Serologic Testing for Syphilis

Comparison of the Traditional and Reverse Screening Algorithms

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Disclosures

None
Objectives

• Describe the treponemal and non-treponemal assays for syphilis screening

• Discuss the advantages and limitations of both the traditional and reverse syphilis screening algorithms

• Result interpretation from the reverse syphilis screening algorithm
Outline

• Syphilis Infection
  • Causative Agent
  • Clinical Manifestations

• Laboratory Tests for Diagnosis of Syphilis
  • Non-treponemal Tests
  • Treponemal Tests

• Traditional Algorithm for Syphilis Screening

• Reverse Algorithm for Syphilis Screening

• Interpretation and Follow-up
**Treponema pallidum - The Agent of Syphilis**

- Spirochete
- Obligate human parasite
- Transmission
  - Sexual
  - Trans-placental
  - Percutaneous following contact with infectious lesions
  - Blood Transfusion
    - No reported cases of transmission since 1964
Syphilis - The “Great Imitator”

• Infectious Dose: ~57 organisms¹
• Incubation Period – 21 days (median)
• 3 clinical stages of syphilis
  • Primary:
    • Painless sore (chancre) at inoculation site
  • Secondary:
    • Rash, Fever, Lymphadenopathy, Malaise
  • Tertiary/ Latent:
    • CNS invasion, organ damage
• “The physician that knows syphilis knows medicine.”
  – Sir William Osler

http://www.cdc.gov/std/syphilis/stdfact-syphilis.htm
Laboratory Diagnosis of Syphilis

The Uncommon Methods

• Rabbit Infectivity Test (RIT)
  • High Sensitivity and Specificity
  • Long turn-around-time
  • Limited to research settings

• Dark Field Microscopy
  • Useful only during primary infection
  • Technician expertise required

• Immunostaining
  • Direct fluorescent antibody or silver stain

• Polymerase Chain Reaction (PCR)
  • Not commercial available
Laboratory Diagnosis of Syphilis
The Common Methods

• Serology
  • Mainstay for syphilis testing
  • Two classes of serologic tests
    • Non-treponemal
    • Treponemal
Serologic Tests for Syphilis: Non- Treponemal Assays

• Principle:
  • *T. pallidum* infection leads to the production of reagin
    • Reagin – Antibodies to substances released from cells damaged by *T. pallidum*
    • Reagin reacts with cardiolipin
    • Cardiolipin – a phospholipid component of certain eukaryotic and prokaryotic membranes

• Examples of non- treponemal tests:
  • Rapid Plasma Reagin (RPR)
  • Venereal Disease Research Laboratory (VDRL)
Serologic Tests for Syphilis: Non-Treponemal Assays

- RPR and VDRL are agglutination assays
Serologic Tests for Syphilis:
Non- Treponemal Assays

• RPR and VDRL are agglutination assays
Non-Treponemal Tests: Advantages

- Rapid turnaround time – Minutes
- Inexpensive
- No specialized instrumentation required
- Usually revert to negative following therapy
  - Can be used to monitor response to therapy
Non-Treponemal Tests: Limitations

- Results are subjective
  - Intra- and Inter-laboratory variability
- Non-specific
  - False positive results can result from other infectious or non-infectious conditions
    - EBV, Lupus, etc.
- Limited sensitivity in early/primary syphilis and in late/latent syphilis
- Low throughput
  - Problematic for high volume laboratories
Non- Treponemal Tests:
Limitations, continued

- Possibility for prozone effect
  - High levels of antibody may inhibit the agglutination reaction
  - To identify prozone, labs must serially dilute samples

Images:
- Undilute
- 1:2
- 1:4
- 1:8
- 1:16
Serologic Tests for Syphilis: Treponemal Assays

• Principle:
  • Infection leads to production of specific antibodies directed against *T. pallidum*

• Treponemal tests detect IgG or total IgM/IgG antibodies directed against *T. pallidum*
Serologic Tests for Syphilis: Treponemal Assays

- Microhemagglutination assay (MHA)
- Fluorescent treponemal antibody (FTA-ABS)
- *Treponema pallidum* particle agglutination (TP-PA)
- Enzyme Immunoassay (EIA)
- Multiplex Flow Immunoassay (MFI)
Treponemal Assays: Multiplex Flow Immunoassays
Treponemal Assays:
Multiplex Flow Immunoassays

Labeled anti-IgM and anti-IgG reporter antibody added

Patient Serum Added
Treponemal Assays: Multiplex Flow Immunoassays

Bound beads are passed through the laser detector

Labeled anti-IgM and anti-IgG reporter antibody added

Patient Serum Added

Syphilis IgM

Syphilis IgG

Laser 1 identifies the bead (IgM vs. IgG)

Laser 2 determines if the target antibody is present (presence or absence of fluor)
Treponemal Assays: Advantages

• High Specificity

• Possibly higher sensitivity during early and late syphilis stages compared to non-treponemal tests

• Newer Methods
  • Objective result interpretation
  • Automation option
  • High throughput
  • High reproducibility/precision
Treponemal Assays: Limitations

• Remain positive despite treatment
  • *Cannot* be used to monitor response to therapy

• Conventional Methods
  • Subjective interpretation requiring technician expertise to read

• Newer Methods
  • Expensive instrumentation
  • Higher cost/test
Syphilis Screening Algorithms: Traditional versus Reverse Screening
Traditional Algorithm

Non-treponemal test (e.g., RPR)
- Reactive
  - Treponemal test (e.g., FTA)
    - Reactive
      - Syphilis
    - Non-reactive
      - Negative for syphilis
- Non-reactive
  - Negative for syphilis
Traditional Algorithm

Non-treponemal test (e.g., RPR)

Reactive

Treponemal test (e.g., FTA)

Reactive

Syphilis

Non-reactive

Non-reactive

Negative for syphilis

Advantages:

- Results show good correlation with disease status
- Rapid, inexpensive screening method
- Excellent option for laboratory with small throughput
- Recommended by the CDC
Disadvantages:

- Manual (RPR) and subjective interpretation
- Screening method is non-specific and may lead to false-positive results
- Not suitable for high throughput laboratories
- Potentially lower sensitivity for detecting early syphilis and late/latent disease
The Traditional Syphilis Algorithm: If it works, why change it?

- Incidence of disease impacts the positive predictive value of the assay
Reverse Algorithm

- Treponemal test (eg, EIA)
  - Reactive
  - Non-reactive

- Non-Treponemal test (eg, RPR)
  - Negative for syphilis

- Non-reactive

- Reactive
  - Syphilis

- Reactive
  - Second Treponemal Test (e.g., TP-PA)
    - Reactive
    - Non-reactive

    - Evaluation Required*
Reverse Algorithm:

Advantages

- Automated treponemal screening assays are available (i.e., EIA, MFI)$^2$
  - > 500 sera/9 hr shift by MFI vs. ~200 sera/9 hr shift by manual methods
- Objective interpretation of results
- Results from EIA or MFI can be interfaced with LIS
- Specific screening test for anti- *T. pallidum* antibodies
- Potentially increased detection of patients with early syphilis$^3$:
  - Among 560 patients with lesions, 18 (3.2%) were EIA (+), DFA (+) and RPR (-)
  - Among 9,137 patients with EIA (+), RPR (-) results, 54 became RPR (+) on follow-up testing
Reverse Algorithm: Limitations

- Higher cost/sample
- Higher assay complexity
- Increased detection of patients with screen (+), RPR (-) results:
  - CDC: ~56% of EIA reactive samples are non-reactive by RPR
  - How do we interpret these results?
Case #1

- 37-year-old with HIV
- Presents to primary care physician with a 2-week history of fatigue, intermittent fever and new rash on palms and soles
- Previously resolved genital lesion
- Syphilis serology ordered
  - Syphilis IgG by EIA: positive
  - RPR: positive, titer of 1:64
Case #1 Conclusion

- No further testing needed on this sample
- **Interpretation:** “Untreated or recently treated syphilis.” Follow CDC treatment guidelines

For treatment follow-up:

- Samples can be tested directly by RPR.
- A 4-fold decrease in RPR titers (eg, 1:64 to 1:16) is interpreted as response to therapy
Case #2

- 23-year-old female
- Evaluated during first-trimester, routine pregnancy visit
- Previously healthy
- Syphilis serology ordered
  - Syphilis IgG by EIA: positive
  - RPR: negative
  - Second treponemal test, TP-PA: negative
Case #2 Conclusion

• **Interpretation**: “Probable false-positive screening test. Negative for syphilis.”

• False-positive serologic tests are not uncommon during pregnancy and confirmatory testing is often required

• Syphilis IgM testing **not** recommended for routine pregnancy screening
Case #3

• 50-year-old immigrant from Somalia
• Pre-kidney transplant evaluation
• Syphilis serology ordered
  • Syphilis IgG by EIA: positive
  • RPR: negative
  • TP-PA: positive
Case #3 Conclusion

• **Interpretation**: “Historical and clinical evaluation required.”

• During evaluation with provider, patient indicates no *known* history of treatment for syphilis.

• Patient treated for *possible* latent syphilis
Case #4

- 30-year-old inmate
- Past history of syphilis (10 years prior)
- Syphilis serology ordered
  - Syphilis IgG by EIA: positive
  - RPR: negative

- Interpretation: “Past, successfully treated syphilis. No further testing for syphilis required.”
### Reverse Syphilis Screening Algorithm: Summary

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Conclusions

• Syphilis is typically diagnosed by serologic means

• Two main classes of syphilis serologic tests:
  • Non-treponemal (e.g., RPR, VDRL)
  • Treponemal (e.g., FTA, TP-PA, EIA, MFI)

• Traditional Algorithm
  • Non-treponemal test first
    • Screen by RPR
    • If RPR positive use treponemal test to confirm
  • Advantages
    • Recommended by CDC
    • Cost-effective
    • Suitable for most lower throughput labs
  • Limitations
    • May miss very early or late/latent infection
Conclusions

• Reverse Algorithm
  • Treponemal test first
    • Screen by EIA or MFI
    • Screen positive samples tested by non-treponemal test: RPR
    • EIA/MFI and RPR discordant samples should be tested by a second treponemal test: TP-PA
  • Advantages
    • Allows for automation and increased sample throughput
  • Limitations
    • Result interpretation can be challenging
    • Good communication with providers is critical
References


Questions & Discussion