Treponemal Based Immunoassay for Primary Syphilis Screening

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Disclaimers

- Studies and grants currently ongoing with:
  - Roche Molecular
  - Bruker Daltonics
  - Pocared

- Personal Disclaimers
  - Roche Molecular Advisory Board – supported by Kaiser Permanente
Objectives

- Background on syphilis
- Challenges in Testing – Available Algorithms
- APHL/CDC Guidance
- Treponemal Testing Options
- Kaiser Experience
- Future
Background on Syphilis
Syphilis

- Sexually transmitted genital ulcerative disease
- Causative agent – *Treponema pallidum subspecies pallidum*
  - Other diseases of *Treponema pallidum* include *yaws* (subspecies *pertenue*), *pinta* (subspecies *carateum*) and *bejel* (subspecies *endemicum*)
- Causes significant complications if untreated
- Facilitates the transmission of HIV infection
- Untreated syphilis in pregnancy
  - Fetal death in up to 40% of the cases
  - If acquired during 4 yrs before pregnancy
    - Infected fetus 80% of time
4 Stages of Syphilis

- **Classic Presentation**
  - **Primary** – chancre (firm, painless, non-itchy skin ulceration)
    - 3-90 days after initial exposure (avg. 21 days)
    - Classic form – evolves macule to papule
  - **Secondary** – diffuse rash involving hands and soles of feet
  - **Latent** – little to no symptoms
  - **Tertiary** – gummas, neurological or cardiac symptoms

- Known as the “great imitator”
  - “Atypical” clinical presentations can occur
Syphilis Statistics from Centers for Disease Control
Syphilis Trends in the United States
Data from Centers for Disease Control and Prevention

Syphilis (Primary and Secondary)

4.5 reported cases per 100,000 people

Once on verge of elimination, syphilis has increased since 2006

2006-2010

+36% since 2006

Syphilis rate

2000 2010

2.1 4.5
CDC Data - Syphilis—Reported Cases by Stage of Infection, United States, 1941–2010

Cases (in thousands)

Year

- Primary and Secondary
- Early Latent
- Total Syphilis
CDC Data - Primary and Secondary Syphilis – Rates by Sex and Male-to-Female Rate Ratios, United States, 1990-2010

Rate (per 100,000 population)

- Male Rate
- Female Rate
- Total Rate
- Male-to-Female Rate Ratio

Rate Ratio (log scale)

- 16:1
- 8:1
- 4:1
- 2:1
- 1:1

Year

Who to Screen/Test for Syphilis – CDC

- **Who to screen** – screen due to asymptomatic nature of disease
  - Individuals at high risk for disease – important to detect latent infection
    - HIV patients
    - MSM
    - Patients with other STD’s
  - Pregnant women
  - Blood donors

- **Who to test**
  - Symptomatic patients
Serodiagnosis

- **Nontreponemal antibodies**
  - Antibodies directed against lipoidal antigens that are released from damaged host cells and (possibly) from treponemal organisms
  - Can be non-reactive in early and late stages of infection
  - Become non-reactive after treatment

- **Treponemal antibodies**
  - Specific against *T. pallidum*
  - Appear earlier during infection
  - Remain detectable for life (even post therapy)
Syphilis Serology

- **Nontreponemal Tests** — methodology - flocculation tests
  - RPR — rapid plasma reagin
  - VDRL — Venereal Disease Research Laboratory

- **Treponemal Tests**
  - TP-PA — *Treponema pallidum* — Particle Agglutination
  - TP-HA — *Treponema pallidum* — Hemagglutination
  - EIA — Enzyme Immunoassay - Treponemal specific
  - FTA-ABS — fluorescent antibody treponemal absorbed
  - Blots
  - PCR — polymerase chain reaction — molecular
Antibody Development – Various Stages of Syphilis Infection

Graph showing the seroactivity over weeks and years post-infection. The stages are:
- Primary syphilis
- Secondary syphilis
- Tertiary or neurosyphilis

Different antibodies are indicated:
- Treponema specific IgG
- Antilipid IgG
- Antilipid IgM
- Treponema specific IgM
Algorithms Available Today for Syphilis Testing

- **Traditional Algorithm**
  - Screen with nontreponemal test
  - Confirmation with treponemal test

- **Reverse Screen Algorithm**
  - Screen with treponemal test
  - Confirm with nontreponemal test
  - Resolving discordants with another treponemal test
Challenges with Serodiagnosis of Syphilis

- **Nontreponemal tests**
  - Biological false positives
    - Presence of anticardiolipin from other diseases
      - Ex. EBV, Hepatitis, connective tissue disease, TB, malaria
    - False positives seen in pregnant women
  - Subjectivity of the nontreponemal test
    - Agglutination assays that must be read and interpreted by technologist
  - Labor intensive

- **Treponemal tests**
  - Can be false positives (suggested to be less than nontreponemal)
  - Remain positive for life – past versus current disease
  - Some EIAs detect IgM – potentially more sensitive than IgG only assays (?)
Challenges with Nontreponemal Tests

- Subjective
- Manual
- No positive patient ID
- LABOR issues
  - Ergonomic issues = impact to work place safety, employee quality of life
  - Labs asked to do more with less
  - Fewer CLS training programs
  - Aging workforce – fewer CLS

Testing challenges spurred development of newer syphilis assays
Current CDC Recommendation

- CDC continues to recommend (TRADITIONAL) serologic screening
  - Initial screening nontreponemal based test
  - Confirm with treponemal based test

NOTE...CDC does NOT recommend the FTA-ABS test for resolution of discordant results

- Historically gold standard – lower sensitivity and specificity noted*

MMWR to Address Newer Syphilis Tests

- MMWR February 11, 2011, Vol. 60 / No. 5 – Discordant Results from Reverse Sequence Syphilis Screening – Five Laboratories, US, 2006-2010
- CDC’s response to newer testing algorithms in clinical setting
- CDC continues to primarily recommend traditional algorithm
- MMWR helps offer “additional recommendations” if reverse screening is used
<table>
<thead>
<tr>
<th>Population type/Laboratory</th>
<th>Treponemal test used</th>
<th>Conjugate type (anti-antibody or antigen)</th>
<th>Total no. of specimens</th>
<th>Reactive EIA/CIA treponemal test</th>
<th>Nonreactive reflex non treponemal RPR test</th>
<th>Nonreactive TP-PA or FTA-ABS confirmatory treponemal test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall</strong></td>
<td></td>
<td></td>
<td>140,176</td>
<td>4,834 (3.4)</td>
<td>2,743 (56.7)</td>
<td>866 (31.6)</td>
</tr>
<tr>
<td><strong>Low prevalence population</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Southern California*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liaison</td>
<td>Trep-Chek</td>
<td>Anti-antibody</td>
<td>47,952</td>
<td>1,278 (2.7)</td>
<td>765 (59.9)</td>
<td>459 (60.0)</td>
</tr>
<tr>
<td>Northern California*</td>
<td></td>
<td></td>
<td>21,623</td>
<td>438 (2.0)</td>
<td>287 (65.5)</td>
<td>88 (30.7)</td>
</tr>
<tr>
<td>Southern California**</td>
<td></td>
<td></td>
<td>57,827</td>
<td>1,268 (2.2)</td>
<td>755 (59.5)</td>
<td>190 (25.2)</td>
</tr>
<tr>
<td><strong>High-prevalence population</strong>††</td>
<td></td>
<td></td>
<td>12,774</td>
<td>1,850 (14.5)</td>
<td>936 (50.6)</td>
<td>129 (14.1)</td>
</tr>
<tr>
<td>New York City***</td>
<td>Trep-Chek</td>
<td>Anti-antibody</td>
<td>7,607</td>
<td>1,165 (15.3)</td>
<td>639 (54.8)</td>
<td>78 (12.2)</td>
</tr>
<tr>
<td>Chicago***</td>
<td>Trep-Sure</td>
<td>Antigen</td>
<td>5,167</td>
<td>685 (13.3)</td>
<td>297 (43.4)</td>
<td>51 (18.6)††</td>
</tr>
</tbody>
</table>

MMWR 2011;60(5):132-137
CDC – Additional recommendations for reverse algorithm

- Specimens reactive by treponemal test
  - TEST reflexively with quantitative nontreponemal test (RPR, VDRL)

- If tests are discordant
  - Reflexively test specimen using the TP-PA as confirmatory treponemal test
    - Discordant results by treponemal test and RPR/VDRL
      - Reactive by TP-PA – considered to have past or present syphilis
      - Non-reactive by TP-PA – syphilis is unlikely
Laboratory Diagnostic Testing for *Treponema pallidum*

Expert Consultation Meeting Summary Report
January 13-15, 2009
Atlanta, GA

This report was produced in cooperation with the Centers for Disease Control and Prevention.
There is a role for Dark Field Microscopy.

Proper serologic diagnosis of syphilis in adults requires both a treponemal test and a nontreponemal test result. A single serologic result is not useful.

The traditional algorithm of screening with a nontreponemal test followed by a treponemal test continues to have value. However, this algorithm is labor intensive. A syphilis testing algorithm using a high throughput treponemal test as the initial screen was proposed by the expert consultation group.
Available Testing Options

<table>
<thead>
<tr>
<th>Sample</th>
<th>Classical assays</th>
<th>INNO-LIA syphilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.D.</td>
<td>Consensus results</td>
<td>INNO-LIA Patterns</td>
</tr>
<tr>
<td>547</td>
<td>indeterminate</td>
<td>3+ 1+ ± 47 17 16</td>
</tr>
<tr>
<td>600</td>
<td>indeterminate</td>
<td></td>
</tr>
<tr>
<td>820</td>
<td>indeterminate</td>
<td></td>
</tr>
<tr>
<td>601</td>
<td>weak positive</td>
<td></td>
</tr>
<tr>
<td>612</td>
<td>weak positive</td>
<td></td>
</tr>
<tr>
<td>688</td>
<td>weak positive</td>
<td></td>
</tr>
<tr>
<td>207</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>515</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>766</td>
<td>Lyme positive</td>
<td></td>
</tr>
<tr>
<td>765</td>
<td>Lyme positive</td>
<td></td>
</tr>
<tr>
<td>770</td>
<td>Lyme positive</td>
<td></td>
</tr>
</tbody>
</table>
Treponemal Syphilis Tests

- Trep Sure (EIA) – Trinity Biotech
- Trep ID (EIA)* – Trinity Biotech - RUC
- Inno-LIA* - Innogenetics - RUC
- Captia (EIA) – Trinity Biotech
- Centaur – Direct chemiluminescence – Siemens
- Bioplex 2200 Syphilis (MIA)– BioRad
- Liaison® Treponema Assay (CLIA) – DiaSorin

List not inclusive, other EIA and WB Not FDA approved or non-US

CLIA – Chemiluminescent Immunoassay; MIA – Multiplex Immunoassay *Not FDA Approved
## Assay Specifics

<table>
<thead>
<tr>
<th>Assay</th>
<th>Antibodies Detected</th>
<th>Recombinant Ag’s</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trep Sure</td>
<td>IgM &amp; IgG</td>
<td>Y</td>
<td>EIA</td>
</tr>
<tr>
<td>Trep ID</td>
<td>IgM &amp; IgG</td>
<td>Y</td>
<td>EIA</td>
</tr>
<tr>
<td>Inno-LIA</td>
<td>IgM &amp; IgG</td>
<td>Y</td>
<td>LIA</td>
</tr>
<tr>
<td>Captia</td>
<td>IgM – separate kit, IgG – separate kit</td>
<td>Native Ag</td>
<td>EIA</td>
</tr>
<tr>
<td>Bioplex</td>
<td>IgM – separate kit, IgG – separate kit</td>
<td>Y</td>
<td>MIA</td>
</tr>
<tr>
<td>Liaison</td>
<td>IgM &amp; IgG</td>
<td>Y</td>
<td>CLIA</td>
</tr>
<tr>
<td>Centaur</td>
<td>IgM &amp; IgG</td>
<td>Y</td>
<td>CLIA</td>
</tr>
</tbody>
</table>
Performance Data


<table>
<thead>
<tr>
<th>Assay</th>
<th>Sensitivity/Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BioPlex IgG</td>
<td>96.9 / 98.5</td>
</tr>
<tr>
<td>TP-PA</td>
<td>95.9 / 97.6</td>
</tr>
<tr>
<td>Trep-Check IgG</td>
<td>95.9 / 98.5</td>
</tr>
<tr>
<td>Trep-Sure</td>
<td>96.9 / 94.7</td>
</tr>
<tr>
<td>Trep-ID</td>
<td>96.9 / 99.0</td>
</tr>
<tr>
<td>ViraBlot IgG</td>
<td>93.8 / 98.5</td>
</tr>
</tbody>
</table>

Compared to FTA; 303 specimens
Kaiser Permanente

- Kaiser Permanente – Integrated health care delivery system
  - 8 regions throughout the United States
- Kaiser Southern California
  - 15 Hospitals [each with Medical Center Lab]
  - 3.5 Million Members
  - ~150 Medical Office Buildings
  - 6000 physicians
  - >200,000 Admissions Per Year
  - 1 Regional Reference Laboratory – Microbiology (Bact, AFB, Mycology, Parasitology, **SEROLOGY** – Infectious Disease/Autoimmune, Virology, Molecular)
Kaiser Syphilis Testing 2004

- **Early 2004** – high volume testing (175K/yr) – looking for solutions
- Newer assays (Trep Check) were available to automate syphilis on open EIA platforms
- Ergonomic issues were affecting our lab and staff
- Work place injuries – repetitive motion with assays like RPR
  - Reason to automate
- CLS pool dwindling
  - Average age of CLS in CA is 57 years old
  - Fewer CLS programs
Adoption of Reverse Screening Algorithm

- Consulted with CDC - 2004
  - Screening with EIA not broadly adopted, needed guidance
  - CDC was willing to engage with us

- Interfaced with Infectious Disease physicians
  - Ultimately supported the laboratory in moving to EIA
  - This support was critical
Syphilis EIA Testing Algorithm

Initial EIA

Positive (P) or Equivocal (Eq)

Repeat EIA
(in duplicate)

Negative (N)

Titer RPR

Nonreactive

TP-PA

“Confirmed Positive for antibodies to Treponema pallidum”

“No evidence of antibodies to T. pallidum”

Positive (P) or Equivocal (Eq)

Negative (N)
Syphilis EIA Testing Algorithm

Initial EIA

Negative (N)

“No evidence of antibodies to T. pallidum”

Positive (P) or Equivocal (Eq)

Repeat EIA (in duplicate)

Negative (N)

3.35%

Positive (P) or Equivocal (Eq)

Titer RPR

Nonreactive

67%

Reactive

33%

TP-PA

Nonreactive

18%

Intermediate

10%

Reactive

72%

“Unable to confirm presence of Treponemal antibodies. In screening a low risk population, this result may represent a “Biological false positive”. However, this could represent early Syphilis in some patients and may be confirmed with repeat testing in 4-6 weeks if clinically indicated.”

“Unable to confirm presence or absence of Treponemal antibodies. If early disease is suspected, treat or repeat EIA in 2-4 weeks, if clinically indicated.”

“Treponemal antibodies present. May be indicative of early infection or past infection. Usually this result represents past infection. Confirmation of adequate prior treatment for Syphilis is warranted. If early disease is suspected, treat or repeat EIA in 2-4 weeks, if clinically indicated.”
Reporting Messages

- **EIA+/RPR-/TPPA-**
  - “Unable to confirm presence of treponemal antibodies. In screening a low risk population this result may represent a “biological false positive”. However this could represent early syphilis in some patients and may be confirmed with repeat testing in 4-6 weeks if clinically indicated”

- **EIA+/RPR-/TPPA IND**
  - “Unable to confirm the presence or absence of treponemal antibodies. If early disease is suspected treat or repeat EIA in 2-4 weeks if clinically indicated”

- **EIA+/RPR-/TPPA+**
  - “Treponemal antibodies present. May be indicative of early infection or past infection. Confirmation of adequate prior syphilis treatment is warranted. If early disease is suspected treat or repeat EIA in 2-4 weeks if clinically indicated”
Benefits after Transition

- Improvement in workflow and ergonomics
- CLS adopted the new assay and automation
- FTE savings
  - Saved almost a 1.5 FTE with reverse algorithm
  - >150K/yr with salary and benefits for CLS
Drawbacks after Transition

- Increase EIA+, RPR-, TP-PA+ *

- Physician discomfort with new test
  - Learning curve for providers
  - Laboratory spent time discussing test results with providers
  - Could not distinguish present with past infection
    - Overtreatment?

- Some patient dissatisfaction
  - Denied they had syphilis
  - Physician still had to manage test results and treat

- Public Health Component

*Not seen with initial validation*
<table>
<thead>
<tr>
<th>Patient Population</th>
<th>Follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prenatal/OB</td>
<td>Nursing follow up</td>
</tr>
<tr>
<td>0-11 years</td>
<td>Nursing follow up</td>
</tr>
<tr>
<td>12-19 years</td>
<td>STD program PHI follow up</td>
</tr>
</tbody>
</table>
| 20-29 years         | Male – no follow up*  
|                     | Female – STD program PHI follow up |
| 30-39 years         | Male – no follow up*  
|                     | Female – district (STD clinic) PHI follow up |
| 40-59, >60          | No follow up |
| Age Unknown         | Male – no follow up*  
|                     | Female – district (STD clinic) PHI follow up |

*Follow-up will be done, however, if a provider reports by CMR that the patient had signs or diagnosis of early syphilis (i.e., primary lesion)
Status of Labs Moving to Reverse Algorithm

- ClinMicroNet – Online consortium of clinical laboratory directors
- Survey performed in April 2012 to assess syphilis screening practices
- 40 respondents
  - 21 laboratories have adopted the reverse screening algorithm
    - Comments
      - Acknowledged challenges algorithm change presented for physicians
  - 19 laboratories evaluated the reverse screening algorithm – opted NOT to adopt
    - Comments
      - Some local or state public health laboratories were not recommending laboratories switch
      - Physician input influenced remaining with RPR as initial test
      - Some laboratories still pondering switch
Next Steps . . . . CDC Study 2012

- Study to assess comparison of several treponemal assays
  - Kaiser Permanente So Cal/No Cal
  - Centers for Disease Control and Prevention
  - CA State Department of Health
  - ~800 specimens that will be tested with all tests below

- Tests to be compared — still in discussions as to full list of assays that will be studied
  - Trep-Sure
  - Liaison
  - Bioplex
  - Inno-LIA
  - TP-PA
  - FTA-ABS

Will include chart review
For more rapid needs... Newer tests on the horizon...
Rapid Syphilis Tests

- Syphilis Health Check (Rapid Immunochromatographic (IC)) – Mod Complex - Trinity Biotech

- DPP® Syphilis Screen and Confirm (Rapid IC) – Chembio Diagnostic Systems
  - Not FDA approved

- Standard Diagnostics, Inc. – (Rapid IC)
  - Not FDA approved
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<th>Method</th>
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</thead>
<tbody>
<tr>
<td>Health Check</td>
<td>IgM &amp; IgG</td>
<td>Y</td>
<td>IC</td>
</tr>
<tr>
<td>DPP</td>
<td>IgM &amp; IgG</td>
<td>Y</td>
<td>IC</td>
</tr>
<tr>
<td>SD Syphilis</td>
<td>IgM &amp; IgG &amp; IgA</td>
<td>Y</td>
<td>IC</td>
</tr>
</tbody>
</table>
DPP Syphilis Screen & Confirm – Chembio

- Point of Care
- Testing performed in 20 min
- CE marked – applying for waived status in US
- Test for nontreponemal and treponemal (G&M)
Syphilis Health Check – Trinity

- Point of care – moderately complex
  - Waiting for CLIA waiver
- FDA Approved
- Results in 10 minutes
- Test for treponemal antibodies
- Finger stick or venipuncture
Syphilis Health Check™ Venipuncture Procedure

1. Collect Venipuncture Sample (WB, serum, plasma)
2. Dispense 1 Drop of Sample into Sample Port (2 drops if WB or FS)
3. Add 4 Drops of Wash Solution
4. Read Test Device between 10 and 15 Minutes
Syphilis Health Check™ Test Interpretation

**Negative (Non-Reactive) Valid Test Result**
- Control Line Present
- Test Line Absent
- Full Red color in the sample port

**Preliminary Positive (Reactive) Valid Test Result**
- Control Line Present
- Test Line Present
- Full Red color in the sample port
POCT

- Potential use of POCT in developing countries

- Potential for use in US
  - More studies are needed to address sensitivity and PPV according to CDC

- Potential for physician offices or clinics where patients may not come back for serologic results
  - Health care clinics
  - Resource poor areas where patients may fail to return for Rx
Conclusion

- Some laboratories have moved to the reverse algorithm, like ours, to gain better efficiency and better standardize testing in the laboratory.

- Physicians must be on board with the change to the reverse algorithm since there will be changes in the patient care setting. Previously treated patients will now be seen that are EIA+ RPR- TP-PA+.

- More studies are needed, such as the upcoming CDC study, to better determine if the reverse algorithm approach is more sensitive at detecting early infection in some patients.

- Additional data is needed on the value and performance of POCT in the US.
References

- **MMWR February 11, 2011**, Vol. 60 / No. 5 – Discordant Results from Reverse Sequence Syphilis Screening – Five Laboratories, US, 2006-2010
End of Speaker’s Presentation
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Trinity Biotech

www.trinitybiotech.com

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