What is the Point in POC Testing for STIs?

Symposium:
Sexually Transmitted Infections: The TEST Matters

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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, Siemens Health Care Diagnostics, Cepheid, and Quidel
Objectives

1. To review clinicians and patient needs for POC STIs tests

2. To discuss current and update new POC tests in the pipeline

3. To discuss the accuracy of older and new POC tests

4. To mention impact of POC testing-advantages and barriers
Overview: Laboratory and Point-of-Care Tests for STIs

*Chlamydia trachomatis* (*CT*)
*Neisseria gonorrhoeae* (*NG*)
*Trichomonas vaginalis* (*TV*)
Syphilis
Herpes Simples Virus (*HSV*)
HIV

Gaydos, C. Rapid Tests for STDs Current Infect Dis Reports 2006;8:115-124
Huppert et al. Point of Care tests for STIs: What’s the Point? Point of Care Journal, 2009
Laboratory Test vs. POC Test

- Laboratory performed test may take 2-4 days turn around time or longer
- Lab test FDA rated highly complex or moderately complex; require a lab
- POC test (CLIA Waived) take 10-20 min-1 hour; minimal equipment
- POC (CLIA Waived) rated simple enough to be performed by a trained health care worker (minimal training)
What Are Current POCs?

- **CT** - Clearview (Inverness); Cx 49.7% sens; vag 32.8% sens
- **NG** - None FDA cleared but Gram Stain
- **Trichomonas** - Wet preparation - OSOM
- **Syphilis** - RPR, VDRL; sensitive, not specific; FS POC
- **HSV** - Tzanck Smear; IsoAmp® HSV Assay
- **HIV** - Oral fluid antibody tests; Many other FS POCs

New POC tests for STIs

- Chlamydia
- Gonorrhea
- Trichomonas
- Syphilis
- HSV
- HIV
Needs Assessment of Clinicians

- For which organisms do Clinicians want a POC test? (Most say chlamydia)
- How sensitive?; (most important -90-99%)
- How specific? (99%)
- How fast does it have to be? (-20 min)
- What about cost? (second most important- $20)
- What about equipment? (no or little equipment)

Forced Choice Questions used in a survey with multivariate analysis

What about Patients Needs?

- Willingness to wait is important
- Willingness to self-collect specimens is important
- Willingness to pay is important

Rompalo et al. Sexual Health 2013;10:541-545
Patient Focus Group and Clinic Questionnaire about POC Tests (N = 371)

<table>
<thead>
<tr>
<th>Specimen Type Preference</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical</td>
<td>15.4%</td>
</tr>
<tr>
<td>Vaginal</td>
<td>50.9%</td>
</tr>
<tr>
<td>Urine</td>
<td>33.7%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Willingness to Wait</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 min</td>
<td>59.0%</td>
</tr>
<tr>
<td>40 min</td>
<td>20.8%</td>
</tr>
<tr>
<td>60 min</td>
<td>10.8%</td>
</tr>
<tr>
<td>90 min</td>
<td>9.4%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Willingness to Pay</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10</td>
<td>46.6%</td>
</tr>
<tr>
<td>$20</td>
<td>31.0%</td>
</tr>
<tr>
<td>$30</td>
<td>10.8%</td>
</tr>
<tr>
<td>$40</td>
<td>2.7%</td>
</tr>
<tr>
<td>$50</td>
<td>8.9%</td>
</tr>
</tbody>
</table>

Self-collected vaginal swabs

- Easy: 80.9%
- Hard: 16.1%
- OK: 3.0%

Barnes et al. 2014 CDC STD Conf, Atlanta GA
Chlamydia trachomatis and Neisseria gonorrhoeae
New: “Near Patient” Test for Chlamydia and Gonorrhea

GeneXpert® CT/NG, (90 minutes)

Urine or female Swab samples in Transport Reagent

Transfer the sample to the cartridge

Insert cartridge and start assay

Total hands-on time: <1 Minute

Results CT/NG 1,722 female & 1,387 males

Xpert CT/NG vs. Patient Infected Status

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

<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></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</td>
<td>Vaginal Male Urine</td>
<td>83.5%</td>
<td>98.9%</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%</td>
<td>97.2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>54.1%</td>
<td>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 compared to NAATs
What about new CT/NG tests coming along?

1. MAMEF-based DNA detection (microwave accelerated metal enhanced fluorescence)
2. Atlas Velox TM System
3. LAMP Assay
4. MobiLab
1. MAMEF-based DNA detection

Microwave-based lysing

Ultra-rapid and sensitive detection of biomolecules

Microwave-Accelerated Metal-Enhanced Fluorescence DNA detection
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

<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>

- 257 vaginal swabs – 245 adolescents and young women

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

JCM 2013;51:2913-2920
Microwave Irradiation on *Neisseria gonorrhoeae*

- Testing of 20 dry vaginal swabs by MAMEF
- Rapid lysis of cells, fragmentation, and detection of target DNA can be carried out in <10 minutes.
- Detection of GC target DNA is mediated by a fluorescent probe-based approach

<table>
<thead>
<tr>
<th>MAMEF</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NAAT</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

1 – DNA ladder
2 – Unlysed GC cells
3 – Lysed GC – lysing chamber
4 – Lysed GC – microfluidic lysing
2. Atlas Velox TM System

Platform/Equipment

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

Disposable cartridge

- Reagent stabilised on card
- 20 minutes
- Simple to use system - designed to meet CLIA Waiver
  - Chlamydia (lead product)
  - Chlamydia & Gonorrhea
- Electrochemical label released from probe hybridised to target by nuclease enzyme
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>positive</td>
<td>49</td>
<td>0</td>
</tr>
<tr>
<td>negative</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>50</strong></td>
<td><strong>50</strong></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>Johns Hopkins Results</th>
<th>GeneProbe/Roche Assay Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlas Genetics Assay Result</td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>105</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
</tr>
</tbody>
</table>

98% sensitivity
98% specificity

3. LAMP At Point of Use

- Single use, totally disposable
- Fully self contained / Self-powered
- Uses proprietary isothermal amplification
- No training needed
- Highly accurate¹
- Fast swab to result in 20 minutes
- Highly economical

¹ 98% specificity 99% sensitivity
4. MobiLab: A low-cost mobile NAAT platform for Chlamydia

- A Smartphone-enabled microfluidic NAAT
- Three units: a droplet pendant microfluidic cartridge, a battery-powered instrument for droplet manipulation and amplification, and a smartphone for user interface, data acquisition and processing
- A single-stream loop-mediated isothermal amplification (LAMP) assay
- Magnetic particles capture nucleic acid targets from sample lysate via electrostatic interaction.
Trichomononas vaginalis

- Wet Preparation - 55%–65% sensitive
- Affirm - 46.3% sensitive
- OSOM POC - 83.3-90% sensitive

OSOM Rapid TV Antigen Test

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

Can this be done by the patient?

Huppert et al, JCM 2005; STI 2007: Sensitivity 83-90%, Specificity 98-100%

5

**POSITIVE**

- A blue Test Line and a red Control Line is a positive result

**NEGATIVE**

- A red Control Line but no blue Test Line is a negative result
AmpliVue® Trichomonas Assay

• Assay combines three steps:
  1) simple sample preparation with one-step dilution/heating
  2) isothermal DNA amplification of target sequences specific to *T. vaginalis* by HDA
  3) lateral-flow strip based colorimetric detection in a self-contained, disposable device

Sensitivity 100%; specificity 98.2% vs. culture/wet prep. Vs. NAAT PPA 87.2-90.1%
Syphilis: Serologic DX requires detection of two types of antibodies

- Non-Treponemal: RPR, VDRL (Can be POC)
- Treponemal: FTA-abs, TPPA, Many new POC

Reactive treponemal test cannot distinguish active from inactive infection
POC Syphilis Health Check™

Syphilis Antibody Rapid Immunochromatographic Test

- Rapid qualitative screening for human TP antibodies in whole blood, serum or plasma
- Results in 10 minutes; 2 steps; room temperature
- 98% agreement to other treponemal tests
- Serum, plasma or whole blood or finger-stick

Negative: 1 colored band in control area
Positive: Colored bands in test area and control area
Inconclusive: No distinct color bands in either area

FDA Cleared
CLIA Waived
Evaluation of an Immunochromatographic Point-of-Care Test for the Simultaneous Detection of Nontreponemal and Treponemal Antibodies in Patients With Syphilis

Rita Castro, MD, PhD,*† Ângela Lopes, Bsc,‡ and Filomena da Luz Martins Pereira, MD, PhD§

### DPP Syphilis Screening & Confirm Assay (SSCA)

<table>
<thead>
<tr>
<th>SSCA</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compared to RPR</td>
<td>98.8%</td>
<td>94.7%</td>
</tr>
<tr>
<td></td>
<td>171/173</td>
<td>71/75</td>
</tr>
<tr>
<td>Compared to TPHA</td>
<td>95.5%</td>
<td>88.8%</td>
</tr>
<tr>
<td></td>
<td>184/185</td>
<td>56/63</td>
</tr>
<tr>
<td>Compared to FTA-abs</td>
<td>98.9%</td>
<td>93.2%</td>
</tr>
<tr>
<td></td>
<td>187/189</td>
<td>55/59</td>
</tr>
</tbody>
</table>
Standard Diagnostics-Alere Determine™ Syphilis TP POC


Detects
Antibodies to *Treponema pallidum* Recombinant TP (15kDa, 17kDa) antigens used as captures and detectors

Rapid
Provides accurate and reliable results in 15 minutes

Convenient
No refrigeration required (storage 2-30°C)
No power or water source is needed to run test

Flexible
Uses serum, plasma or whole blood by venipuncture or finger prick
What about Combination Syphilis and HIV POC tests?

These are coming soon—stay tuned

Here is a preview
Chembio Diagnostic Systems has developed a dual HIV 1/2 and Syphilis Treponemal antibodies POC test (Dual Path Platform (DPP®) technology)

- Immunochromatographic rapid screening POC test
- Fingerstick whole blood, venous whole blood, serum, and plasma

Video whole Blood Sample

http://www.youtube.com/watch?v=DE4Wxy4byQE&x-yt-ts=1401912551

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

3 minute test procedure
Whole blood, serum or plasma specimens
No specialized training required
Built-in procedural and reagent control line
DPP HIV-1/2 Assay: Now CLIA Waived

- Moderate complexity for serum, plasma, oral fluid
- “Sample Tainer” = residual specimen after testing
- FDA-approved Dec 21, 2012
- CLIA for Fingerstick or Oral fluid
## Sensitivities and Specificities for POC Tests for HIV

<table>
<thead>
<tr>
<th>Organism</th>
<th>Test</th>
<th>Sample Type</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV*</td>
<td>OraQuick Advance Rapid HIV-1/2 Antibody Test</td>
<td>Oral Fluid, Whole blood/Serum</td>
<td>99.6%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Reveal G3 Rapid HIV-1 Antibody Test</td>
<td>Serum/Plasma</td>
<td>99.8%</td>
<td>99.9%</td>
</tr>
<tr>
<td></td>
<td>Multispot HIV-1/HIV-2 Rapid Test</td>
<td>Serum/Plasma</td>
<td>100%</td>
<td>99.9%</td>
</tr>
<tr>
<td></td>
<td>Uni-Gold Recombigen HIV Test</td>
<td>Whole blood/Serum/Plasma</td>
<td>100%</td>
<td>99.7%</td>
</tr>
<tr>
<td></td>
<td>Clearview HIV-1/2 Stat-Pak or Clearview Complete HIV ½</td>
<td>Whole blood/Serum/Plasma</td>
<td>99.7%</td>
<td>99.9%</td>
</tr>
<tr>
<td></td>
<td>Chembio DPP HIV1/2 Assay</td>
<td>Oral Fluid, Whole blood/Serum/Plasma</td>
<td>99.8%</td>
<td>99.9%</td>
</tr>
<tr>
<td></td>
<td>INSTI HIV-1 Antibody Test</td>
<td>Whole blood/Plasma</td>
<td>99.8%</td>
<td>99.5%</td>
</tr>
</tbody>
</table>


*Adapted and Updated from Huppert et. al. (2010) and Branson (2007)
HSV
# Sensitivities and Specificities for Serology & POC Diagnostics for HSV-2

<table>
<thead>
<tr>
<th>Herpes Simplex Virus 2 (HSV-2)</th>
<th>Test</th>
<th>Sample Type</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serology</strong></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></td>
<td>Kalon HSV-2 gG2</td>
<td>Serum</td>
<td>84-98.6%*</td>
<td>83.2-100%*</td>
</tr>
<tr>
<td><strong>Virus</strong></td>
<td>Rapid Real-Time PCR LDT ABI7500 Fast</td>
<td>Genital lesions</td>
<td>96.7%***</td>
<td>99.6%***</td>
</tr>
<tr>
<td><strong>Virus</strong></td>
<td>Qx PCR (BD)</td>
<td>Lesion vs. PIS</td>
<td>95.9-97.3%†</td>
<td>95.7-100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lesion vs. PCR</td>
<td>95.7-100%</td>
<td>95.8-100%</td>
</tr>
<tr>
<td><strong>Virus</strong></td>
<td>IsoAMP HSV POC <em>(Biohelix)</em></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. **Adapted from Zahariadis et. al. (2010); ***Adapted from Gardella et. al. (2010); †Van Der Pol et al. J Clin Microbiol 2012;51:3466-3471. ****Adapted from Lemieux et. al. (2012)
New Virus POC HSV Test- IsoAMP

- FDA cleared POC assay for the detection of HSV in lesions; IsoAMP® HSV (Biohelix)
- Technology utilizes isothermal helicase dependent amplification (HAD), which uses Bst DNA polymerase, and by obviating the nucleic acid extraction process, with results in 1.5 hours
- From 5 sites in the U.S., and after discrepant analysis, overall agreement of IsoAmp with ELVIS was 98.8%, with a 37.0% overall prevalence
- 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. (5.5 and 34.1 copies/reaction for HSV-1-2)

The IsoAmp® HSV Assay (Biohelix Corp)

- FDA-cleared for HSV in genital and oral lesions
- The IsoAmp HSV has a test-to-result time of <1.5 hr.
- Isothermal helicase-dependent amplification (HDA) technique; no nucleic acid extraction
- The rapid and simple characteristics of the IsoAmp HSV assay make it potentially suitable for POC testing

Lemieux et al. Expert Reviews Ltd. 437-443, 2012;
Why do POCTs?

• Improve patient satisfaction
• Treat patients before leave clinic
• Provide counseling on risk reduction
• Decrease interval of disease spread
• Improve clinical practice efficiency
• Improve medical outcomes

Published data to substantiate these claims are rare to date
Barriers to implementation of POCTs

- Financial viability
- Money for instruments and consumables
- Obtaining CLIA certificate
- Validating the new test(s)
- Policies and procedures (training manuals)
- Operator training (recertification, proficiency)
- Getting results into the EMR (interface- $7K?)
- Space
- Work Flow Disruption
- Billing and Reimbursement
Conclusions

• POCTs in primary/STI care have great potential

• But there are barriers to successful implementation that need to be overcome which can be costly, time consuming, and require learning new skill sets

• Better POC tests are coming; the future is promising
Acknowledgements

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• Laura Dize
• Perry Barnes
• Billie Masek

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