td>
<td>1</td>
<td>0</td>
<td>262</td>
<td>99.5%</td>
</tr>
<tr>
<td>Total</td>
<td>503</td>
<td>2</td>
<td>5</td>
<td>508</td>
<td>99.2%</td>
</tr>
</tbody>
</table>

2. Medically Diagnosed

<table>
<thead>
<tr>
<th>Population</th>
<th>Reactive</th>
<th>Equivocal</th>
<th>Non-Reactive</th>
<th>Total</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult not Pregnant</td>
<td>230</td>
<td>0</td>
<td>3</td>
<td>233</td>
<td>99.7%</td>
</tr>
<tr>
<td>Total</td>
<td>233</td>
<td>0</td>
<td>3</td>
<td>236</td>
<td>99.7%</td>
</tr>
</tbody>
</table>

Sensitivity = 99.6% and Specificity = 99.5%

Laboratory Testing: Treponemal Tests

**ADVANTAGES**

- T. Pallidum specific antibodies (qualitative)
- Indicates Treponemal infection both past and present
- Useful for screening in low prevalence populations
- Automated EIA testing options are available

**DISADVANTAGES**

- Only used qualitatively

The Treponemal test is positive for life in most patients

- Up to 85% of successfully treated patients remain positive by treponemal tests

Time after exposure (days, weeks, months, years)
**Why Run both Tests? Role of Non-treponemal Tests**

- A positive Non-treponemal test suggests
  - This patient may have syphilis or some other cause for tissue damage
  - The process is active, they could benefit from treatment
  - But the unanswered question is: Do they really have syphilis?

**Why Run both Tests? Role of Treponemal Tests**

- A positive Treponemal test suggests
  - This patient has/had syphilis
  - This patient has another treponemal infection
  - But the unanswered questions are
    - Is this infection active, resolved, or acute?
    - Is this reinfection?
    - Were they treated?

**Why both? Each Provides Essential Information**

- Both tests are needed to determine if ...
- The patient is likely (with a high PPV) to have a treponemal or non-treponemal infection
- If they need to be treated for a non-treponemal infection

**Sensitivity and Specificity of Common Tests by Disease Stage**

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity by Stage of Untreated Syphilis (%)</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-treponemal Tests</td>
<td></td>
</tr>
<tr>
<td>VDRL</td>
<td>Primary 78</td>
<td>Secondary 100</td>
</tr>
<tr>
<td>RPR Card</td>
<td>86</td>
<td>100</td>
</tr>
<tr>
<td>USR</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>TRUST</td>
<td>85</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Treponemal Tests</td>
<td></td>
</tr>
<tr>
<td>FTA-ABS</td>
<td>84</td>
<td>100</td>
</tr>
<tr>
<td>MHA-TP</td>
<td>76</td>
<td>100</td>
</tr>
<tr>
<td>FTA-ABS DS</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>TP-PA</td>
<td>88</td>
<td>100</td>
</tr>
</tbody>
</table>

**Untreated Syphilis: Treponemal vs. Non-treponemal Tests and Disease Stage**

- In late disease (latent and tertiary) nontreponemal tests can be negative

**Nontreponemal Serological Profiles: Untreated**

RPR a Nontreponemal Test

Macroscopic flocculation test

Reactive

Nonreactive

RPR: Each patient sample must be spotted to card

RPR Card (Rapid Plasma Reagin)

Each spot must be visually examined to determine results and interpretation recorded

RPR Advantages and Disadvantages

<table>
<thead>
<tr>
<th>ADVANTAGES</th>
<th>DISADVANTAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indicates active infection</td>
<td>Manual test</td>
</tr>
<tr>
<td>Widely accepted</td>
<td>Causes of False Positives</td>
</tr>
<tr>
<td>Useful to monitor therapy</td>
<td>Acute viral infections</td>
</tr>
<tr>
<td>Able to detect re-infection</td>
<td>Autoimmune disease</td>
</tr>
<tr>
<td>Inexpensive</td>
<td>IV drug abuse</td>
</tr>
</tbody>
</table>

Traditional Serology Screening

For syphilis screening, a new reflex testing algorithm

For syphilis screening, a new reflex testing algorithm

Nancy Cortin, MD, The Pathology Center. Director

August 2007

Screening with a Treponemal Test

CDC MMWR

Improved Serology Screening

Un-diagnosed tertiary syphilis

Up to 30% may not be reactive in non-treponemal tests, whereas treponemal tests are almost always reactive.
Results of Reverse Sequence Syphilis Screening

- **EIA Non-Reactive**: 135,242 (96.6%)
- **EIA Reactive**: 4,834 (3.4%)
- **RPR Non-Reactive**: 2,743 (56.7%)
- **RPR Reactive**: 2,091 (43.3%)
- **TP-PA or FTA-ABS Non-Reactive**: 866 (41.4%)
- **TP-PA or FTA-ABS Reactive**: 1,225 (58.6%)

Treponemal screening advantages may include:
- Detection of Previously Undiagnosed Disease
- Treatment can prevent late-stage complications
- Treatment can reverse gummas

Why Screen with an EIA Treponemal Test?

Journal Watch Infectious Disease, August 27, 2008

“Adoption of the new algorithm may reduce undiagnosed cases of latent syphilis associated with false negative results on traditional non-treponemal screening tests.”

Reverse Algorithm Example

- **Early or No Syphilis**: Nonreactive
- **Indeterminate**: TP-PA or FTA-ABS
- **Likely Syphilis**: Reactive
- **Performs Titer**: TP-PA or FTA-ABS
- **No Syphilis**: Nonreactive

Automated Treponemal EIA Advantages

- **Diagnosis**: Can diagnose cases missed by non-treponemal tests, e.g., latent and tertiary syphilis
- **Workflow**: Significant reduction in labor time and labor costs
- **Automation allows for increased test volume and faster turnaround time**
- **Decreased opportunity for error / subjective interpretation**
Syphilis infections are increasing

Treponemal and non-Treponemal tests are important in the accurate diagnosis of syphilis

Treponemal tests can be successfully used as a first-line screening test

Treponemal tests can detect latent and tertiary cases that may be missed by non-Treponemal tests

Automated Treponemal tests can offer significant workflow and productivity advantages