Syphilis Automation: Updates on automated nontreponemal Rapid plasma Reagin (RPR) tests

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Conflict of Interest

- No conflicts of interest or financial disclosures
The diagnosis of syphilis is challenging and determining a correct stage requires “laboratory results” along with “clinical presentations/history of the disease”

Serological tests are the most commonly used for syphilis and are of two types; nontreponemal (RPR, VDRL) and treponemal tests (EIA, TP-PA)

Manual syphilis tests (e.g. RPR, TP-PA) require skilled staff to setup, perform and interpret results

Automated syphilis tests reduce hands-on time and repetitive motion injuries, minimize subjectivity with result interpretation, and benefit labs with documentation
Syphilis automation

- High-volume laboratories seeking to improve workflow lean towards “automation”
  - Reverse algorithm; automated treponemal tests
- FDA-cleared automated treponemal tests are widely used for syphilis testing
- Now we have “automated nontreponemal RPR” tests
Automated nontreponemal RPR tests

- Automated nontreponemal RPR tests were recently introduced in the United States for syphilis
  - **BioPlex 2200 Syphilis Total & RPR** (Bio-Rad Laboratories, Inc., CA)
  - **AIX 1000 agglutination RPR analyzer** (Gold Standard Diagnostics, Inc., CA)
  - **ASI Evolution automated RPR syphilis test** (Arlington Scientific, Inc., UT)
Automated nontreponemal RPR tests

- BioPlex 2200 syphilis total & RPR is based on flow immunoassay principle
- Detects treponemal (TP47/TP17) and nontreponemal (cardiolipin) antibodies
- Offers qualitative results for treponemal assay and qualitative/titer for nontreponemal assay

Source: Respective manufacturer’s website
Automated nontreponemal RPR tests

- Based on flocculation reaction like RPR
- Use cameras and proprietary hardware/software tools
- Offers qualitative/titer for nontreponemal assay

AIX 1000 RPR (Gold Standard Diagnostics, CA)

ASI Evolution (Arlington Scientific, UT)

Source: Respective manufacturer’s website
Automated nontreponemal RPR tests

**Table 1** Key features of the automated RPR tests

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BioPlex RPR</th>
<th>AIX 1000</th>
<th>ASI Evolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turnaround (tests/hour)</td>
<td>200</td>
<td>192</td>
<td>190</td>
</tr>
<tr>
<td>Specimen vial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type</td>
<td>Polystyrene / polypropylene</td>
<td>Polystyrene / polypropylene</td>
<td>Polystyrene / polypropylene</td>
</tr>
<tr>
<td>Dimension (mm)</td>
<td>12 x 75</td>
<td>16 x 100</td>
<td>12 x 75</td>
</tr>
<tr>
<td>Specimen volume</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Qualitative / Screening (µL)</td>
<td>240</td>
<td>305</td>
<td>300</td>
</tr>
<tr>
<td>Quantitative low titer (µL)</td>
<td>285</td>
<td>394</td>
<td>290</td>
</tr>
<tr>
<td>Quantitative high titer (µL)</td>
<td>405</td>
<td>220</td>
<td>140</td>
</tr>
<tr>
<td>Titer range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low titer</td>
<td>1:4 to 1:64</td>
<td>1:1 to 1:16</td>
<td>1:1 to 1:64</td>
</tr>
<tr>
<td>High titer</td>
<td>1:128 to 1:2048</td>
<td>1:16 to 1:256</td>
<td>1:128 to 1:2048</td>
</tr>
</tbody>
</table>
Automated nontreponemal RPR tests

- These three platforms automate sample testing/reporting procedure, hence circumventing the subjectivity in interpretation
  - Record results electronically; BioPlex RPR provides antibody index value and AIX 1000/ASI Evolution capture test well images, offering added benefit with lab documentation (digital result output)
  - Minimize subjectivity in result interpretation

- Only a handful of studies are available evaluating automated RPR tests

- CDC and APHL collaborated to evaluate the performance of three automated RPR test systems assessing reproducibility, qualitative and quantitative/titer reporting
Automated nontreponemal RPR tests

- Reproducibility panel testing
  - A reproducibility panel comprised of 15 nonreactive and reactive sera (RPR titer 1:1 to 1:64) was prepared
  - Each specimen was tested 10 times at participating public health labs
  - Data analyzed and Point estimate calculated; 69 to 95%

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Nonreactive (n=2)</th>
<th>Low titer (n=4)</th>
<th>Moderate titer (n=7)</th>
<th>High titer (n=2)</th>
<th>Point Estimate % [95%CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPR Titer range</td>
<td>-</td>
<td>1:1 to 1:2</td>
<td>1:4 to 1:16</td>
<td>1:64</td>
<td></td>
</tr>
<tr>
<td>Automated RPR tests</td>
<td>% Agreement to manual RPR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BioPlex RPR</td>
<td>100</td>
<td>60 to 100</td>
<td>40 to 100*</td>
<td>100</td>
<td>68.7 (60.9 to 75.5)</td>
</tr>
<tr>
<td>AIX 1000</td>
<td>100</td>
<td>100</td>
<td>100**</td>
<td>90 to 100</td>
<td>94.7 (89.8 to 97.3)</td>
</tr>
<tr>
<td>ASI Evolution</td>
<td>100</td>
<td>30 to 100</td>
<td>70 to 100</td>
<td>80</td>
<td>86.0 (79.5 to 90.7)</td>
</tr>
</tbody>
</table>

*Two sera (1:16) were out of range (gave 4 to 8 fold higher titer) for all 10 repeated runs

**One serum (1:16) gave 4 fold lower titer for 7 out of 10 repeated runs
Automated nontreponemal RPR tests

- Quantitative Panel testing
  - A quantitative panel comprised of 50 syphilis reactive sera (RPR titer 1:64 to 1:1024) was prepared
  - For data analysis, following criteria were kept:
    • Within range: 2-fold (1 dilution) to manual RPR titer
    • Out of range: Not within 2-fold (1 dilution) to manual RPR titer

### Table 3
Results of the quantitative panel testing for the automated RPR tests

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BioPlex RPR</th>
<th>Automated RPR test</th>
<th>ASI Evolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPR titer range</td>
<td></td>
<td>AIX 1000 1:64 to 1:1024 (n=50)</td>
<td></td>
</tr>
<tr>
<td>Within range n(%)</td>
<td>32 (64)</td>
<td>47 (94)</td>
<td>34 (68)</td>
</tr>
<tr>
<td>Out of range n(%)</td>
<td>18 (36)</td>
<td>3 (6)</td>
<td>16 (32)</td>
</tr>
<tr>
<td>Spearman’s correlation coefficient (p-value)</td>
<td>0.746 (p = 5.2 × 10⁻¹⁰)</td>
<td>0.893 (p = 3.1 × 10⁻¹⁸)</td>
<td>0.162 (p = 0.262)</td>
</tr>
</tbody>
</table>
Automated nontreponemal RPR tests

### Qualitative Panel testing

<table>
<thead>
<tr>
<th>Status</th>
<th>Specimen n</th>
<th>Manual RPR</th>
<th>BioPlex RPR</th>
<th>AIX 1000</th>
<th>ASI Evolution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Status</td>
<td>R n(%)</td>
<td>NR n(%)</td>
<td>R n(%)</td>
</tr>
<tr>
<td>Primary</td>
<td>24</td>
<td>R 19</td>
<td>18 (100)</td>
<td>0 (0)</td>
<td>18 (94.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NR 5</td>
<td>1 (25)</td>
<td>3 (75)</td>
<td>3 (60)</td>
</tr>
<tr>
<td>Secondary</td>
<td>43</td>
<td>R 41</td>
<td>37 (100)</td>
<td>0 (0)</td>
<td>40 (97.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NR 2</td>
<td>0 (0)</td>
<td>2 (100)</td>
<td>2 (100)</td>
</tr>
<tr>
<td>Early Latent</td>
<td>38</td>
<td>R 34</td>
<td>33 (97.1)</td>
<td>1 (2.9)</td>
<td>33 (97.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NR 4</td>
<td>1 (25)</td>
<td>3 (75)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Early NPNS</td>
<td>14</td>
<td>R 14</td>
<td>11 (84.6)</td>
<td>2 (15.4)</td>
<td>14 (100)</td>
</tr>
<tr>
<td>Late Latent</td>
<td>66</td>
<td>R 57</td>
<td>47 (83.9)</td>
<td>9 (16.1)</td>
<td>55 (96.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NR 9</td>
<td>1 (11.1)</td>
<td>8 (88.9)</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Syphilis specimens with unknown stage</td>
<td>140</td>
<td>R 64</td>
<td>41 (82)</td>
<td>9 (18)</td>
<td>64 (100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NR 76</td>
<td>7 (14)</td>
<td>43 (86)</td>
<td>16 (21.1)</td>
</tr>
<tr>
<td>Non-reactive or not diagnosed with syphilis</td>
<td>409</td>
<td>R 2</td>
<td>1 (100)</td>
<td>0 (0)</td>
<td>1 (50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NR 407</td>
<td>8 (3.2)</td>
<td>240 (96.8)</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Total</td>
<td>734</td>
<td>-</td>
<td>-</td>
<td>206 (33.9)</td>
<td>320 (60.8)</td>
</tr>
</tbody>
</table>

**Overall Concordance % (95% CI)**

- **92.6 (90.3 to 94.8)**
- **95.9 (94.5 to 97.3)**
- **94.6 (92.9 to 96.2)**

**PPA % (95% CI)**

- **90.6 (87.7 to 93.5)**
- **93.6 (91.5 to 95)**
- **91.3 (88.6 to 94)**

**NPA % (95% CI)**

- **93.9 (92 to 95.8)**
- **97 (95.9 to 98)**
- **96 (94.8 to 97.2)**

**Kappa correlation % (95% CI)**

- **84.5 (79.8 to 89.2)**
- **90.7 (87.5 to 94)**
- **87.3 (83.5 to 91.2)**
Summary
Summary

- Automated RPR tests have a relatively rapid turnaround of approximately 200 tests per hour (qualitative testing)
- A higher test reproducibility was recorded for AIX 1000 compared to ASI Evolution and BioPlex RPR
- Our evaluation and prior published reports collectively show promising performance of automated RPR tests, particularly for qualitative testing
- For quantitative testing, variabilities in titer reporting were recorded for automated RPR tests
- A variability in RPR titer of 4-fold or greater could have significant clinical implications
Summary

- A laboratory should know the “titer capabilities” for automated RPR tests as it varies from one automated RPR test to another.

- All reactive specimens should be diluted until an endpoint titer is achieved;
  - either by automated RPR test, or manual RPR if cannot be tittered on an automated RPR test due to instrument’s titer range limitations.

- Inaccuracy with titer reporting as < 1:4 or > 1:256 could lead to unnecessary treatment, or follow-up lab visits.
Summary

- Titer between an automated and a manual RPR should concord
- More robust the correlation between these two RPR methods, the greater confidence clinicians will have in the results when managing syphilis
- Laboratories considering a switch from manual to automated RPR should ensure that they communicate with clinicians about the potential differences between automated and manual RPR titers
Limitations

- Frozen sera (two freeze-thaw cycles) were used; warrants further testing using freshly collected sera
- Syphilis staging based on records from submitting judications, and accuracy could not be verified
- Prozone specimens were not available at the time of testing
- COVID-19 vaccines were not available at the time of testing, hence its potential interference on assay performance is negligible
- BioPlex RPR’s reagent stability issue was not evaluated (FDA Class 2 device recall 2021)
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Q&A