Reverse Sequence Syphilis Screening: Frequently Asked Questions

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1) Treponemal immunoassays: description and test performance

What are the similarities and differences between the EIA, CIA and MBIA? Are there any preliminary data available on the performance of the MBIA?

ASSAY	Antigen	Solid support	Reaction	Multiplex Testing
Enzyme Immunoassay (EIA)	*T. pallidum* lysates or Recombinant antigens	Polystyrene microtiter plate	Color	No
Chemiluminescence Immunoassay (CIA)	Recombinant antigens	Polystyrene microtiter plate	Light	No
Microbead Immunoassay (MBIA)	Recombinant antigens	Polystyrene microbeads	Light	Yes

MBIA is the newest immunoassay technology and allows for detection of antibodies elicited by more than one microorganism in the same specimen, permitting multiplex testing. For further information on the performance of MBIA, please refer to the following recently published studies from Binnicker M et al. in the *Journal of Clinical Microbiology*, 2011 and Gomez E et al. in *Clinical and Vaccine Immunology*, 2010.

It seems like the Trep-Chek EIA (Trinity Biotech) is less specific than the Trep-Sure EIA (Trinity Biotech). Has this been confirmed?

Based on data from Southern California Kaiser Permanente, a large integrated healthcare organization (published in the CDC Morbidity and Mortality Weekly Report) 60 percent of specimens that were EIA-positive, RPR-negative with the Trep-Chek were not confirmed when tested with the TP-PA (Treponema pallidum-Particle Agglutination). With the Trep-Sure this percentage was only 25 percent. The Trep-Chek test utilizes a different format than the Trep-Sure, and this may account for difference in specificity.

2) Discordant EIA/CIA serology

My laboratory initially tests specimens with a treponemal test (e.g., EIA or TP-PA) followed by an RPR. How should I manage patients with a positive treponemal test and negative RPR (discordant serology)?

Patients with discordant serology should receive a clinical evaluation, including an assessment of sexual risk, prior syphilis history, and history of prior treatment for

syphilis. If results of a second treponemal test are available, they can be helpful in guiding therapy.

Per CDC recommendations, for asymptomatic individuals who are EIA+/RPR-/TP-PA+ who have previously treated syphilis and do not have ongoing sexual risk, no further treatment is necessary. Individuals who have never been treated for syphilis should be staged and treated with appropriate antibiotic therapy (many will be diagnosed with late latent syphilis). Though individuals with late latent syphilis are not thought to be infectious, goals of treatment are to prevent the sequelae of late syphilis.

If a second treponemal test is not available, patients with EIA+/RPR- serology should receive a clinical evaluation and sexual risk assessment. Those with previously treated syphilis who do not have ongoing sexual risk do not need further treatment. Individuals with symptoms suggestive of early syphilis should be presumptively treated for syphilis, and repeat RPR testing should conducted one week after treatment to document if seroconversion occurred. For asymptomatic individuals without a history of syphilis, clinicians may either choose to postpone treatment and perform repeat testing, or treat for potential latent disease, depending on the clinician's assessment of the patient's risk.

If the EIA is reactive, RPR is nonreactive, TP-PA is reactive and the patient gets treated, how will we follow-up to determine if the treatment is effective?

Many patients with an EIA+/RPR-/TP-PA+ result may have been previously treated for syphilis, may have late latent syphilis, or latent syphilis of unknown duration. A very small proportion of patients with these test results might have early primary syphilis. A quantitative nontreponemal test should be performed at a follow-up visit to ensure that the patient had not recently acquired syphilis and to establish a baseline titer to monitor the response to treatment.

Do you recommend that specimens that are EIA+/RPR-/TP-PA- be retested? Could these specimens be tested with a third treponemal test?

There are several approaches to retesting specimens that have discordant EIA and TP-PA results. One approach would be to repeat the EIA testing sequence again in four weeks. It is possible that the patient's repeat EIA may be negative, or the patient may seroconvert to EIA-positive and RPR-positive during that time. Those that remain persistently EIA+/RPR-/TP-PA- are likely false positive EIAs, and clinicians may choose to continue observing these individuals without treatment.

Performing a third treponemal test on EIA+/RPR-/TP-PA- specimens is not currently recommended for several reasons. A third treponemal test should ideally use a different platform and have similar test performance to the EIA and TP-PA. Though the FTA-ABS (fluorescent treponemal antibody absorption) uses a different platform, it is not recommended by the CDC as a confirmatory test due to issues with test performance. (See section 5, "Other treponemal tests (TP-PA, FTA-ABS)".) Furthermore, laboratories would either need to maintain reagents for three separate

treponemal tests, or specimens would need to be sent out to another laboratory, which may cause delays in provider reporting.

Performing a second treponemal test for discordant (EIA+/RPR-) specimens adds potentially significant costs to laboratory budgets, and the overall proportion of individuals with EIA-positive, RPR-negative serology is small compared to the total number of tests being performed. Is the second treponemal test really necessary?

The number of individuals with discordant serology (EIA or CIA+/RPR-) may be small compared to the total number of tests performed; however, in published studies of the treponemal immunoassay, discordant serology comprised more than half of all EIA/CIA+ positive results (Park IU et al., *Journal of Infectious Diseases*, 2011). Most laboratories do not have extensive experience with EIA for clinical diagnostic use. Until more data are available about the performance of treponemal immunoassays in routine clinical use, the CDC recommends that a second treponemal test (e.g., TP-PA) be performed for specimens that are EIA+/RPR-. The rationale behind this recommendation is that although treponemal immunoassays appear to have excellent analytic sensitivity, their specificity appears to be lower. Data published in the February 11, 2011 CDC Morbidity and Mortality Weekly Report reported that the percentage of initially discordant specimens that did not confirm with a second treponemal test ranged from 12 to 60 percent (mean 31.6 percent), implying that the initial EIA result may have been a false positive. False positive results can have significant repercussions, especially in populations such as pregnant women.

One area under investigation is whether the quantitative EIA index value can be utilized in lieu of performing a second treponemal test. There are data to suggest that specimens with high EIA index values are less likely to be false positive EIA results; however, further studies are needed. (See section 8 “Laboratory issues”.) CDC currently does not recommend the use of EIA or CIA index values to confirm discordant sera.

What do you think is the biological reason for EIA+/RPR-/TP-PA- results?

Syphilis is unlikely in patients whose sera have unconfirmed EIA/CIA results (e.g., EIA+/ RPR-/TP-PA-), especially in a low-risk individual. Research is needed to increase understanding of the cause of the false-positive EIA/CIAs, but might be due to cross-reacting antibodies in sera. However, if a patient with an EIA+/ RPR-/TP-PA- result is at risk for syphilis, the RPR should be repeated in several weeks, as the positive EIA may indicate early syphilis.

The confirmatory TP-PA and FTA-ABS tests have lower sensitivity than the EIA, so how can it be said that discordant results are false positive EIAs?

EIA/CIAs appear to have lower specificity than would be expected in tests that use recombinant treponemal antigens, and false-positive test results can occur with tests

that have low specificity. Based on published estimates of test performance, EIA/CIAs and the TP-PA test have similar sensitivities, but the sensitivity of the FTA-ABS is lower. The TP-PA is recommended as a confirmatory treponemal test because its sensitivity is comparable to the screening EIA/CIA sensitivity, and its specificity is higher.

3) False positive and false negative EIA/CIA results

I have a high-risk patient who was previously RPR-positive and FTA-ABS-positive. He was recently tested with the EIA and his test result was negative. What happened?

Given that this is a high risk individual who had positive non-treponemal and treponemal tests in the past, the current EIA result is likely a false-negative EIA. Though the treponemal immunoassays are very sensitive, no test ever demonstrates perfect sensitivity. Prior syphilis history, risk factors, and current symptoms should always be taken into account in the interpretation of syphilis serology results.

What is the possibility that a patient with Lyme disease could have a false positive EIA?

Lyme disease is unlikely to cause a false-positive EIA result, because most treponemal EIA/CIA tests use recombinant *T. pallidum* antigens that do not cross react with antibodies directed against *Borrelia burgdorferi* antigens. However, serologic tests for Lyme disease utilize native antigens from whole *B. burgdorferi* organisms, and *T. pallidum* serum antibodies can cross react in these Lyme disease tests.

Is pregnancy one of the factors that cause false positives with the EIA test?

Pregnancy is unlikely to cause a false positive EIA test result. However, the low specificity of certain EIA/CIAs can lead to a low positive predictive value when used to test persons in low-prevalence populations such as many populations of pregnant women. Reflexive nontreponemal testing is required to confirm the treponemal screening test and to detect active infection.

Are there biological factors other than previous treponemal infection that can result in a positive EIA? If so, can the CDC make these factors available to providers?

False-positive treponemal tests have been reported to occur with some of the older treponemal tests (FTA-ABS) for medical conditions unrelated to syphilis such as autoimmune diseases, viral infections, and other chronic diseases. Studies are needed to understand the cause of false-positive EIAs. For that reason, we continue to recommend nontreponemal testing of all sera with a reactive treponemal EIA screening test, to confirm syphilis and to identify active infection. Reflexive testing of discordant sera (e.g., EIA+/RPR-) with a TP-PA test confirms the treponemal reactivity of the EIA/CIA screening test.

4) Reverse sequence screening algorithm

What is the head-to-head performance between traditional sequence and reverse sequence testing? Is it ok to continue using the traditional sequence, or should my laboratory switch to reverse sequence screening?

To date there are no prospective studies that directly compare head-to-head performance of the traditional sequence versus the reverse sequence algorithm. Based on cost effectiveness modeling performed by Owusu-Edusei K et al. and published in *Sexually Transmitted Diseases* (2011), the reverse sequence algorithm may identify more cases of syphilis but would also cost more per case detected and result in more overtreatment.

CDC recommends that laboratories continue to use the traditional non-treponemal testing algorithm. However, there has been a trend towards reverse sequence screening for laboratories that test large numbers of specimens, given that the treponemal immunoassays are automatable, resulting in higher throughput and less hands-on microbiologist time.

With reverse sequence testing, isn't the high rate of false-positive RPRs or VDRLs virtually eliminated, which makes sense medically?

Reverse sequence testing with EIA or CIA identifies persons with presumed treponemal antibodies, but requires confirmation with an RPR or VDRL test. This algorithm results in essentially no biologic false-positive RPR or VDRL test results, because a nontreponemal test is not routinely performed if the screening EIA or CIA is negative.

However, the reverse sequence algorithm can be problematic. The use of reverse sequence testing identifies a large number of persons with EIA-positive, RPR-negative sera in whom management is uncertain and who require a second treponemal test, increasing testing costs and the workload of health departments. These patients would not be identified using the traditional algorithm.

When using reverse sequence testing, a treponemal test alone cannot be used to diagnose syphilis. Serum with a reactive treponemal test must be reflexively tested with a nontreponemal test, to confirm infection and to identify active infection. Discordant sera (e.g., EIA+/RPR-) must be reflexively tested with a TP-PA test to confirm the treponemal reactivity of the EIA/CIA result.

I realize the point of the work involved is essentially attempting to clarify a discordant test result for the CIA/EIA and RPR; however, there is a conceptual difficulty with the sequential logic of using a (CIA/EIA) treponemal test followed by a non-treponemal test (RPR), and followed again by a treponemal test (TP-PA). Do the results, for example, make statistical sense with regard to the sensitivity/specificity of each of the tests sequentially?

A nontreponemal test (e.g., RPR) must be performed reflexively if the screening treponemal test is positive to identify active infection and as a baseline for monitoring the response to treatment. A positive treponemal EIA/CIA alone is not sufficient to make the diagnosis of syphilis, regardless of the EIA/CIA's sensitivity/specificity.

If a reflexive RPR is positive, then a second, different treponemal test is not needed. If the two tests are discordant (e.g., EIA+/RPR-), then the TP-PA is performed reflexively to "break the tie" and to confirm the screening EIA/CIA. The TP-PA is recommended as a confirmatory treponemal test because its sensitivity is comparable to the screening EIA/CIA and its specificity is higher.

5) Other treponemal tests (TP-PA, FTA-ABS)

How was the cutoff for TP-PA (reactive versus non-reactive) established?

The cutoff for TP-PA reactivity was established by the manufacturer of the assay (Fujirebio Diagnostics, Inc., Malvern, PA, US). A specimen is considered TP-PA reactive when it is both 1) reactive with particle agglutination in the presence of sensitized particles at any dilution of 1:80 or over, and 2) non-reactive (no agglutination) in the presence of unsensitized particles (control). For more details about the TP-PA, please consult the manufacturer's package insert available online from Fujirebio.

Why is the FTA-ABS not recommended as a confirmatory test for discordant sera with an EIA+/RPR- result?

The TP-PA test should be used as the confirmatory treponemal test because the FTA-ABS is less sensitive and specific, is inherently subjective, and requires more expensive instrumentation.

6) Non-treponemal tests (RPR, VDRL)

Which non-treponemal test is recommended for syphilis testing, VDRL or RPR?

The non-treponemal tests (VDRL and RPR) are similar in that they are both low cost and have similar sensitivity and specificity. VDRL requires the use of microscopy to interpret the results, whereas RPR can be read macroscopically and can therefore more readily be performed on a point-of-care basis in clinical settings. However, the same assay should be used for serial testing to monitor the response to treatment, preferably performed in the same laboratory.

What is the current thinking on reasons for biologic false positives with the RPR or VDRL tests?

Patients with medical conditions unrelated to syphilis (such pregnancy, other infections, connective tissue diseases, malignancies, drug dependence, and advanced age) might have serum antibodies that cross-react with the lipoidal antigens used in nontreponemal tests.

7) Clinical management of syphilis

Should a cerebrospinal fluid (CSF) examination be done on patient with no history of previous treatment for syphilis and serological results of EIA+, RPR-, and TP-PA+ before treating this patient?

Patients diagnosed with syphilis at any stage with evidence of neurologic involvement should receive a CSF examination. However, routine CSF examination is not recommended for EIA+/RPR-/TP-PA+ individuals without neurologic symptoms. Recommendations for neurosyphilis diagnosis and treatments can be found in the 2010 STD Treatment Guidelines, available at http://www.cdc.gov/std/treatment/2010/.

What are the recommendations for testing neonates with the EIA/CIA?

Use of treponemal tests for diagnosis of congenital syphilis including EIA/CIA is not recommended. Recommendations for the diagnosis of congenital syphilis can be found in the 2010 STD Treatment Guidelines, available at http://www.cdc.gov/std/treatment/2010/

Do we need to repeat the EIA if a patient is presumptively treated based on clinical presentation and sexual history and if the initial EIA is negative?

Yes, serologic testing should be repeated in several weeks to confirm the diagnosis of syphilis and to establish a baseline RPR titer to monitor the response to treatment.

8) Laboratory issues

Should index values of EIA/CIA tests be reported, or should they be reported as a range of values (e.g., low, medium, high)?

The utility of index values in predicting subsequent TP-PA results or in predicting disease is an area of interest for future investigation. Typically, treponemal immunoassays are qualitative and reported to the provider as positive or negative without the quantitative index values. It is unclear whether the quantitative index value, reported either alone or within a range of values, has any clinical utility.

There are two studies (Wong E et al, *Sexually Transmitted Diseases*, 2011, and Park IU et al., *Journal of Infectious Diseases*, 2011*)* that evaluated quantitative index values for

two treponemal immunoassays and determined that nearly all individuals with high cutoff index values were subsequently TP-PA positive, implying that a TP-PA might not be needed in individuals with high index values. Of note, the range of index values differs from assay to assay, and the clinical utility of these index values is still uncertain.

How many labs in this country use automated EIA, CIA, or MBIA technology to perform reverse screening?

A survey of public health laboratories was conducted by CDC and the Association of Public Health Laboratories in 2007, and the results of this survey are available at http://www.cdc.gov/std/general/LabSurveyReport-2011.pdf. The survey found that most syphilis screening was performed using nontreponemal tests. Insufficient data exists to assess the syphilis screening tests being used in private laboratories.

Are any treponemal immunoglobin M (IgM) tests cleared by the FDA? Which IgM do you suggest we use for early infection?

Although IgM tests are commercially available in the United States, there are insufficient data to recommend their use for the diagnosis of early syphilis.

Are there any special time or processing requirements for transport of serum for the new tests?

Specimen type, processing, storage, and freeze and thaw recommendations vary by test, so it is important to read the manufacturer's package insert. Serum is the specimen of choice for use in EIA/CIA/MBIA; some tests permit the use of ethylenediaminetetraacetic acid (EDTA) or citrated plasma. Specimens should be transported with a cold pack, stored at 2-8°C for a varying number of days, and stored at -20°C for longer periods of time.

9) MISCELLANEOUS

Please define the threshold for high-prevalence populations versus low-prevalence populations.

Defining high- and low-prevalence populations presents a challenge as there can be substantial variability within a population or geographic region. A specific cutoff for defining low versus high syphilis prevalence for a geographic region has not been defined by CDC. Consult your local infectious disease expert or local health jurisdiction's STD Controller to determine whether you are practicing in a high-prevalence area or setting.