Point-of-care testing for Sexually Transmitted Diseases

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Disclosures

• Nothing to disclose
• No conflicts of interest
Special Thanks

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For sharing slides and data
Objectives

• At the end of this presentation, attendees should be able to:
  – Identify current options available for STD POCTs
  – Discuss promising future STD POCT options for
    • Gonorrhea
    • Chlamydia
    • HSV
    • Syphilis
Background: World Estimates

- 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
Background: U.S. Estimates

Estimated **Prevalence** of Sexually Transmitted Infections in the U.S.  
(Total 110,197,000)

Estimated **New** Sexually Transmitted Infections in the U.S.  
(Total 19,738,800/Year)

What do Clinicians want?

- First Priority of Needs Assessment Survey (N=218)
  - Chlamydia (62%); HIV – Early Seroconversion (14%)
  - Syphilis (8%)

- Overall, participants selected sensitivity as their top priority, followed by cost, specificity, and time

- Choices (statistically significant)
  - Sensitivity: 90-99% > 80-90% > 70-80%
  - Cost: $20 > $35 > $50
  - Specificity: 99% > 95% > 90%
  - Time: 5 > 15 > 25 minutes

- *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><strong>Chlamydia trachomatis</strong></td>
<td>Biostar OIA Chlamydia test</td>
<td>Cervical</td>
<td>59.4-73.8%</td>
<td>98.4-100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male Urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clearview Chlamydia</td>
<td>Cervical</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</td>
<td>74.2%</td>
<td>95.7%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male Urine</td>
<td>41.4%</td>
<td>89.0%</td>
</tr>
<tr>
<td></td>
<td>X-pert CT/NG</td>
<td>Cervical</td>
<td>97.4%</td>
<td>99.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vaginal</td>
<td>98.7%</td>
<td>99.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female Urine</td>
<td>97.6%</td>
<td>99.8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male Urine</td>
<td>97.8%</td>
<td>99.9%</td>
</tr>
<tr>
<td><strong>Neisseria gonorrhoeae</strong></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</td>
<td>70%</td>
<td>97.2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vaginal</td>
<td>54.1%</td>
<td>98.25</td>
</tr>
<tr>
<td></td>
<td>X-pert CT/NG</td>
<td>Cervical</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vaginal</td>
<td>100%</td>
<td>99.9%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female Urine</td>
<td>95.6%</td>
<td>99.9%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male Urine</td>
<td>98.9%</td>
<td>99.9%</td>
</tr>
</tbody>
</table>

Adapted from Huppret et al. (2010). * Sensitivity and specificity Vs. NAATs; **Hurly STI Mar 2014
### Current 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>

Future Chlamydia POCTs:
Microwave-accelerated metal-enhanced fluorescence (MAMEF)

- Microwave-based lysing
- Microwave-accelerated metal-enhanced DNA detection

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

Johan 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
GC Promise  MAMEF-based detection

Target – OPA gene

MAMEF assay # 1

<table>
<thead>
<tr>
<th>TAMRA</th>
<th>Fluorescent Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH</td>
<td>Anchor Probe</td>
</tr>
<tr>
<td>GCCGTCGTAAGTTAAACAAAGG</td>
<td>GTCGTTCAAGCGGATATGCGGAC</td>
</tr>
<tr>
<td>CGGCAGCATTCAATTTTGTTCCGAGTCAAAAACAGCAAGTCCGCCTATACGCCTG</td>
<td></td>
</tr>
</tbody>
</table>

MAMEF assay # 2

<table>
<thead>
<tr>
<th>TAMRA</th>
<th>Fluorescent Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH</td>
<td>Anchor Probe</td>
</tr>
<tr>
<td>GCCGTCGTAAGTTAAACAAAGG</td>
<td>TCAGTTTTGTCGTTCAAGCGGATATGCGGAG</td>
</tr>
<tr>
<td>CGGCAGCATTCAATTTTGTTCCGAGTCAAAAACAGCAAGTCCGCCTATACGCCTG</td>
<td></td>
</tr>
</tbody>
</table>

Target Probe

Green = Perfect match to N. meningitidis

Melendez CDC STD Conf.
Atlanta GA Poster WP 147;
June 9-12, 2014
Detection of GC resistance markers
Surface plasmon resonance (SPR)

- Refractive index-based monitoring of interactions between molecules
- Low limits of detection

Original position of dark line
Angular shift
Promise: Single Use, Disposable Lysing and Detection Chip

- Au
- Mw shrinkable plastic
- 0.5 micron Cell debris filtering system
- Drain
- MAMEF Assay
- Disposable lysing and detection Microfluidic Chip

1" x 3" dimensions
Promise: Atlas Velox TM System

Instrument
- Small footprint
- Low cost
- No reagents onboard
- No fragile optical sensors
- Portable – Robust reader for POC settings

Disposable card
- All reagent stabilised on card
- 20 minutes
- Simple to use system - designed to meet CLIA Waiver
  - Chlamydia (lead product)
  - Chlamydia & Gonorrhea
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
# Chlamydia assay performance

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

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

98% 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

<table>
<thead>
<tr>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>98.1</td>
<td>98.0</td>
</tr>
</tbody>
</table>
Trichomonas vaginalis
Reality: OSOM Rapid TV Antigen Test

- Immunochromatographic detection
- TV membrane proteins
- Mouse antibodies
- Latex beads/capillary action

Huppert, 2005; 2007: Sensitivity 83-90%, Specificity 98-100%

- A blue Test Line and a red Control Line is a positive result
- A red Control Line but no blue Test Line is a negative result.
Herpes simplex 1-2 (HSV)
<table>
<thead>
<tr>
<th>Organism</th>
<th>Test</th>
<th>Sample Type</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV 2 Serology</td>
<td>HerpeSelect</td>
<td>Serum</td>
<td>80-100%*</td>
<td>41-100%*</td>
</tr>
<tr>
<td></td>
<td>HerpeSelect</td>
<td>Serum</td>
<td>91%**</td>
<td>97%**</td>
</tr>
<tr>
<td>HSV 2 Serology</td>
<td>Kalon HSV-2 gG2</td>
<td>Serum</td>
<td>84-98.6%*</td>
<td>83.2-100%*</td>
</tr>
<tr>
<td>HSV POC Serology</td>
<td>Biokit</td>
<td>Blood/Serum</td>
<td>80.7-94.5%^</td>
<td>92.4-94.1%^</td>
</tr>
<tr>
<td>HSV Organism PCR</td>
<td>Rapid Real-Time PCR</td>
<td>Genital secretions,</td>
<td>96.7%***</td>
<td>99.6%***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genital lesions, Buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>solutions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV Organism POC</td>
<td>IsoAMP HSV POC</td>
<td>Genital swabs</td>
<td>97.1%****</td>
<td>93.4%****</td>
</tr>
</tbody>
</table>


*Adapted from Biaro et al. (2011). Sensitivity and specificity are expressed as a range from multiple studies over multiple years from the meta-analysis performed in the publication

**Adapted from Zahariadis et. al. (2010); ***Adapted from Gardella et. al. (2010)

****Adapted from Lemieux et. al. (2012); ^ Unpublished
• Analytical sensitivity of the assays was 5.5 and 34.1 copies/reaction for HSV-1-2

• Viral culture was used as the reference standard, the clinical sensitivity and specificity of the IsoAmp® HSV assay were 100.0% and 96.3% respectively.
### IsoAmp® HSV virus assay workflow.

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
</table>
| **1. Sample Prep** (5 min) | 1. Add 25 µL sample to the dilution tube and invert to mix  
2. Transfer 25 µL diluted sample to 0.2 mL Amp. tube |
| **2. Amplification** (65 min) | 2.1. Make Master Mix by adding 25 µL Enzyme to 100 µL Amplification Reagent  
2.2. Add 25 µL Master Mix to the 0.2 mL tube from step 1.2.  
2.3. Add 50 µL mineral oil and Place the tube in a 64°C heat block for 60 min |
| **3. Detection** (20 min) | 3.1. Place the tube in a cassette  
3.2. Close the lid to release the liquid  
3.3. Read results after 15 min |
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 requires detection of two types of antibodies

- **Non-Treponemal**: RPR, VDRL
- **Treponemal**: FTA-abs, TPPA, EIA/CIA, Many POCTs

- 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
Second generation treponemal tests utilize recombinant antigens

- Recombinant *T. pallidum* antigens developed in the 1980s
  - High test specificity
  - Recombinant antigens as solid-phase immunoassays
  - High test sensitivity
- Over the years, several POCTs, EIAs, CIAs, and MFIs have become commercially available
Syphilis serologic screening algorithms

**Traditional**

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

**Reverse sequence**

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

*If at risk for syphilis, repeat RPR in several weeks*
Sensitivities and Specificities for Point-of-Care Diagnostics for Syphilis

<table>
<thead>
<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>

A Non-treponemal & Treponemal Combo Test

Source: Chembio Diagnostic Systems Inc., DPP® Syphilis Screen & Confirm product information sheet, 2009
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)

5 Syphilis POC tests Study at CDC
[ongoing study SFDH, KPNC, KPSC]
N = 591/1700 tested
1. SD BIOLINE Syphilis (Standard Diagnostics)

http://www.standardia.com

- 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.
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

<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 Diagnostics)
Conclusions

- Better, Cheaper POC tests; self testing possible
- Testing outside a laboratory to near patient (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