Developments in STI Testing and Implications for Practice

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April 20, 2016
During the webinar you may e-mail your questions for the presenter to:

maphtc@jhu.edu

Questions will be answered at the end of the presentation
Disclosures

• Nothing to disclose
• No conflicts of interest
Special thanks to Dr. Charlotte Gaydos for sharing her slides
Objectives

At the end of this presentation, attendees should be able to:

• Discuss current options available for point of care STI/HIV testing

• Identify pros and cons to new syphilis rapid tests and the reverse syphilis testing algorithm

• Discuss the importance of extra-genital STI testing
Case Study

• Alyssa is a 17 year old female runaway who presents complaining of 5 days of vaginal discharge
  – Discharge smells funny, is itchy, but not any blood seen
  – Also has mild abdominal pain mostly during sex
• She’s been on the streets for 6 months living with her new boyfriend, Derek
• She has never had an STI
• She only has sex with Derek
  – oral, vaginal and anal sex
• She is not using birth control and her last menstrual period was about 6-8 weeks ago
• She only engaged in injection drug use once and shared her boyfriend’s needle
Even before you examine her, what is on your differential diagnosis list?

### Funny Vaginal Discharge
- Chlamydia
- Gonorrhea
- Bacterial vaginosis
- Trichomonas
- Candidiasis
- PID

### Other things to consider
- Sites of infection
- HIV
- Pregnancy
- Co-morbidities
  - Drug use
  - Abuse
  - Psychiatric issues

And what about that Derek?
Facts to remember

• The anatomy, physiology and microbiome of the vagina are age-dependent
  – This results in different causes of vaginitis in the neonate, infant, prepubertal girl and pre and postmenopausal women

• There are two types of cervicitis:
  – Endocervicitis: CT, NG, MG, and HSV
  – Ectocervicitis: TV, HSV
## Poor Correlation Between Symptoms and Diagnosis

<table>
<thead>
<tr>
<th></th>
<th>Abn Discharge</th>
<th>Pruritis</th>
<th>Burning</th>
<th>Odor</th>
<th>Dysuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trich</td>
<td>12%</td>
<td>11%</td>
<td>15%</td>
<td>17%</td>
<td>15%</td>
</tr>
<tr>
<td>BV</td>
<td>42%</td>
<td>31%</td>
<td>24%</td>
<td>62%</td>
<td>21%</td>
</tr>
<tr>
<td>Yeast</td>
<td>17%</td>
<td>23%</td>
<td>24%</td>
<td>18%</td>
<td>18%</td>
</tr>
<tr>
<td>No Infection</td>
<td>19%</td>
<td>25%</td>
<td>34%</td>
<td>23%</td>
<td>32%</td>
</tr>
</tbody>
</table>

Landers, Wiesenfeld, Heine et al. AJOG 2004
How do I get to the diagnosis?

What do I have available to me right now?
What laboratory tests do I need to order?
What specimens do I need to collect?
Near Patient or Point of Care Tests for work-up of discharge

Vaginal Discharge
- Look at it
- Test the pH
- Add 10% KOH and smell for “fishy odor”
- Examine it under a light microscope

Cervical Discharge
- Look at it
- Gram’s Stain it and examine it under a light microscope
What do I have available to me right now?

**Cervicitis**
- Gonorrhea
  - Culture
  - Gram’s Stain
- Chlamydia
  - DFA stain
  - Tissue culture in McCoy Cells
- **BOTH = NAATS (Nucleic Acid Amplification Tests)**
  - Real time PCR (Roche)
  - TMA (Transcription Mediated Amplification) (Gen-Probe)
  - SDA (Strand Displacement Amplification) (Becton Dickinson)
  - Real Time PCR (m2000) (Abbott)
  - Real Time PCR (GenXpert) (Cepheid)
  - Robotics: Tigress, Viper, m2000, Infinity 80

**Vaginitis**
- Bacterial vaginosis
  - Amsel’s Criteria
  - Nugent’s score
- Candida
  - KOH preparation
  - Culture
- Trichomonas
  - Wet preparation
  - Culture
  - OSOM
  - Affirm
  - NAATS

**POCT**

**NAATs** 90-97% sensitivity; 98-100% specificity
Now, what about gonorrhea and chlamydia?

How do I get to the diagnosis?

What do I have available to me right now?

Are there any POCTs available to me right now?

What laboratory tests do I need to order?

What specimens do I need to collect?
## 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 Huppert et al. (2010). * Sensitivity and specificity Vs. NAATs; **Hurly STI Mar 2014
Biostar: 19 minutes  $29.95 (per web)
QuickVue: 12 minutes: $15.93 (per web)
Chlamydia Rapid Test:
10 minutes: $28.95 (per web)
## 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>

So what’s the upfront cost?

GeneXpert XVI-8
- 8 independent testing units (modules)
- 8 module upgrade capacity available
- EACH Cartridge includes all reagents required
- EACH Cartridge includes internal controls run with every specimen

Established reimbursement rates for frequently ordered tests

<table>
<thead>
<tr>
<th>Test</th>
<th>CPT Code</th>
<th>Medicare National Limitation Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT/NG</td>
<td>CT 87491, NG 87591</td>
<td>$48.24, $48.24</td>
</tr>
<tr>
<td>GBS LB (Antepartum)</td>
<td>87150</td>
<td>$48.24</td>
</tr>
<tr>
<td>Flu</td>
<td>Flu A and B 87502, H1N1 87503</td>
<td>$116.96, $28.55</td>
</tr>
</tbody>
</table>
NAATs Only Test Type Recommended by CDC for CT and NG

NAATs are FDA cleared for:

- Male urethral swabs
- Female cervical swabs
- Male and female urine specimens
- Self and clinician administered vaginal swabs
- Liquid PAP medium
- Symptomatic and asymptomatic patients

CDC recommends the vaginal swab as the preferred sample type for females and urines for males

Prepared by John R. Papp, Julius Schachter, Charlotte A. Gaydos, Barbara Van Der Pol
What do I have available to me right now?

**Cervicitis**
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  - Culture
  - Gram’s Stain
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- Bacterial vaginosis
  - Amsel’s Criteria
  - Nugent’s score
- Candida
  - KOH preparation
  - Culture
- Trichomonas
  - Wet preparation
  - Culture
  - OSOM
  - Affirm
  - NAATS
    - Research PCRs
    - Aptima *T. vaginalis* (ATV)
    - Becton Dickinson (TVQ)
    - Cepheid
    - AmpliVue

**POCT**

**NAATs 90-97% sensitivity; 98-100% specificity**
### Table 1: Overview and characteristics of diagnostic assays for *Trichomonas vaginalis*

<table>
<thead>
<tr>
<th>Diagnostic Test</th>
<th>Technique</th>
<th>Time to Result</th>
<th>Specimen</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet mount</td>
<td>Microscopic visualization</td>
<td>Minutes</td>
<td>Vaginal or urethral discharge</td>
<td>51–65%</td>
<td>up to 100%</td>
<td>Inexpensive; technician-dependent</td>
</tr>
<tr>
<td>Culture</td>
<td>Culture media</td>
<td>24–120 hours</td>
<td>Vaginal or urethral swab</td>
<td>75–96%</td>
<td>up to 100%</td>
<td>Considered diagnostic gold standard in the past</td>
</tr>
<tr>
<td>OSOM Trichomonas Rapid Test</td>
<td>Immunochromatographic capillary-flow enzyme immunoassay dipstick</td>
<td>10 minutes</td>
<td>Vaginal swabs or saline for wet mount</td>
<td>82–95%</td>
<td>97–100%</td>
<td>CLIA-waived for females; can be used at the point-of-care</td>
</tr>
</tbody>
</table>

American Public Health Laboratory (APHL) issues in brief. Laboratory Detection of Trichomonas August 2013
<table>
<thead>
<tr>
<th>Test Description</th>
<th>Methodology</th>
<th>Time</th>
<th>Sample Type</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Affirm VPIII Microbial Identification Test</strong></td>
<td>Nucleic acid probe test</td>
<td>45 min</td>
<td>Vaginal swabs</td>
<td>63%</td>
<td>99.9%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>APTIMA Trichomonas vaginalis Assay</strong></td>
<td>Transcription Mediated Amplification (TMA)</td>
<td>Hours</td>
<td>Urine specimens, endocervical and vaginal swabs, and specimens collected in PreservCyt Solution</td>
<td>95–100%</td>
<td>95–100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BD ProbeTec Trichomonas vaginalis Qx Amplified DNA Assay</strong></td>
<td>Strand Displacement Amplification (SDA)</td>
<td>Hours</td>
<td>Not an FDA-cleared product</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PCR</strong></td>
<td>Polymerase Chain Reaction</td>
<td>Hours</td>
<td>No FDA-cleared kit</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Moderately complex same-day test; FDA-cleared for use with specimens from females; also detects <em>Gardnerella vaginalis</em> and <em>Candida albicans</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NAATs are the most sensitive tests; FDA-cleared for use with specimens from symptomatic or asymptomatic females</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Variety of female specimens have been tested</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Variety of male and female specimens have been tested</td>
<td></td>
</tr>
</tbody>
</table>
# Trichomonas Test Comparisons

<table>
<thead>
<tr>
<th>Test Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet prep</td>
<td>55%-65%</td>
<td>100%</td>
</tr>
<tr>
<td>Culture</td>
<td>75%</td>
<td>100%</td>
</tr>
<tr>
<td>POCT (OSOM)</td>
<td>&gt;83%</td>
<td>&gt;97%</td>
</tr>
<tr>
<td>PCR (LDT)</td>
<td>83-92%</td>
<td>100%</td>
</tr>
<tr>
<td>TMA AptimaTV</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>ProbTec TVQ</td>
<td>98.3%</td>
<td>98.3%</td>
</tr>
<tr>
<td>Cepheid Xpert POC</td>
<td>98.4%</td>
<td>99.7%</td>
</tr>
<tr>
<td>AmpliVue</td>
<td>100%</td>
<td>98.2%</td>
</tr>
</tbody>
</table>

- Van Der Pol; Schwebke; Taylor: Posters STI & AIDS, 2013
Research Comparison of Wet Mount, Culture, Antigen OSOM, and APTIMA for TV

- **Wet mount**: 50.8%
- **Culture**: 75.4%
- **OSOM**: 82.0%
- **APTIMA**: 98.4%

Trichomonas Vaginalis Test Availability in PHLs, 2010

Number of Laboratories

Not Available
Available by Referral
Available In-house

Diagnostic Test
Culture (InPouch)
Culture (Other)
Wet Mount
Affirm VPill
PCR
XeroStrip (now OSOM)
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%

**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.
**OSOM® Test Stick**

*Positive test result*

A blue Test Line and a red Control Line is a positive result for the detection of Trichomonas antigen.
- On examination you note homogeneous white vaginal discharge, mucopurulent cervical discharge, mild bilateral adnexal tenderness and have a cervical Gram’s stain showing Gram negative intracellular diplococci. Otherwise exam is normal.

- Her urine pregnancy test is positive.

- The wet prep of her vaginal secretions show trichomonads and her vaginal pH is 6.0.
But didn’t Alyssa have pharyngeal and receptive rectal sex?

• I am already doing cervical testing on Alyssa, so how important is doing extragenital testing anyway?
• What do I do about testing in these sites?
• What tests do I order?
• Can I bill for these tests?
Why do extragenital testing?

• From July 2003 until February 2007, 441 rectal test sets were collected from individuals attending a sexually transmitted disease clinic and three HIV clinics who gave a history of anal intercourse or were women at high risk for *Neisseria gonorrhoeae* or *Chlamydia trachomatis* infections.

• Over 60% and 80% of gonococcal and chlamydial infections, respectively, among men who have sex with men and over 20% of chlamydial infections in women would have been missed if the rectal site had not been tested.

Proportion of extragenital gonorrhea and chlamydia infections associated with concurrent negative urethral tests

- Positive Pharyngeal Gonorrhea Tests: 73.8%
- Positive Rectal Gonorrhea Tests: 71.8%
- Positive Pharyngeal Chlamydia Tests: 92.2%
- Positive Rectal Chlamydia Tests: 88.3%

21,994 MSM attending 42 STD Clinic in US 2011-2012

NAAT tests are not FDA-cleared for with rectal, oropharyngeal, and conjunctival specimens; however, some laboratories have met CLIA requirements and established performance specifications for using NAAT with rectal and oropharyngeal swab specimens, thereby allowing results to be used for clinical management.
Don’t forget to test extragenital sites!

### NAAT Laboratory Ordering and Billing Codes

<table>
<thead>
<tr>
<th>Company-Specific Ordering Codes for Combined GC/CT Nucleic Acid Amplified Tests (NAATs)</th>
<th>Company-Specific Ordering Codes for CT test only</th>
</tr>
</thead>
<tbody>
<tr>
<td>LabCorp*</td>
<td>Quest*</td>
</tr>
<tr>
<td>Rectal</td>
<td>188672</td>
</tr>
<tr>
<td>Pharyngeal</td>
<td>188698</td>
</tr>
</tbody>
</table>

NAATs are offered at (or from) any location in the country with these two codes.

For information on specimen collection and transportation, clinicians should contact the local reference laboratory representative.

### CPT Billing Codes

<table>
<thead>
<tr>
<th>CT detection by NAAT</th>
<th>GC detection by NAAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>87491</td>
<td>87591</td>
</tr>
</tbody>
</table>

* CDC does not endorse these laboratories, however, they represent the largest laboratories nationally. There may be other private laboratories that have verified rectal and pharyngeal testing with NAATs. Many PHLs have also verified rectal and pharyngeal testing.
Alyssa’s test results...

- GC NAATS: Positive cervical, oropharynx, rectum
- CT NAATS: Positive cervical
- Serologic test for syphilis: pending
- HIV: pending
- Hepatitis C: pending
• Derek is a 19 year old homeless male who presents as a contact to PID.
• He has no symptom complaints.
• He says he practices “survival sex” and has had over 20 partners, both male and female, over the past 6 months, but only one regular partner.
• He has had unprotected oral, receptive and insertive anal, and vaginal insertive sex.
• He injects heroine when he has enough money, but is not addicted.
• He has had multiple STDs in the past, but last HIV test 9 months ago was negative
Physical exam findings
POCT tests available in the clinic

- Rapid Plasma Reagin (RPR) test: Positive
- Rapid Syphilis Test: Positive
- Determine Rapid HIV1/2 Ag/Ab test: Ag positive; Ab positive
His test results....

- Urine NAATS = + GC, - CT
- Pharyngeal NAATS = +GC, -CT
- Rectal NAATS = +GC, +CT
- Serologic Tests for Syphilis = RPR+ 1:512/FTA positive
- CD4 count 250; HIV VL > 1 million
- Hepatitis screen = HEP A and B negative; anti-HCV +/ HCV RNA +
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

<table>
<thead>
<tr>
<th>Type</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Treponemal</td>
<td>RPR, VDRL</td>
</tr>
<tr>
<td>Treponemal</td>
<td>FTA-abs, TPPA, EIA/CIA, Many POCTs</td>
</tr>
</tbody>
</table>

- 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
Serologic reactivity in syphilis patients
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**

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

**Reverse sequence**

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

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

CDC recommended algorithm for reverse sequence syphilis screening followed by nontreponemal test confirmation.
Discordant Syphilis Immunoassays in Pregnancy: Perinatal Outcomes and Implications for Clinical Management

- 3 year study at Kaiser Permanente Northern CA
- All pregnant women with discordant treponemal serology underwent reflexive testing with TPPA
- 194 women:
  - 38 (20%) were CIA+/RPR-/TPPA+
  - 156 (80) were CIA+/RPR-/TPPA-
- 77 were retested: 53% became CIA
- More likely to be older, have a prior history of STD, receive tx for syphilis during pregnancy
- 189/194 live births: no difference in birth outcomes according to TPPA status and no stillbirths

Routine retesting of pregnant women with CIA+/RPR-/TPPA- serology and reflexive testing of CIA+/RPR- specimens with a second treponemal test is useful given the high likelihood of false-positive CIA results in pregnancy.
### 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><em>Treponema pallidum</em></td>
<td><em>Abbott Determine</em></td>
<td>Whole blood/Serum</td>
<td>59.6-100%</td>
<td>95.7-100%</td>
</tr>
<tr>
<td><em>Syphilis</em></td>
<td><em>Omega Visitect</em></td>
<td>Whole blood/Serum</td>
<td>72.7-98.2%</td>
<td>98.1-100%</td>
</tr>
<tr>
<td></td>
<td><em>Qualpro Syphicheck</em></td>
<td>Whole blood/Serum</td>
<td>64-97.6%</td>
<td>98.4-99.7%</td>
</tr>
<tr>
<td></td>
<td><em>Standard Bioline</em></td>
<td>Whole blood/Serum</td>
<td>85.7-100%</td>
<td>95.5-99.4%</td>
</tr>
<tr>
<td></td>
<td><em>Trinity Syphilis Health Check</em></td>
<td>Whole blood/Serum</td>
<td>98.2%**</td>
<td>97.3%**</td>
</tr>
</tbody>
</table>

Trinity Rapid Syphilis Test
(Syphilis Health Check)

• December 15, 2014: FDA grants CLIA waver
  – New release states that test can be distributed “to a variety of nontraditional lab sites, including physicians’ offices, Emergency rooms, maternity wards, other health care facilities, health department clinics, outreach sites, community-based organizations and other freestanding counseling and testing sites.”
What is Syphilis Health Check™?

- Qualitative rapid assay for the detection of T. pallidum antibodies in human whole blood, serum or plasma
- Detects BOTH IgG and IgM
- 10 minute, 2-step procedure using fingerstick sample
- Room temperature kit storage
- FDA clearance and CLIA waved
- 98% agreement with reference treponemal assays
- 100% agreement with clinically diagnosed samples

Read within 10 to 15 minutes – NOT after so there is a window.
Syphilis Health Check™ Test Interpretation

**Negative**
- Valid Test Result
- Control Line Present
- Test Line Absent
- Full Red color in the sample well

**Positive**
- Valid Test Result
- Control Line Present
- Test Line Present
- Full Red color in the sample well
Syphilis Health Check......Information

- Syphilis Health Check Kit #VSC-11-1  20 tests
  - List:$400.00/kit or $20.00/test

- Syphilis Health Check Controls # VSC-11-2  pos/neg
  - List $53.05/box

- CPT Code: #86780
- LOINC Code:24110-9
- HCPCS Code:86592 (Medicare Part A)
- Reimbursement: $18.06/test
Do NOT use this test when

• Someone has a past history of known syphilis-treated or untreated
And what about HIV?
Evolution of HIV Tests

- 1\textsuperscript{st} generation: whole viral lysate, detects IgG antibody
- 2\textsuperscript{nd} generation: synthetic peptides, detects IgG antibody
- 3\textsuperscript{rd} generation: detects IgM and IgG antibody
- 4\textsuperscript{th} generation: detects IgM, IgG antibodies, p24 antigen

- “Combi” tests: detect both HIV-1 and HIV-2 antibodies
- Nucleic Acid tests: detect HIV RNA
After Infection

- After infection, virus replicates “unchecked”—becomes detectable by RNA ~ day 10-12; load becomes very high; correlates with viral load in semen
- After ~ 3 weeks antibody develops, becomes detectable
Reduction of Window Period

- Source: Das, G. et al. (2010). *BMJ*, 341:bmj.c4583
Commonly used CLIA-Waived Point-of-Care Rapid HIV Tests (2nd generation)

- OraQuick Advance
- Clearview Complete
- Uni-Gold Recombigen
- INSTI
- Clearview Stat Pak
<table>
<thead>
<tr>
<th>HIV*</th>
<th>Test</th>
<th>Sample type</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></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 1/2</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/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).

DPP (Dual Path Platform) HIV-1/2 Assay

- CLIA moderate complexity for serum, plasma, oral fluid
- SampleTainer = residual specimen after testing
- 15 minutes
- Antibodies to HIV1 & 2
- Oral Sensitivity = 98.9; specificity 99.9
Alere Determine HIV-1/2 Ag/Ab

Test in 3 Easy Steps

1. Prepare Test
   Bend along the perforation, then tear one strip from the right and remove cover.

2. Add Sample
   - **Fingerstick Whole Blood**
     Apply 50 μL sample by touching the tip of the capillary tube to the Sample Pad, wait 1 minute, then add Chase Buffer.
   - **Venous Whole Blood**
     Apply 50 μL of sample by touching the tip of the precision pipette to the Sample Pad, wait 1 minute, then add Chase Buffer.
   - **Serum or Plasma**
     Apply 50 μL of sample by touching the tip of the precision pipette to the Sample Pad.

3. Read Results
   Read the results — for both the free HIV-1 p24 antigen (Ag) and HIV-1/2 antibodies (Ab)—in just 20 minutes.

<table>
<thead>
<tr>
<th>Line</th>
<th>Reactive</th>
<th>Nonreactive</th>
<th>Invalid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ag</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ab</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Result Key**
- Patient Identification Area
- Lot Number
- Product Name
- Control Line Area
- Antigen Results Area
- Antibody Results Area
- Sampling Area
New 3rd, 4th HIV Generation Tests

- ADVIA® Centaur™ HIV 1/O/2 Enhanced
- APTIMA Qualitative HIV-1 RNA
- Ortho VITROS ECi/ECiQ
- Abbott Architect 4th Generation Ag/Ab Combo Assay
- Bio-Rad GS HIV Combo Ag/Ab EIA
Sequence of Test Positivity Relative to WB (Plasma)

166 specimens, 17 Seroconverters - 50% Positive Cumulative Frequency

Days before WB positive


Luo et al, J Clin Virol 2013
CDC/APHL Proposed New HIV Testing Algorithm

4th generation HIV-1/2 immunoassay

(+)  
(-)  

Negative for HIV-1 and HIV-2 antibodies and p24 Ag

HIV-1/HIV-2 antibody differentiation immunoassay

HIV-1 (+)  
HIV-2 (-)  

HIV-1 antibodies detected

HIV-1 (-)  
HIV-2 (+)  

HIV-2 antibodies detected

HIV-1 (+)  
HIV-2 (+)  

HIV antibodies detected

HIV-1 (-) or indeterminate

HIV-2 (-)  

NAT

NAT (+)  
Acute HIV-1 infection

NAT (-)  
Negative for HIV-1

NAT: nucleic acid test (e.g., RNA)


Updated: cdc.gov/hiv June 27, 2014
Questions for the speaker?

Email questions to maphtc@jhu.edu