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Centers for Disease Control and Prevention Syphilis Summit—Diagnostics and Laboratory Issues

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Abstract

Syphilis, caused by the bacterium *Treponema pallidum*, is on the rise in the United States particularly among men who have sex with men. The disease is complex with varied clinical manifestations and challenges remain in the laboratory diagnostic setting because *T. pallidum* is noncultivable and no single test can accurately diagnose all stages of the disease. There are missed opportunities for the use of direct detection tests in primary and secondary syphilis. The increasing use of different reverse sequence algorithms for serology testing without validation in populations with varying risks for syphilis makes the interpretation of test results difficult; this has led to concerns about diagnostic errors or overtreatment. On the other hand, the traditional algorithm may miss some early primary syphilis cases, which is of concern in high-risk populations. The potential utility of rapid syphilis serology tests in different settings or populations remains to be determined. The implementation of better tests and appropriate testing algorithms together with laboratory guidelines for test use in general will lead to better diagnostic options for syphilis.

Syphilis is a disseminated acute and chronic infection caused by *Treponema pallidum* subspecies *pallidum* (hereafter *T. pallidum*). The laboratory diagnosis of syphilis remains a challenge because the disease has multiple stages with varied clinical manifestations and, no single test can accurately diagnose all stages. The inability to cultivate *T. pallidum* on routine laboratory media has hindered diagnosis and test development. The diagnosis of syphilis relies on serological and, the infrequently available, direct detection tests. Thus, serologic tests remain the mainstay for diagnosis. Laboratories are adapting the reverse sequence algorithm for serology testing without validation in some cases, leading to concerns about diagnostic errors or overtreatment of patients.[1,2] Although the majority of laboratories continue to use the traditional algorithm, there are concerns about this approach missing cases of early syphilis when the nontreponemal screening test is nonreactive.[3,4] The Syphilis Health Check (Trinity Biotech, US) is the only Food and Drug Administration (FDA)-cleared Rapid Syphilis Test (RST) for use in the United States, but the role of RSTs in the United States is still unclear.[5]

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Despite penicillin being an effective first-line treatment option for syphilis, the disease continues to spread in the United States. From a laboratory perspective, this may be due, in part, to the following reasons. First, there is no single test that can accurately diagnose all stages of syphilis, and there are missed opportunities for the use of direct detection tests in early primary syphilis because nontreponemal and treponemal serological tests are insensitive and may be nonreactive in up to 47% of patients.[6–10] Second, because treponemal serological tests cannot be quantified and usually stay positive for life after successful treatment and nontreponemal titers may decline slowly or remain serofast after treatment, it is difficult to distinguish between treated and new infections in high-risk individuals with a history of treated syphilis, such as men who have sex with men (MSM). Lastly, primary lesions are painless and may go unnoticed in the vagina of women and the rectum of MSM allowing the infection to be transmitted to other sex partners. This review focuses on the diagnostic and laboratory issues relating to the use of direct detection and serology tests for syphilis that were discussed at the Centers for Disease Control and Prevention (CDC) Syphilis Summit in 2016.

Direct Detection Tests for Syphilis

Unlike many other bacterial pathogens, *T. pallidum* cannot be grown on routine laboratory culture media. Darkfield (DF) microscopy, polymerase chain reaction (PCR), silver staining, and immunostaining (which consists of immunofluorescent staining based on the direct fluorescent antibody for *T. pallidum* [DFA-TP] test and immunohistochemistry [IHC]) are direct detection tests for *T. pallidum*. Darkfield microscopy, PCR, or immunofluorescent staining are useful tests for infectious lesions such as ulcerative lesions, erosive rashes, condyloma lata and biopsies of skin, placenta, and other organs. Darkfield microscopy relies on examining live treponemes and is a morphology and motility-based test. Silver staining is a morphology-based test, whereas both immunofluorescent staining and IHC are both immunologically and morphology-based. Polymerase chain reaction tests detect *T. pallidum*-specific nucleic acid sequences.

Darkfield microscopy is useful for moist lesions of primary or secondary syphilis where treponemes can be readily found. The test is generally not used on cerebrospinal fluid (CSF), lymph node aspirate, and other body fluids. Because *T. pallidum* is difficult to distinguish from other spirochetes in the mouth, DF is not recommended for specimens from this site.[11] Darkfield microscopy is a useful point-of-care test in STD clinics especially for patients with moist primary or secondary lesions; however, successful testing relies on an adequately trained microscopist who maintains performance proficiency in the test and the ability to perform the test before motility is compromised. The sensitivity of DF on primary lesions approaches 80% compared with immunofluorescent staining,[12,13] 88% compared with PCR,[14] and 97% versus serology[15]; however, each of these tests will miss cases when the other is used as a comparator in the absence of an acceptable gold standard. In 2011, the Association of Public Health Laboratories conducted a survey on STD Testing Practices in US public health laboratories (PHLs) and found that only 19% (11/58) of laboratories had DF capability. Because DF is more often done in STD clinics and emergency departments, it would be more helpful to survey these testing sites versus PHLs. The CDC supports proficiency testing of DF microscopy in settings where the test can be performed according

to specifications and provides live *T. pallidum* for training purposes. Requests should be sent to stdlaboratoryspecim@cdc.gov.

The DFA-TP test was first described by Yobs and colleagues[16] but is no longer available in the United States. Immunofluorescent detection, which is based on the DFA-TP test, uses in-house monoclonal or polyclonal antibodies to detect *T. pallidum* antigens in the same specimen types as DF except that they have to be fixed on a microscope slide and body fluids require centrifugation to concentrate the bacteria. A fluorescent microscope is required to read the test. Immunofluorescent detection has similar sensitivity to DF when fresh lesion material is examined and the *T. pallidum* antibodies are of high quality.[13,17] Although monoclonal and polyclonal antibodies are available commercially, these reagents are not approved for diagnostic testing because they have either not been validated for clinical use or quality control is not performed routinely. Silver staining and IHC are 2 techniques used for observing *T. pallidum* in paraffin-embedded tissue sections with samples from the brain, placenta, umbilical cord, or skin (other than primary lesions) being the most frequently tested. These staining techniques are performed in clinical pathology laboratories.

There are a number of laboratory developed PCR tests for *T. pallidum*, based on different gene targets, but an FDA-cleared test is not available.[18] In the absence of a gold standard, the sensitivity of PCR on samples from primary syphilis is about 87% compared with DF and approaches 95% versus serology.[19,20] Polymerase chain reaction sensitivity in secondary syphilis ranges from 50% to 84% on specimens from mucous patches, condyloma lata, and maculopapular rash, with the lower detection rates most likely reflecting inadequate sampling of patients with skin rash.[19,21]

Given the unprecedented transmission of infectious syphilis in the United States, there is a lack of a sensitive test to diagnose the most infectious lesions at the primary stage thus impeding timely detection and treatment. A commercial nucleic acid amplification-based test is urgently needed for detecting *T. pallidum* in moist lesions of primary or secondary syphilis. Only 1 laboratory within the Association of Public Health Laboratories network reported using a PCR test for syphilis. In addition, Quest Diagnostics offers a laboratory developed test based on real-time PCR assay targeting the flagellar biosynthetic protein (*FlhB*) gene. The PCR has been validated for use on CSF and genital lesion swabs and has an analytical sensitivity of 100 genomic copies/mL. The Laboratory Reference and Research Branch within the Division of STD Prevention at the CDC validated a multiplex PCR for detecting *T. pallidum*, herpes simplex virus (HSV) 1 and 2, and *Haemophilus ducreyi*, the causes of genital ulcer disease (GUD) in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory. This assay has an analytical sensitivity of 10 to 100 genomic copies per reaction.[22] Since *H. ducreyi* is rarely encountered in the United States these days, modifying the assay to detect *T. pallidum* and HSV could be 1 approach to improve the etiologic diagnosis of GUD. As of December 2017, there are 12 FDA-cleared tests to detect HSV 1 and 2 genital infections in the United States. Modifying these tests to include *T. pallidum* detection in a GUD multiplex PCR might be a more practical and faster approach for the development of an FDA-cleared syphilis and herpes GUD PCR test.

Because *T. pallidum* numbers are usually lower in CSF, blood, ocular fluid, amniotic fluid, lymph node aspirate, earlobe scraping, and some tissue biopsies compared with moist lesions of primary and secondary syphilis, nucleic acid amplification tests with better sensitivity than existing PCR assays are needed for these specimen types. A study by Yang and colleagues[23] showed that swabs of the oral cavity, in the absence of visible syphilitic lesions, are useful for detecting *T. pallidum* in MSM engaged in unprotected oral sex practices. In addition, *T. pallidum* has been detected in urine by PCR.[24] These noninvasive specimen types increase the repertoire of samples for PCR diagnosis and should be evaluated further.

Until an FDA-approved PCR test for GUD becomes available, it is hoped that PHLs can implement the CDC CLIA-validated multiplex GUD PCR assay for testing genital lesions swabs from sexually active patients. A nucleic acid amplification test that is available at the point of care would be particularly useful for clinics with a high burden of GUD. Specimen quality is vital for accurate PCR diagnosis. Swabs, body fluids, and tissue samples should be collected and shipped appropriately for testing as described elsewhere.[25] A specimen bank including samples from genital ulcers, well-defined primary and secondary lesions, blood from all stages of syphilis, CSF, ocular fluid and congenital syphilis specimens is needed for PCR validation purposes. Other innovative methods such as RNA-based assays for increased sensitivity and next-generation sequencing methods should be explored for direct detection of *T. pallidum*.

Serological Diagnosis of Syphilis

Serological tests for syphilis are divided into 2 categories, tests that detect treponema-specific antibodies and those that detect antibodies to lipoidal (nontreponemal) material as a result of treponemal infection. The most common nontreponemal tests in use in the United States are the rapid plasma reagin (RPR) and the Venereal Disease Research Laboratory (VDRL) test. Of the FDA-cleared treponemal antibody tests, the fluorescent treponemal antibody-absorption (FTA-ABS) uses whole *T. pallidum*, the *T. pallidum* particle agglutination assay (TP-PA) uses a *T. pallidum* lysate and the enzyme immunosorbent assay (EIA)-based, chemiluminescence (CIA) assays and multiplex flow immunoassays use recombinant *T. pallidum* antigens (eg, 15, 17, 47 kDa, recombinant *T. pallidum*) to detect either IgG, IgM, or both antibodies.

Nontreponemal tests detect both IgG and IgM antibodies using a complex of cardiolipin, lecithin, and cholesterol. Positive treponemal tests indicate lifetime exposure to syphilis, whereas nontreponemal tests are more reliable indicators of untreated infection. Quantitative nontreponemal test titers are used to monitor response to treatment with a treatment response in syphilis patients defined by resolution of disease manifestations, a 4-fold decline in nontreponemal titers (or a change in 2 dilutions, eg, from 1:16 to 1:4) using the same assay, or seroreversion of a nontreponemal test to a nonreactive test.[26] Nontreponemal test titers usually decline after treatment and can become nonreactive with time; however, in some treated persons, nontreponemal antibodies can persist for a long period of time, a response referred to as the “serofast reaction.”[26]

False-positive reactions, defined as reactivity in a nontreponemal test with a negative treponemal test in conditions other than treponemal infection occur in 1% to 2% of the United States population.[27] False-positive nontreponemal test results can occur in patients with advancing age, malaria, brucellosis, mononucleosis, viral hepatitis, lymphogranuloma venereum, chickenpox, viral pneumonias, tuberculosis, chancroid, systemic lupus erythematosus, human immunodeficiency virus infection, intravenous drug use, leprosy, and in pregnancy.[11,25] False-positive treponemal tests can occur in patients with endemic treponematoses (yaws, bejel, pinta) and other infections caused by spirochetes, such as Lyme disease.[11] False positive reactions in both nontreponemal and treponemal tests are also seen in the sera of healthy individuals, albeit rarely.[1,25] The factors associated with false positives using the newer treponemal EIA, CIA and other immunoassays for screening in the reverse sequence algorithm are not well defined.[28,29]

Optimization of the Reverse Sequence Algorithm to Minimize Diagnostic False Positives Leading to Overtreatment

The traditional syphilis testing algorithm uses a nontreponemal test to detect untreated syphilitic infection followed by confirming with a treponemal test. With the advent of fully automated high-throughput treponemal immunoassays that are FDA-cleared for screening, reverse screening algorithms are increasingly being used in the United States. Reverse algorithms use a treponemal test (EIA, CIA, or multiplex flow immunoassay) as a screening test, followed by a nontreponemal test for reactive samples. If the nontreponemal test is negative (discordant) then a different treponemal test is recommended.[26]

In 2011, 11.7% (7/53) of PHLs in the United States reported using the reverse algorithm but the algorithm and testing practices appear tovary among laboratories. For example, a review conducted at 4 laboratories in New York City found that, at 2 of the laboratories, specimens that were discordant by EIA and RPR testing were retested with a different treponemal test (TP-PA or FTA-ABS); at the third lab, specimens that were reactive by both EIA and RPR were retested by TP-PA or FTA-ABS; and in the fourth lab, no further testing was done after the EIA and RPR tests.[2] Implementation of algorithms that have not been validated for specific populations with varying incidence and prevalence of syphilis infection leads to confusion among clinicians regarding the interpretation of these tests and the need for a second confirmatory treponemal test in the reverse algorithm. There is also a lack of consistency in the way laboratories report results; for example, treponemal and nontreponemal results are not always reported together, which makes it difficult for clinicians to interpret results when only 1 test result is available.[2]

In another study, analysis of reverse screening data from several low- and high-prevalence settings in the United States showed that overall, 56.7% of reactive EIA/CIA were nonreactive by RPR and of those, 31.6% were nonreactive with a confirmatory treponemal test.[29] The percentage of discordant samples that were nonreactive by TP-PA or FTA-ABS was 2.9 times greater (40.8% vs 14.1%) in the low- versus high-prevalence populations, suggesting false positive screening test results. There are several reasons for discordant results (ie, EIA or CIA positive/RPR or VDRL negative). First, this may indicate past treated

syphilis because treponemal tests may remain reactive for life. Second, it may indicate early primary syphilis since treponemal antibody test may be slightly more sensitive than nontreponemal tests at this stage because treponemal antibodies can be detected before nontreponemal antibodies.[8,11] Lastly, if reflex testing with a treponemal test such as TP-PA is nonreactive, then this may indicate a false-positive EIA/CIA screening test result in low-risk populations or a true positive in high-risk populations[30]; however, differences in performance characteristics among the treponemal screening tests and the treponemal tests used to adjudicate discordant results can also have a bearing on false or true positive results.

With the increased use of the reverse algorithm in the United States, higher rates of false positives by CIA/EIA are being seen in low prevalence settings, such as in pregnant women.[31s] False-positive results can lead to overtreatment, treatment concerns in pregnant women and in patients with penicillin allergies, and anxiety in both patients, their partners, and clinicians especially if in a monogamous relationship and the partner is negative or if retesting before treatment is suggested for a pregnant woman. The CDC recommends a quantitative nontreponemal test be performed if the treponemal screening test is positive to guide patient management decisions, and if the nontreponemal test is negative, then a different treponemal test (preferably one based on different antigens and a different platform than the original test such as the TP-PA) should be used to adjudicate the results of the screening treponemal test.[26] More studies are needed to determine if other factors are associated with false- or true-positive treponemal antibody results, especially with the newer treponemal tests.

Optimization of Comparisons of Traditional and Reverse Algorithms Using Clinically Defined Specimens

Studies comparing the traditional and reverse testing algorithms using specimens from various stages of syphilis in high- versus low-prevalence settings in the United States and the consequent effect on patient management are lacking. Reverse algorithms may not be appropriate for all settings and treatment delay is of concern in some high incidence populations in the United States. Additional studies are needed to determine whether EIA or CIA IgM-specific screening tests may be useful in guiding patient management. A study by Jost and colleagues showed that the analytic sensitivities of 9 approved commercial treponemal tests varied considerably, suggesting that the choice of screening and second treponemal tests in the reverse algorithm can affect testing outcome.[32s] Ideally, the second treponemal test should have an equivalent or higher sensitivity and specificity than the initial screening test, and target a different antigen and testing platform. With respect to use of the traditional algorithm, there is concern among clinicians that this approach misses some cases of primary syphilis particularly among high-risk populations because the VDRL and RPR screening tests generate more false-negative results due to decreased sensitivity compared to treponemal screening tests.[3,4,8,33s]

A few reports suggest that a higher antibody index value or optical density index cutoff of the initial screening test may help to predict a reactive second treponemal test result in the reverse algorithm, which may reduce the need for the second treponemal test, especially in

lower prevalence settings.[34s–36s] Reporting the index value cutoff may be helpful in clinical management decisions; however, use of such values may also cause confusion with respect to their interpretation. Therefore, validation of the use of these values along with clear guidelines for laboratorians and clinicians are needed.

Value of RSTs for Diagnosis of Syphilis in the United States

There are a number of RSTs being used worldwide, and RSTs are particularly promoted for settings where risk of infection is high (eg, screening in pregnancy) and where laboratory capacity is limited or results return late (so patients were lost to follow up).[37s–40s] Most RSTs detect only treponemal antibodies; thus, they cannot distinguish between recent and old or previously treated infections. Several RSTs have undergone quality assessments (eg, CE Mark, WHO Prequalification, Brazilian ANVISA) and might be appropriate for introduction to the United States. As of December, 2017, the Syphilis Health Check (Trinity Biotech, US) is the only FDA-cleared RST for use in the United States but published performance data of this test in the field is still limited.[5] Rapid Syphilis Tests are designed for use with fingerstick blood as part of a clinic visit, and their simplicity allows performance by health providers who are not trained technologists; however, the sensitivities of a number of tests have been shown to be higher with serum.[37s] The performance of RST use versus laboratory-based screening algorithms needs to be evaluated in well-defined populations together with quality assurance programs in various settings, such as public and private clinics, emergency departments or urgent care clinics, and outbreak and community outreach in the United States.

CONCLUSIONS

In light of the rising incidence of syphilis in the United States, there are many missed opportunities for use of direct detection tests during early syphilis, when the disease is most infectious. Polymerase chain reaction tests and DF for *T. pallidum* are needed in early primary syphilis because serological tests may not yet be reactive at this stage. Few STD clinics in the United States have DF capability and no FDA-cleared PCR test is available. As more laboratories consider using the reverse algorithm for serology testing, it is imperative that they validate algorithms in their particular setting before implementation. Also, more comparative data on the 2 algorithms are needed to address concerns about the traditional algorithm missing some early primary cases. More data are needed on RSTs with regard to appropriate settings for use, interpretation of tests results, and the need for additional laboratory-based confirmatory testing.

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