Making the Reverse Algorithm Work For Kentucky

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Dr. Jeffrey D. Howard, Commissioner
The Department for Public Health (DPH) is dedicated to improving health and safety of Kentuckians through prevention, promotion, and protection.

As a major component of the Cabinet for Health and Family Services, DPH provides guidance and support for health departments in all 120 counties.

Serving as Kentucky’s dedicated public health resource, DPH is responsible for identifying and allocating resources to communities and public health institutions in an effort to prevent and protect against diseases, outbreaks, hazards statewide.
Kentucky Public Health Laboratory
What is syphilis?

- **Merriam-Webster**: a chronic contagious usually venereal and often congenital disease caused by a spirochete (*Treponema pallidum*) and if left untreated producing chancre, rashes, and systemic lesions in a clinical course with three stages continued over many years.
KY DLS Serology Team 2000
Serologic Methods: Algorithms

• I am not here to advocate for either algorithm:
• Laboratories should take into account their patient population and syphilis risk when considering a traditional or reverse sequence screening algorithm.
• Decisions based on cost should take into account total costs at both the laboratory level and the system level.
• The test results report should include a brief description of the method(s) and algorithm used.
• Lastly, the use of only one type of serologic test is insufficient for diagnosis and can result in false-negative results in persons tested during primary syphilis and false-positive results in persons without syphilis.
Syphilis Testing Methods?
Venereal Disease Research Laboratory Assay (VDRL)

- Original non-treponemal testing method
- Qualitative and Quantitative test results
- Microscopic slide flocculation
- Only FDA-approved non-treponemal testing method for CSF specimens

LIMITATIONS OF THE PROCEDURE

- VDRL test is non-specific for syphilis. All Reactive samples should be retested with treponemal methods such as IgG, TPPA and/or FTA-Abs to confirm the results.
- A Non-Reactive result by itself does not exclude a diagnosis of syphilis.
- False-positive results have been reported in diseases such as toxoplasmosis, autoimmune diseases, infectious mononucleosis, viral pneumonia and pregnancy.
Non-Treponemal Assays - Advantages

• Considered inexpensive in regards to required test supplies
• Rapid results
• High Sensitivity
• Does not detect past infections
• Requires little equipment for test analysis
• Requires only one reflex test, typically
• Change in titer is useful for treatment monitoring
Non-Treponemal Assays - Disadvantages

• Lower specificity
• Labor intensive and time consuming
• Subjective results
• Manual data manipulations
• Detection of late latent syphilis

• False-positive non-treponemal test results have been associated with various medical conditions and factors unrelated to syphilis, such as other infections (e.g., HIV), autoimmune conditions, immunizations, pregnancy, injection-drug use, and older age.

• Although rare, a situation called the “prozone effect” may cause a false-negative reaction. It is most often found during secondary syphilis, when the body is overwhelmed by syphilis antibody excess.
Treponemal Assays

• Detect specific antibody against T. pallidum
• Qualitative test results only
• Reactivity can persist over lifetime
• Traditionally utilized for confirmation
Treponemal Assays - Advantages

• Detection of specific treponemal antibody
• Objective results (EIA, CIA, and MIA) – can be instrument generated
• Less labor intensive (if automated)
• Ability to detect early primary infection
Treponemal Assays - Disadvantages

• Cannot differentiate between active and past infection
• Subjective results (TPPA, FTA)
• More costly than non-treponemal, per test
• If IgG-only assay utilized, can miss early primary infections
Types of Treponemal Tests

• Immunoassays
  • – Enzyme Immunoassay (EIA)
  • – Chemiluminescent Immunoassay (CIA)
  • – Microbead Immunoassay (MIA)

• Treponemal Pallidum Particle Agglutination Assay (TP-PA)

• Fluorescent Treponemal Antibody Absorption (FTA-ABS)
Immunoassays (EIA, CIA, MIA)

• Qualitative assays that measure IgG, IgM, or both
• Commonly used as screening assays for reverse algorithm
• Can be automated
• Objective interpretation based on cutoff values of specific assay – Reactive, Non-reactive, Equivocal
TPPA - *Treponema pallidum* particle agglutination assay

- Qualitative particle agglutination assay
- Subjective interpretation

A reactive treponemal test indicates past or present infection and usually remains reactive for life.
FTA-ABS - fluorescent treponemal antibody absorption

- Blood test that detects the presence of antibodies to *Treponema pallidum* bacteria.
- FTA-ABS turns positive earlier and remains positive longer than VDRL.
- Other treponemes, such as *T. pertenue*, may also produce a positive FTA-ABS.
- The ABS suffix refers to the processing step used to remove nonspecific antispirochetal antibodies present in normal serum.
- Currently, CDC recommends TPPA over FTA.
### Possible interpretations of different syphilis test results.

<table>
<thead>
<tr>
<th>EIA</th>
<th>RPR /VDRL</th>
<th>TPPA</th>
<th>INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>NT</td>
<td>NT</td>
<td>No evidence of treponemal infection. (Repeat test in 4 weeks if clinically indicated).</td>
</tr>
<tr>
<td>R</td>
<td>N</td>
<td>N</td>
<td>Possible early primary infection, or a false positive EIA, or very longstanding syphilis (either treated or untreated)</td>
</tr>
<tr>
<td>R</td>
<td>R</td>
<td>N</td>
<td>Possible early primary syphilis, or false positive EIA and false positive RPR.</td>
</tr>
<tr>
<td>R</td>
<td>N</td>
<td>R</td>
<td>Possible longstanding syphilis (either treated or untreated) or possible early syphilis (with the RPR yet to become reactive).</td>
</tr>
<tr>
<td>R</td>
<td>R</td>
<td>R</td>
<td>Possible current syphilis (either treated or untreated), or possible old syphilis (either treated or untreated). (If the syphilis is old and/or treated one would expect a low titre RPR)</td>
</tr>
</tbody>
</table>
What is the Reverse Syphilis Algorithm?

• It is the opposite of the “traditional” syphilis testing algorithm
• It begins with a treponemal screening assay...
• Followed by a non-treponemal confirmatory assay
• A third assay will be utilized to decide upon discrepant results between the screening and confirmatory assay
Syphilis Testing Team 2019

Virology!
Reverse Algorithm for Syphilis Diagnosis
Kentucky Reverse Algorithm

Syphilis Flow Chart

- Syphilis IgG EIA
  - If Negative: Report Results
  - If Reactive or Equivocal on repeat Testing: Perform VDRL

- Perform VDRL
  - VDRL Reactive
    - EIA Reactive: Report Results
    - EIA Equivocal: Confirm with TP-PA
  - VDRL Negative
    - EIA Reactive or Equivocal: Report Results

TP-PA Detected: Report Results
TP-PA Not Detected: Report Results
Syphilis EIA

**EIA+**
- Quantitative RPR or VDRL

**EIA−**
- Negative for syphilis antibodies

**EIA+ RPR−**

**EIA+ RPR+**
- Consistent with past or current syphilis

**TPPA or FTA-ABS**
- (Different platform and target antigens)

**EIA+ RPR− TPPA+**
- Possible syphilis infection. Requires historical and clinical evaluation.

**EIA+ RPR− TPPA−**
- Unconfirmed EIA. Unlikely to be syphilis. If pt is at risk for syphilis, retest in 1 month.
Lab and Epidemiology – Why/How in Kentucky?

• Merging of two Microbiology sections, staffing
• After internal meetings....
• DLS met with our EPI partners
  • What about the old, treated cases that lab will send for us to investigate?
  • Strong support both ways from each Division.
  • Many calls/meetings.
  • Kentucky Division of Epidemiology wanted the same results without having to chase old infections.
Some Questions KY DLS Faced:

1. What is the traditional Syphilis testing algorithm?

2. What is the Reverse Sequence Syphilis Screening Algorithm?

3. Why is the laboratory changing to this new algorithm?

4. What is the turnaround time (TAT) for this new algorithm?

5. What types of clinical specimens are suitable for the new algorithm?

6. Will specimen submission in OUTREACH be the same for the new algorithm?

7. Non-electronic Ordering: Will the requisition form for this new algorithm be the same?

8. Is this test algorithm applicable for patients previously treated for Syphilis?

9. How will the lab report reflect the new assay findings?

Answers:

1. The traditional Syphilis screening algorithm consists of using a non-treponemal test (VDRL), as its initial screen, with reactive tests confirmed by treponemal testing (EIA) and *Treponema pallidum* particle agglutination (TPPA) test for discordant results.

2. This algorithm utilizes a treponemal test (EIA) as its initial screening test and reactives are confirmed by using a quantitative non-treponemal test (VDRL). If test results are discordant (reactive IgG EIA and non-reactive VDRL) then specimen is reflexed to the TPPA test.

3. The initiation of this new Syphilis testing algorithm is expected to reduce false positives from the VDRL and decrease scientist manual processes.

4. Nonreactive results will be completed within 24 hours after specimen receipt. Confirmed reactive samples will be completed with 48 hours from specimen receipt and samples requiring TPPA testing will be complete within 72 hours of receipt.

5. There will be no change in sample requirements for the Reverse algorithm. Blood or serum in a non-preservation collection tube, approximately 2-3 ml. If additional tests are requested, such as Hepatitis B or HIV, please increase volume.

6. The only change will be that when the edit clinical order que is selected the test ordered will be Syphilis and not VDRL Syphilis Screen. This can be easily accomplished by typing “Syphilis” in the FullName box next to the Run que. This will bring up the Syphilis IgG (EIA) test code “IGGE” which can then be ordered.

7. Yes, the serodiagnosis submission form #213 for Syphilis testing can be downloaded from the State Lab Website.

8. Yes, if a patient has been treated for Syphilis and is in need of follow up testing then the new algorithm will be able to provide quantitative VDRL confirmation results to assess the patient treatment status.

9. The first test on the lab report will be the Syphilis IgG EIA. The VDRL (Quantitative) will follow only when the Syphilis IgG screen is reactive. The TPPA will be performed only if there is a discordant result between the treponemal IgG and the non-treponemal VDRL.
The Decision

• DLS and EPI came to a joint decision in late 2012...
• Completed a study (became the verification) ~ 1,500 specimens.
• Results were acceptable by both Divisions.
• Move forward with the implementation of the Reverse Syphilis Algorithm.
• Email/letter was sent to submitters and partners to inform them of the testing change.
Kentucky DLS Syphilis Study
VDRL Annual Testing Volume
Annual Percent Positive VDRL

<table>
<thead>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Positive</td>
<td>2.5%</td>
<td>1.2%</td>
<td>1.2%</td>
<td>0.8%</td>
<td>1.1%</td>
<td>1.4%</td>
<td>41.1%</td>
<td>41.1%</td>
<td>38.8%</td>
<td>41.2%</td>
<td>43.1%</td>
<td>58.7%</td>
<td>48.6%</td>
</tr>
</tbody>
</table>
Syphilis IgG Annual Testing Volume
Annual Percent Positive Syphilis IgG
TPPA Annual Testing Volume
Annual Percent Positive TPPA
Annual Syphilis Test Requests

<table>
<thead>
<tr>
<th>Year</th>
<th>Requests</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>30018</td>
</tr>
<tr>
<td>2007</td>
<td>29808</td>
</tr>
<tr>
<td>2008</td>
<td>28536</td>
</tr>
<tr>
<td>2009</td>
<td>27580</td>
</tr>
<tr>
<td>2010</td>
<td>28600</td>
</tr>
<tr>
<td>2011</td>
<td>27892</td>
</tr>
<tr>
<td>2012</td>
<td>25694</td>
</tr>
<tr>
<td>2013</td>
<td>22521</td>
</tr>
<tr>
<td>2014</td>
<td>18881</td>
</tr>
<tr>
<td>2015</td>
<td>16108</td>
</tr>
<tr>
<td>2016</td>
<td>14548</td>
</tr>
<tr>
<td>2017</td>
<td>14000</td>
</tr>
<tr>
<td>2018</td>
<td>13245</td>
</tr>
</tbody>
</table>
Annual Confirmed Syphilis Test Results

<table>
<thead>
<tr>
<th>Year</th>
<th>Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>1.61</td>
</tr>
<tr>
<td>2007</td>
<td>0.76</td>
</tr>
<tr>
<td>2008</td>
<td>0.71</td>
</tr>
<tr>
<td>2009</td>
<td>0.72</td>
</tr>
<tr>
<td>2010</td>
<td>0.61</td>
</tr>
<tr>
<td>2011</td>
<td>0.72</td>
</tr>
<tr>
<td>2012</td>
<td>1.24</td>
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<tr>
<td>2013</td>
<td>1.84</td>
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<td>2014</td>
<td>1.73</td>
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<tr>
<td>2015</td>
<td>1.99</td>
</tr>
<tr>
<td>2016</td>
<td>2.09</td>
</tr>
<tr>
<td>2017</td>
<td>2.36</td>
</tr>
<tr>
<td>2018</td>
<td>2.72</td>
</tr>
</tbody>
</table>
Results after Algorithm Change

• DLS did receive some calls initially
  • From submitters – what do these results mean?
  • From EPI – what do these results mean? Is this an old infection?

This lasted for a month or two AND then the calls stopped....
for the most part 😊.
Considerations for Algorithm Selection

• – Testing volume.
• – Laboratory workflow and staffing.
• – Prevalence and incidence of syphilis in your testing population.
• – Cost and reimbursement of testing.
• – Programmatic and clinical input.
Points to Remember, Conclusions:

• Syphilis immunoassays remain important and commonly requested tests, both in low-risk populations and in populations at higher risk, especially with national syphilis positivity rates on the rise.

• Traditional testing for syphilis (non-treponemal screening followed by treponemal testing for confirmation) is a long-held practice that is generally well known to clinicians.

• It is important for laboratories to investigate the potential use of the Reverse Syphilis Algorithm.

• Treponemal testing does not differentiate between treated and untreated infections. As such, patients with discordant results should be carefully considered for treatment, especially if the initial treponemal-test result is confirmed with a second treponemal assay.
Further Points to Remember, Conclusions:

• At this time, CDC recommends use of the TPPA test in resolving samples that fail to show reactivity with a non-treponemal test following a reactive treponemal result.

• The traditional screening algorithm is thought to be more suitable for laboratories with low-volume samples due to the lower false-reactive/treated infection rate and its cost-effectiveness.

• If your laboratory decides to implement the Reverse Algorithm, submitting facilities should be informed and educated on both the benefits and interpretive changes of this Syphilis algorithm.

• It is important for laboratories to be prepared to explain the interpretation of individual tests and the overall algorithm interpretation.
Final Thoughts

• Again, I am not advocating for either testing algorithm.
• Currently, the Reverse Algorithm works for KY.
• However, with the automated non-treponemal testing platforms available,...KY DLS may be performing another Syphilis testing study in the near future.
Thank you!
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https://chfs.ky.gov/agencies/dph/dls/Pages/default.aspx