What does Lyme and Syphilis have in common…

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Disclosures

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**Syphilis Testing in the Clinical Lab**

Alfredo Villarreal  
CID Marketing
Outline

- Introduction / Background
- Epidemiology
- Syphilis Diagnostic Techniques
- Syphilis Testing Algorithms
- The latest CDC guidelines
• Syphilis
  – Sexually transmitted disease
  – Caused by bacterium: *Treponema pallidum* (spirochete)
  – Classed as one of the World’s 50 most prevalent diseases (WHO, 1990)

• Infection
  – Infection progression through different stages
  – Transmission
    • Sexual transmission
    • Through blood transfusion
    • From mother to fetus (congenital Syphilis)
  – Easily treated with antibiotics (penicillin)

**Infection Course**

- **Primary Syphilis**
  - Painless Chancre
  - Spontaneously resolves
  - 9-90 days

- **Secondary Syphilis**
  - Diffuse Rash, Swollen Glands
  - Spontaneously resolves
  - 6 wks – 1 yr

- **Latent Syphilis**
  - Asymptomatic
  - Spontaneously resolves
  - 2 – 4 yr

- **Tertiary Syphilis**
  - Cardiovascular, Cutaneous Diseases, Neurosyphilis
  - Spontaneously resolves
  - 3 – 20 yr

**Infectious Stages**

**Non Infectious Stages**
Infection Course

• Primary syphilis (infectious)
  – Silent incubation
  – Clinical symptoms appear after ~3 weeks incubation
  – Clinical symptoms: painless chancre, located mainly on the external genitals, vagina, anus, in the rectum or extra-genitals (mouth, amygdales …)

• Secondary syphilis (very infectious)
  – ~1 month to 1 year after contamination
  – Septicemic phase of the infection
  – Clinical symptoms: diffuse rash, swollen glands

Infection Course

• Early latent syphilis (infectious)
  – No clinical symptoms are associated
  – Usually ~1-2 years after syphilis infection

• Late latent Syphilis (non infectious)

• Tertiary Syphilis (non infectious)
  Almost eradicated in industrialized countries
  – Cutaneous diseases
  – Neurosyphilis
  – Cardiovascular disease

• HIV associated Syphilis
  – Few specific symptoms but quicker progression to Neurosyphilis
Syphilis and Pregnancy

- **Congenital Syphilis**
  - Rarely seen in industrialized countries
  - Fetal infection when mother’s active syphilis is not detected or detected too late
  - Can cause premature delivery, stillbirth or neonatal death
  - Possible infant disorders (appearing a few weeks after birth):
    - Deafness
    - Neurological impairment
    - Bone deformities

Quiz

- In what clinical stage is syphilis most infectious?
  - Primary
  - Secondary
  - Latent
  - Tertiary
Treponema pallidum has at least four subspecies that cause treponemal diseases:

- Venereal Syphilis – *T. pallidum* subsp. *pallidum*

- Non-venereal diseases caused by:
  - *T. pallidum* subsp. *pertenue* – Yaws
  - *T. pallidum* subsp. *endemicum* – Bejel
  - *T. carateum* – Pinta

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Treponemes (non-sexual disease)

- Yaws and Pinta
  - like syphilis, begin with skin symptoms

- Pinta affects only the skin
  - Begins as flat, itchy, reddened areas on the hands, feet, legs, arms, face, or neck.

- Bejel begins with mouth sores
  - The symptoms subside, and after a period with few or no symptoms, but new symptoms may develop
The bacteria is a helical coiled, corkscrew-shaped cell, 6-15um x 0.1-0.2um wide

- It is Gram negative
- It consists of: outer membrane, peptidoglycan cytoplasmic membrane and a protoplasmic membrane
- Replication is by binary fission

Treponema pallidum

Electron micrograph of Treponema pallidum on cultures of cotton-tail rabbit epithelium cells (Sf1Ep)

Spirochetes

Electron micrograph of Treponema pallidum on cultures of cotton-tail rabbit epithelium cells (Sf1Ep)

Photo credit: CDC / Dr. David Cox
Quiz

Is *treponemal pallidum* Gram

- Positive
- Negative

Epidemiology
Epidemiology

Rates of Reported Cases by Stage of Infection, United States, 1941–2017

- Primary and Secondary
- Early Latent
- Total Syphilis

10.5% increase compared with 2016 (8.6 cases per 100,000 population)
72.7% increase compared with 2013 (5.5 cases per 100,000 population).

Primary and Secondary Syphilis — Rates of Reported Cases by Region, United States, 2008–2017

- West
- Midwest
- Northeast
- South

9.4 cases per 100,000 population
The total rate of primary and secondary syphilis for the United States and outlying areas (Guam, Puerto Rico, and Virgin Islands) was 4.5 per 100,000 population.

The total rate of reported cases of primary and secondary syphilis for the United States and outlying areas (Guam, Puerto Rico, and Virgin Islands) was 9.5 per 100,000 population.
Syphilis Diagnostic Techniques

Bacteriological Diagnosis

- **Direct Diagnosis**
  - To reveal the bacterium from genital, anal or oral lesion
  - *Treponema pallidum* can also be found in CSF in case of neurosyphilis
  - Typically only performed in reference and specialized labs
    - **Methods**
      - Dark field microscopy (potential false positives)
      - Immuno-Fluorescence / Silver Stain (more specific)

- **Indirect Diagnosis**
  - Detection of antibodies in serum, plasma and possibly CSF
  - Always a silent serological phase (beginning of infection)
  - Types of antigens used
    - Non syphilis specific (Lipoidal / Cardiolipin Ag)
    - Treponemal Ag (lysate or recombinant)
Direct Visualization

Darkfield Microscopy

Silver Stain
Syphilis Serology

• Non treponemal specific antibodies
  – Lipoidal / Cardiolipin Ag
  – Passive agglutination reactions
  – Assays
    • VDRL (Venereal Disease Reagent Laboratory)
    • RPR (Rapid Plasma Reagin)
  – Lipoidal substances are found in numerous organs and typically in compounds of *T. pallidum*
    • Thus nonspecific and susceptible to false positives
      – Malaria, Lyme disease, Chancroid, TB, HIV, Pregnancy, IV drug abuse, Rheumatoid arthritis, Lupus, etc.

Non-Treponemal Tests

  – VDRL / RPR are considered good assays for treatment efficiency and follow-up (how rapid a sample turns negative after treatment)
    • However, turns positive only after the treponemal methods
  – The tests are highly subjective and require experienced operators
  – Positive in primary and secondary syphilis
Syphilis Serology

• Specific Antibodies - Reaction with a Treponemal Ag
  – TPHA (Treponema Pallidum Haemagglutination Assay) or TPPA (Treponema Pallidum Particle Agglutination Assay)
    • Detection of Ab in serum / plasma through coated red cells agglutination
    • Is positive ~3-4 weeks after infection (~1 week after chancre)
    • Usually still positive after recovery or treatment
  – FTA-Abs (Fluorescent Treponemal Antibody Absorption Assay)
    • Detection on slide
    • Same Ab kinetics as TPHA / TPPA before treatment but FTA-Abs typically turns negative after treatment
    • Currently used as a confirmatory test in cases where a sample is positive from a diagnostic screen test (non-treponemal)

Specific Antibodies

– Western Blot (immunotransfer)
  • Electrophoresis separate *T. pallidum* proteins transferred on membrane
  • Most typically used Ag’s are:
    – Tp 15.5kDa, Tp 17kDa, Tp 47kDa and Tmp A
– EIA / CIA / MFI Assays*
  • Based on purified *T. pallidum* Ag or recombinant proteins
    – e.g. Tp 17kDa, 47kDa
  • Considered the most sensitive screening assays

* Enzyme immunoassay
  Chemiluminescence immunoassay
  Multiplex fluorescence immunoassay
Syphilis Serology – non treated

31 Müller F, Hagedorn HJ. Syphilis in: Clinical Laboratory Diagnostics, Thomas L; TH Books Frankfurt 1998;1203-12

Quiz

Name at least two treponemal Ag tests

- TPHA
- TPPA
- FTA-Abs
- Wester Blot
- EIA / CIA / MFI
Name a drawback of non treponemal specific tests

- Subjective
- Doesn’t measure causative agent
  - Measures cardiolipin antibodies
- Subject to false
Qualitative RPR Test

Quantitative RPR Test

End-Point Titer (1:64)
Syphilis Testing Algorithms

Traditional vs. Reverse Sequence

- **Traditional algorithm**
  - Inexpensive, low throughput, mostly manual
  - Reliable for detection of active infection
  - RPR interpretation is highly subjective

- **Reverse Sequence algorithm**
  - High throughput (automated), less manual labor
  - Detection of early syphilis and untreated syphilis
  - Objective interpretation
  - Patient management issues regarding discordant results (EIA+/RPR-)
Traditional Test Algorithm

Non-treponemal test (RPR or VDRL)

- Reactive
- Non-reactive

Confirmatory treponemal test (FTA-ABS, TP-PA, or EIA)

- Positive => Syphilis
- Negative => Not Syphilis or very recent infection

Reverse Sequence Algorithm

Treponemal test (EIA, CIA, or MFI)

- Reactive
- Non-reactive

- Non-treponemal test (RPR)

- Positive = Syphilis
- Negative = ?

2nd Treponemal test (FTA-ABS, TP-PA)
Comparison of 3 Algorithms

• Compared classical, reverse and European algorithms (24,124 patients)
  – Classical algorithm- 76% accuracy
  – Reverse algorithm- 99.9% accuracy
  – European algorithm- 99.6% accuracy

Tong, et al., Clin Infect Dis. 2014 Apr;58(8):1116-24

Discordant Results with Reverse Sequence

• Positive treponemal test and negative non-treponemal test (RPR/VDRL)
  – Previously treated syphilis
  – Late latent syphilis
  – Early infection with syphilis
  – False positive treponemal test
What are some advantages to the reverse sequence algorithm?

– High throughput (automated)
– Less manual labor
– Detection of early syphilis and untreated syphilis
– Objective

The latest CDC guidelines
Syphilis screening is of great importance

- Controlling the spread of the disease
- Preventing the transmission of congenital syphilis
- Preventing irreversible tissue damage (cardiovascular and neurological)

### Syphilis Screening Recommendations and Considerations

**Pregnant Women**
- All pregnant women at the first prenatal visit
- Retest early in the third trimester and at delivery if at high risk
- Repeat as needed
- Stillborn infant mother should be tested

**Men Who have Sex With Men (MSM)**
- At least annually for sexually active MSM
- Every 3 to 6 months if at increased risk

**Persons with HIV**
- For sexually active individuals, screen at first HIV evaluation, and at least annually thereafter
- More frequent screening might be appropriate depending on individual risk behaviors and the local epidemiology

The routine screening of adolescents who are asymptomatic for certain STDs (e.g., syphilis, trichomoniasis, BV, HSV, HPV, HAV, and HBV) is not generally recommended. However, and pregnant adolescent females should be screened for syphilis.
Discordant Results with Reverse Sequence Testing


Composite Results from Four Laboratories

Initial Syphilis EIA (N=116,822)
- 6,587 positive (6%)
- 110,235 negative (94%)

RPR (n=6,548)
- 2,884 positive (44%)
- 3,664 negative (56%)

Not Syphilis or very recent infection

2nd Treponemal test?
Treponemal- Non-treponemal Test Discordance

**EI A+/RPR- (n=3,664)**

- 2nd Treponemal test (n=2512)
  - 2,079 positive (83%)
  - Treat if no history of prior treatment
  - **False Positive EIA?**
  - 433 negative (17%)
  - Possible early infection?
- No further testing unless requested by the clinician

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2015 Recommendations (CDC) on implementing the Reverse Testing Algorithm

- Non-Trep test be repeated in 2-4 weeks for patients at risk for primary syphilis
- Optimally the 2nd test should be a TPPA (when the Trep & non-Trep tests are discordant
- Non-Trep test is recommended to monitor patient treatment progress
Does the CDC recommend general population screening for syphilis?

- No

CDC Guidelines for Interpretation
CDC Guidelines

- Since treponemal tests may remain active for life in adequately treated patients, a positive T PALLIDUM IGG + IGM [86781E] indicates exposure to syphilis and it does not indicate untreated syphilis.
- If the RPR is also positive (especially at >1:8) and there is no history of treatment for syphilis, a diagnosis of syphilis is made and the patient should receive treatment.
- Most people become negative for RPR with adequate treatment, though some patients who present with later stage disease may maintain a low titer RPR (<1:8) for life despite adequate treatment. This is the serofast* state.

* Patients who fail to achieve serological cure

CDC Guidelines

- Initial screening may be negative in early primary syphilis. If the history is strongly suggestive of syphilis then an RPR should be done and/or repeat T PALLIDUM IGG + IGM [86781E] in 3 - 4 weeks.
  - The most common cause of a false negative syphilis serologic test is performance prior to the development of diagnostic antibodies
- Positive T PALLIDUM IGG + IGM [86781E] with a non-reactive RPR and non-reactive TPPA is most likely a false positive T PALLIDUM IGG + IGM [86781E] result. If clinical history suggests a risk for syphilis then T PALLIDUM IGG + IGM [86781E] should be repeated in 3-4 weeks.
CDC Guidelines

- Positive T PALLIDUM IGG + IGM [86781E] with a non-reactive RPR and REACTIVE TPPA is most consistent with old treated syphilis. If there is no clear history of syphilis treatment then 3 weekly shots of 2.4 million units of benzathine penicillin should be considered. Clinical correlation is required as in rare cases of late latent or tertiary syphilis the RPR may be negative.
- The diagnosis of syphilis should not be made on the basis of a single test result. Clinical history, findings and symptoms should be taken into consideration.

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Takeaways
Conclusions

- Syphilis remains a diagnosis requiring a combination of laboratory tests and keen clinical observation
- Reverse sequence syphilis testing has several key advantages over the conventional algorithm (CCF)
- In the early phase of implementation, laboratorians must be prepared to educate/inform clinicians
- Laboratorians must be open to altering the testing algorithm to best serve the clinicians and patient population to conserve resources

What if there a way to have both treponemal and non-treponemal results simultaneously?
Bio-Rad BioPlex 2200 System

BioPlex 2200 Syphilis Total & RPR Assay  
(Multiplex Treponemal and RPR dual assay)

Syphilis Total & RPR Assay

*Detects and differentiates* the presence of treponemal and non-treponemal antibodies

- Reportable results:
  - Qualitative Syphilis Total
  - Qualitative RPR
  - RPR Titer/dilutions
    - 1:4 - 1:64 directly from BioPlex
    - 1:2 & 1:128 - 1:2048 after one simple off-line dilution*

- 100 test reagent pack
- Multiplexed calibrators and controls

* Requires BioPlex 2200 Sample Dilution Buffer (P/N 12006863)
This assay can serve as a one-step universal method to aid in the diagnosis of syphilis infection.