EVALUATION OF FIVE RAPID POINT-OF-CARE TESTS FOR SYPHILIS: TWO TREPONEMAL ONLY, AND THREE DUAL TREPONEMAL/ HIV ASSAYS

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Syphilis Quick Facts
✓ Caused by - Treponema pallidum subspecies pallidum (spirochete bacterium)
✓ Transmission: Mostly by Sexual, or maternal – fetal route, rarely by blood transfusion
✓ Various stages of disease – Primary, Secondary, Latent (early or Late), and Tertiary syphilis.
✓ Syphilis - in pregnancy -- miscarriage, stillbirth, prematurity and organ damage, low birth weight, congenital syphilis.
✓ Syphilis increases risk of acquiring HIV in high risk population.
✓ Syphilis can be easily treated and adverse outcomes can be prevented (benzathine penicillin G)

Background
✓ WHO, PAHO, UNICEF - Global initiative to prevent MTCT of HIV/Syphilis
✓ Traditional syphilis and HIV screening strategies require laboratory capacity.
✓ Affordable, rapid point-of-care tests (RPOCT), high sensitivity and specificity.
✓ Same-day testing and referral for treatment of syphilis and HIV in pregnant women.

Global incidence of Syphilis
• WHO - Global problem with an estimated 12 million people infected each year.
• Estimated two million pregnancies are affected annually.
  ▪ ~ 25% of these pregnancies end in stillbirth or spontaneous abortion
  ▪ ~ 25% the newborn has a low birth weight or serious infection, both of which are associated with an increased risk of perinatal death.

Congenital Syphilis – Reported Cases Among Infants by Year of Birth and Rates of Primary and Secondary Syphilis Among Women, United States, 2001–2010
http://www.cdc.gov/std/stats10/figures/47.htm

Objectives
✓ Primary - To compare the sensitivity and specificity of two point-of-care (POC) treponemal tests and three treponemal/HIV tests for detection of treponemal antibodies in sera.
✓ Secondary - To determine the sensitivity and specificity of three POC treponemal/HIV tests for detection of HIV antibodies in sera.

Methods – Test Population

Test Population
• 1186 – Previously characterized sera for syphilis from Kaiser Permanente Northern California (KPNC)
  ▪ CIA+, RPR - TPPA+
  ▪ CIA+, RPR - TPPA-
  ▪ CIA+, RPR+
  ▪ CIA-
• 1623 – Kaiser Permanente Southern California (KPSC)
  ▪ EIA+, RPR - TPPA+
  ▪ EIA+, RPR - TPPA-
  ▪ EIA+, RPR+
  ▪ EIA-
• 437 – San Francisco Department of Public Health
  ▪ Sera from patients with diagnoses of primary and secondary syphilis.
• 457 – Known HIV positive samples – CDC Atlanta/ HIV
• Total – 1623

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Methods – Rapid tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Multiplo TP</td>
<td>77.2%</td>
<td>98.05%</td>
</tr>
<tr>
<td>2</td>
<td>Chembio TP</td>
<td>83.68%</td>
<td>97.07%</td>
</tr>
<tr>
<td>3</td>
<td>Multiplo TP</td>
<td>79.92%</td>
<td>91.58%</td>
</tr>
<tr>
<td>4</td>
<td>SD 3.0</td>
<td>75.31%</td>
<td>98.05%</td>
</tr>
<tr>
<td>5</td>
<td>Determine TP</td>
<td>89.96%</td>
<td>97.78%</td>
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All 1623 sera - tested by all rapid tests by CDC technicians blind.

Methods – RPOCT devices

- Treponemal/HIV POC tests
  - SD BIOLINE HIV Syphilis Duo (Standard Diagnostics)
  - Multiplo TP/HIV (MedMira*)
  - DPP HIV-syphilis Assay (Chembio)

- Treponemal RPOCT:
  - SD Syphilis 3.0 (Standard Diagnostics)
  - Determine SyphilisTP (Alere)

Results

Treponemal results
- 1623 samples tested.
- 1606 with all results.
- Only 27 samples known to be cases of P&S syphilis so far.
- An overall agreement on reactivities of 84.1% among the 5 tests on TP results.
- Traditional treponemal results (EIA, CIA or TPPA)
  - Reactive – 30.3% (487/1606)
  - Nonreactive – 69.7% (1119/1606)

HIV results
- 1623 samples - agreement of 96.6% among the 3 tests of 1569/1623.
- Only 437 samples had a known HIV status – there was 100% agreement among all 3 devices and known status.

Need all patient data to come in before sensitivity and specificity can be calculated.

Sensitivity and specificity of treponemal results from rapid tests compared to previous treponemal results

<table>
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Sensitivity of treponemal results vs known P&S cases

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<tr>
<td>Multiplo TP</td>
<td>100%</td>
</tr>
<tr>
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<td>92.69%</td>
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<td>Determine TP</td>
<td>96.3%</td>
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N=1606
Sensitivity and Specificity of 5 Rapid point-of-Care tests compared to consensus traditional treponemal result when testing syphilis PT samples

<table>
<thead>
<tr>
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<th>SD 2d</th>
<th>Determine</th>
<th>SD Day</th>
<th>Chemo</th>
<th>Multipi</th>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>94.7%</td>
<td>100.00%</td>
<td>94.7%</td>
<td>94.7%</td>
<td>95.7%</td>
</tr>
<tr>
<td>Specificity</td>
<td>94.4%</td>
<td>100.00%</td>
<td>100.00%</td>
<td>100.00</td>
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SD PT serum samples repeated once a week for 3 weeks.
Known reactivities previously confirmed with TPPA, FTA-ABS and EIA.

Challenges and Limitations
- Flow issues with some devices.
- Control lines not properly formed.
- Extremely faint lines – difficult to read.
- Complexity – Multiplo most challenging and Determine easiest to use.
- Serum samples of unknown freeze thaw cycles.

Summary
- Positive agreement was greater for HIV antibodies than for treponemal antibodies.
- Using banked sera could have affected performance of treponemal assays.
- Further prospective studies need to be performed in field to better characterize performance of RPOCT treponemal tests.
- Need more studies to generate independent data to provide to countries’ in the selection of RPOCTs for syphilis and HIV.

Co-authors
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- Philip S, San Francisco Department of Public Health

Many thanks
- Standard diagnostics – Alere
- Medmira
- Chembio

Save the Date!
2016 STD Prevention Conference
Atlanta, GA | September 2016
www.cdc.gov/std/prevention