The Reverse Sequence Syphilis Screening Webinar will begin shortly

The dial-in number for the call is 800-908-9207

Questions can be submitted during the Webinar via the chat function.

Due to the volume of Webinar participants and the time we have allotted, we may not be able to provide live answers to all of the submitted questions.

We will compile and answer these questions and will post them online at www.cdc.gov/std/syphilis/treatment as soon as we can.

Instructions on how to receive CME credits are available at:
http://www.surveymonkey.com/s/SyphilisScreeningWebinar

An archived version of the Webinar will be available at www.cdc.gov/std/syphilis/treatment within a few days.

If you have questions about reverse sequence syphilis screening following the Webinar you may submit them to stdtraining@cdc.gov
CME

The Denver PTC has collected information from our planners and speakers to ensure that you are aware of any potential conflicts of interest that may lead to commercial bias. These include:

• Gail Bolan, MD – No disclosures
• Ina Park, MD, MS - No disclosures
• Karen Hoover, MD, MPH - No disclosures
• Rheta Barnes, MSN, MPH – No disclosures
• Helen Burnside, MS – No disclosures
• John Fitch, LPN – No disclosures

The Denver STD/HIV Prevention Training Center is accredited by the Colorado Medical Society to provide continuing medical education for physicians.

The Denver STD/HIV Prevention Training Center designates this educational activity for a maximum of 1 AMA PRA Category 1 Credit™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

Reverse Sequence Syphilis Screening

An Overview by CDC
Learning objectives

- Describe the evaluation and management of persons with a reactive treponemal enzyme immunoassay (EIA+) result
- Identify three possible explanations for discordant test results (i.e., EIA+ and RPR-) with reverse sequence screening
- Discuss the management of a person who has a discordant test result (i.e., EIA+ and RPR-) that is nonreactive by confirmatory treponemal testing with a *Treponema pallidum* particle agglutination (TP-PA) test
- Compare the performance of the reverse sequence algorithm in populations with high and low prevalence of infection
Target Audience

- Clinicians who provide screening, diagnosis, and clinical care for persons at risk for or infected with syphilis
- Other health care professionals, such as laboratorians, epidemiologists and public health program staff, whose work involves syphilis screening or management of persons at risk for or infected with syphilis

Insert Audience polling questions here

- If you are watching in a group, how many people are in your group?
What is your primary profession/discipline *(select ONE)*?

- Advanced practice nurse
- Registered nurse
- Licensed practical nurse
- Physician
- Physician assistant
- Public health professional
- Laboratorian
- Other

What is your principal employment setting *(select one)*?

- Academic health center
- College/university
- Community health center (federally qualified)
- Other non-profit health center
- Correctional facility
- HMO/managed care organization
- Hospital/Hospital-affiliated clinic
- Military health system/ Veterans Health Admin facility
- Private practice (solo/group)
- State/local health department
- Tribal/Indian Health Service facility
- Other
- Not working
Presenters

Karen Hoover, MD, MPH
Medical Epidemiologist
Division of STD Prevention
Centers for Disease Control and Prevention

Ina Park, MD, MS
Chief
Medical and Scientific Affairs Unit
STD Control Branch
California Department of Public Health

Webinar Overview

- **Syphilis screening with serologic tests**
- **Enzyme immunoassays (EIA) and chemiluminescence immunoassays (CIA)** increasingly used as syphilis screening tests
  - Large proportion of EIA+/RPR- results
  - Confusion about patient management
- **Performance and clinical data for the use of reverse sequence screening**
  - MMWR 2011
  - JID 2011 (under review)
- **CDC recommendations for the use of EIA/CIA to screen for syphilis**
- **Research needs to provide an evidence basis for future guidelines**
Diagnosis of syphilis

- Treponema pallidum cannot be cultured
- Ideally, early syphilis would be diagnosed using direct detection methods
  - Darkfield microscopy
  - Polymerase chain reaction (PCR)
  - Direct fluorescent antibody test for T. pallidum (DFA-TP)
- Direct detection methods are not widely available
- Direct detection methods can miss cases
  - Fail to detect up to 30% of primary cases
- Most persons present without symptoms or signs of syphilis
  - Healed early lesions
  - Inapparent lesions
- Syphilis is usually diagnosed with serologic tests

Serologic diagnosis of syphilis

- Serologic diagnosis always requires detection of two types of antibodies
  - Nontreponemal antibodies
    - Antibodies directed against lipoidal antigens
      - Damaged host cells
      - Possibly from treponemes
  - Treponemal antibodies
    - Antibodies directed against T. pallidum proteins
Serologic diagnosis of syphilis

- **Nontreponemal tests**
  - Rapid plasma reagin (RPR) test
  - Venereal Disease Research Laboratory (VDRL) test
  - Toluidine red unheated serum test (TRUST)

- **Treponemal tests**
  - Fluorescent treponemal antibody absorbed (FTA-ABS) test
  - Treponema pallidum particle agglutination (TP-PA) test
  - Enzyme immunoassays (EIAs)
    - Trep-Check
    - Trep-Sure
  - Chemiluminescence immunoassays (CIAs)
    - LIAISON
    - Architect
  - Microbead immunoassays (MBIA)
    - BioPlex 2200 Syphilis IgM and IgG

Common patterns of serologic reactivity in syphilis patients
Syphilis serologic screening algorithms

**Traditional**
- Quantitative RPR
  - RPR+
    - TP-PA+
      - Sp. or other trep. test
    - TP-PA-
  - RPR-
  - TP-PA+ (past or present)
  - TP-PA- (past or present)

**Reverse sequence**
- EIA or CIA
  - EIA/CIA+
  - Quantitative RPR
    - RPR+
      - TP-PA+ (past or present)
    - RPR-
  - TP-PA-
  - TP-PA+ (past or present)

Which algorithm?

- **Traditional algorithm**
  - Detects active infection
  - High rate of biologic false positives
    - Confirmation with treponemal test
      - Use of both tests results in a high positive predictive value
  - Can miss early primary and treated infection

- **Reverse sequence algorithm**
  - Detects early primary and treated infection that might be missed with traditional screening
  - Nontreponemal test needed to detect active infection
  - Ideally, EIAs and CIAs should have perfect specificity
    - EIAs and CIAs are nonspecific
    - High rate of false positive results
    - Varies by risk of population
Symphilis Screening Paradigm

**EIA/CIA AS SYMPHILIS SCREENING TESTS**

**Non-treponemal tests (RPR, VDRL)**
- Non-specific antibody against lipoidal antigens
- Quantitative
- Reactivity declines with time

**Treponemal tests (TP-PA, FTA-Abs)**
- Specific antibody against *T. pallidum*
- Qualitative
- Reactivity persists over lifetime

**reflex to**
Traditional Use of Treponemal Tests

• Confirming reactive non-treponemal tests
• Screening the blood supply

Syphilis Screening Paradigm

EMERGING / NEW…

Treponemal tests (EIA, CIA, MBIA)
• SPECIFIC ANTIBODY TO T. pallidum
• QUALITATIVE
• REACTIVITY PERSISTS OVER LIFETIME

reflex to

Non-treponemal tests (RPR, VDRL)
• NON-SPECIFIC ANTIBODY AGAINST LIPOIDAL ANTIGENS
• QUANTITATIVE
• REACTIVITY DECLINES WITH TIME
**Treponemal Immunoassay: A timeline**

1980s

- EIA is FDA cleared for use as confirmatory test & in blood bank screening

2000

- UK Public Health Laboratory Guidelines: EIA “appropriate alternative” to VDRL/RPR + TPHA

2001

- EIA is FDA cleared for clinical diagnostic use

2008

- EU Guidelines: EIA/TPPA recommended for screening, VDRL and RPR no longer recommended

2009

- CDC-APHL Report: Presents algorithm for screening with Trep EIA

---

**Why switch to EIA/CIA?**

- Automated (high throughput)
- Low cost in high volume settings
- Less lab occupational hazard (pipetting)
- No false negatives due to prozone reaction
- Objective results
- Some EIA/CIAs detect IgM antibodies; potentially useful for diagnosis of early syphilis
Why switch to EIA/CIA?

180 tests per hour, no manual pipetting

Syphilis Tests by Test Type, 1996-2009

California Department of Public Health, STD Control Branch, 2009
Challenges and limitations of the EIA/CIA

- Cannot distinguish between active disease and old disease (treated/untreated)
- Studies to compare test performance with other serologic tests are lacking
- Studies evaluating performance of EIA/CIA to detect IgM antibodies in early syphilis are lacking
- Confusion re: management of patients with discrepant serology (e.g., positive EIA/CIA and a negative RPR)

PERFORMANCE AND CLINICAL DATA FOR THE USE OF THESE TESTS
Discordant Results from Reverse Sequence Syphilis Screening
Five Laboratories, United States, 2006–2010

Methods

- Analyzed data from five laboratories that used reverse sequence screening during 2006-2010
  - 140,176 sera screened with a treponemal EIA/CIA
  - Data from sera with equivocal EIA/CIA test results were not included as reactive tests

- EIA tests
  - Trep-Chek
  - Trep-Sure

- CIA test
  - LIAISON

- Reflex nontreponemal test
  - RPR

- Confirmatory treponemal tests
  - TP-PA
  - FTA-ABS
Methods

- Three sites served patient populations with low prevalence for syphilis
  - Large managed-care organizations
- Two sites served patient populations with high prevalence
  - MSM
  - HIV-infected patients
- Calculated
  - Reactive EIA/CIAs among all sera (i.e., EIA+)
  - Discordant sera among reactive EIA/CIAs (i.e., EIA+/RPR-)
  - Nonreactive confirmatory TP-PA or FTA-ABS tests among discordant sera (i.e., EIA+/RPR-/TP-PA-)

Results of reverse sequence syphilis screening — five laboratories, United States, 2006 – 2010

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*Nonreactive confirmatory treponemal test includes TP-PA or FTA-ABS.
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**Reasons for discordant test results (i.e., EIA/CIA+ / RPR-)**

- **False-positive EIA/CIA**
  - EIAs and CIAs are very sensitive
  - But have lower specificity

- **Treated syphilis**
  - Treponemal antibodies are detected by sensitive EIAs and CIAs
  - Seroreversion of nontreponemal antibodies

- **Early primary syphilis**
  - Treponemal antibody titer rises before nontreponemal antibody titer
Conclusions

- EIA/CIA have high sensitivity but lower specificity
- All reactive EIA/CIA must be reflexly tested with a quantitative RPR
  - Confirm reactive EIA/CIA
  - Detect active infection
- Although test performance varies by prevalence of syphilis in the population, all discordant specimens (EIA+/RPR-) must be confirmed with a confirmatory treponemal test
- Confirmatory treponemal test must have at least equivalent sensitivity and higher specificity compared to the screening treponemal test
  - TP-PA recommended
  - FTA-ABS not recommended

Screening for syphilis with the treponemal immunoassay:

*Analysis of discordant serology results and implications for clinical management*
## Methods

- Cross sectional analysis of individuals tested with Diasorin LIAISON chemiluminescence immunoassay (CIA) at Kaiser Permanente Northern California Regional Laboratory from Aug-Oct 2007

- Data abstracted from electronic medical records (laboratory and clinical) using standardized protocol

- HIV-status, sexual orientation, pregnancy status, prior syphilis history and CIA index values were compared for all CIA-positive, RPR-negative patients according to TP-PA status.

### CIA Testing Results

- **N=21,623 specimens**
- CIA+ = 2%
- CIA+ / RPR− = 1.3%

**CIA**

- + 98%
- − 2%

**RPR**

- + 34%
- − 66%

Managed like prior RPR screening algorithm

**TPPA**

- + 72%
- − 28%

- *N=255*  
- *33 duplicate or infant tests*
Demographics

<table>
<thead>
<tr>
<th>CIA+/RPR- TP-PA+ (N=184)</th>
<th>CIA+/RPR- TP-PA- (N=71)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean age ( SD)</strong></td>
<td></td>
</tr>
<tr>
<td>50 ( 14)</td>
<td>42 ( 16)</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
</tr>
<tr>
<td>149 (81%)</td>
<td>33 (47%)</td>
</tr>
<tr>
<td><strong>MSM</strong></td>
<td></td>
</tr>
<tr>
<td>60 (33%)</td>
<td>7 (10%)</td>
</tr>
<tr>
<td><strong>Heterosexual</strong></td>
<td></td>
</tr>
<tr>
<td>15 (8%)</td>
<td>8 (11%)</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
</tr>
<tr>
<td>35 (19%)</td>
<td>38 (53%)</td>
</tr>
<tr>
<td><strong>Pregnant</strong></td>
<td></td>
</tr>
<tr>
<td>12 (34%)</td>
<td>16 (42%)</td>
</tr>
<tr>
<td><strong>HIV-positive</strong></td>
<td></td>
</tr>
<tr>
<td>86 (47%)</td>
<td>14 (20%)</td>
</tr>
<tr>
<td><strong>Prior syphilis</strong></td>
<td></td>
</tr>
<tr>
<td>105 (57%)</td>
<td>6 (9%)</td>
</tr>
</tbody>
</table>

*P= <0.0001

Management based on initial serology and syphilis history

CIA+ / RPR- / TP-PA+ N=184

Prior treated syphilis N=105 (57%)
• 10 (9%) received repeat treatment
• 95 (91%) no antibiotic treatment

No prior syphilis N=79 (43%)
• 51 (65%) received treatment
• 28 (35%) no antibiotic treatment
Management based on initial serology and syphilis history

CIA+ / RPR- / TP-PA-
N=71

Prior treated syphilis
N=6 (8%)
- 2 (33%) received repeat treatment
- 4 (66%) no antibiotic treatment

No prior syphilis
N=65 (92%)
- 7 (11%) received treatment
- 58 (89%) no antibiotic treatment

Repeat Serology Testing Results

CIA+ / RPR- / TP-PA+
N=184

Repeat Serology
N=78

Initially treated n=31 (64%)
- 0 seroverted to CIA-
- 27 (87%) remained CIA+/RPR-/TPPA+
- 4 (13%) seroconverted to CIA+/RPR+

Not treated initially n=47 (36%)
- 0 seroverted to CIA-
- 41 (87%) remained CIA+/RPR-/TPPA+
- 6 (13%) seroconverted to CIA+/RPR+
Repeat Serology Testing Results

CIA+/ RPR-/ TP-PA-
N=71

Initially treated N=6 (19%)
• 0 seroreverted to CIA-
• 6 (100%) remained CIA+/RPR-/TPPA+
• 0 seroconverted to CIA+/RPR+

Not treated initially N=25 (81%)
7 (28%) seroreverted to CIA-
17 (68%) remained CIA+/RPR-/TPPA+
1 (4%) seroconverted to CIA+/RPR+

High EIA/CIA index values may predict TP-PA positivity (n=255)

N=79 individuals with CIA index value >12.0; 100% were TP-PA positive

Park IU et al. unpublished data
Kaiser Study Conclusions

- Among CIA+/RPR- patients, performance of a second treponemal test is useful in low prevalence settings to guide treatment decisions.

- Conflicting treponemal results (CIA+/TP-PA-) may represent false positives, especially if low CIA index value. Repeat testing should be considered.

- Among CIA+/RPR- patients at high risk, repeat testing should be performed to rule out early syphilis.

Acknowledgments

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Joan Chow, MPH, DrPH
Gail Bolan, MD
Denise Gilson
Michael Samuel DrPH

Kaiser Permanente
Northern California Regional Laboratory
Jeffrey Schapiro, MD
Jen Shieh, PhD
Mark Stanley, MPH

San Francisco DPH: Kyle Bernstein PhD
Los Angeles DPH: Monica Muñoz, Sarah Guerry MD
CDC RECOMMENDATIONS FOR THE USE OF EIA/CIA

Recommended algorithm for reverse sequence syphilis screening

- EIA or CIA
- EIA/CIA+
- EIA/CIA-
- Quantitative RPR
  - RPR+ Syphilis (past or present)
  - RPR- TP-PA
- TP-PA
  - TP-PA+ Syphilis (past or present)
  - TP-PA- Syphilis unlikely
Recommended algorithm for reverse sequence syphilis screening

EIA or CIA

EIA/CIA+
EIA/CIA-

Quantitative RPR

RPR+
Syphilis (past or present)

RPR-

TP-PA

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

If incubating or early primary syphilis is suspected, treat with benzathine penicillin G 2.4 million units IM x 1

Evaluate clinically, determine if treated for syphilis in the past, assess risk of infection, and administer therapy according to CDC’s STD Treatment Guidelines if not previously treated.

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If at risk for syphilis, repeat RPR in several weeks.
RESEARCH NEEDS TO PROVIDE AN EVIDENCE BASIS FOR FUTURE GUIDELINES

Research needs

- Compare head-to-head the performance of EIAs, CIAs, TP-PA, FTA-ABS test, and microbead immunoassay
  - Well-defined patient populations whose clinical history and syphilis risk are known
    - HIV-infected persons
    - Pregnant women
- Characterize discordant sera with nonreactive confirmatory treponemal tests by immunoblotting
  - Define reactivities with *T. pallidum* antigens
- Study utility of immunoglobulin M treponemal testing
  - Diagnosis of early primary syphilis
  - Evaluation of infection in asymptomatic, seropositive, untreated persons
Question and Answer Session

• Questions may be submitted during the Webinar via the chat function.

• If you have questions about the reverse sequence syphilis screening following the Webinar you may submit them to stdtraining@cdc.gov

Continuing Medical Education (CME) Information

To receive CME, an evaluation and request for certificate must be completed at: http://www.surveymonkey.com/s/SyphilisScreeningWebinar

You must complete the evaluation by April 30, 2011 to receive CME credit

Certificates will be sent out via email starting April 15, 2011.

Continuing Nursing Education (CNE) and other types of Continuing Education (CE) are not available for this program.

If you have CME questions, please contact John Fitch at John.Fitch@dhha.org
April is National STD Awareness Month

Visit our web sites for updated tools, materials, and resources to help support your local STD prevention efforts.

- www.cdc.gov/std/sam
- www.cdcnpin.org/stdawareness
- provider.gytnow.org
- www.findstdtest.org

Educational and Training Resources

National Network of STD/HIV Prevention Training Centers
- www.nnptc.org

CDC Division of STD Prevention
- www.cdc.gov/std/training
- stdtraining@cdc.gov or 404.639.8360
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For more information please contact Centers for Disease Control and Prevention  
1600 Clifton Road NE, Atlanta, GA 30333  
Telephone, 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348  
E-mail: cdcinfo@cdc.gov  Web: www.cdc.gov  
The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.
High EIA/CIA index values may predict TP-PA positivity (n=674)