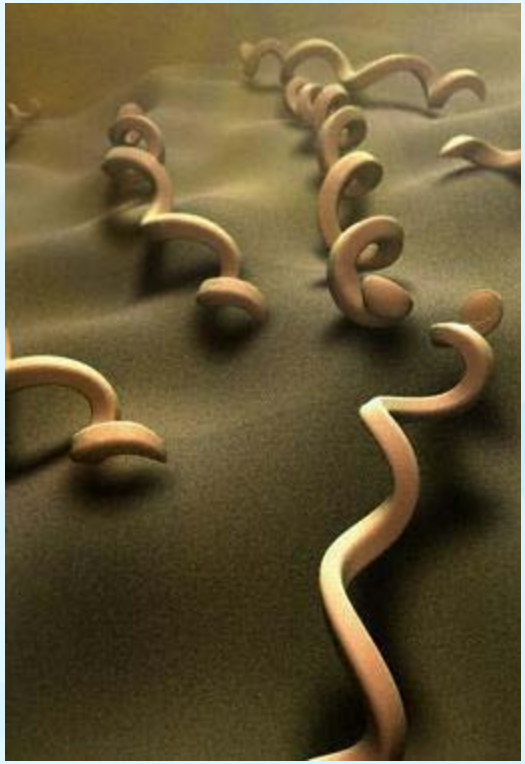
Reverse Sequence Syphilis Screening

An Overview by CDC



National Center for HIV/AIDS, Viral Hepatitis, STD , and TB Prevention
Division of STD Prevention

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

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 February 11, 2011 / 60(05);133-137
  - 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
  - Latent infection
- Syphilis is usually diagnosed with serologic tests

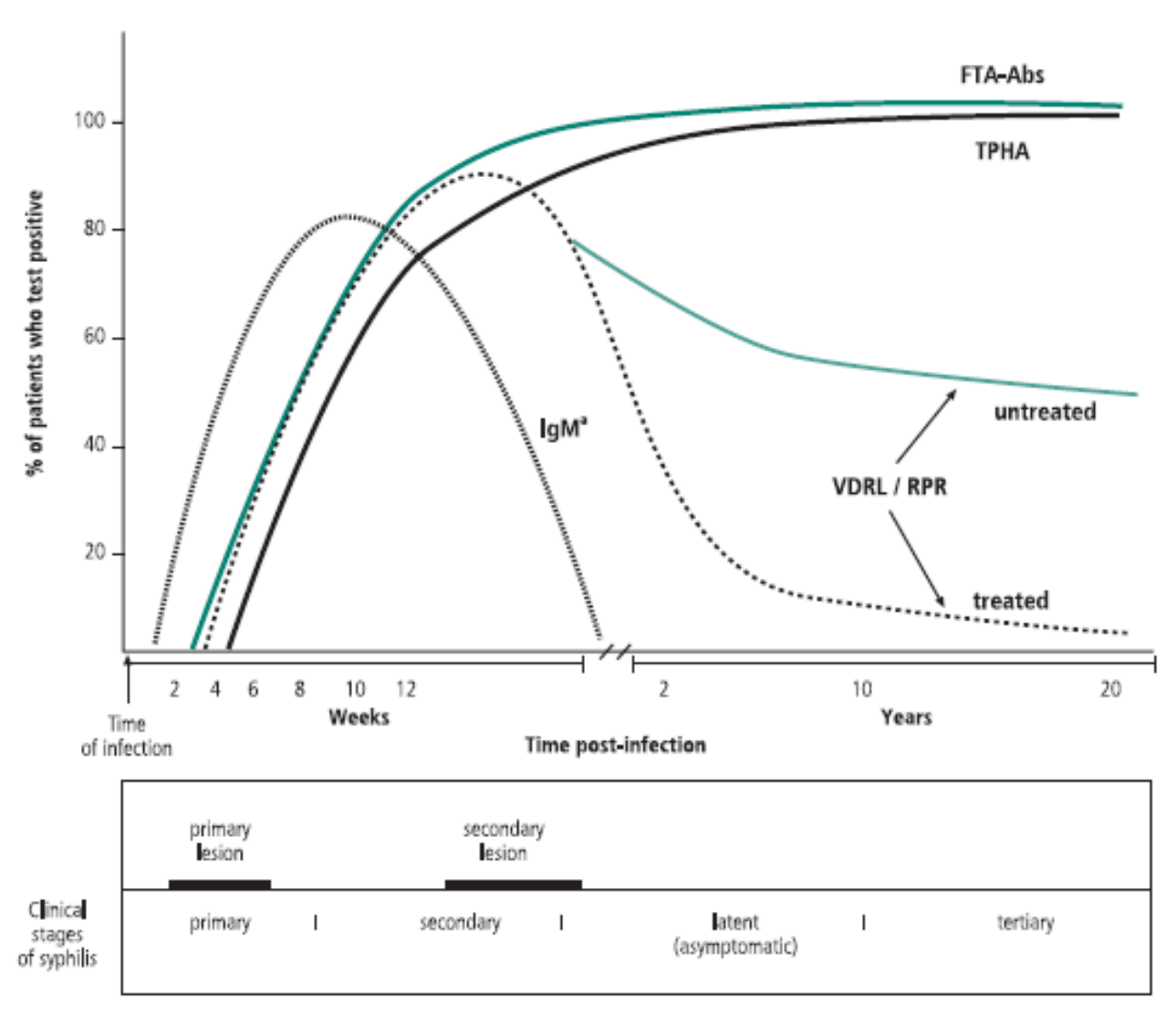
Serologic diagnosis of syphilis

- **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 article agglutination (TP-PA) test
  - Enzyme immunoassays (EIAs)
    - Trep-Chek
    - Trep-Sure
  - Chemiluminescence immunoassays (CIAs)
    - LIAISON
    - Architect
  - Microbead immunoassays (MBIA)
    - BioPlex 2200 Syphilis IgM and IgG

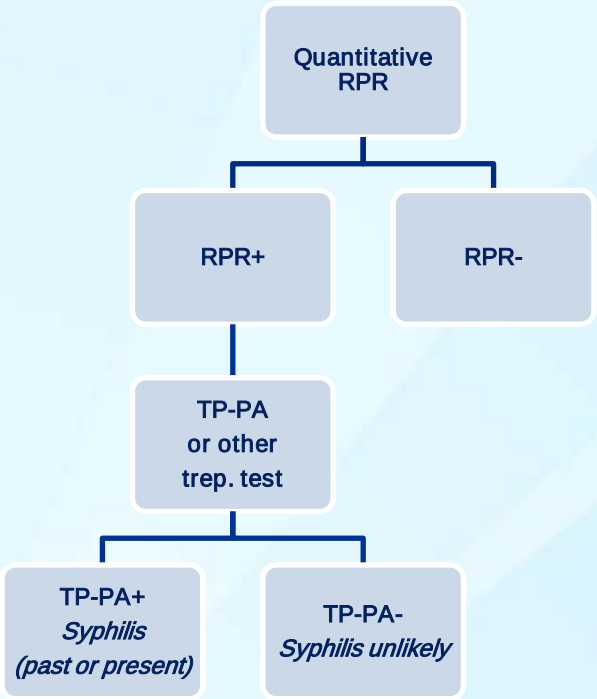
Serologic reactivity in syphilis patients



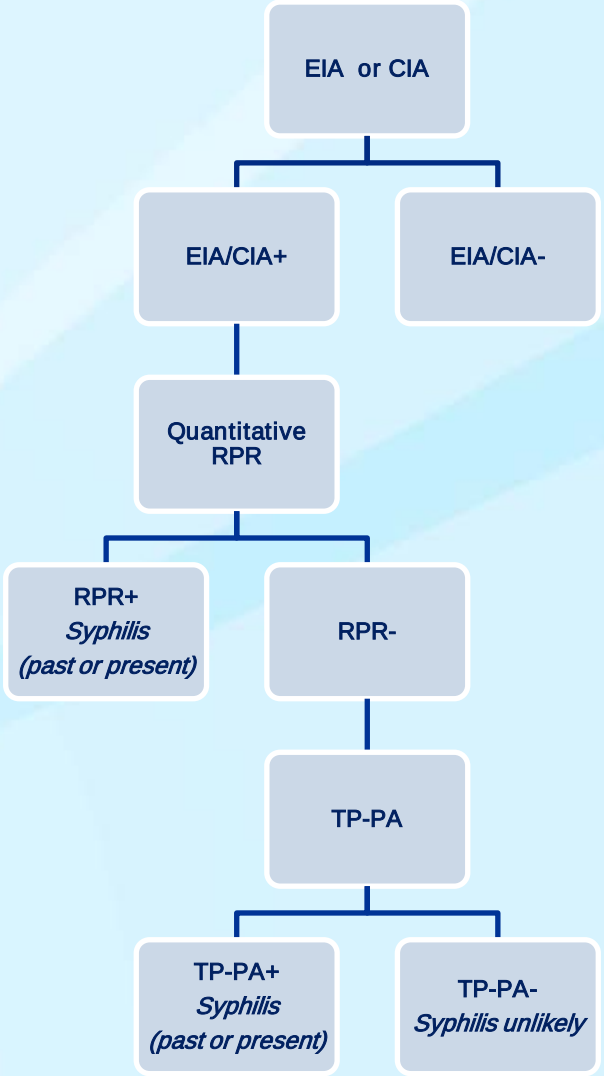
Peeling et al. / Bulletin of the World Health Organization / 2004 / Vol. 82 / No. 6

Syphilis serologic screening algorithms

Traditional



Reverse sequence



MMWR R&R / in preparation

MWR / February 11, 2011 / Vol. 60 / No. 5

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

EIA/CIA AS SYPHILIS SCREENING TESTS

Syphilis Screening Paradigm

TRADITIONAL

Non-treponemal tests (RPR, VDRL)

- NON-SPECIFIC ANTIBODY AGAINST LIPOIDAL ANTIGENS
- QUANTITATIVE
- REACTIVITY DECLINES WITH TIME

reflex to

Treponemal tests (TP-PA, FTA-Abs)

- SPECIFIC ANTIBODY AGAINST *T pallidum*
- QUALITATIVE
- REACTIVITY PERSISTS OVER LIFETIME

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*

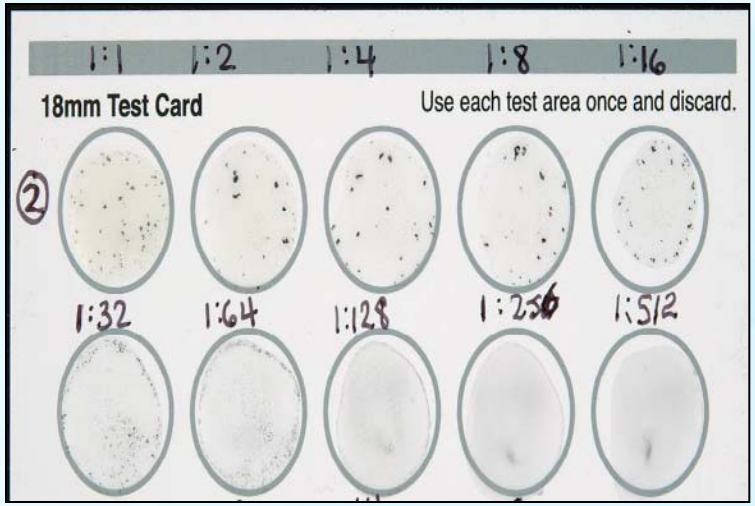
2009

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

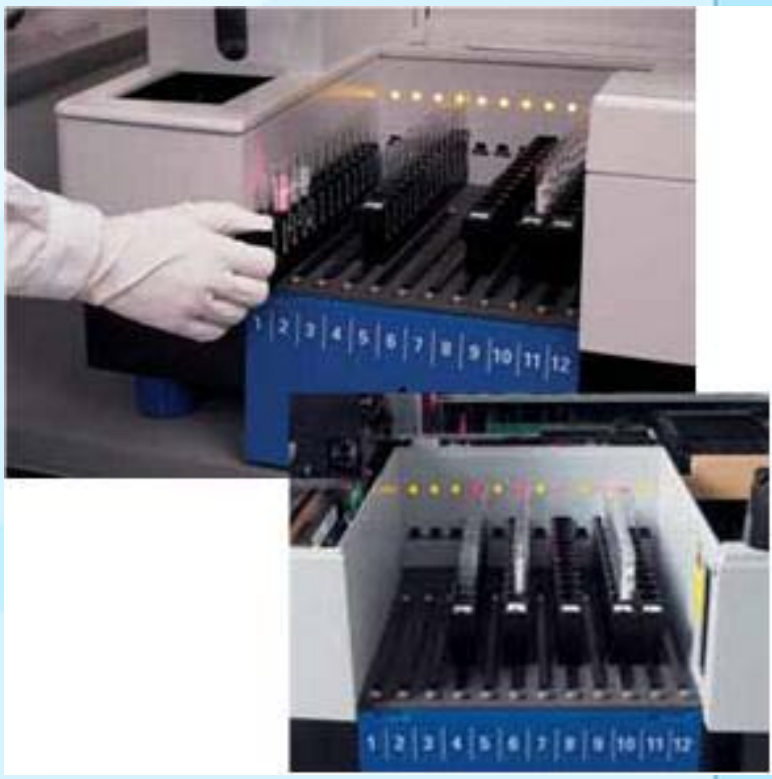
Why switch to EIA/CIA?

- 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?

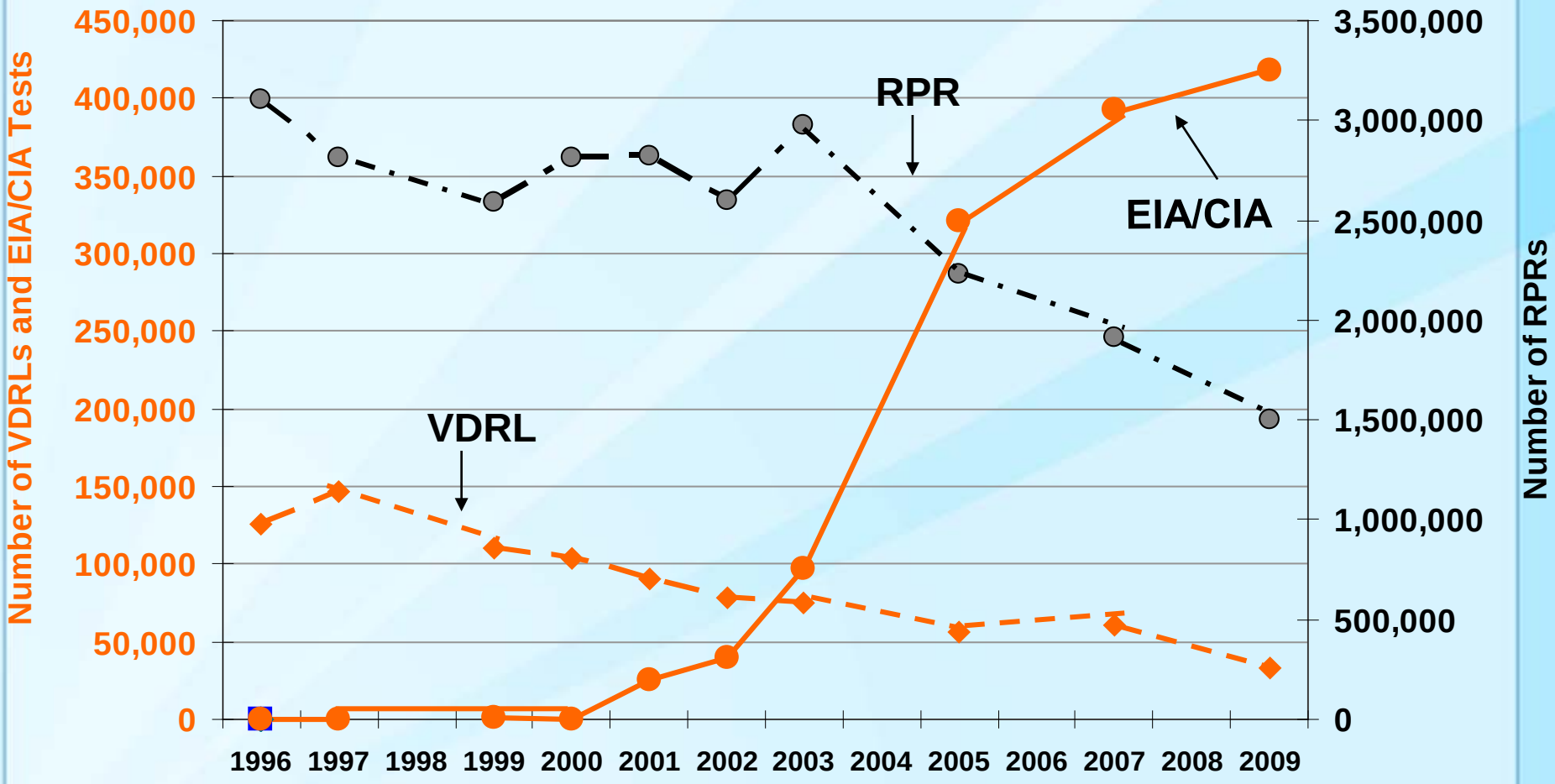


VS



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

Population	Test	Total	Reactive EIA/CIA		Nonreactive reflex RPR		Nonreactive confirmatory treponemal test*	
		N	n	% total	n	% EIA/CIA+	n	% RPR–
Overall		**140,176**	**4,834**	**3.4**	**2,743**	**56.7**	**866**	**31.6**
Low prevalence		**127,402**	**2,984**	**2.3**	**1,807**	**60.6**	**737**	**40.8**
Southern California	Trep-Chek	47,952	1,278	2.7	765	59.9	459	60.0
Northern California	Liaison	21,623	438	2.0	287	65.5	88	30.7
Southern California	Trep-Sure	57,827	1,268	2.2	755	59.5	190	25.2
High prevalence		**12,774**	**1,850**	**14.5**	**936**	**50.6**	**129**	**14.1**
New York City	Trep-Chek	7,607	1,165	15.3	639	54.8	78	12.2
Chicago	Trep-Sure	5,167	685	13.3	297	43.4	51	18.6

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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

KAISER PERMANENTE®

CDPH
California Department of
PublicHealth

JID 2011, under review

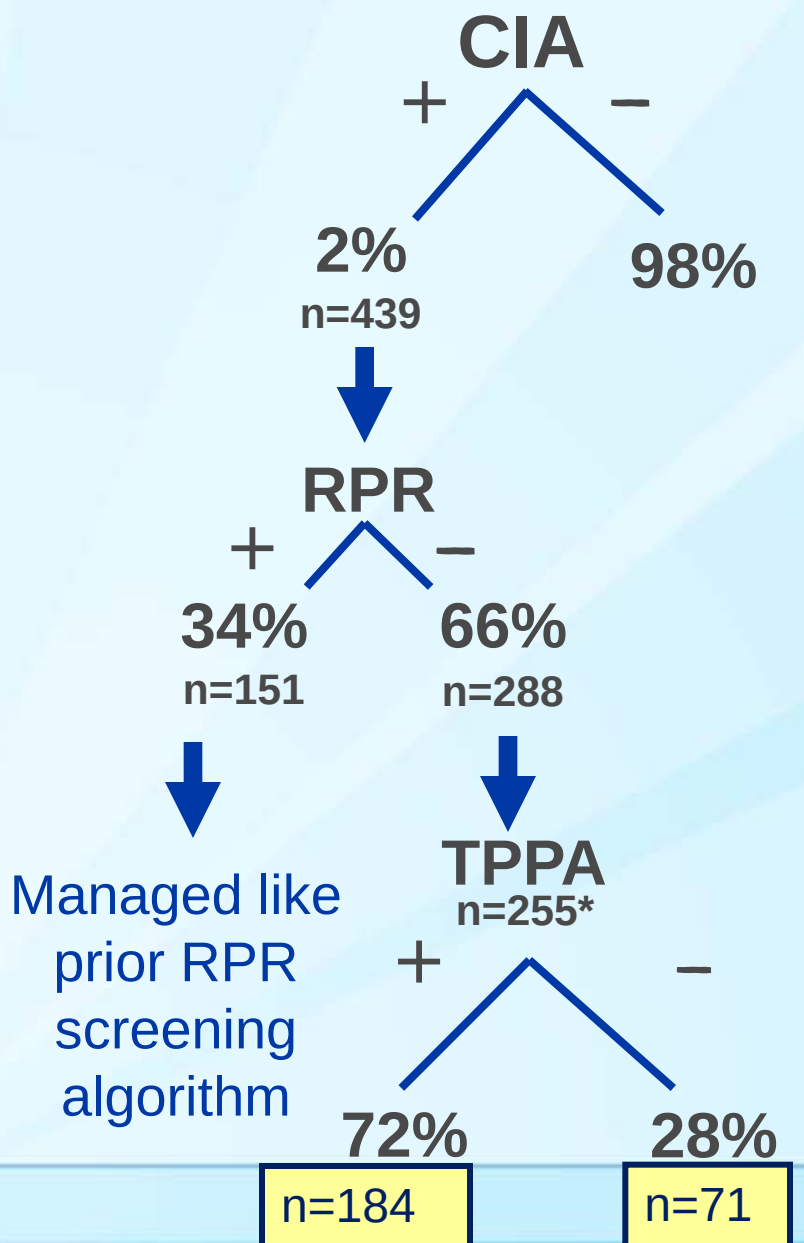
Methods

- 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%

* 33 duplicate or infant tests

Demographics

	CIA+/RPR-TPPA+ (N=184)	CIA+/RPR-TPPA- (N=71)
Mean age (SD)*	50 (14)	42 (16)
Males*	149 (81%)	33 (47%)
MSM*	60 (33%)	7 (10%)
Heterosexual	15 (8%)	8 (11%)
Females	35 (19%)	38 (53%)
Pregnant	12 (34%)	16 (42%)
HIV-positive*	86 (47%)	14 (20%)
Prior syphilis*	105 (57%)	6 (9%)

*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 seroreverted to CIA-
- 27 (87%) remained CIA+/RPR-/TPPA+
- 4 (13%) seroconverted to CIA+/RPR+

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

Repeat Serology Testing Results

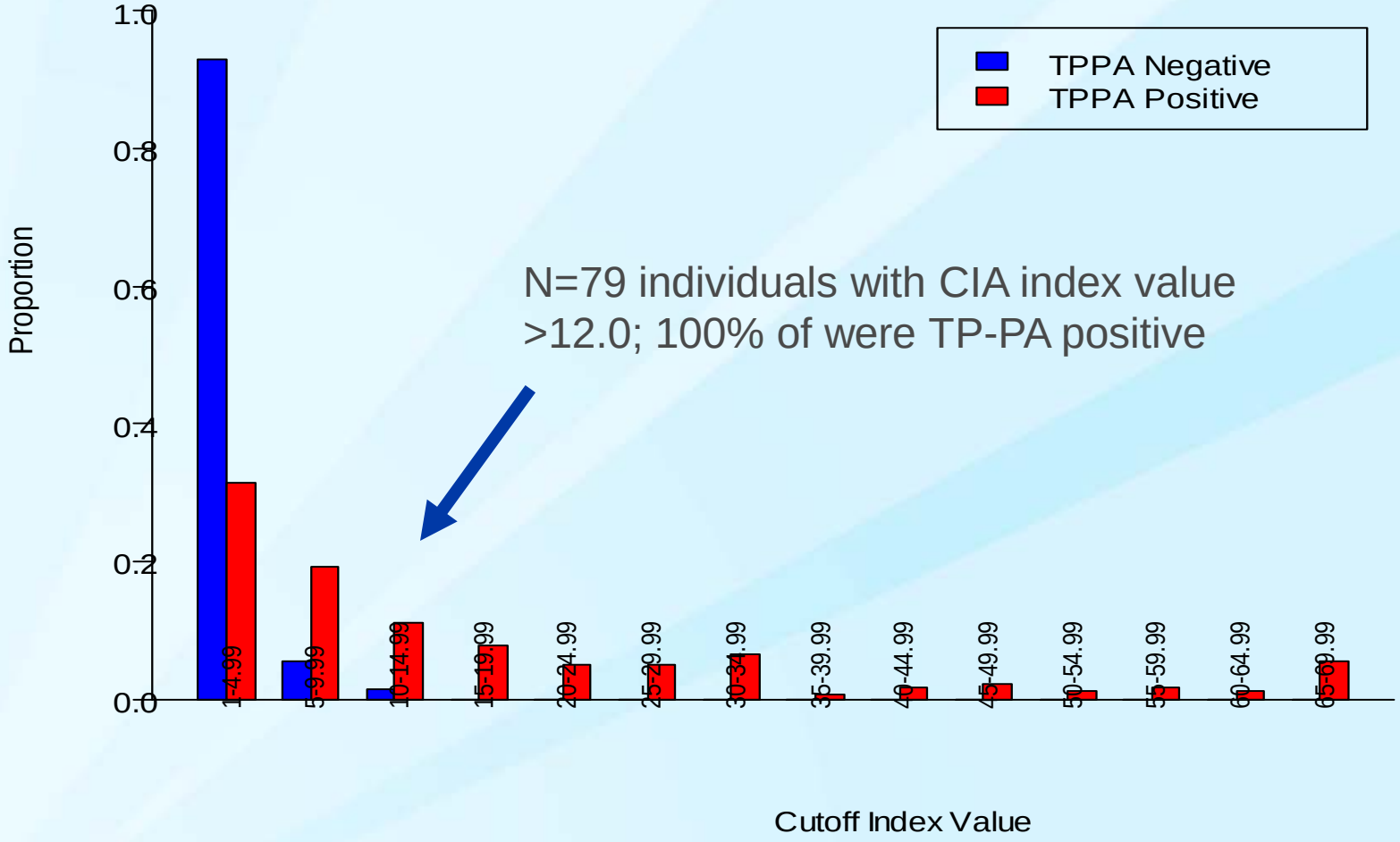
CIA+/ RPR- / TP-PA-
N=71

Repeat Serology
N=31

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

Not treated initally 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)



Park IU et al. unpublished data

Kaiser Study Conclusions

- Among CIA+/RPR- patients, reflex testing with a second treponemal test is useful in low prevalence settings to guide treatment decisions
- Conflicting treponemal results (CIA+/TP-PA-) and isolated CIA+ results with low index values may represent false positives. Repeat testing should be considered
- Among CIA+/RPR- patients at high risk, repeat testing should be performed to rule out early syphilis

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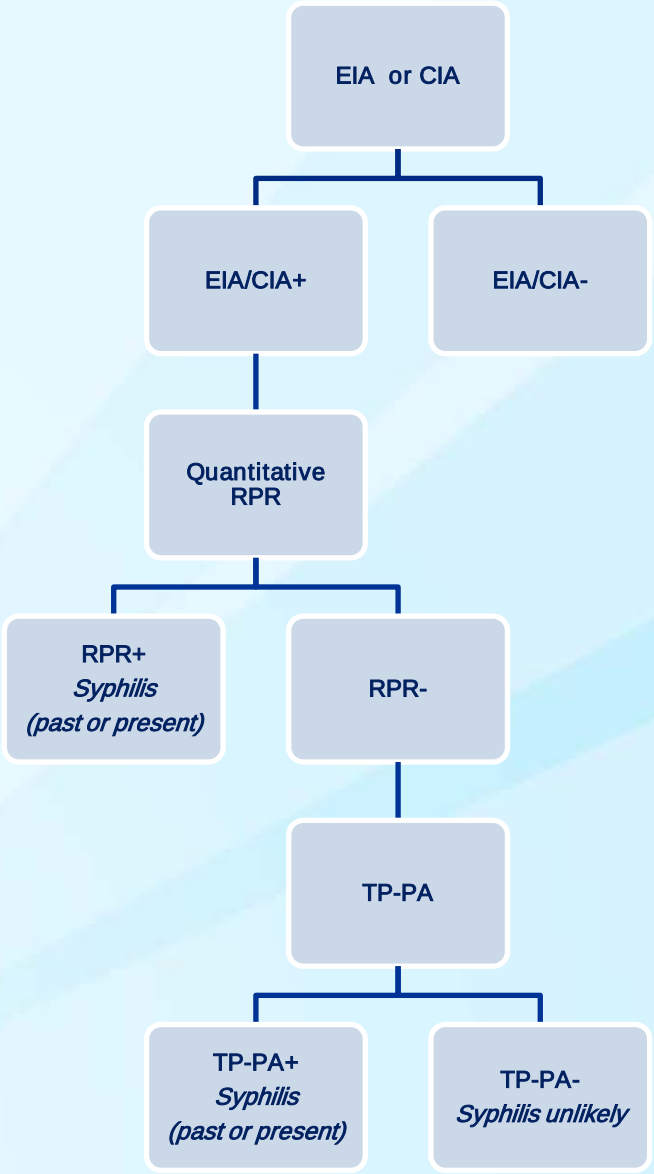
Mark Stanley, MPH

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



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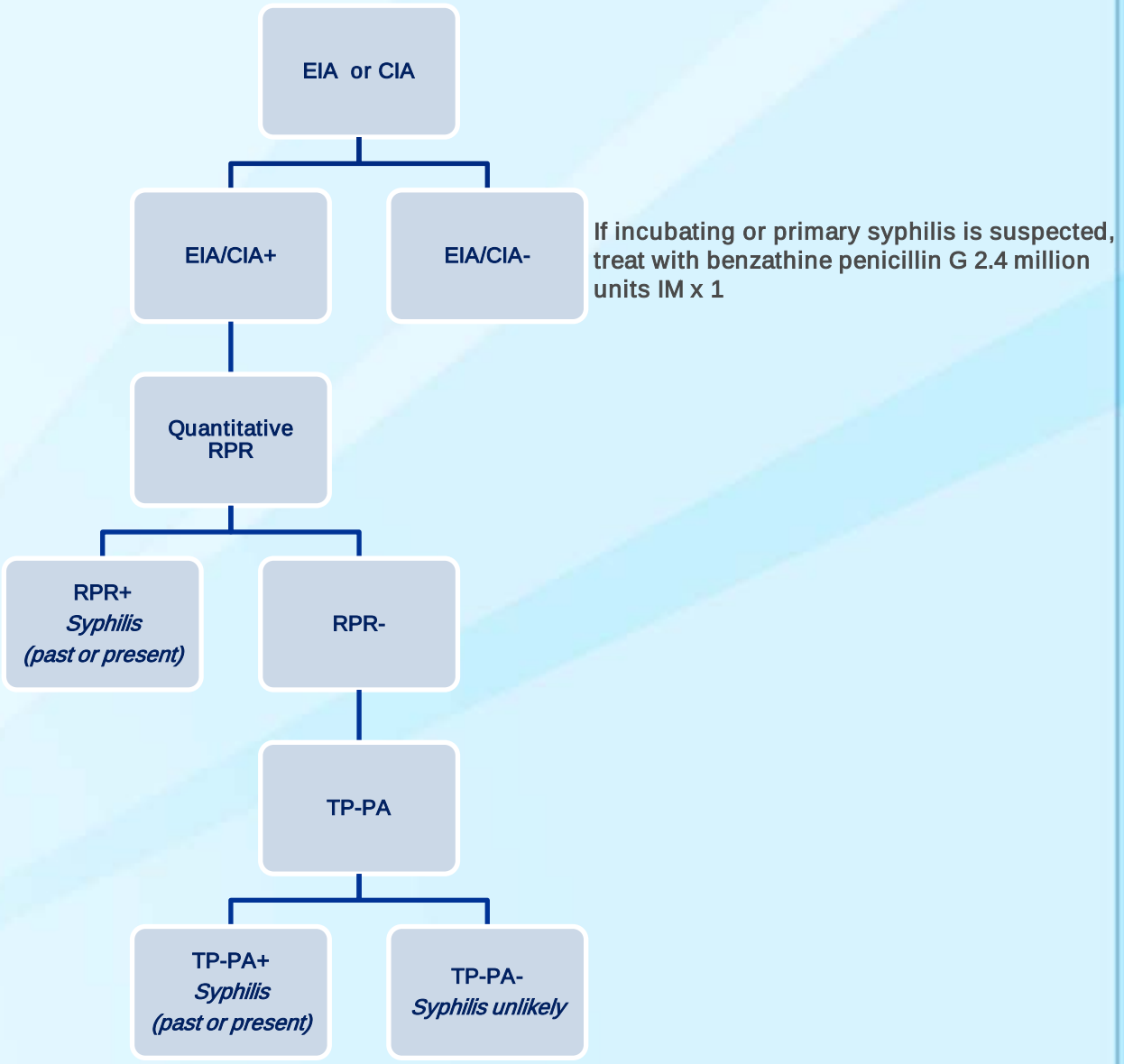

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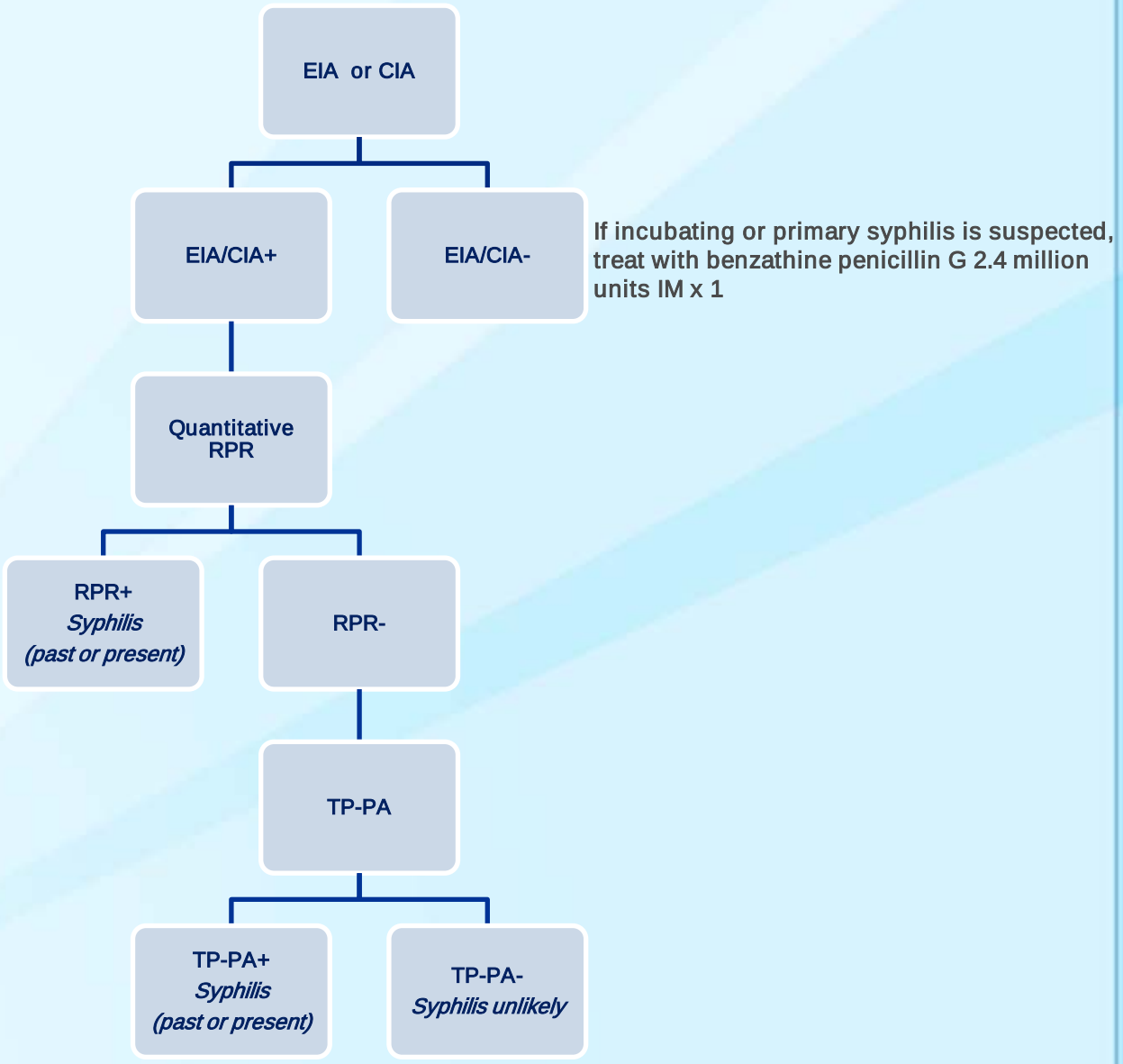

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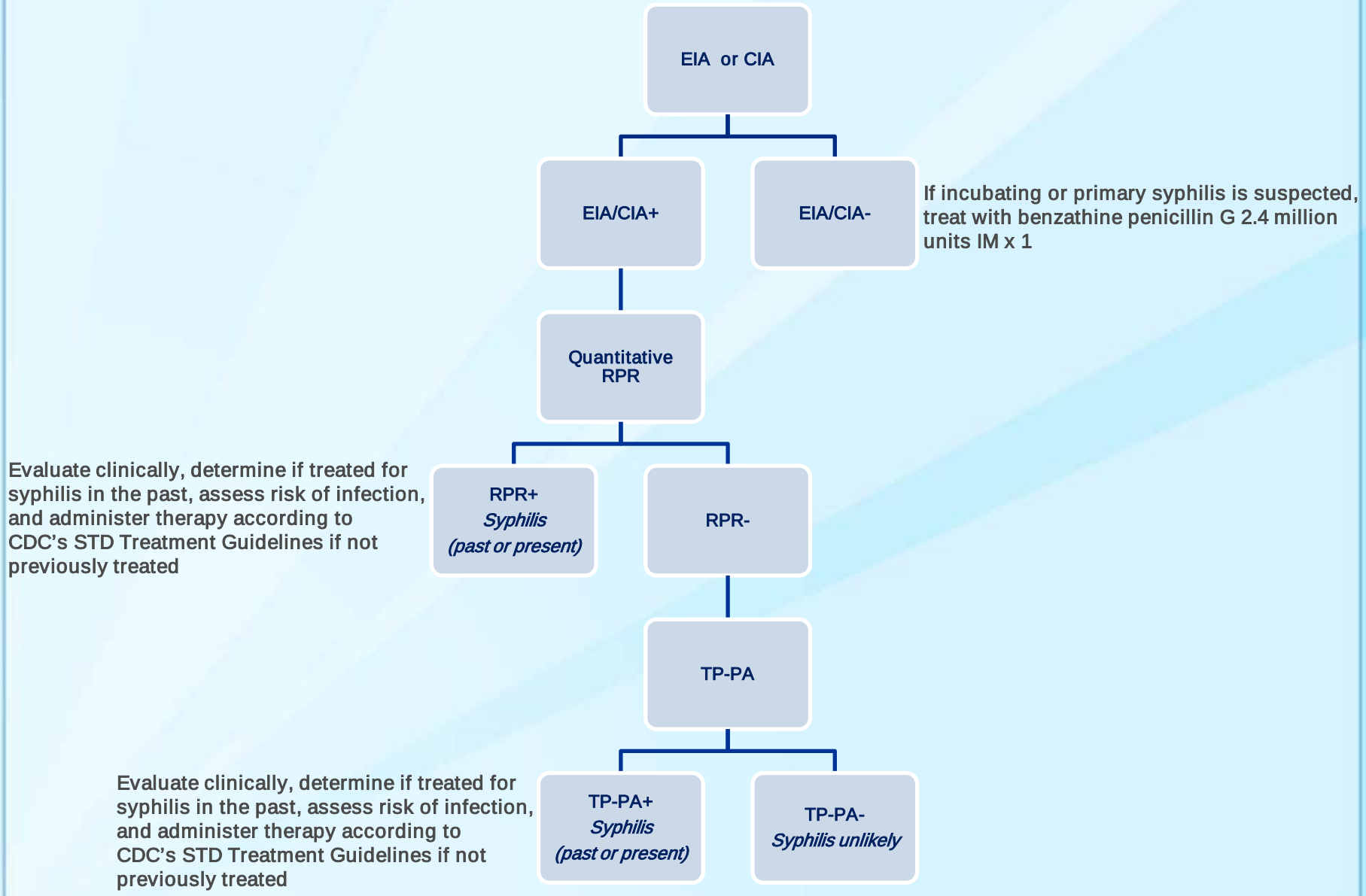

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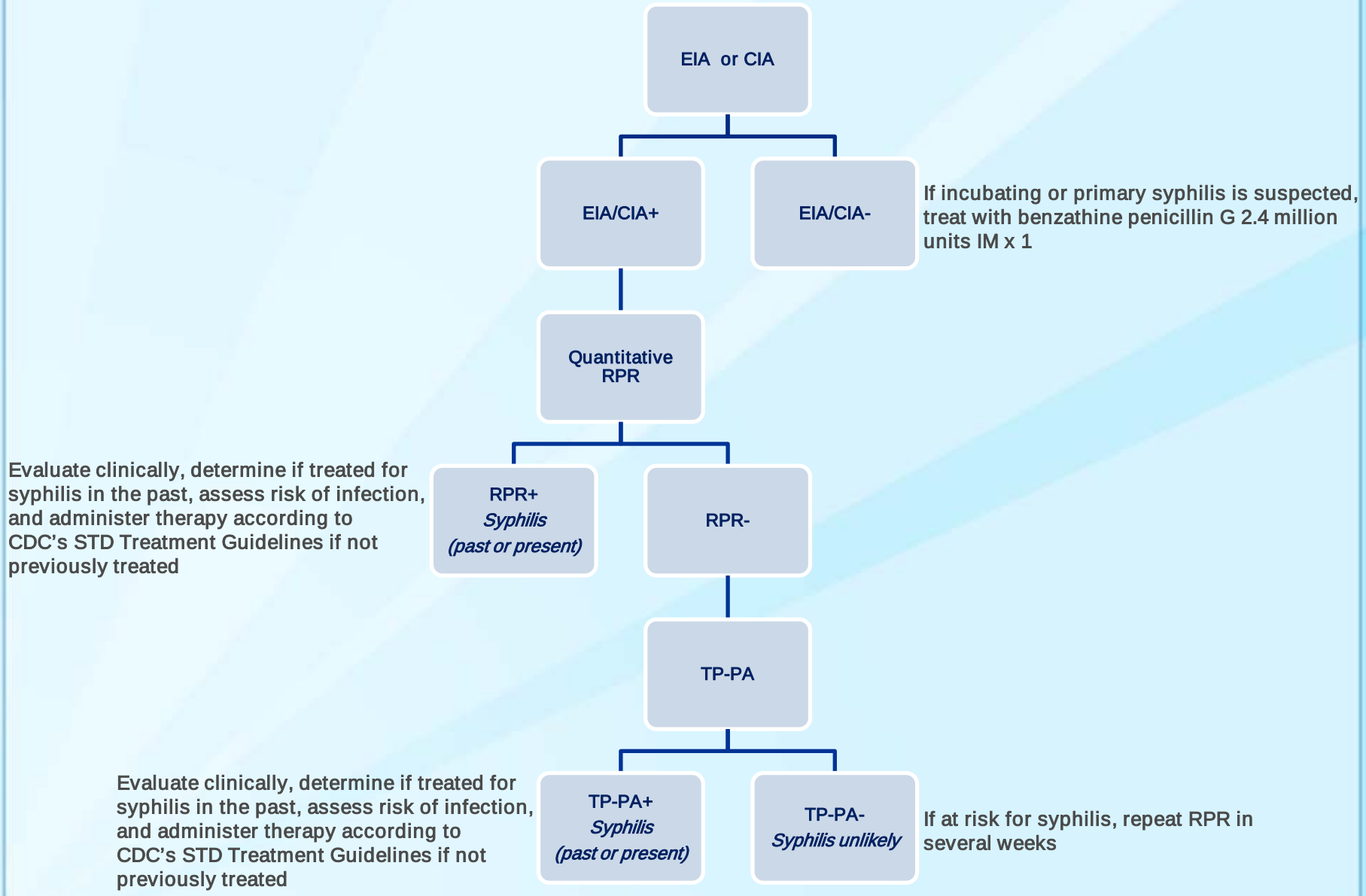

RESEARCH NEEDS TO PROVIDE AN EVIDENCE BASIS FOR FUTURE GUIDELINES

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

Acknowledgments

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Susie Zanto
Kim Workowski

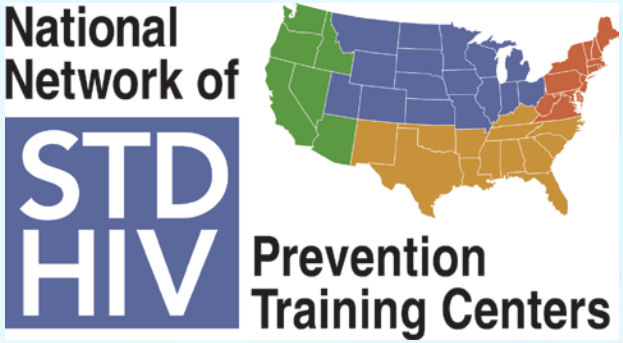
Clinician Resources

- 2010 STD Treatment Guidelines
  - www.cdc.gov/std/treatment/2010
- Condoms and STDs: Fact Sheet for Public Health Personnel
  - www.cdc.gov/condomeffectiveness/latex.htm
- Expedited Partner Therapy
  - www.cdc.gov/std/ept
- Get Yourself Tested
  - www.itsyoursexlife.com/gyt

Educational and Training Resources

National Network of STD/HIV Prevention Training Centers

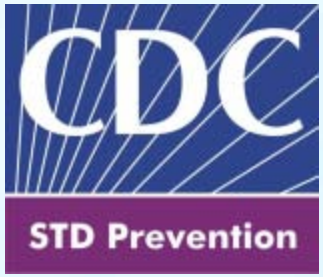
www.nnptc.org

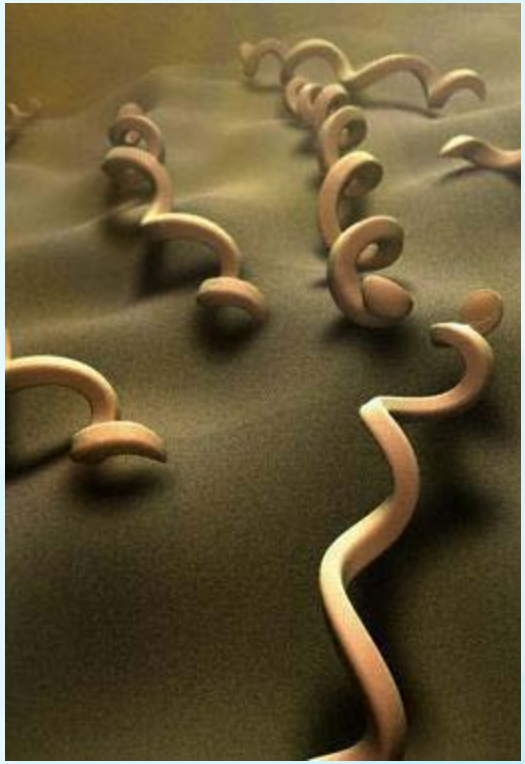


CDC Division of STD Prevention

www.cdc.gov/std/training

stdtraining@cdc.gov or 404.639.8360





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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.