SeroLogic Testing for Syphilis with Traditional and Reverse Algorithms

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You’ll leave knowing something about…

- “Treponemal” and “non-treponemal” tests for syphilis

- “Traditional” and “reverse syphilis” screening

- Rapid diagnostic tests for syphilis
Outline

• Syphilis
  • Causative agent
  • Key facts about syphilis

• 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
• New rapid diagnostic test for syphilis
Many slides adapted from...

Rates of early syphilis by sex and year—Oregon, 2000–2015

*for 10 months....will be higher by 25–30%
*Treponema pallidum*

- **Bacterium**
  - “Spirochete”
  - Motile ("corkscrew")
  - Can’t culture in lab
- **Transmission**
  - Sexual
  - Trans-placental
  - Percutaneous following contact with infectious lesions
  - Bloodborn
    - Extremely rare
• Spirochaetes
  • Spirochaetaceae
    • Treponema
      • *pallidum* (syphilis, yaws), *carateum* (pinta), *denticola*
    • Borrelia
      • *burgdorferii/afzelii* (lyme), *hermsii/duttoni/parkeri* (tick-borne relapsing fever)
  • Leptospiaceae
    • Leptospira
      • *interrogans* (leptospirosis)
  • Spirillaceae
    • Spirillum
      • *minus* (rat-bite fever)
Syphilis—a few key concepts

- Highly infectious
  - Infectious Dose ~ 57 organisms
  - Attack rate 1/3

- Incubation – 21 days (median)

- 3 clinical stages
  - Primary:
    - Painless sore (chancre) at inoculation site
  - Secondary:
    - Rash, fever, lymphadenopathy, malaise
  - Symptomatic Late/Tertiary:
    - Dementia, tabes dorsalis, cardiovascular disease
Lab Diagnosis—uncommon methods

Rabbit Infectivity Test (RIT)

Dark field microscopy

Immunostaining

Polymerase Chain Reaction (PCR)
Lab Diagnosis—common methods

• Serology (tests for antibodies produced upon syphilis infection)
  • Mainstay for syphilis testing
  • Two kinds
    • Non-treponemal
    • Treponemal
Non-treponemal serologic tests

- *T. pallidum* causes cells to release cardiolipin
- Reagin = antibody to cardiolipin
- Non-treponemal tests measure levels of reagin:
  - Rapid Plasma Reagin (RPR)
  - Venereal Disease Research Laboratory (VDRL)
  - Toluidine red unheated serum test (TRUST)
RPR and VDRL are agglutination assays

- Reagent is carbon particles + cardioliopin
- No reagin present, no agglutination
Reagin present...agglutination of the charcoal
Non-Treponemal Test 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 Test Limitations

- Results are subjective
  - Intra- and Inter-laboratory variability
- False positives (lower specificity)
  - Lupus, pregnancy, viral hepatitis
- Might be negative (lower sensitivity) in very early syphilis and late syphilis even if never treated
- Low “throughput” = can’t be “batched”
Treponemal serologic tests

- Syphilis $\Rightarrow$ Antibodies against *T. pallidum*
- Tests detect ‘treponeme specific’ antibodies
  - Fluorescent treponemal antibody absorption test (FTA-ABS)
  - Microhemagglutination assay (MHA)
  - *T. pallidum* particle agglutination (TP-PA)
  - Enzyme Immunoassay (EIA)
  - Immunochromatographic strips (ICS…point of care tests)
Treponemal Test Advantages

- Few false positives (high specificity)
- Fewer false negatives (more sensitive) especially during early and late syphilis
- Objective result interpretation
- Automation option
- High throughput = “batchable”
- High reproducibility/precision
Treponemal Test Limitations

- Remain positive for life
  - Cannot be used to monitor response to therapy
- Conventional (older) versions (e.g. FTA-ABS, TP-PA)
  - Subjective interpretation like non-treponemal tests
- Newer versions
  - Expensive instrumentation
  - Higher cost/test
Syphilis Screening Algorithms: Traditional versus ‘Reverse’
Traditional Algorithm

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

- Reactive
  - Treponemal test (e.g., FTA)
    - Reactive: Syphilis
    - Non-reactive: Not syphilis
  - Non-reactive: Not syphilis

- Non-reactive: Not syphilis
Traditional algorithm pros and cons

- **Pros**
  - Familiar
  - One confirmation test, typically done reflexively, leads to clear result
  - Rapid, inexpensive
  - Recommended by CDC

- **Cons**
  - Manual
  - Subjective interpretation
  - False-positives
  - False negatives, especially late syphilis
Reverse Algorithm

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

2. Non-Treponemal test (eg, RPR)
   - Reactive
     - Syphilis
   - Non-reactive
     - Second Treponemal Test (e.g., FTA)
       - Reactive
         - Probably syphilis
       - Non-reactive
         - Not syphilis

3. Not syphilis
Reverse algorithm pros and cons

- **Pros**
  - Objective
  - Can be batched for high volume labs
  - Recommended by public health agencies in Europe and Canada
  - More sensitive and more specific...more cases of syphilis diagnosed and treated

- **Cons**
  - Unfamiliar
  - Cost
  - Complexity – often second confirmatory test needed, not yet typically done reflexively
  - Disfavored by CDC
Interpreting reverse algorithm

Case #1

- 37-year-old man with HIV
- 2-weeks of fatigue, fever and rash on palms and soles
- Previously resolved genital lesion
- Syphilis IgG by EIA: positive
- RPR: positive, titer of 1:64
Interpreting reverse algorithm

Case #1 Conclusion

• Untreated or recently treated syphilis
• Follow treatment guidelines
• No further testing needed on this sample
• For follow-up after treatment
  • RPR titers only, should fall 4-fold (2 dilutions, e.g. 1:64 to 1:16)
Interpreting reverse algorithm

Case #2

- 23-year-old female
- First-trimester pregnancy screening
- Previously healthy
- Syphilis IgG by EIA: positive
- RPR: negative
- Second treponemal test, FTA: negative
Interpreting reverse algorithm

Case #2 conclusion

- False positive EIA
- Not syphilis
- No further screening at this time
- Consider screening again at 28 weeks and delivery if syphilis prevalent in community
Interpreting reverse algorithm

Case #3

- 50-year-old Somalian immigrant
- Kidney transplant evaluation
- No known history of syphilis or treatment
- Syphilis IgG by EIA: positive
- RPR: negative
- FTA: positive
Case #3 Conclusion

- Possible latent syphilis
- Evaluate and treat according to current guidelines
- Consider lumbar puncture if neurologic symptoms consistent with late neurosyphilis
Interpreting reverse algorithm

Case #4

- 30-year-old inmate
- Past history of treated syphilis (10 years prior)
- Syphilis IgG by EIA: positive
- RPR: negative
Interpreting reverse algorithm

Case #4 Conclusion

• Consistent with successfully treated syphilis
• No additional testing needed
Summary

- Syphilis usually diagnosed by serology
  - Non-treponemal (e.g., RPR, VDRL)
  - Treponemal (e.g., FTA, TP-PA, EIA, MFI)

- Traditional Algorithm
  - Non-treponemal test (RPR) first
  - 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
Summary

• Reverse Algorithm
  • Treponemal test first
  • Confirm with RPR
  • If RPR negative, use different treponemal ‘tiebreaker’ test
• Advantages
  • High volume throughput
  • More sensitive, same specificity
• Limitations
  • Result interpretation can be challenging
  • ‘Tiebreaker’ test not yet reflexive in most labs