Improved diagnosis of syphilis at the point-of-care

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Diagnostic tests for syphilis in Resource constrained settings

A combination of **2 tests** to confirm syphilis infection

1) **Treponemal tests** (TPHA) for treponemal antibodies
2) **Non-treponemal tests** (RPR) distinguish **current** from **past** infection

- Require expensive lab equipment, technical expertise, seldom available outside reference labs.
- Significant barrier to effective control syphilis in resource constrained settings.

**Rapid-point-of-care (RPOC) treponemal antibody tests**

- Currently used for on site screening in primary health care settings.
- Address lack of access to a laboratory and the low patient return rates
- **Cannot** be used to distinguish active infection from **past/treated** infection
- Cannot monitor effectiveness of treatment.
- Reluctance to implement these tests exists.
Syphilis RPOC Target product profile

Diagnostic development pathway:

1. **Develop** a simple to use, low-cost ($2.50 per test), instrument-free, sensitive and specific POC diagnostic test for active syphilis that produces accurate results within 30 minutes

2. To **optimise** the prototype test until it meets minimum clinical sensitivity for active syphilis >95% and specificity of >80% when tested with the reference method (TPHA+/RPR tire ≥1/8)

3. To independently **evaluate** prototype test performance in the laboratory using stored patient samples.
1. Evaluation of novel active syphilis biomarkers by ELISA

- Detection of anti-syphilis IgA and IgM were evaluated as potential biomarkers of active syphilis infection

<table>
<thead>
<tr>
<th></th>
<th>IgM pos</th>
<th>IgM neg</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPR + &gt; 8</td>
<td>23</td>
<td>11</td>
</tr>
<tr>
<td>RPR - &lt; 8</td>
<td>12</td>
<td>54</td>
</tr>
<tr>
<td>total 100 patients</td>
<td></td>
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</tbody>
</table>

Sensitivity %: 67.85
Specificity %: 81.82
Predictive pos %: 65.71
Predictive neg %: 83.08
Test Efficiency %: 77.00

<table>
<thead>
<tr>
<th></th>
<th>IgA pos</th>
<th>IgA neg</th>
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</thead>
<tbody>
<tr>
<td>RPR + &gt; 8</td>
<td>29</td>
<td>2</td>
</tr>
<tr>
<td>RPR - &lt; 8</td>
<td>13</td>
<td>54</td>
</tr>
<tr>
<td>total 100 patients</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity %: 85.29
Specificity %: 80.30
Predictive pos %: 69.05
Predictive neg %: 91.38
Test Efficiency %: 82.00

1. Improving IgA performance using 2 Tp antigens

Combining two syphilis antigens in an ELISA assay increased the sensitivity of detecting active/early syphilis significantly but specificity has decreased marginally.

<table>
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<tr>
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<th>IgA neg</th>
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</thead>
<tbody>
<tr>
<td>RPR + &gt; 8</td>
<td>33</td>
<td>2</td>
</tr>
<tr>
<td>RPR - &lt; 8</td>
<td>16</td>
<td>50</td>
</tr>
<tr>
<td>total 100 patients</td>
<td></td>
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</tr>
</tbody>
</table>

Sensitivity %: 97.1
Specificity %: 75.8
Predictive pos %: 67.3
Predictive neg %: 98.04
Test Efficiency %: 83.0

Enough data to proceed with transition from ELISA to rapid test.
The Syphilis IgA rapid Test

- Qualitative lateral flow assay
- *Treponema pallidum* antigens are immobilized onto Test line (T)
- A procedural control (C) is included in the test to determine that the assay has been run correctly and to indicate whether the sample is IgA deficient.
- Colloidal gold-labelled anti-human IgA antibody detects *T. pallidum* specific IgA in the patient's sample.
- Visual readout any visible line in Test area=positive result
- Test time is 30 minutes using 5ul of serum, plasma or whole blood.

Test Procedure

1. Add 5ul of plasma or whole blood to 1st well. 1 drop of running Buffer. Incubate 10 minutes.
2. 4 drops of buffer to 20 minutes.
3. Visually interpret results.
4. 5ul applicator with whole blood.
Laboratory evaluation of the rapid IgA RPOCT

- Preliminary laboratory evaluation to assess its ability to identify active syphilis from a population of syphilis antibody positive and negative serum samples.
- National Center for Sexually Transmitted Disease Control, Nanjing, China in a ‘blinded’ study using (n=458) stored serum samples.
- Classified by rapid plasma reagin (RPR) and *Treponema pallidum* Haemagglutination (TPHA) serology
- HREC approval granted by Alfred Health and NCSTD Nanjing

458 serum samples were classified into the following groups:
- 154 active syphilis samples (TPHA positive + RPR titre ≥8)
- 153 past treated syphilis infection (TPHA positive, RPR negative)
- 151 healthy controls (TPHA and RPR negative)

IgA RPOCT differentiates Past/treated from active syphilis

<table>
<thead>
<tr>
<th>Reference test</th>
<th>IgA Confirm RPOCT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Active infection (TPHA + RPR≥8)</td>
<td>148</td>
</tr>
<tr>
<td>Past/treated (TPHA+/RPR-)</td>
<td>43</td>
</tr>
<tr>
<td>Negative (TPHA-/RPR-)</td>
<td>3</td>
</tr>
</tbody>
</table>

*Three results were indeterminate

<table>
<thead>
<tr>
<th>Percent (95% CI)</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>96.1% (91.6-98.4)</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>84.7% (80.2-88.6)</td>
<td></td>
</tr>
<tr>
<td>Specificity (past/treated)</td>
<td>71.3% (63.4-78.4)</td>
<td></td>
</tr>
<tr>
<td>Specificity (negative)</td>
<td>98.0% (94.3-99.6)</td>
<td></td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>76.3% (69.8-81.8)</td>
<td></td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>97.7% (95.0-99.1)</td>
<td></td>
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</tbody>
</table>
### Combining IgA RPOC + Determine™ Syphilis TP RPOC

#### IgA + total Ab RPOC classifies active, past/treated & negative

<table>
<thead>
<tr>
<th>Reference</th>
<th>active +/-</th>
<th>Past/treated +/-</th>
<th>negative +/-</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPHA + RPR</td>
<td>148</td>
<td>6</td>
<td>0</td>
<td>154</td>
</tr>
<tr>
<td>TPHA+/RPR-ve</td>
<td>43</td>
<td>107</td>
<td>0</td>
<td>150</td>
</tr>
<tr>
<td>RPR+ve/TPHA-ve</td>
<td>3</td>
<td>1</td>
<td>146</td>
<td>150</td>
</tr>
</tbody>
</table>

% Sensitivity active + Past/treated 100.0 97.1 - 100
% Specificity Past/treated 71.3 63.6 - 78.0
% Specificity negative 97.3 93.1 - 99.2

*4 indeterminates (95% CI)

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### In summary

- **Anti-treponemal IgA** is a potential marker for syphilis infections
- Can be converted to a RPOC device
- Met the WHO TPP performance requirement
- Used in combination with a rapid screening syphilis test, it can further classify 71.3% (107/150) of TP antibody positive samples as past/treated
- Immediate access to diagnosis and increased syphilis treatment uptake
- Further studies need to be undertaken using whole blood on high risk populations in a clinical setting.
- Low incidence hard to acquire performance data on blood
- Is IgA is detectable in newborn samples
- Validation using fingerprick blood
- Proof of concept to product requires resources, and long term investment (>5years)
- ISO13485 accredited facilities for design phase for commercialisation (time/$$)
- Extensive clinical trials to meet regulatory requirements.
Thank you to everyone...

• Stanley Luchters
• David Anderson
• Huy Van
• Yasmin Mohammed
• NCSTD: Han Yan and Mrs Wei. Prof Chen