Session III
Syphilis

1. Syphilis Testing Overview – Megan Crumpler
2. Syphilis Automation – Mayur Shukla
3. Syphilis Case Studies and Discussion – Eric Tang
Syphilis Testing Overview

HIV, HCV and Syphilis Diagnostic Testing Workshop
ID Lab Con 2023
March 12, 2023
Megan Crumpler, PhD, HCLD
Outline

1. General Syphilis Overview and Statistics
2. Review Stages of Syphilis
3. Review Syphilis Testing Algorithms
4. Overview of Syphilis Testing Procedures
5. Syphilis Reporting
Syphilis Overview

- Sexually transmitted infection caused by the spirochete *Treponema pallidum*
- Spread by:
  - direct contact with a syphilis sore during vaginal, anal, or oral sex
  - Infected mother to fetus
- Causes a systemic infection and might lead to serious sequelae in multiple organ systems, including the CNS
- Vertical transmission can cause congenital syphilis
  - May results in spontaneous abortions, miscarriages, or stillbirths
  - infants with congenital syphilis can present with clinical signs of infection at birth or months to years after birth
- Treated with single or three weekly IM injections of benzathine penicillin G, depending on stage of infection
Syphilis — Rates of Reported Cases by Stage of Infection, United States, 1941–2020

Rate* (per 100,000)

Year


Total Syphilis
Early Non-Primary
Non-Secondary
Primary and
Secondary

Content source: Division of STD Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention
Syphilis (All Stages) — Reported 2020 Cases as a Percentage of 2019 by MMWR Week, United States

Percentage of Previous Year
(2020 ÷ 2019)

NOTE: The MMWR week is the week of the epidemiologic year for which the case is assigned by the reporting local or state health department.

Adapted from Pagaoa et al, Sexually Transmitted Diseases, 2021
Primary and Secondary Syphilis — Rates of Reported Cases by County, United States, 2020

Rate* * Per 100,000

- 0.0
- 0.1–5.2
- 5.3–11.0
- 11.1–142.4

Content source: Division of STD Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention
Congenital Syphilis — Reported Cases by Year of Birth and Rates of Reported Cases of Primary and Secondary Syphilis Among Women Aged 15–44 Years, United States, 2011–2020

ACRONYMS: CS = Congenital syphilis; P&S = Primary and secondary syphilis

* Per 100,000
## Stages of Syphilis

### Table 1. Stages, Time Course, and Manifestations of Syphilis

<table>
<thead>
<tr>
<th>Stage</th>
<th>Time</th>
<th>Manifestations</th>
<th>Uncommon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>10 to 90 days</td>
<td>Chancre</td>
<td>Local lymphadenopathy</td>
</tr>
<tr>
<td>Secondary</td>
<td>1 to 3 months</td>
<td>Arthralgia, condylomata lata, fatigue, generalized lymphadenopathy, headache, maculopapular/papulosquamous exantheme, myalgia, pharyngitis</td>
<td>Annular syphilis, iritis, pustular syphilis, pyrexia, syphilitic alopecia, ulceronodular syphilis</td>
</tr>
<tr>
<td>Early latent</td>
<td>After primary or secondary stages, 1 year or less of no symptoms</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Late latent</td>
<td>More than 1 year of no symptoms</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Tertiary</td>
<td>Months to years</td>
<td>Late neurosyphilis*</td>
<td>Cardiovascular syphilis, gummatous syphilis</td>
</tr>
</tbody>
</table>

*—Neurosyphilis may occur at any stage of infection.

Information from reference 5.

Syphilis Diagnostic Tests
Syphilis Darkfield

- Definitive method for diagnosing syphilis
- Examine exudate from lesion with darkfield microscope
- Rarely performed
  - Slide must be ready within 30 minutes of collection
  - Training and experience necessary
  - Cannot be used for oral specimens
Common Syphilis Serology Tests

• Non-treponemal tests
  – VDRL (Venereal Disease Research Laboratory)
  – RPR (Rapid Plasma Reagin)

• Treponemal tests
  – TP-PA (Treponema Pallidum Particle Agglutination)
  – FTA-abs (Fluorescent Treponemal Antibody - Absorbed)
  – Immunoassay
Coming soon!

- *Laboratory Recommendations for Syphilis Testing in the United States*
- CDC, Division of STD Prevention
- In 2017 APHL assisted with the literature review for development of evidence-based recommendations for syphilis testing in the US
- Will be posted for public comment period soon
Syphilis stages & possible test results*

- **Primary Chancre**
  - Dark Field +
  - RPR +/-
  - VDRL+/-

- **Secondary Eruptions**
  - RPR +
  - VDRL+
  - TP-PA+
  - AIA+
  - FTA+

- **Tertiary Disease**
  - RPR +/-
  - VDRL+/-
  - TP-PA+
  - AIA+
  - FTA+

- 10-90 days
- 6 weeks to 6 months
- 10-30 years after primary (Period of latency)

*excludes CSF

*Courtesy: Michael Pentella, PhD*

https://labtestsonline.org/tests/syphilis-tests
## Table 3. Sensitivity and Specificity of Commonly Used Syphilis Tests

<table>
<thead>
<tr>
<th>Syphilis screening test</th>
<th>Mixed</th>
<th>Sensitivity by stage of untreated syphilis, % (range)†</th>
<th>Specificity % (range)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Primary</td>
<td>Secondary</td>
</tr>
<tr>
<td><strong>Nontreponemal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VDL†</td>
<td>79 (71-97)</td>
<td>100</td>
<td>96 (88-100)</td>
</tr>
<tr>
<td>RPR†</td>
<td>86 (77-99)</td>
<td>100</td>
<td>98 (95-100)</td>
</tr>
<tr>
<td>TRUST†</td>
<td>85 (77-86)</td>
<td>100</td>
<td>98 (95-100)</td>
</tr>
<tr>
<td>USR†</td>
<td>80 (72-88)</td>
<td>100</td>
<td>95 (88-100)</td>
</tr>
<tr>
<td><strong>Treponemal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FTA-ABS†</td>
<td>84 (70-100)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>TPPA†</td>
<td>88 (86-100)</td>
<td>100</td>
<td>97 (97-100)</td>
</tr>
<tr>
<td>EIA:</td>
<td>(77-100)</td>
<td>(85-100)</td>
<td></td>
</tr>
<tr>
<td>Trep-Chek</td>
<td>95.9</td>
<td>96.9</td>
<td></td>
</tr>
<tr>
<td>Trep-Sure</td>
<td>95.9</td>
<td>96.9</td>
<td></td>
</tr>
<tr>
<td>CIA:</td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>LIAISON†</td>
<td>99.2</td>
<td>98</td>
<td>100</td>
</tr>
<tr>
<td>MFI:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BioPlex 2200</td>
<td>96.9</td>
<td>98.5**</td>
<td></td>
</tr>
<tr>
<td>Syphilis IgG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syphilis Health Check</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* This is not a comprehensive list of tests available in the United States.
† Sensitivity and specificity of tests also depends on the disease prevalence in the population tested, and may vary considerably by manufacturer or the standard used as a comparison.
‡ Unknown reference standard.
§ When compared with FTA-ABS test results.
‖ When compared with results from western blotting.
¶ When compared with nontreponemal test results.
** When compared with treponemal test results.
Traditional Syphilis Testing Algorithm

Figure 1: Traditional Syphilis Serology Testing Algorithm

- **Nontreponemal** (e.g., RPR or VDRL)
  - Reactive
    - Treponemal (e.g., TP-PA)
      - Reactive
        - Consistent with past or current (potentially early) syphilis
      - Nonreactive
        - Syphilis unlikely; biological false positive possible
  - Nonreactive
    - No laboratory evidence of syphilis

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a. Perform a quantitative nontreponemal test to determine the end-point titer. b. Clinical correlations, including past titer(s), is necessary to determine whether the infection is past, current or potentially early.
Reverse Syphilis Testing Algorithm

Figure 2: Reverse Syphilis Serology Testing Algorithm

- Treponemal (e.g., EIA, CIA, CMIA or MBIA)
  - Reactive
    - Nontreponemal (e.g., RPR or VDRL)
      - Reactive
        - Consistent with current or past syphilis
      - Nonreactive
        - Treponemal
          - Reactive
            - Consistent with past or current (potentially early) syphilis
          - Nonreactive
            - Inconclusive for syphilis
        - Nonreactive
          - No laboratory evidence of syphilis

Abbreviations:
- Immunoassay
- RPR/VRDL
- TP-PA/FTA-ABS
Nontreponemal Syphilis Assays

• Detect “reagin”, a non-specific antibody-like substance present in serum or plasma in persons infected with syphilis

• Both qualitative and quantitative

• Used for:
  — Screening
  — Evaluation of patients with symptoms or possible re-infection
    — 4 fold rise in titer indicates reinfection
  — Follow-up assessment after treatment
    — 4-fold drop in titer
Venereal Disease Research Laboratory (VDRL)

- Flocculation test, antigen consists of very fine particles that precipitate out in the presence of reagin.
  - Antigen must be made up fresh daily and is very dependent on proper technique.
- Acceptable specimens: serum or CSF
  - Only FDA-approved test available for CSF specimens to test for neurosyphilis
  - Serum must be heated to 56 C for 30 minutes to remove anti-complementary activity which may cause false positive
VDRL

- Read microscopically at 100x and grade reaction if positive.
- Perform titer on positive samples, report out titer.
Rapid Plasma Reagin (RPR) Test

- RPR antigen suspension is a carbon particle cardiolipin antigen which detects "reagin"
  - The reagin binds to the test antigen, is bound to charcoal, causing macroscopic flocculation.
- Antigen is more stable than VDRL and does not need to be made daily
- Acceptable specimens: serum and plasma
- Read with naked eye under a high intensity incandescent lamp or strong daylight.
- Qualitative and quantitative:
  - Used to monitor patient titer in response to treatment
- Instruments for automation are now available
Rapid Plasma Reagin (RPR) Test

• Reading and reporting:
  • Reactive - showing characteristic clumping ranging from slight but definite (minimum-to-moderate) to marked and intense.
    • Reactive minimal-to-moderate (showing slight, but definite clumping) is always reported as Reactive
  • Nonreactive - Showing no clumping. Note: There are only two possible final reports with the Card Test: Reactive or Nonreactive, regardless of the degree of reactivity.

From ASI Package Insert:
Nontreponemal Test Limitations

• False negative due to prozone
  • occurs in 1% to 2% of patients with secondary syphilis
  • may exhibit a nonreactive pattern that is slightly granular or “rough”
  • Should be repeated using an alternative procedure and/or titer for quantification.

• Biological False Positives
  • infectious mononucleosis, leprosy and malaria, lupus erythematosus, vaccinia and virus pneumonia.
  • pregnancy, narcotic addiction and autoimmune diseases
  • Pinta, yaws, bejel and other treponemal diseases
  • Typically below a titer of 1:8

• Lipemic or hemolyzed specimens: as a general rule, should be able to read newsprint through the serum.
Treponemal Syphilis Assays

• Measure antibody (IgM and/or IgG) directed against *T. pallidum* antigens

• Qualitative

• Reactivity can persist over lifetime

• More expensive and labor intensive

• Can not quantitate - not useful for following response to treatment

• Traditionally used for confirmation
CLIA-Waived Rapid Testing

• Two FDA-cleared, CLIA-waived for the detection of *T. pallidum* antibodies:
  • Syphilis Health Check (Trinity Biotech)
  • Dual Path Platform (DPP) HIV-Syphilis assay (Chembio Diagnostics, Inc)
    • Requires DPP Micro Reader optical analyzer

• Could be utilized as the treponemal test in a syphilis testing algorithm to expedite results
FUJIREBIO SERODIA TP-PA

- Qualitative gelatin particle agglutination assay intended to be used for the detection of *Treponema pallidum* antibodies

- Acceptable specimens: Serum and EDTA, Sodium citrate or heparin plasma

- Visually observe the pattern of agglutination in each well.
  - A plate viewer may be used with indirect lighting.

- Ensure each of the Unsensitized Particle wells is non-reactive and interpret the agglutination pattern of the Sensitized Particles (next slide)
<table>
<thead>
<tr>
<th>Settling Patterns of Particles</th>
<th>Reading</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particles are concentrated in the shape of a button at the center of the well with a smooth round outer margin.</td>
<td>( - )</td>
<td>Non- Reactive</td>
</tr>
<tr>
<td>Particles are concentrated in the shape of a compact-ring with a <strong>very small</strong> &quot;hole&quot; in the center and a smooth round outer margin.</td>
<td>( - )</td>
<td>Non- Reactive</td>
</tr>
<tr>
<td>Particles are concentrated in the shape of a compact ring with a &quot;hole&quot; in the center and a smooth round outer margin.</td>
<td>( ± )</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>Defined large ring with a rough multiform outer margin and peripheral agglutination.</td>
<td>( + )</td>
<td>Reactive</td>
</tr>
<tr>
<td>Agglutinated particles spread out covering the bottom of the Well uniformly, edges sometimes folded.</td>
<td>( ++ )</td>
<td></td>
</tr>
</tbody>
</table>
FTA-ABS

- Alternative to TP-PA as confirmatory assay
- Serum is layered on slide that is coated with *T. pallidum* and FITC-labeled antihuman IG is added
- Requires experienced microscopist
- Subjective interpretation
Syphilis Immunoassays

- Qualitative assays that detect anti-treponemal IgM and/or IgG antibodies
- Most are on automated platforms
- Examples:
  - Bio-Rad Syphilis IgG on the EVOLIS
  - Abbott Syphilis TP on the Architect
  - Elecsys Syphilis on the Cobas (IgM and IgG)
- Commonly used as the 1st test in the reverse algorithm
Reporting Language for Syphilis Serological Testing

Use this slide to transition between topics
Syphilis Reporting

- With multiple tests being reported for the same specimen, it is important to review all results before reporting.
- Look for discrepancies.
  - For example:
    - RPR Reactive, TPPA Negative
    - RPR Nonreactive, TPPA positive
- If possible, refer to previous result
  - Use this to identify discrepancies
    - TP-PA previous positive, now negative
    - RPR titer increase from previous result when patient has been treated

Traditional Syphilis Testing Algorithm

- Qualitative Non-Treponemal
  - Reactive
    - RPR/VRDL
  - Non-reactive

- Quantitative Non-Treponemal
  - Treponemal
    - Reactive
      - Consistent with current syphilis infection*
    - Non-reactive
      - Syphilis infection unlikely; biological false positive likely
      - No laboratory evidence of syphilis infection

*If titer <1:4 consider these values associated with possible serofast condition. Serofast is used to refer to those persons with early syphilis with non-treponemal titers that neither increase nor decrease 4-fold after treatment.
Reverse Syphilis Testing Algorithm

1. Treponemal (Immunoassay)
   - Reactive
   - Non-reactive

2. Non-Treponemal
   - Reactive
   - Non-reactive

3. Supplemental Treponemal*
   - Reactive
   - Non-reactive

Lab Interpretation:
- Consistent with current or past syphilis infection
- Consistent with past or potential early syphilis infection
- Inconclusive for syphilis infection; potentially early infection or false positive
- No laboratory evidence of syphilis infection

Tests:
- RPR/VRDL
- TP-PA/FTA-ABS
Questions and Discussion