Point-of-care testing for STIs: myth or reality?

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Disclosures

• I have received funding for research grants and/or have been a lecturer for Becton Dickinson, Gen-Probe/Hologic, Abbott Molecular Diagnostics, Siemens Health Care Diagnostics, and Cepheid
• 350 million (M) prevalent cases of curable STIs are estimated worldwide:
  ✓ 100M chlamydia (CT)
  ✓ 36M gonorrhea (NG)
  ✓ 187M trichomonas
  ✓ 36M cases of syphilis
  ✓ 34M HIV infections

• Include viral STIs:
  2,993,200,000
Objective: Some Myths; Some Reality

- To discern where we are heading for STI POC diagnostics for research & practice
  - *Chlamydia trachomatis*
  - *Neisseria gonorrhoeae*
  - *Syphilis (Treponema pallidum)*

- The Promising Future
Overview

• The **Myth**: What is bad/poor
• The **Reality**: What is reality
• The **Promise**: What we can expect

*Chlamydia trachomatis*

*Neisseria gonorrhoeae*
Sensitivity and Specificity of POC/near patient tests for *CT & NG*

<table>
<thead>
<tr>
<th>Organism</th>
<th>Test</th>
<th>Sample Type</th>
<th>Sensitivity*</th>
<th>Specificity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlamydia trachomatis</td>
<td>Biostar OIA Chlamydia test</td>
<td>Cervical, Male Urine</td>
<td>59.4-73.8%</td>
<td>98.4-100%</td>
</tr>
<tr>
<td></td>
<td>Clearview Chlamydia</td>
<td>Cervical, Vaginal</td>
<td>49.7%</td>
<td>97.9%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vaginal</td>
<td>32.8%</td>
<td>99.2%</td>
</tr>
<tr>
<td></td>
<td>Quick Vue</td>
<td>Cervical</td>
<td>25-65%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Chlamydia Rapid Test** (CRT)</td>
<td>Vaginal, Male Urine</td>
<td>74.2%, 41.4%</td>
<td>95.7%, 89.0%</td>
</tr>
<tr>
<td></td>
<td>X-pert CT/NG</td>
<td>Cervical, Vaginal, Female Urine, Male Urine</td>
<td>97.4%, 98.7%, 97.6%, 97.8%</td>
<td>99.6%, 99.4%, 99.8%, 99.9%</td>
</tr>
<tr>
<td>Neisseria gonorrhoeae</td>
<td>Biostar OIA GC test</td>
<td>Cervical</td>
<td>60%</td>
<td>89.9%</td>
</tr>
<tr>
<td></td>
<td>PATH GC-Check</td>
<td>Cervical, Vaginal</td>
<td>70%, 54.1%</td>
<td>97.2%, 98.25</td>
</tr>
<tr>
<td></td>
<td>X-pert CT/NG</td>
<td>Cervical, Vaginal, Female Urine, Male Urine</td>
<td>100%, 100%, 95.6%, 98.9%</td>
<td>100%, 99.9%, 99.9%, 99.9%</td>
</tr>
</tbody>
</table>

Adapted from Huppert et al. (2010). * Sensitivity and specificity Vs. NAATs; **Hurly STI Mar 2014
## Reality: Results CT/NG

### Xpert CT/NG vs. Patient Infected Status

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CT</strong> Cervical</td>
<td>97.4%</td>
<td>99.6%</td>
</tr>
<tr>
<td><strong>CT</strong> Vaginal</td>
<td>98.7%</td>
<td>99.4%</td>
</tr>
<tr>
<td><strong>CT</strong> Female Urine</td>
<td>97.6%</td>
<td>99.8%</td>
</tr>
<tr>
<td><strong>NG</strong> Cervical</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>NG</strong> Vaginal</td>
<td>100%</td>
<td>99.9%</td>
</tr>
<tr>
<td><strong>NG</strong> Female Urine</td>
<td>95.6%</td>
<td>99.9%</td>
</tr>
<tr>
<td><strong>CT</strong> Male Urine</td>
<td>97.5%</td>
<td>99.9%</td>
</tr>
<tr>
<td><strong>NG</strong> Male Urine</td>
<td>98.9%</td>
<td>99.9%</td>
</tr>
</tbody>
</table>

Chlamydia Promise:
Microwave-accelerated metal-enhanced fluorescence (MAMEF)

Microwave-based lysing

Ultra-rapid and sensitive detection of biomolecules
Clinical evaluation of CT MAMEF

Blind Evaluation of the Microwave-Accelerated Metal-Enhanced Fluorescence Ultrarapid and Sensitive *Chlamydia trachomatis* Test by Use of Clinical Samples

Johann H. Melendez, Jill S. Huppert, Mary Jett-Goheen, Elizabeth A. Hesse, Nicole Quinn, Charlotte A. Gaydos, Chris D. Geddes

Institute of Fluorescence and Department of Chemistry and Biochemistry, University of Maryland Baltimore County, Baltimore, Maryland, USA; Cincinnati Children’s Hospital Medical Center, Division of Gynecology, Cincinnati, Ohio, USA; Division of Infectious Diseases, Johns Hopkins University Medical School, Baltimore, Maryland, USA

- 257 vaginal swabs – 245 adolescents and young women

<table>
<thead>
<tr>
<th></th>
<th>NAAT+ / MAMEF+</th>
<th>NAAT+ / MAMEF-</th>
<th>NAAT- / MAMEF+</th>
<th>NAAT- / MAMEF-</th>
<th>Clinical Sensitivity (%)</th>
<th>Concordance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptic plasmid</td>
<td>37</td>
<td>8</td>
<td>15</td>
<td>197</td>
<td>82.2</td>
<td>91.1</td>
</tr>
<tr>
<td>16S rRNA</td>
<td>34</td>
<td>11</td>
<td>15</td>
<td>197</td>
<td>75.5</td>
<td>89.9</td>
</tr>
<tr>
<td>Both assays</td>
<td>33</td>
<td>12</td>
<td>15</td>
<td>197</td>
<td>77.3</td>
<td>89.5</td>
</tr>
</tbody>
</table>

-Less than 10 minutes       $1.50 per test       $2,500 reader

JCM 2013;51:2913-2920
GC Promise  MAMEF-based detection

Target – OPA gene

MAMEF assay # 1

SH Anchor Probe
GCCGTCGTAAGTTAAACAAGG
CGGCAGCATTCAATTTGGTTCCGAGTCAAAACAGCAAGTCCGCCTATACGCCTG
TAMRA Fluorescent Probe
GTCTTTCAGGCAGATATGCGGAC
Target Probe

MAMEF assay # 2

SH Anchor Probe
GCCGTCGTAAGTTAAACAAGG
CGGCAGCATTCAATTTGGTTCCGAGTCAAAACAGCAAGTCCGCCTATACGCCTG
TAMRA Fluorescent Probe
TCAGTTTTGTCTTCAAGGCAGATATGCGGAG
Target Probe

Green = Perfect match to N. meningitidis

MAMEF
+ 5 1
- 1 13
Promise: Single Use, Disposable Lysing and Detection Chip for CT and NG

- Au
- 0.5 micron Cell debris filtering system
- Mw shrinkable plastic
- Drain
- MAMEF Assay
- Disposable lysing and detection Microfluidic Chip
- DNA
- 3”
- 1”
Promise: Atlas Velox Point-of-Care

- User simply adds sample to card
- All other functions performed by system (on card)
  - DNA extraction
  - PCR amplification
  - Detection of target
- Perform test & treat in single clinic appointment
- Rapid results in 20 minutes

Principle of Detection

Electrochemical label released from probe hybridised to target by nuclease enzyme

- Nuclease double strand specific, so no label release in absence of target
- Voltage applied to carbon electrode
- At a known potential the electrochemical label oxidises generating measurable current

![Diagram of electrochemical process]

Positive Result

Negative Result
Chlamydia assay performance

100 patient samples determined to be positive or negative for Chlamydia using the BD test

<table>
<thead>
<tr>
<th></th>
<th>Disease Present</th>
<th>Disease Absent</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test +ve</td>
<td>49</td>
<td>0</td>
<td>49</td>
</tr>
<tr>
<td>Test -ve</td>
<td>1</td>
<td>50</td>
<td>51</td>
</tr>
<tr>
<td>Totals</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

98% sensitivity
100% specificity

306 patient samples determined to be positive or negative for Chlamydia using Roche or Gen-Probe test

<table>
<thead>
<tr>
<th>Atlas Genetics Assay Result</th>
<th>Johns Hopkins Results</th>
<th>GeneProbe/Roche Assay Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>105</td>
<td>4</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>195</td>
</tr>
</tbody>
</table>

98% sensitivity
98% specificity

Sensitivity (%) 98.1
Specificity (%) 98.0
Syphilis

Reverse Algorithm testing has been introduced in the U.S.

New POC serology tests for diagnosing syphilis have proliferated

Their use is important to syphilis elimination programs worldwide, especially MTCT
Serologic diagnosis **syphilis** requires detection of two types of antibodies

- **Non-Treponemal**  
  RPR, VDRL

- **Treponemal**  
  FTA-abs, TPPA, EIA/CIA,  
  Many POC developed

✓ Both test types have imperfect specificity

✓ Biologic false positive non-treponemal test

✓ Falsely reactive treponemal test due to cross-reacting serum antibodies

- Reactive treponemal test cannot distinguish active from inactive infection
Syphilis serologic screening algorithms

**Traditional**

- **Quantitative RPR**
  - **RPR+**
    - TP-PA
      - TP-PA+
        - **Syphilis** (past or present)
      - TP-PA-
        - **Syphilis unlikely**
  - **RPR-**

**Reverse sequence**

- **EIA or CIA**
  - **EIA/CIA+**
    - **RPR+**
      - **Syphilis** (past or present)
      - Evaluate clinically
    - **TP-PA**
  - **EIA/CIA-**
    - **Quantitative RPR**
      - **RPR-**
      - **TP-PA**
      - **TP-PA+**
        - **Syphilis** (past or present)
      - **TP-PA-**
        - **Syphilis unlikely**

- **CDC recommended algorithm for reverse sequence syphilis screening followed by nontreponemal test confirmation**

If at risk for syphilis, repeat RPR in several weeks.

Evaluate clinically.
# Sensitivities and Specificities for Point-of-Care Diagnostics for Syphilis

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<tr>
<th>Organism</th>
<th>Test</th>
<th>Sample Type</th>
<th>Sensitivity *</th>
<th>Specificity *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treponema pallidum (Syphilis)</td>
<td>Abbott Determine</td>
<td>Whole blood/Serum</td>
<td>59.6-100%</td>
<td>95.7-100%</td>
</tr>
<tr>
<td></td>
<td>Omega Visitect</td>
<td>Whole blood/Serum</td>
<td>72.7-98.2%</td>
<td>98.1-100%</td>
</tr>
<tr>
<td></td>
<td>Qualpro Syphicheck</td>
<td>Whole blood/Serum</td>
<td>64-97.6%</td>
<td>98.4-99.7%</td>
</tr>
<tr>
<td></td>
<td>Standard Bioline</td>
<td>Whole blood/Serum</td>
<td>85.7-100%</td>
<td>95.5-99.4%</td>
</tr>
<tr>
<td></td>
<td>Trinity Syphilis Health Check</td>
<td>Whole blood/Serum</td>
<td>98.2%**</td>
<td>97.3%**</td>
</tr>
</tbody>
</table>

5 Syphilis POC tests Study at CDC
[ongoing study SFDH, KPNC, KPSC]

N = 591/1700 tested

• Treponemal POC tests:
  1. SD Syphilis 3.0 (Standard Diagnostics)
  2. Determine Syphilis TP (Standard Diagnostics-Alere)

COMBO/DUAL

• Treponemal/HIV POC tests:
  3. Multiplo TP/HIV (MedMira)
  4. DPP HIV-syphilis Assay (Chembio)
  5. SD BIOLINE HIV Syphilis Duo (Standard Diagnostics)
The SD BIOLINE Syphilis 3.0 test is a solid phase immunochromatographic assay for the qualitative detection of antibodies of all isotypes (IgG, IgM, IgA) against Treponema pallidum (TP) simultaneously in human serum, plasma, or whole blood.

- Recombinant TP, 15kDa, 17kDa antigens used as captures and detectors; 2-30°C Storage
The Alere Determine™ Syphilis TP test empowers healthcare professionals to detect antibodies to *Treponema pallidum* at the point of care. This rapid, *in vitro*, qualitative immunoassay provides a result in just 15 minutes, meaning patients can be tested and treated in the same visit.

**Rapid**
The test provides accurate and reliable results in just 15 minutes.

**Convenient**
It is easy to transport and store with no refrigeration required (storage conditions are 2-30°C). No power or water source is needed to run the test.

**Flexible**
Sampling can be done using serum, plasma or whole blood by finger prick or venipuncture.
3 minute test procedure
Whole blood, serum or plasma specimens
No specialized training required
Built-in procedural and reagent control line

18 month shelf-life at 2-30°C
No refrigeration or cold chain required
No timers required
Results are easy to interpret
No specialized equipment required
Chembio Diagnostic Systems has developed the first dual HIV 1/2 and Syphilis Treponemal antibodies Point-of-Care (POC) test utilizing Chembio’s patented Dual Path Platform (DPP®) technology.

The Chembio DPP® HIV-Syphilis Assay is single-use immunochromatographic rapid screening test for the detection of antibodies to Human Immunodeficiency Virus Types 1 and 2 (HIV 1/2) and Syphilis Treponema pallidum in fingerstick whole blood, venous whole blood, serum, and plasma.

[Video whole Blood Sample](http://www.youtube.com/watch?v=DE4Wxy4byQE&feature=player_embedded&x-yt-ts=1401912551)
The SD BIOLINE HIV/Syphilis Duo test is a solid phase immunochromatographic assay for the qualitative detection of antibodies to all isotypes (IgG, IgM, and IgA) specific to HIV-1/2 and/or Treponema pallidum (TP) simultaneously in human serum, plasma, or whole blood.

1-30°C for 24 months
**Promise: Syphilis and HIV Preliminary Results**

- **Sera tested n= 591/1700**
- Of the Treponemal tests, 84.4% agreement all 5 assays
- (Reactive 333, non-reactive 166), with 92 results discordant
- TPPA used as the Gold Standard

### Tests for Syphilis

<table>
<thead>
<tr>
<th>Tests for Syphilis</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chembio DPP HIV-syphilis</td>
<td>90.37</td>
<td>95.71</td>
<td>92.49</td>
<td>94.44</td>
</tr>
<tr>
<td>SD BIOLINE HIV Syphilis Duo</td>
<td>84.40</td>
<td>95.71</td>
<td>92.0</td>
<td>91.30</td>
</tr>
<tr>
<td>Multiplo TP/HIV</td>
<td>84.09</td>
<td>95.44</td>
<td>91.58</td>
<td>91.05</td>
</tr>
<tr>
<td>SD Syphilis 3.0</td>
<td>83.49</td>
<td>97.32</td>
<td>94.79</td>
<td>90.98</td>
</tr>
<tr>
<td>Determine Syphilis TP</td>
<td>97.71</td>
<td>95.71</td>
<td>93.01</td>
<td>98.62</td>
</tr>
</tbody>
</table>

- **HIV results for the 3 assays with DUAL HIV/ Syphilis**
  - All 3 assays were HIV reactive in 207 samples, while non-reactive in 357 and discordant in 27 (n= 591)
  - There was a Cohen’s kappa value of 0.95, indicating a good agreement among the 3 assays
More Promise: Combined HIV/Syphilis POC Tests in Development

- INSTI Combined HIV/Syphilis Test (Biolytical Laboratories)
- mChip Assay
  (Junco Labs and Columbia University in collaboration with OPKO Health, Inc.)
- Uni-Gold™ HIV/Syphilis Assay (Trinity Biotech)
- PreventIt (Research Consortium)
- Combination HIV/Syphilis Assay (MBio DiagnosticsR)
Conclusions
The Reality of a Promising Future

• Better Cheaper POC tests; self testing possible
• Testing outside a laboratory to now in the clinic & outreach (regulatory issues remain barriers)

• Use of research to remove barriers to testing
• Learning how to effectively use these new tools and new research can improve the detection of STIs and provide cost-effective ways to increase the number of patients being treated
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