Laboratory Recommendations for Syphilis Testing in the United States

DIVISION OF STD PREVENTION

CENTERS FOR DISEASE CONTROL AND PREVENTION

Contents

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Summary

This report provides new recommendations for tests that can support a diagnosis of syphilis, including serologic testing and methods for the identification of the causative agent *Treponema pallidum*. Laboratory testing for syphilis has traditionally been based on serologic algorithms to detect a humoral immune response to *T. pallidum*. These tests can be divided into so-called "nontreponemal" and "treponemal" serologic tests depending on whether they detect antibodies that are broadly reactive to lipoidal antigens shared by both host and *T. pallidum* or antibodies specific to *T. pallidum*, respectively. Both types of tests must be used in conjunction to help distinguish between an untreated infection or a past infection that has been treated. Newer serologic tests allow for laboratory automation but must be used in an algorithm, which can also involve older manual serologic tests. Direct detection of *T. pallidum* continues to evolve from microscopic examination of material from lesions for visualization of *T. pallidum* to molecular detection of the organism. There are limited point-of-care (POC) tests for syphilis available in the United States; availability of sensitive and specific POC tests could facilitate expansion of screening programs and reduce the time from test result to treatment.

These recommendations are intended for use by clinical laboratory directors, laboratory staff, clinicians, and disease control personnel who must choose among the multiple available testing methods, establish standard operating procedures for collecting and processing specimens, interpret test results for laboratory reporting, and counsel and treat patients.

Introduction

Background

Treponema pallidum subsp. *pallidum*, primarily transmitted through sexual contact, is among four pathogenic species in the genus *Treponema*, which is in the family *Treponemataceae* (*1*) The other three pathogenic *Treponema* species cause skin diseases mostly transmitted by direct skin-to-skin contact. Yaws is caused by *T. pallidum* subsp. *pertenue* and found in tropical areas in Africa, Asia, and Latin America (*2*). *T. carateum* infection results in pinta, which although rare, is found in tropical areas of Latin America (*3*). Endemic syphilis or bejel, caused by *T. pallidum* subsp. *endemicum*, occurs mostly in children and is mainly found in the eastern Mediterranean, West Africa, and Cuba (*4*,*5*). However, phylogenic analysis of lesion specimens from some patients outside of bejel-endemic areas who had received a diagnosis of syphilis revealed that *T. pallidum* subsp. *endemicum* might be sexually transmitted with a clinical presentation like syphilis (*5*-*8*). For this report, *T. pallidum* subsp. *pallidum* will be abbreviated to *T. pallidum* unless further distinction between the subspecies is necessary.

T. pallidum causes a systemic infection and might lead to serious sequalae in multiple organ systems, including the central nervous system and the ocular and otic systems. Vertical transmission can cause congenital syphilis, which might result in spontaneous abortions, miscarriages, or stillbirths; infants with congenital syphilis can present with clinical signs of infection at birth or months to years after birth. Clinical features in adults progress through different stages, beginning with primary syphilis, that often appear about 3 weeks after exposure, with an incubation period of 10–90 days (*9*). Primary syphilis is characterized by single or multiple ulcerative-like lesions called chancres that are often painless, and therefore might be unnoticed when they occur inside the mouth, vagina, or rectum. Chancres can persist

for 2–6 weeks before healing spontaneously. Secondary syphilis typically begins 2–24 weeks after most primary lesions heal and is commonly characterized by a mucocutaneous rash appearing on the trunk, palms, and soles; mucous patches of the mouth or condylomata lata on the genitals or rectum occur in about a quarter of patients. Primary and secondary syphilis symptoms can occur concurrently, which is more likely in persons with HIV. Moist primary and secondary syphilis lesions contain infectious *T. pallidum* that can be transmitted through sexual contact to susceptible people. Secondary clinical manifestations can also consist of lymphadenopathy, alopecia, and occasionally neurological and ocular manifestations. Signs and symptoms of secondary syphilis typically resolve in approximately 3 months, with a range of 1–12 months (*10*,*11*) but can periodically recur for the first several years of infection in ≤25% of untreated individuals (*12*).

The interval between primary to secondary and secondary to tertiary syphilis is known as latency when no symptoms or signs of syphilis are present. The interval from secondary to tertiary syphilis can last for years or decades before symptoms appear. In up to two-thirds of patients, the disease can remain latent for life and never progress to tertiary syphilis (*13*-*15*). Latent asymptomatic syphilis is divided into early latent infections thought to have been acquired within the past year, late latent infections thought to be longer than one-year duration or latent syphilis of unknown duration where the timing of acquisition cannot be determined based on available clinical, historical, or laboratory data. Clinical signs of tertiary syphilis, a rare condition, include cardiovascular syphilis, with aneurysms or stenosis resulting from multiplication of treponemal spirochetes in the thoracic aorta or coronary arteries; syphilitic gummas, with soft granulomatous growths that can cause tissue destruction in any organ system, including bones and cartilage; and neurosyphilis, with late neurologic manifestations including tabes dorsalis and general paresis. Neurosyphilis can occur during any stage of syphilis and can be asymptomatic or symptomatic during early stages of infection.

Rationale

Syphilis, a notifiable disease with over 130,000 cases in the United States reported to the CDC in 2020 (*16*) and over 6 million new cases occurring worldwide (*17*), is caused by *T. pallidum* subspecies *pallidum*. The United States is currently experiencing a syphilis epidemic, with sustained increases in primary and secondary syphilis with 5979 cases reported in 2000 to 133,945 cases reported in 2020, a 2,140% increase (*16*,*18*). The epidemic is characterized by health disparities, particularly among sexual and gender minority populations, intersections with the HIV and substance use epidemics, and increased morbidity and mortality attributable to congenital syphilis infections (*16*).

Laboratories play a critical role in the public health response to the syphilis epidemic. The responsibility of the laboratory is to test specimens and report results in a timely manner, allowing clinicians to efficiently make clinical diagnoses for patient management. Public health reporting by laboratories also allows local health departments and CDC to conduct surveillance and monitoring of disease trends. This report details CDC's recommendations for syphilis testing, including laboratory-based tests, point-of-care (POC) tests, processing of samples, and reporting of test results to aid laboratorians and clinicians in the diagnosis of syphilis.

Scope and Audience

The primary audiences for these recommendations are clinical laboratory directors, laboratory staff, clinicians, and disease control personnel interested in better understanding syphilis laboratory diagnostics. These recommendations are meant to serve as a laboratory guide for test selection and to assist with interpretation of test results for clinical diagnosis and management of syphilis.

Methods

These recommendations were developed by CDC staff based on evidence published in peer-reviewed scientific journals. Data available in FDA-cleared syphilis diagnostic test inserts were reviewed and assessed for consistency with published findings. In 2017, the Association of Public Health Laboratories (APHL) assisted with the literature review through an independent work group formed to evaluate the scientific literature for CDC to consider in the development of evidence-based recommendations for syphilis testing in the United States. APHL work group members were selected based on expertise in the field of syphilis and represented public health and commercial laboratory directors, public- and private-sector providers, and academic researchers. The workgroup leads were experienced in conducting systematic reviews of the literature. Potential conflicts of interest were disclosed to APHL and are listed at the end of the work group section (Appendix 1).

CDC identified key questions regarding syphilis testing in the United States that should be addressed during the literature review process and shared these questions with the APHL work group members in March 2017. Work group members were assigned key questions to review (Appendix 1) and, with the assistance of CDC and APHL staff, conducted an extensive literature search on Medline, Embase, Scopus, Cochrane Library, and CINAHL; combinations of search terms for each key question were used to search for literature published during 1960–June 30, 2017(Appendix 1). The wide time interval was necessary because some tests have been used for almost a century. In November 2017, work group members presented their reviews to CDC and APHL staff. Key questions and pertinent publications were reviewed for strengths, weaknesses, and relevance and were openly discussed by individual work group members. The discussions were informal and not designed to reach consensus; no formal rating system was used. Background papers summarizing the evidence reviewed were peer reviewed and

published in July 2020 (*19-23*). Subsequently, CDC staff used the same search criteria and evidence review ranking methods described above and in Appendix 1 to identify articles published through September 1, 2022.

Following the meeting, the APHL work group was disbanded, and CDC staff reviewed the scientific evidence and ranked the evidence as high, medium, and low based on each study's strengths and weaknesses as outlined by the U.S. Preventive Services Task Force Ratings (https://www.uspreventiveservicestaskforce.org/uspstf/us-preventive-services-task-force-ratings). Publications were rated as an "A" if they were high quality using clinically characterized specimens, stratified by stage, larger sample size, prospective or a well-done cross sectional or retrospective study. "B" rated studies were good to moderate quality with large sample sizes, clinically characterized but not stratified by stage, or characterized but unclear exactly how it was done, mild methodological issues. A fair, "C" rated study included those with small sample sizes, moderate methodological issues, single lab test as gold standard, or descriptive. Poor, "D" rated studies were those with major methodological issues or small sample sizes. Case reports or small case studies were rated as "I." Studies that were not relevant to the key question were assigned as "NR" and not further rated. The recommendations were based on high-ranking scientific evidence from "A" and "B" ranked studies that would result in a net benefit for the diagnosis of syphilis and ultimately patient care. Studies with misleading or poor data that may lead to a net harm for patient care because of inaccurate laboratory testing were not included in formulating these recommendations.

Draft recommendations were peer reviewed as defined by the Office of Management and Budget for influential scientific information. In February 2022, draft recommendations were peer reviewed by five experts in the field of syphilis who were not United States federal employees, were not funded by CDC for syphilis research, and were not involved in the development of these recommendations (Appendix

2). Comments submitted during the external peer review were addressed and the document was open for a 60-day public comment period beginning April 5, 2023. Draft recommendations were reviewed by key subject matter experts and stakeholders, including the APHL, American Society for Microbiology, Centers for Medicare and Medicaid Services (CMS), and FDA. Following the public comment and stakeholder review, CDC considered all comments in the development of final testing recommendations for syphilis.

Principles for Syphilis Diagnosis

Indications for syphilis testing include identification of individual, population, or community risk factors for exposure to *T. pallidum;* suggestive signs and symptoms; or a known sexual contact of someone who has syphilis. The selection of laboratory tests and interpretation of results varies by stage of syphilis and prior treatment history. Once diagnosis and staging has occurred, benzathine penicillin G is the recommended therapy for clinical resolution of infection and avoidance of sequelae (*24*). Patients with a history of penicillin allergy should be managed according to CDC's *Sexually Transmitted Infections Treatment Guidelines, 2021* (*24*).

Testing for syphilis is based on the detection of reactive antibodies, (typically in serum or cerebrospinal fluid [CSF]) suggestive of exposure to *T. pallidum,* direct observation of the organism by darkfield or fluorescent microscopy of lesion fluids or exudate, or histologic assessment of infected tissues or amplification of *T. pallidum* specific nucleic acid sequences in fluids, exudate, or tissue biopsy material. Conventional microscopy used to examine Gram-stained smears is insufficient to visualize *T. pallidum* because of the bacterium's slender morphology and poor uptake of aniline dyes (*25*). There are no

commercially available nucleic acid amplification tests (NAAT) in the United States, and culture for *T. pallidum* is cumbersome and is available only in selected research laboratories.

Nontreponemal (lipoidal antigen) serologic tests are most suitable for screening or diagnosis in conjunction with a medical history and physical examination when antibody titers are important to determine recent exposure to infection, a presumptive diagnosis in persons with suspected signs or symptoms, or to determine response to treatment.

Treponemal serologic tests target specific *T. pallidum* antigens, either intact or sonicated *T. pallidum* or defined recombinant proteins; these tests were traditionally used to confirm that a reactive nontreponemal (lipoidal antigen) serologic test is the result of *T. pallidum* infection (*25*). Treponemal antibodies generally persist after treatment and cannot be used to distinguish between a current infection or previously treated infection. None of the nontreponemal (lipoidal antigen) or treponemal serologic tests can distinguish infections caused by other *T. pallidum* subspecies. Several finger-stick immunoassays have been developed as rapid tests and might offer some diagnostic aid in clinical, public health, or nonclinical settings.

Direct detection tests of *T. pallidum* are limited to darkfield microscopic examination of lesion fluids, staining of lesion fluid or exudate smears or tissue sections obtained by biopsy for treponemal spirochetes, or amplification of specific nucleic acid sequences by validated laboratory methods.

Nontreponemal (lipoidal antigen) serologic tests. In general, nontreponemal (lipoidal antigen) serologic tests have been used as a screening test for syphilis, a diagnostic test when patients present with signs or symptoms suggestive of syphilis or have a known sexual contact, when assessing possible reinfections, or when monitoring treatment outcome. Rapid plasma reagin (RPR) and Venereal Disease Research Laboratory (VDRL) serologic tests are still the primary screening methods currently used in public health laboratories in the United States (*26*); other FDA-cleared nontreponemal (lipoidal antigen)

serologic tests, such as the Toluidine red unheated serum test (TRUST) and unheated serum reagin test (USR), are also available (Table 1) but are less commonly used in the United States. Importantly, regardless of which test method is applied, serum antibody titers from RPR, VDRL, and other nontreponemal (lipoidal antigen) serologic tests should not be used interchangeably to manage patients because they are different test methods, and the subjective titer results can vary by laboratory. Therefore, patient specimens should be tested using the same nontreponemal serologic test method, specimen type and, ideally, by the same laboratory.

The manual nontreponemal (lipoidal antigen) serologic tests are flocculation tests that detect antibody-antigen complexes that fall out of solution as a precipitate. Microscopic or macroscopic procedures have been developed to detect the precipitate that forms following specific binding of antibodies to a combination of cardiolipin, cholesterol and/or phosphatidylcholine that are used as antigens in nontreponemal (lipoidal antigen) serologic tests (Table 1). VDRL tests are read microscopically at 100x magnification (*25*). Charcoal is used in the RPR test to aid in detection of the flocculant and the results can be read macroscopically because the antigen-antibody lattice traps the charcoal particles. The TRUST test replaces charcoal with toluidine red dye.

Nontreponemal (lipoidal antigen) serologic tests are usually performed manually, but the RPR has recently been automated for higher throughput (Table 1). The automated systems digitally analyze the density and size of antibody-antigen flocculation and store results for future retrieval (*27-29*). Results from any nontreponemal (lipoidal antigen) serologic test should be reported as an endpoint titer, and not with greater or less than values, to allow for optimal clinical interpretation. Some automated RPR serologic tests have a constrained serum dilution range (e.g., between 1:4 and 1:64) that might be incapable of generating an endpoint titer beyond this range. In these situations, the titer range of the

automated test must be considered, and specimens might require reflex testing using a manual RPR procedure to establish an endpoint titer at either the lower or upper bounds prior to reporting.

Whether automated or manual, performance depends on several factors, including specimen type and quality, stage of syphilis, autoimmune or other diseases, and infections or coinfections with organisms other than *T. pallidum*. Nontreponemal (lipoidal antigen) serologic tests might be less sensitive than treponemal tests in early primary syphilis and tend to wane with time, regardless of treatment. Prior to testing, test and specimen type should be carefully considered because serum and plasma cannot always be used interchangeably, and certain nontreponemal (lipoidal antigen) serologic tests require heat treatment of specimens (Table 1).

The subjective nature of results interpretation for manual tests as well as variability among laboratories and technicians pose challenges to clinicians who compare titers with stage syphilis for treatment purposes, especially when assessing possible reinfection or to monitor treatment outcomes. One of the main caveats of nontreponemal (lipoidal antigen) serologic tests is that a reactive result could be a false positive because of recent conditions such as infections, immunizations or injection drug use (IDU), or underlying autoimmune or other chronic conditions. Nonetheless, when performed by an experienced laboratory technician and used in conjunction with treponemal serologic tests, clinical history, physical examination, and contact history, nontreponemal (lipoidal antigen) serologic tests remain a highly reliable testing method for screening and determining the endpoint titer for subsequent serological monitoring post treatment.

Serologic response following treatment. Nontreponemal antibody titers usually decrease at least four-fold in the 12 months after syphilis treatment, particularly among persons treated during the early stages of infection, and might become nonreactive over time, especially among patients treated before the secondary stage of syphilis (*30-32*). However, in some persons, the decrease in nontreponemal antibody

titers is less than four-fold despite appropriate treatment. A prospective study by Rolfs and colleagues (n = 541) found that 14% of patients with early syphilis had a <4-fold serologic titer decline 12 months post treatment; patients living with HIV who had primary or secondary syphilis were more likely to have an inadequate response compared with those without HIV (*30*). Additionally, titers might not serorevert to a nonreactive result after treatment and remain persistently reactive, often referred to as the serofast state. This is most common in persons treated ≥1 years after acquiring syphilis or in persons with multiple episodes of syphilis. Titers are generally ≤1:8, but higher titers have also been observed (*33,34*). Additional recommendations regarding clinical interpretation of nontreponemal titers can be found in CDC's *Sexually Transmitted Infections Treatment Guidelines, 2021* (*24*). Clinicians with complex cases of titer interpretation may consult with the STD Clinical Consultation Network for assistance (https://stdccn.org).

Box 1. Recommendation for Endpoint Titers

Endpoint titers should be determined and clearly reported when testing serum with nontreponemal assays that detect antibodies to lipoidal antigens (i.e., RPR and VDRL). Reports should not contain mathematical symbols such as > (greater than) or < (less than) signs.

Comment and Evidence Summary. Antibody titers measured by nontreponemal (lipoidal antigen) tests can correlate with infection status and are the only tests available to monitor treatment outcome (*30,32*). A fourfold change in titer between two results with the same nontreponemal (lipoidal antigen) tests is considered clinically significant (*24*). Titers need to be reported for appropriate clinical management. Serum samples tested with some automated RPR serologic tests are outside the dilution range of the test should be reflex tested using a manual RPR.

Prozone. The detection of antigen-antibody interactions in agglutination or flocculation assays is dependent on the formation of antigen-antibody complexes that clump cells in agglutination tests or aggregates of small particles known as floccules. There are many epitopes on an antigen that can be bound by an antibody specific to the antigen and each antibody has two binding sites that can possibly bind two antigens. As these interactions continue, a lattice structure can develop and become sufficiently large to cause agglutination or flocculation. The level of agglutination or flocculation varies depending on the relative concentrations of antigen and specific antibodies. Agglutination and flocculation assays standardize the antigen concentrations to maximize the formation of a lattice in a reactive test. Excess antibodies in serum can interfere with the development of a lattice if each antibody molecule binds to a single (instead of two) antigen epitope. In this case, cross-linking fails to occur, and a lattice will not form. This can occur especially in an undiluted serum specimen. This "false-negative" phenomenon is referred to as a prozone because it occurs before the zone of equivalence where the concentration of antibodies and antigens are sufficient for agglutination or flocculation. A prozone can be avoided if the serum sample is diluted prior to testing. False-negative results attributable to a prozone have been reported for nontreponemal but not for agglutination-based treponemal serologic tests (*25*,*35*).

In two studies of 4,328 and 46,856 patients that had specimens referred for syphilis testing, false-negative RPR tests caused by a prozone were rare (<0.85%) (*35*,*36*). In a study by Lui et al., prozone in an RPR test occurred at all stages of syphilis but was more common during primary and secondary syphilis (4.7% and 1.8%, respectively) (*35*). Diluting serum can remove the prozone but there are no specific titration values that can ensure all effects of a prozone are removed. In the study by Lui and colleagues, among 36 serum samples with a prozone, 11 required serial dilutions from 1:8 to 1:16 to remove the prozone; 22 of these 36 samples required dilutions ranging from 1:32 to 1:128 for the

optimal concentration of antibodies and antigens for agglutination (*35*). There were two samples that continued to have a prozone until they were diluted to 1:256 and one to 1:512. Because the prozone phenomenon is considered rare in a general population screened for syphilis, it is not recommended to routinely dilute all nonreactive, undiluted nontreponemal serologic tests. However, laboratories should rule out a prozone using a dilution series for a nontreponemal serologic test when requested by a clinician who should request a prozone rule out if a patient with signs or symptoms suggestive of syphilis has a nonreactive, undiluted nontreponemal serologic test result or when unusual graininess is observed in the test of undiluted serum.

Biological false positive. A nontreponemal (lipoidal antigen) serologic test that is reactive for conditions other than syphilis is referred to as a biological false-positive (BFP). Persons with antibodies that are reactive in the nontreponemal (lipoidal antigen) serologic tests, but are nonreactive in a confirmatory treponemal test, are defined as BFP reactors. Health departments frequently retain records of persons with known BFP reactions; these data can assist clinicians in a future evaluation of possible syphilis infection in such persons. Reactive nontreponemal (lipoidal antigen) serologic tests attributable to BFP have been estimated to occur in 0.2%–0.8% of the population and are associated with medical conditions other than syphilis (*37-41*). BFP reactions attributable to other infections include malaria, leprosy, and HIV, as well as recent immunizations, autoimmune disorders, and IDU (*25*).

Treponemal serologic tests. Treponemal serologic tests are clinically used to confirm results of reactive nontreponemal (lipoidal antigen) serologic tests and evaluate patients with signs suggestive of syphilis in early primary infection when nontreponemal serologic tests might not yet be reactive. Treponemal serologic tests can also be automated for high throughput screening in blood banks and in large laboratories for routine screening using the reverse sequence algorithm. Antibodies detected in treponemal serologic tests typically persist for life despite treatment, unless treatment occurs early in the

course of infection; approximately 15%–25% of patients treated for primary syphilis can revert to a nonreactive treponemal serologic test (fluorescent treponemal antibody-absorption [FTA-ABS] and MHA-TP) result within 2–3 years after treatment (*31*,*32*). In these two studies, no patients treated for secondary syphilis or stages of longer duration of infection seroreverted the reactive treponemal test. Seroreversion of treponemal serologic tests can also occur in patients with advanced HIV disease and AIDS, albeit rarely (*42*,*43*).

There are no published data examining whether reversion to a nonreactive treponemal serologic test occurs with enzyme immunoassays (EIA) or chemiluminescence immunoassays (CIA). Treponemal serologic tests, unlike nontreponemal (lipoidal antigen) serologic tests, cannot be used to monitor response to therapy because they remain reactive indefinitely. In patients with a history of treated syphilis and reactive treponemal test results, additional treponemal testing is not helpful for detecting reinfection and is not recommended. In this case, nontreponemal testing titers along with clinical history of syphilis, physical examination, and sexual risk assessment, including contact history, must be used to determine infection status.

Manual treponemal serologic tests include the FTA-ABS, *T. pallidum* particle agglutination (TPPA), Captia Syphilis IgG EIA, Trep-Sure EIA, and Zeus Scientific EIA (Table 1). The assay mechanism, sample types, antigens, and antibodies are described in Table 1. Manual assays are typically used as reflex tests to confirm reactive nontreponemal specimens in the traditional testing algorithm. The FTA-ABS test is a florescence microscopy-based test that uses a fluorescein isothiocyanate-labeled antihuman immunoglobulin to detect antibody binding to whole *T. pallidum* that has been fixed on a glass slide. The TPPA is an indirect agglutination assay with *T. pallidum* antigens bound to gelatin particles.

The manual treponemal *T. pallidum* hemagglutination assay (TPHA) and microhemaggluntination assay for antibodies to *T. pallidum* (MHA-TP) tests are no longer available for in vitro diagnostics in the

United States but are still used in some international settings. The TPHA and MHA-TP are indirect agglutination with *T. pallidum* antigens bound to avian or ovine erythrocytes. The MHA-TP is a microplate version of the TPHA.

As of December 31, 2021, there were 12 FDA-cleared automated treponemal immunoassays for clinical use, including EIA, CIA, and multiplex flow (microbead) immunoassays (MFIA) (Table 1). In contrast to the manual assays, the treponemal immunoassays are often run as the initial test, in a reverse sequence screening algorithm. All FDA-cleared treponemal serologic tests can be performed on serum, and some can also be performed on plasma, including heparinized, EDTA, and citrate plasma. Some laboratories have also validated use of treponemal serologic tests with dried blood spots (DBS); however, no currently available tests have been FDA cleared for this specimen type, nor are there published data on DBS specimens collected in the United States to aid in the diagnosis of syphilis.

The reading output is typically an index value calculated as a signal to cutoff ratio (S/CO) or fluorescence ratio based on values between the specimen and calibrator controls. Equivocal results should be retested according to algorithms in the package insert. The raw reading outputs and index values can be stored for future retrieval. The strength of the S/CO from immunoassays is an estimate of relative binding between molecules in the assay and has been researched as a predictor for positivity in hepatitis C and HIV confirmatory tests (*44-48*). When applied to treponemal immunoassays, several studies reported strong correlation between increasing index value strength and reactive results from an independent treponemal test or a combination of nontreponemal (lipoidal antigen) and treponemal serologic tests, with most studies demonstrating 91%–100% correlation between S/CO cutoffs and TPPA positivity (*49-54*). Additional research is needed to establish test-specific cutoff values that are likely to be true positives for each of the FDA-cleared immunoassays. S/CO cutoff values could

eliminate the need to adjudicate discrepant results between treponemal immunoassays and nontreponemal (lipoidal antigen) serologic tests with a second TPPA.

For discordant nontreponemal and treponemal test results, an additional treponemal testing is recommended using a different type of treponemal test assay and target, such as the TPPA. Until further data are available regarding the role of S/CO cutoffs in a screening algorithm, the cutoff value could be an additional data point to assess likelihood of infection in complex situations, such as among pregnant persons with low risk for syphilis. Clinicians with these types of cases should contact the STD Clinical Consultation Network for assistance (https://stdccn.org).

Blood bank screening: Blood donations are required to be tested for antibodies to *T. pallidum* as outlined in 21 CFR 610.40(a)(2). Individuals that donate blood found to be serologically reactive are deferred (21 CFR 610.41(a)) and notified (21 CFR 630.40). The FDA updated recommendations for screening blood donors for syphilis are available at https://www.fda.gov/media/85283/download. The current list of tests to screen blood donations for infectious agents can be viewed at https://www.fda.gov/vaccines-blood-biologics/complete-list-donor-screening-assays-infectious-agents-and-hiv-diagnostic-assays.

Traditional and Reverse Algorithms for Syphilis Screening

The traditional algorithm for syphilis serologic screening begins with a nontreponemal serologic test and any reactive specimens are tested for confirmation by a treponemal serologic test (Figure 1). This sequence has been widely used for decades, as nontreponemal serologic tests were relatively inexpensive, and treponemal serologic tests were manual, labor intensive, more costly, and limited in number. However, automated treponemal immunoassays, which were originally FDA cleared for blood

bank screening are now FDA cleared for clinical screening, leading to the reverse sequence algorithm. Initial screening with an automated treponemal serologic test of a sample with a positive result must be followed by a quantitative nontreponemal serologic test. When the reverse sequence algorithm is used, any discordant results should be adjudicated by a second treponemal assay, such as TPPA, which has a different format and includes different antigens (*55*). POC serologic tests should only be used as a confirmatory test in either traditional or reverse algorithm when laboratory-based treponemal testing (e.g., TPPA or automated treponemal immunoassays) is not available in a timely manner and urgent results are needed to guide clinical management (e.g., labor and delivery).

The number of clinical laboratories performing traditional, reverse, or both algorithms was assessed among 2,360 laboratories participating in the 2015 College of American Pathologists syphilis serology proficiency testing program in the United States (*56*). Of the 1,911 laboratories that responded, 81.1% (n = 1,550) offered only one algorithm, 9.5% offered different algorithms depending on patient demographics or clinician preference, and 9.4% reported being uncertain if a single algorithm was offered. Almost two-thirds of laboratories (63.1%; n = 1,205) used the traditional algorithm, 15.9% (n = 304) reported using the reverse sequence algorithm, 2.5% (n = 47) reported using both algorithms, 5.9% reported that they did not know, and 3.9% reported "other." Surprisingly, 8.8% (n = 169) of responding laboratories stated that they did not reflexively perform a confirmation test.

A prospective comparison of 1,000 patient samples from a population with a low prevalence of syphilis tested with both algorithms revealed 15 (1.5%) that were reactive by the reverse sequence algorithm starting with the BioPlex IgG and 4 (0.4%) that were reactive by the traditional algorithm with the RPR as the first test (*57*). The four samples that were reactive by RPR were confirmed to be positive by TPPA. The false-positive EIA rate (e.g., EIA reactive, RPR nonreactive, TPPA nonreactive) was higher in the reverse sequence algorithm than the traditional algorithm (0.6% versus 0%). CDC reported a

similar false-positive rate for treponemal immunoassay (0.6%; 866 of 140,176) when using the reverse sequence algorithm during 2006–2010 (*55*).

Data are conflicting regarding the cost-effectiveness of the traditional versus the reverse sequence algorithm. The traditional algorithm might be more cost effective (lower cost per adverse event prevented) in settings with a low prevalence of syphilis (approximately 0.5%) and cost saving in higher prevalence settings (approximately 10%) (*58*,*59*). These data are not consistent with a study that reported the reverse sequence algorithm as being cost effective when applied to screening prenatal and non-prenatal lower prevalence populations with a syphilis prevalence of 0.076% and 1.94%, respectively (*60*). In an economic impact model on a local sexually transmitted disease (STD) program in Los Angeles County, the reverse algorithm was less expensive and identified more patients for treatment if the cost of the treponemal test was $1.67 less than the nontreponemal test cost of $5.80 (*61*). Testing, treatment, and follow-up costs were included in the analysis. Applying 2015 test costs from the 2015 CMS laboratory fee schedule, in which treponemal serologic tests costs were three times more costly than nontreponemal (lipoidal antigen) serologic tests, the reverse sequence algorithm was more costly than the traditional algorithm. It was estimated that each additional syphilis case detected would cost $1,242.17 when using reverse sequence algorithm with 2015 CMS test costs. These data highlight the need to carefully consider local costs, including testing, treatment, and follow-up costs, when choosing the best algorithm for syphilis screening.

Each algorithm has advantages and disadvantages and are equally recommended (Table 2). The traditional algorithm might be less sensitive in detecting early or late latent syphilis, while there might be an increase in false positives when applying the reverse algorithm in low prevalence populations (*22*). The main advantage of automated treponemal immunoassays in high volume laboratories is automation to increase throughput and reduce labor costs. Considerations for test/algorithm selection include cost,

labor, volume of specimen test requests, throughput, laboratory space, and turnaround time. In addition, clinicians and state and local public health STD programs need the nontreponemal test results coupled with the treponemal test results for timely clinical management and public health reporting. If one test result in the algorithm is delayed and needs to be coupled with the initial test by the clinician or the STD program, matching errors can occur, and clinical management and reporting can be delayed. The laboratory processing the initial screening test should ensure the second or third (if necessary) test results, especially if performed in a different lab, are linked with the screening test result when the report is sent to the ordering clinician and public health department.

Box 2. Recommendation for Syphilis Serologic Testing Algorithm

Serologic tests that measure antibodies to both nontreponemal (lipoidal) and treponemal antigens related to syphilitic infections should be used in combination, when the primary test is reactive, to aid in the diagnosis of syphilis (Figure 1). Sole reliance on one reactive serologic test result can misclassify a patient's syphilis status. Both the traditional syphilis screening algorithm (initial screening with nontreponemal serologic assays) and the reverse syphilis screening algorithm (initial screening with treponemal serologic immunoassays) are acceptable. The preferred algorithm should be based on laboratory resources, including staff, space and costs, test volume, and patient populations served.

Comment and Evidence Summary. Both traditional and reverse syphilis testing algorithms are widely used in the United States (*56*) and have about 99% concurrence between the two approaches (*55*,*57*). The cost-effectiveness of the two algorithms may vary by laboratory setting (*58-61*) and need to be considered by individual laboratories.

Serologic and CSF Antibody Specimen Collection and Storage

Serum, plasma, and CSF are specimen types that have been used in syphilis assays that detect antibodies against *T. pallidum*. This section is provided as a general guide because the information is summarized from various sources including product inserts and manuals on standard laboratory practices (*25,62*). Product inserts should be reviewed for optimal specimen type, transport, and storage, as these vary by test (Table 1).

Serum collection devices and storage. Serum, the liquid fraction of whole blood that is collected after the blood has clotted, is the most common specimen used for syphilis serologic assays. Whole blood is collected by a trained phlebotomist using a vacutainer tube without an anticoagulant, coagulants, or a serum separator component. The use of vacuum tubes with serum separators or coagulants has not been widely evaluated with syphilis serology tests and should be avoided unless stated as an acceptable collection device in the test's product insert. The volume of whole blood collected should be approximately 2.5 times the volume of serum required for the test. Approximately 1 ml of serum is enough to process both nontreponemal and treponemal syphilis serology tests, with extra reserved for repeat testing if needed. Consideration should be given for collecting more serum if tests for conditions other than syphilis tests are requested. Following collection of whole blood, the tube should be left undisturbed at room temperature for approximately 15– 30 minutes to allow for clot formation. Vacutainer tube or other tubes containing whole blood should not be refrigerated because lower temperatures will increase clotting time. Serum can be aspirated if the clot has retracted or following centrifugation at 1,000 to 2,000 xg for 10 minutes. Serum should be transferred into a clean polypropylene tube for shipping or storage. In general, serum should be stored at 2°C–8°C and tested within 5 days or frozen at ≤–20°C for longer storage. Serum should not be stored in "frost-free" freezers because the freeze-thaw cycles in these appliances are detrimental to the stability of frozen serum

samples. However, recommended storage conditions vary among tests, as summarized in Table 1, and the current product insert should be reviewed for up-to-date information. Samples should be free of hemolysis (www.cdc.gov/ncezid/dvbd/stories/research-lab-diagnostics/hemolysis-palette), icterus, bacterial contamination, and lipemia. Serum should be aliquoted for storage to avoid repeated freeze-thaw cycles that could result in diminished antibody reactivity because of protein degradation and denaturation.

Plasma collection devices and storage. Plasma, the liquid fraction of whole blood that remains when clotting is prevented but cells are removed, is acceptable for some qualitative syphilis serologic assays. Whole blood is collected by a trained phlebotomist using a vacutainer tube with an anticoagulant, including EDTA-treated, citrate-treated, or heparinized tubes. The blood volume collected should be approximately 2.5 times the volume of plasma required. Approximately 1 ml is enough plasma to process both nontreponemal and treponemal syphilis serology tests, with extra reserved for repeat testing if needed. Cells are removed from plasma by centrifugation at 1,000–2,000 xg for 10 minutes. The supernatant plasma should be immediately transferred to a clean polypropylene tube and tested 1–5 days after collection, depending on the test (Table 1). In general, the time that plasma can be successfully stored is shorter than for serum, but storage conditions, as summarized in Table 1, vary among tests. The current product insert should be reviewed for up-to-date information. Samples should be free of hemolysis, icterus, bacterial contamination, and lipemia. Plasma should be aliquoted for storage to avoid repeated freeze-thaw cycles that could result in diminished antibody reactivity by tests as a result of protein degradation and denaturation.

CSF collection devices and storage. Only medical personnel qualified to perform lumbar puncture can collect CSF. Approximately 1 ml of CSF, placed into a clean polypropylene tube, is enough CSF for syphilis serologic testing, with extra remaining for repeat testing if needed. A larger volume of CSF

might be required for additional tests, such as protein, cell count, gram stain, or culture. If testing is delayed more than 4 hours, store the CSF sample between 2°C–8°C for ≤5 days. After 5 days, CSF should be stored frozen at ≤–20°C. Blood contamination, which could cause a false-positive result because of the presence of serum-derived antibodies rather than CSF-produced antibodies, should be avoided when collecting CSF specimens.

Serologic and CSF Antibody Test Performance

Sensitivity of serologic tests for primary syphilis. Estimating the sensitivity of nontreponemal serologic tests during primary syphilis is best assessed when direct detection of *T. pallidum* is used as the comparator test to ensure proper staging of syphilis for the analysis. The sensitivity of RPR when compared with darkfield microscopy ranged from 48.7% to 76.1% (*63-69*); one study, however, reported a sensitivity of 92.7% (n = 109 patients) (*70*) (Table 3). VDRL had a similar sensitivity range (50.0%–78.4%) (*63-67,70-75*). One head to head comparison study of RPR and VDRL nontreponemal (lipoidal antigen) serologic tests from 76 patients with primary syphilis confirmed by darkfield microscopy showed a sensitivity of 48.7% and 50.0% for RPR and VDRL, respectively (*69*). Studies that used a NAAT to detect *T. pallidum* nucleic acid from a lesion swab and staged primary syphilis based on clinical exam findings and a positive NAAT reported that nontreponemal (lipoidal antigen) serologic test sensitivity ranged from 80% to 95% (*76-80*). Studies using the NAAT as the reference standard rather than darkfield microscopy in lesions suggestive of primary syphilis suggest that nontreponemal serologic tests might be more sensitive than previously thought.

The sensitivity for manual treponemal serologic tests in primary syphilis has been estimated from studies that used reference standards such as darkfield microscopy (*63,70,81-83*), clinical findings (*84-*

551 *86*), or stored serum collected from patients staged as having primary syphilis, although the criteria used
552 to stage the disease were not fully described (*87-91*) (Table 3). The MHA-TP had a sensitivity of 53.0%,
553 72.5%, and 88.6% in studies that used darkfield microscopy as the reference standard (*70,81,86*). In
554 studies that used stored sera collected from patients that were clinically classified as having primary
555 syphilis, MHA-TP had a sensitivity of 45.9%, 64% and, 88.6% (*82,86,91*). A 2019 study by Park and
556 colleagues involving 959 patients and which classified 55 as having primary syphilis (based on serology,
557 physical findings, and positive/negative darkfield microscopy) reported a sensitivity of 78.2% (95%
558 confidence interval [CI]: 65.0%– 88.2%) and 94.5% (95% CI: 84.9%–98.9%) for the FTA-ABS and
559 TPPA, respectively (*83*). Other studies with fewer patients and/or different reference standards are more
560 difficult to compare; sensitivities for FTA-ABS and TPPA have ranged from 88.4% to 100% and 86.2%
561 to 100%, respectively, for primary syphilis (*70,81,82,85,86,90-95*).

562 Among the automated treponemal immunoassays, there are few published data on test performance
563 stratified by stage. Park and colleagues found similar sensitivity for the ADVIA Centaur, Bioplex 2200
564 Syphilis IgG, Diasorin LIAISON, and Trep-Sure in primary syphilis compared with TPPA and FTA-
565 ABS (*83*), however another study of 52 patients found poorer sensitivity of Trep-Sure in primary
566 syphilis (53.8%; 95% CI: 39.5%–67.8%) (*89*).

567 Nontreponemal (lipoidal antigen) and treponemal serologic tests might not yet be reactive in some
568 persons with primary syphilis, particularly those with very recently appearing lesions. Using darkfield
569 microscopy as the sole comparator will skew results toward lower sensitivities, as persons with early
570 lesions are more likely to test positive by darkfield microscopy and be seronegative. Lesions of longer
571 duration might become negative by darkfield microscopy because of immune clearance, but these
572 persons are more likely to be seropositive. NAATs might be positive in both early and older lesions
573 because this test method is not dependent on visualization of motile organisms. Additional studies of

genital, anal, and oral lesions using both darkfield microscopy and NAATs as the reference standard, including studies that assess age of lesions, are needed to better refine the sensitivity estimates of nontreponemal (lipoidal antigen) and treponemal serologic tests for primary syphilis.

Sensitivity of serologic tests for secondary syphilis. In studies that classified secondary syphilis based on clinical diagnosis that included rash, mucocutaneous lesions or patchy alopecia, mucous patches, or condylomata lata; clinical diagnosis with visualized spirochetes on darkfield microscopy; or clinical diagnosis with reactive nontreponemal and treponemal serology, the sensitivity of both the RPR and the VDRL was 100% (*64-67,69,71,73,96-99*) (Table 3). Only two studies reported an RPR sensitivity of <100% (91% and 97.2%) (*67,69*).

The sensitivity of the treponemal serologic assay, MHA-TP, for secondary syphilis ranged from 96% to 100%, except in one study that reported 90% sensitivity (*81,82,86,91*) (Table 3). The estimated sensitivity of FTA-ABS was >92% with six out of eight studies reporting 100% (*81-83,85,91-93,95*). Of the two studies that found sensitivity to be <100% (*83,92*), the FTA-ABS sensitivity was reported to be 92.8% (95% CI: 85.7%–97.0%) and 95.0% (95% CI: 76.4%–99.1%). The TPPA was 100% sensitive in five studies (*83,84,92,94,100*). Among the automated treponemal immunoassays, there are few published data on test performance stratified by stage, but the sensitivity of five treponemal immunoassays (LIAISON, TrepSure, Bioplex 2200, ADVIA Centaur, INNO-LIA) was estimated at 100% for secondary syphilis in one study of 98 patients (*83*).

The sensitivity of both nontreponemal (lipoidal antigen) and treponemal serologic tests approaches 100% because of higher antibody titers during the secondary stage of syphilis. A prozone should be ruled out in specimens from patients suspected of having secondary syphilis and are nonreactive in nontreponemal (lipoidal antigen) serologic tests. Because laboratorians generally do not know the patient's stage of syphilis when the serologic specimen is submitted, clinicians should specifically

request to assess for prozone when clinically indicated (e.g., in patients who have signs and symptoms of syphilis and nonreactive nontreponemal serologic test results).

Sensitivity of serologic tests for latent syphilis. There are limited data on nontreponemal (lipoidal antigen) serologic test performance in early latent and late latent stages of syphilis, with limited information regarding reference standards, previous treatment status, patient population risk for syphilis, and specific stage of latency (*96-99,101-103*). Furthermore, some international studies use different definitions of early and late syphilis than are used in the United States.

No studies involving RPR test performance for latent syphilis have been conducted in the United States. Two international studies conducted more than 10 years ago and without stratification by duration of latency (i.e., early latent of less than one year versus late latent of greater than one year) make estimates of sensitivities difficult (*96,102*). Three international studies on the performance of VDRL in cases of latent syphilis reported sensitivities that ranged from 82.1% to 100% for early latent syphilis of <1 year and 63% to 66% for late latent syphilis of >1 year or of unknown duration; however, all of the studies were limited by small samples sizes (n≤72), making the results difficult to interpret (*97,99,101*) (Table 3).

The sensitivity of the manual treponemal serologic tests, FTA-ABS, TPPA, and MHA-TP, ranged from 94.4% to 100% for the diagnosis of early latent syphilis; a wider range for late latent syphilis than early latent syphilis (84.5%–100%) has been reported (*81,83,84,86,88,92*) (Table 3). Among the treponemal immunoassays, sensitivity ranged from 95% to 100% for early latent syphilis and 91.7% to 100% for late latent syphilis (*83,87,88,104*) (Table 3). Although the sensitivity of treponemal serologic tests is generally high for early latent and late latent syphilis, the range of sensitivities identified in these studies suggests that additional studies are needed in larger samples where the duration of infection is better characterized. The duration of latency is often difficult to pinpoint; some patients staged as late latent

could have unknown latency duration, whereas other patients classified as late latent could have recently acquired their syphilis infection. This misclassification of duration of infection could falsely elevate the syphilis test performance sensitivity in patients with late latent syphilis.

The sensitivity of nontreponemal (lipoidal antigen) serologic tests decreases during latent syphilis of longer duration because the antibody detected by these test titers diminishes over time. In general, treponemal serologic tests remain reactive during latent syphilis.

Sensitivity of serologic tests for tertiary syphilis. Because tertiary syphilis is rare in the post-antibiotic era, there are very limited published data on the performance of serologic tests for diagnosis of tertiary syphilis (e.g., gummatous disease, late neurosyphilis, cardiovascular syphilis); further studies are unlikely to be done. One study estimated the sensitivities of the FTA-ABS and VDRL at 70.6% and 47%, respectively, in 17 patients with tertiary syphilis (*101*), although the criteria for the stage of diagnosis were not stated. There were several studies that examined sensitivity of treponemal serologic tests (LIAISON CIA, CAPTIA EIA, FTA-ABS) for detection of cardiovascular syphilis. All studies estimated sensitivity to be 100%, however, sample sizes were extremely small (n = 1–21 cases) (*87,88,91,105,106*). The largest study of cardiovascular syphilis included 21 patients and found sensitivities of the MHA-TP and FTA-ABS were 89.5% and 100%, respectively (*82*). Like latent syphilis, nontreponemal (lipoidal antigen) serologic tests are often nonreactive during tertiary syphilis, while treponemal serologic tests remain reactive.

Specificity of serologic tests. Reference standards for specificity analyses varied widely and included: 1) apparently healthy volunteers, 2) antenatal patients, 3) syphilis-negative blood donors who are not living with HIV, and 4) patients clinically characterized as not having syphilis (from serum banks or based on prior test results or chart review). Some studies of treponemal test specificity also used results

from a different treponemal test or a consensus of a panel of treponemal serologic tests as the reference standard.

Few head-to-head studies compare the specificity of RPR with VDRL specificity on well-characterized specimens. A study among 500 antenatal serum samples found little difference in specificity between VDRL and RPR (2 versus 1 false positive, respectively) (*107*). Another study among 200 blood donors found VDRL was slightly less specific than RPR (98.5%, with RPR as the gold standard) (*108*).

For manual treponemal serologic tests, while one study found the specificity of FTA-ABS to be 87% (n = 128 patients) (*109*), the specificity range of FTA-ABS and TPPA (95%–100% and 94%–100%, respectively) were similar in older studies (*70,81-83,85,86,90-95*). The specificity of the FTA-ABS serologic test can be limited by laboratory expertise and quality control measures. For these reasons and based on the recent high-quality, head-to-head study demonstrating superior TPPA test performance characteristics, the manual serologic TPPA test is preferred over the serologic FTA-ABS test. As discussed below, the CSF FTA-ABS can still help in excluding a neurosyphilis diagnosis because of its negative predictive value when performed in a laboratory experienced in the off-label use of this test. The immunoassays demonstrated specificity ranging from 94.5% to 100% (*87-89,105,110-117*); however, Trep-Sure was 82.6% (95% CI: 78.4%–86.1%) specific, significantly lower than the other immunoassays evaluated in a single head-to-head study of 959 patients (*83*).

Box 3. Recommendations for Serologic Syphilis Testing

Nontreponemal (lipoidal antigen) serologic tests (e.g., RPR or VDRL) are not interchangeable when used to determine antibody titers; testing on follow-up samples must be performed with the same type of test.

The TPPA test is the preferred manual treponemal serologic test.

Comment and Evidence Summary. Sensitivity and specificity estimates of RPR and VDRL were similar but not exact in head-to-head studies and studies that used similar reference standards (*63-67,69-72,74-76,79,80,107*). When assessing changes in antibody titers using nontreponemal (lipoidal antigen) tests, it's critical that the same test be used because titers are used by clinicians to classify the infection status of a patient and follow treatment response (*24*).

A recent study with 959 patients estimated the sensitivity of FTA-ABS and TPPA to be 78.2% and 94.5%, respectively, when testing specimens from patients with primary syphilis (*83*). Two studies that tested specimens from patients with secondary syphilis reported a sensitivity of 92.8% to 95.0% compared to 100% for TPPA (*83,92*).

Many automated treponemal immunoassays are similar in sensitivity, and some are slightly less specific when compared with the manual TPPA, except for the Trep-Sure test which has inferior specificity. Among the other immunoassays, there are insufficient data to recommend one assay based on test performance.

CSF antibody tests for neurosyphilis. There are several challenges associated with the diagnosis of neurosyphilis. These include a lack of consensus on the clinical implications of abnormal CSF findings in patients with no neurological symptoms or signs but with serologic evidence of syphilis, and poor distinction between asymptomatic and symptomatic patients in studies evaluating laboratory tests to aid in the diagnosis of neurosyphilis. In addition, the wide variation in reference standards that included CSF VDRL, CSF protein elevation and pleocytosis, CSF NAAT, CSF FTA-ABS, or other CSF treponemal and nontreponemal (lipoidal antigen) serologic tests, limited direct comparisons of CSF

antibody test performance among neurosyphilis studies. Lastly, the CSF VDRL is the only FDA-cleared test recommended to aid in the diagnosis of neurosyphilis. While no treponemal test is FDA cleared to aid in the diagnosis of neurosyphilis, the CSF FTA-ABS has been used off-label for years in unique clinical circumstances for its negative predictive value (e.g., in patients with nonspecific neurologic signs or symptoms, reactive serologic tests, and a negative CSF VDRL, even if CSF lymphocytic pleocytosis and elevated CSF protein are present).

Because asymptomatic or symptomatic central nervous system (CNS) invasion can occur in persons with primary, secondary, latent, or tertiary disease, serum examination can confirm the presence of syphilis but does not address CNS invasion or involvement. Examination of CSF is required to confirm CNS invasion but is only recommended in patients with reactive serologic tests and signs or symptoms suggestive of neurosyphilis; the clinical significant of CSF laboratory abnormalities in patients without any neurologic findings is unknown (*24*).

Nontreponemal (lipoidal antigen) tests for neurosyphilis. Manual nontreponemal serologic tests have been used to test CSF as an adjunct in cases of neurosyphilis, but performance estimates can vary widely depending on the reference standard. In three studies with a reference standard of: 1) detection of *T. pallidum* nucleic acid by NAAT on CSF; 2) hearing or vision loss or neurologic signs and symptoms suggestive of neurosyphilis with a reactive CSF TPPA; or 3) presence of at least 10 white blood cells in CSF and a positive CSF TPPA, CSF VDRL sensitivity and specificity ranged from 66.7% to 85.7%, and 78.2% to 86.7%, respectively, in 149–154 patients with neurosyphilis symptoms (*118,119*) (Table 4). In these studies, CSF RPR sensitivity and specificity was 51.5%–81.8% and 89.7%–90.2%, respectively (*118,119*). The CSF VDRL is the only FDA-cleared test to aid in the diagnosis of neurosyphilis.

Another study using a reference standard of reactive CSF FTS-ABS, increased CSF protein >45 mg/dL, and CSF pleocytosis ≥10 cells/mm^3 estimated the CSF VDRL sensitivity in eight patients with

symptomatic neurosyphilis to be 87.5% (*120*). The study did not report CSF VDRL specificity stratified by asymptomatic and symptomatic neurosyphilis, but the combined specificity was 99%. The sensitivity of CSF RPR in this study was estimated to be 100% in symptomatic patients. The combined specificity estimate for CSF RPR was 99.3%. There are no data currently available for the performance of automated nontreponemal (lipoidal antigen) RPR tests on CSF samples. Additional head-to-head studies with comparable high-quality, agreed-upon reference standards and well-characterized patient symptom status are needed to better understand CSF nontreponemal (lipoidal antigen) test performance.

Treponemal tests for neurosyphilis. The lack of a definitive diagnosis standard makes it difficult to interpret studies of the use of treponemal tests to support neurosyphilis diagnosis. Studies of treponemal test sensitivity in CSF included patients with symptomatic and asymptomatic neurosyphilis; a variety of laboratory tests were used for the reference standard, including CSF white blood cell count, protein, and CSF-VDRL (*121*). Studies of test specificity included patients without syphilis as well as patients with syphilis but no symptoms suggestive of neurosyphilis. The variation in reference standards limits the ability to compare sensitivity and specificity estimates among studies. No CSF treponemal antibody tests are cleared by FDA to aid in the diagnosis of neurosyphilis.

Thirteen studies describing CSF FTA-ABS test performance were summarized in a prior systematic review (*122*). Sensitivity varied depending on whether the reference standard required reactive CSF-VDRL to meet the case definition (definitive neurosyphilis) or a combination of other criteria (presumptive neurosyphilis), including reactive nontreponemal or treponemal CSF, other CSF indices (pleocytosis, elevated protein), rabbit inoculation, or clinical signs/symptoms.

In studies of definitive neurosyphilis, sensitivity of CSF FTA-ABS was 90.9%–100% (*123-125*). In the two largest studies of presumptive neurosyphilis (n = 60, n = 156), CSF FTA-ABS demonstrated 100% sensitivity (*126,127*).

CSF FTA-ABS specificity varied greatly depending on whether true negatives were patients without syphilis or patients with syphilis but not symptomatic neurosyphilis. Six studies included patients without syphilis as true negatives, and CSF FTA-ABS specificity was 100%. In 11 studies that included patients with syphilis but not symptomatic neurosyphilis, the specificity ranged from 55% to 100% (*122*), likely because of passive diffusion of serum antibodies across an inflamed blood-brain barrier. This wide range of specificity in patients with syphilis but without neurologic symptoms could lead to false-positive results and overtreatment in these patients and patients with nonspecific neurologic symptoms where the diagnosis of neurosyphilis is unlikely. A negative of CSF FTA-ABS can be clinically helpful to exclude neurosyphilis in complex cases where the cause of nonspecific neurologic signs or symptoms is mostly likely from other conditions.

There are limited data on the use of CSF TPPA in public health and commercial laboratories and no studies on the performance of automated treponemal immunoassays in CSF. For CSF TPPA, three studies reported sensitivities of 75.6–95.0%; the highest sensitivities ranged from 83.3% to 95.0%, when a reactive CSF-VDRL was the reference standard for neurosyphilis (*128-130*). CSF TPPA specificity increased from 75.6 to 93.9% with increasing CSF TPPA titers from ≥1:160 to ≥1:640, respectively, when neurosyphilis was defined as a reactive CSF-VDRL or as new vision or hearing loss (*130*) (Table 4). Based on these limited data, CSF TPPA might have similar sensitivity performance to CSF FTA-ABS in studies of patients with definitive or presumptive symptomatic neurosyphilis (*24*). However, further studies on CSF TPPA test performance and titers before this treponemal test can be recommended for off-label use in unique clinical situations to aid in the diagnosis of neurosyphilis.

No other treponemal antibody tests have been evaluated in the CSF in studies of sufficient sample size to determine their performance characteristics in CSF. Therefore, off-label use of TPPA or treponemal immunoassays to aid in the diagnosis of neurosyphilis is currently not recommended. The only current

off-label CSF treponemal antibody test that can be considered in unique clinical circumstances is the CSF FTA-ABS.

CSF antibody tests for ocular syphilis and otosyphilis. Ocular syphilis and otosyphilis diagnoses are difficult, and there are very limited data on CSF nontreponemal (lipoidal antigen) and treponemal test performance in these clinical scenarios. Existing studies are largely retrospective with small sample sizes (N < 50) and use of CSF VDRL testing, with low sensitivity for both ocular syphilis (<50%) and otosyphilis (<10%) when compared with clinical manifestations and serological evidence of syphilis as reference standards (*131-141*). Currently, the CDC STI Treatment Guidelines state that CSF analysis, including a cell count, protein determination, and CSF-VDRL, might be helpful in diagnosis of suspected ocular syphilis for patients without neurologic symptoms and no evidence of ocular infection on examination; however, it is not recommended in suspected otosyphilis among persons with isolated auditory symptoms and a normal neurologic exam (*24*).

There are no published data of CSF treponemal test performance in ocular syphilis, and limited studies of CSF treponemal tests in patients with otosyphilis include insufficient sample sizes and unsuitable reference standards. No CSF treponemal tests are currently recommended for off-label use in patients with suspected ocular syphilis or otosyphilis and no symptoms or signs suggestive of neurosyphilis.

Serologic tests for congenital syphilis. Passive transfer of maternal antibody can cause positive treponemal test serologic results in neonates and infants for >1 year (*142*). Performing a treponemal test (i.e., TPPA, FTA-ABS, or immunoassay) on neonatal serum is not currently recommended because interpreting these results is difficult (*24*). While some studies have found good correlation between IgM FTA-ABS or ELISA and clinical congenital syphilis findings or other reactive serology in neonates, (*143,144*) these studies were not performed with commercially available IgM tests. Currently, there is no IgM test recommended to aid in the diagnosis of congenital syphilis. Quantitative nontreponemal

(lipoidal antigen) serologic tests (e.g., RPR or VDRL) are recommended for use in newborns born to mothers with positive syphilis serologies during pregnancy (*24*). Nontreponemal (lipoidal antigen) serologic tests should be performed on serum and not cord blood. The same nontreponemal (lipoidal antigen) serologic test should be used in the infant that was used in the mother at delivery so titer levels can be compared.

Serologic test performance in pregnant persons. A 1995 study evaluating RPR serologic testing of 265 specimens from obstetric patients immediately after delivery showed a sensitivity and specificity of 100% and 97.6%, respectively, when using clinical diagnosis and FTA-ABS and/or CAPTIA Syphilis G as reference standards (*145*). Similar to the low incidence of BFPs in the general population (<0.85%) (*35*), false positives are low among pregnant persons (0.6%); all initial reactive nontreponemal tests should be reflexed to a confirmatory treponemal antibody serologic test (*36*).

Treponemal serologic test performance data during pregnancy are limited. Based on a single study that included 2,000 patients, manual treponemal serologic test specificity using concordance among both tests as the reference standard (e.g., FTA-ABS, TPHA) was high for both tests (99.8% and 99.95%, respectively) for pregnant persons; however, there was no control group in this study (*146*). For manual treponemal immunoassays, one study of CAPTIA EIA used TPPA as the reference standard and included 9,896 pregnant patients and 24,346 nonpregnant persons who were screened at an institution that screens high-prevalence populations, including persons living with HIV and men who have sex with men (MSM) (*147*). Discordant immunoassay results (e.g., EIA positive, RP negative, TPPA negative) were more common for pregnant than nonpregnant persons (71.4% versus 43.5%). This is likely related to the lower prevalence of syphilis among pregnant persons screened compared with higher risk nonpregnant persons screened. A retrospective study of over 100,000 pregnant persons screened with an automated immunoassay found 194 women with discordant immunoassay results; 156 of these women

had a reactive LIAISON CIA result, nonreactive RPR, and nonreactive TPPA (isolated CIA reactive), while 38 women had a reactive LIAISON CIA, nonreactive RPR, and reactive TPPA (*148*). Among 77 women with an isolated CIA-reactive result who were retested by their provider, 41 (53%) seroreverted to nonreactive within 12 months.

Box 4. Recommendation for Syphilis Serologic Testing in Pregnant Persons

Nontreponemal (lipoidal antigen) and treponemal serologic tests should be interpreted in the same manner regardless of pregnancy status.

Comment and Evidence Summary. Based on existing data, treponemal serologic tests perform no differently in pregnant persons and should be interpreted in the same manner as for nonpregnant persons (*145,147,148*). However, given the lower prevalence of syphilis in pregnant persons in many areas of the United States, discordant immunoassay results identified with the reverse sequence screening algorithm need to be adjudicated with the TPPA and managed according to the 2021 CDC STI Treatment Guidelines *(24)*. False-positive nontreponemal (lipoidal antigen) serologic tests in pregnancy occur at a similar rate to the general population (*35,36*).

Serologic test performance in persons living with HIV and AIDS. There are limited data on nontreponemal (lipoidal antigen) serologic test performance for persons with HIV as a distinct group; with most studies report RPR and VDRL sensitivity in general populations that include HIV-positive individuals or HIV in the context of neurosyphilis or syphilitic posterior uveitis. A 2007 cross-sectional study of 868 patients with genital ulcer disease showed that RPR serologic test sensitivity and specificity for patients with HIV was 81.8% and 90.6%, respectively, which was comparable to results observed for

the cohort without HIV (*149*). In addition, a 2017 study found no significant difference in sensitivity or specificity based on HIV status when evaluating 571 specimens using CSF VDRL and CSF PCR with clinical neurologic symptoms as reference standards (*130*); using laboratory and clinical diagnostic criteria, CSF-VDRL sensitivity ranged from 49% to 68% and specificity ranged from 90% to 91%. Other studies of populations with varying levels of HIV prevalence found overall sensitivities of 72.5%–85% for serum RPR, 68.8% for CSF RPR, 13.3%–62.5% for CSF VDRL, and 72.6%–91.2% for serum VDRL (*63,120,131,137,150*).

Although data suggest that nontreponemal (lipoidal antigen) serologic test performance sensitivities do not significantly differ between people living with and without HIV, studies have reported increased likelihood of BFP in HIV-positive individuals. In studies with samples sizes that ranged from 789 to 300,000, serum testing by VDRL or RPR showed that the rate of BFP results was 2.5–34.5 times higher among HIV-positive individuals than HIV-negative individuals (*37-39,151,152*). These studies were conducted in populations before antiretroviral therapy (ART) was widely available or in populations where viral load was not assessed. BFP rates in persons living with HIV who are virally suppressed have not been studied.

Treponemal test positivity generally persists after prior treated infection, unless the infection is treated before the secondary stage, as has been previously described in persons without HIV infection. Prior to modern ART, seroreversion of either the MHA-TP or FTA-ABS serologic test was found to vary by severity of HIV disease in two studies and was lower for asymptomatic HIV infection (5 of 69 patients) than symptomatic HIV and AIDS (8 of 21 patients) in one study (*32*). In another study, seroreversion was identified in 14% of 29 patients with asymptomatic HIV and 41% of 29 patients with symptomatic HIV (*42*). However, two subsequent studies including 31 and 104 patients found no difference in seroreversion of treponemal serologic tests by HIV status in patients previously treated for syphilis

(*81*,*153*). In a more recent study of 294 patients with prior syphilis followed for ≥6 months after treatment and with no signs of syphilis during the follow-up interval, 87% were reactive for FTA-ABS, 92% for TPPA, and 96%–99% for one of four treponemal immunoassays (*83*). Treponemal immunoassays were significantly more likely to remain reactive compared with FTA-ABS (*83*).

Box 5. Recommendation for Syphilis Serologic Testing in Persons Living with HIV and AIDS

Nontreponemal (lipoidal antigen) and treponemal serologic tests should be interpreted in the same manner regardless of HIV status.

Comment and Evidence Summary. Based on existing data, nontreponemal (lipoidal antigen) and treponemal serologic tests should be interpreted the same for patients with and without HIV. (*63*,*83*,*120*,*130*,*149*).

Direct Detection Tests for *T. pallidum*

Darkfield microscopy. Darkfield microscopy has been the most widely used direct detection method for *T. pallidum,* but over time, has become less widely available in the United States as the health care delivery system has evolved (*26*,*154*). It is a morphology- and motility-based test that relies on examining live treponemal spirochetes and must be performed within 20 minutes of specimen collection (*25*,*62*). The test is useful for moist lesions of suspected anogenital primary or suspected secondary syphilis where treponemal spirochetes can be readily found (e.g., ulcerative lesions, condylomata lata). Suspected lesions of the external and internal genitalia (including the cervix) and rectum can be

867 examined if serous fluid is collected according to established procedures for darkfield microscopy
868 specimen collection, as outlined below (*25*). Darkfield microscopy on oral lesions is difficult to interpret
869 because of the presence of oral commensal treponemes, which are easily confused with *T. pallidum*;
870 therefore, it is not recommended to use darkfield microscopy on oral lesions.

871 An optimal specimen for darkfield microscopy is serous fluid that is free of red blood cells collected on
872 a microscope slide by using a touch preparation or sterile bacteriological loop. The lesion should be
873 gently cleaned and abraded with a sterile gauze pad or a swab dipped in saline. Serous fluid will appear
874 when slight pressure is applied to the base of the ulcer. A microscope slide should be used to collect the
875 exudate, and a coverslip should be applied in a manner that avoids trapping air bubbles. Alternatively, a
876 sterile bacteriological loop can be used to transfer the exudate to a slide. For cervical, intravaginal, and
877 rectal lesions, serous fluid specimens can be collected with a moist swab and transferred to a glass slide.

878 Darkfield microscopic capability should be maintained or established in clinics in areas with high
879 burden of syphilis; rapid onsite detection of primary syphilis results in timelier treatment that benefits
880 both patient care and public health. A well-trained microscopist and a darkfield microscope are required
881 onsite so the sample can be examined within 20 minutes of collection before motility is compromised.
882 Proficiency testing of darkfield microscopy should be ongoing, and training is provided by the National
883 Network of STD Clinical Training Centers (https://www.nnptc.org). The use of commensal *Treponema*
884 *refringens* and *Treponema denticola* for darkfield microscopy training is not recommended because
885 these spirochetes can easily be confused with *T. pallidum* (*25*). Proficiency with darkfield microscopy
886 requires the ability to distinguish *T. pallidum* from other commensal spirochetes based on motility and
887 morphology.

888 The sensitivity and specificity of darkfield microscopy, defined by clinical presentation and laboratory
889 findings (i.e., serology or PCR), ranges from 75% to 100% and 94% to 100% for primary lesions and

58% to 71% and 100% on secondary lesions, respectively (*109,155-159*). Because serological tests can be negative in early infection, darkfield microscopic examination of anogenital lesions suspected of being primary syphilis can result in a definitive diagnosis (*154*). The variation in darkfield microscopy sensitivity for primary lesions might be related to the duration of the lesion because most studies do not assess the age of the lesion when conducting performance studies for primary syphilis. Darkfield microscopy may still be used as a POC test to definitive diagnosis in any patient presenting with anogenital lesions suggestive of primary syphilis.

The sensitivity of serology at the secondary stage of syphilis in adults is superior to darkfield microscopy; therefore, darkfield microscopy is not routinely recommended in suspected secondary syphilis, except for condylomata lata when POC serology is not available or negative and a definitive diagnosis is warranted. If available, darkfield testing might also be very useful for testing moist lesions of congenital syphilis such as bullous rashes and snuffles. The sensitivity of darkfield microscopy compared with rabbit infectivity testing (RIT) (previous gold standard) on amniotic fluid for congenital syphilis diagnosis varies from 42% to 86% with a specificity of 100% (*160,161*). Because data are limited, darkfield testing on amniotic fluid is generally not recommended.

Darkfield testing is not recommended for oral lesions, CSF, lymph node aspirate, and other body fluids because of the lack of specificity in oral lesions and lack of scientific evidence for use with these specimen types. A list of test performance, specimen types, storage, and transportation-related guidance for direct detection syphilis tests is provided (Tables 5 and 6).

Box 6. Recommendation for the Direct Detection of *T. pallidum* by Darkfield Microscopy

Darkfield microscopy should be maintained if already in use or established in STD clinics where a POC test for primary or secondary syphilis diagnosis would be beneficial for timely patient treatment.

Comment and Evidence Summary. The sensitivity of darkfield microscopy in detecting *T. pallidum* from primary lesions ranges from 94% to 100% and 81% to 100% from secondary lesions when compared to NAATs (*109,155-159*). Darkfield microscopy can be more sensitive than serologic tests at the primary stage and offers the advantage of timely detection and rapid treatment of primary syphilis (*154*). The procedure is classified as moderately complex by CLIA, and settings implementing the darkfield microscopy will require CLIA certification for such a test.

Immunofluorescent antibody staining for *T. pallidum* detection. The direct fluorescent antibody test for *T. pallidum* (DFA-TP) method uses fluorescence-tagged specific antibodies to visualize *T. pallidum* in specimens from primary and secondary syphilis lesions. This test specimen collection method is similar to darkfield microscopy except that after the specimen is placed on the microscope slide it is fixed and sent to a laboratory for processing. Generally, the DFA-TP test is equivalent in sensitivity to darkfield microscopy (*156,158*); however, whereas darkfield test performance to assess motility might decline with time, DFA-TP might be more sensitive in older primary lesions. DFA-TP also has the advantage that is does not require motile organisms to detect *T. pallidum*, and the reading of the results is more objective. The main disadvantages are that results take 1–2 days because they must be processed in a laboratory, and the commercial, FDA-cleared DFA-TP test is no longer available in the United States (*162*). Fluorescence-tagged monoclonal or polyclonal antibodies are commercially available but

are not FDA cleared. For use in diagnostics, these reagents would need to be validated for clinical diagnostic testing and routine quality control would need to be performed.

Immunohistochemistry and silver staining. Immunohistochemistry (IHC) and silver staining are direct detection methods that have been used to stain and examine formalin-fixed, paraffin-embedded (FFPE) tissue biopsies from the skin, brain, placenta, umbilical cord, or other tissues. Biopsies can help identify the cause of atypical ulcers or skin lesions or those that do not respond to initial therapy (*24*). Silver staining (Warthin-Starry, Steiner stains) is a morphology-based test, whereas IHC is both immunologically and morphology based.

For IHC, the avidin-biotin peroxidase complex (ABC) technique has been the most frequently evaluated method for tissue sections. The method involves heat-induced epitope exposure and incubation with rabbit anti-*T. pallidum* immunoglobulin antibodies. Subsequently, biotinylated anti-rabbit immunoglobulin antibodies are added, followed by incubation with peroxidase-conjugated avidin-biotin complex and visualization of the stained treponemal spirochetes. The main difference between the indirect immunofluorescence (IIF) method and IHC ABC is that the secondary antibody is labelled with a fluorescent dye in IIF.

Compared with a clinical or serological diagnosis of secondary syphilis, the IHC ABC method shows 100% specificity across four studies, with sensitivity ranging from 64% to 94% (*155,159,163,164*). In one of these studies, the sensitivity of IHC ABC was compared with IIF on 37 tissue samples; the sensitivity was 95% and 89%, respectively (*159*).

The sensitivity of silver staining of FFPE skin biopsies reported in four studies ranged from 0% to 41% compared with darkfield microscopy, clinical diagnosis and staging based on presentation, and serology (*163-166*). While specificity was not addressed in these studies, several papers reported challenges with interpreting stained sections because background staining of artifacts and reticulum fibers in skin tissue

made it difficult to visualize treponemal spirochetes (*164,167*). Another study evaluated silver staining and an IIF assay on FFPE tissue sections from 17 cases of fetal demise attributable to congenital syphilis and found the test sensitivities were 41% (7/17) and 88% (15/17), respectively (*168*). Given both low sensitivity and challenges with distinguishing spirochetes, use of silver staining for direct detection of *T. pallidum* is no longer recommended for any type of FFPE tissue specimens (*163*).

IHC ABC should be used for evaluating atypical lesions and tissue biopsies for suspected syphilis (primary, secondary, congenital, and gummatous) when the diagnosis remains uncertain. Polyclonal antibodies used with IHC ABC might cross-react with intestinal or other spirochetes (e.g., *Borrelia burgdorferi*) (*164,169*). Further studies comparing the test performance of IIF with IHC ABC are needed.

For congenital syphilis testing, placenta and umbilical cord samples should be tested with the IHC ABC technique or IIF but not with silver stain. Placenta tissue samples should be taken at the periphery and close to where the cord is attached. A cord sample approximately 3– 4 cm long should be obtained from a section distal to the placenta soon after delivery; the tissue should not be cleaned with antimicrobial-containing solution prior to sample collection (*169*). Tissue samples should be fixed in 10% buffered formalin at room temperature immediately upon collection and sent to a pathology laboratory for paraffin embedding and sectioning.

Box 7. Recommendation for Direct Detection of *T. pallidum* by Immunohistochemistry (IHC) and Silver Staining

IHC is preferred over silver staining for (FFPE) tissue sections.

Comment and Evidence Summary. The sensitivity of IHC ranges from 64% to 94% (*155,159,163,164*) while silver stain had a sensitivity of 0% to 41% (*163-166*). Two studies reported difficulties in visualizing treponemal spirochetes because of background artifacts in sliver-stained sections (*164,167*).

NAATs. While NAATs hold great promise for syphilis diagnosis, especially for primary syphilis, there are currently no FDA-cleared NAATs for syphilis. Most laboratory developed NAATs are based on the *tpp47* (*tp074*) or *polA* (*tp0105*) genes with varying sensitivities depending on the stage of syphilis and specimen type (*161,165,170-172*). A highly sensitive reverse transcriptase PCR test that targets a region of the 16S rRNA gene has also been described (*173*) and used on CSF in research studies (*174-176*). In addition, a research use only, real-time, transcription-mediated assay that targets the 23S rRNA gene (Hologic TMA; Hologic Inc, San Diego, CA) has been used to evaluate the presence of *T. pallidum* in rectal and pharyngeal specimens (*76*). Quest Diagnostics (Secaucus, New Jersey) offers a real-time PCR test for *T. pallidum* that has been CLIA-validated for genital lesions and CSF. A digital droplet PCR test was recently used to evaluate the presence of *T. pallidum* in saliva (*177*).

The sensitivity of *tpp47* and *polA* targets varies across studies, from 72% to 95% on lesion exudate of primary syphilis and 20% to 86% on secondary lesion swabs based on lesion type sampled (skin rash versus condylomata lata). These studies are limited by small sample sizes and different reference standards that include some combination of the following: syphilis clinical diagnosis, serologic findings,

or darkfield microscopy results (*77,78,157,171,172,178,179*). If both a darkfield microscopy and a NAAT are performed on the same lesion, the specimen for darkfield microscopy should be collected first. Detailed information on specimen type and collection, transport, and storage requirements for NAAT specimens drawn from references in this document are summarized in Table 5.

A NAAT that targets the *polA* gene had a sensitivity of 84% when tested from maculopapular lesions that were scraped from patients with secondary syphilis using the noncutting edge of a sterilized blade (*80*). The previously described low sensitivity of NAATs in detecting *T. pallidum* from maculopapular lesions might have been attributable to inadequate sampling, but more studies using this scraping technique for direct detection of *T. pallidum* in skin lesions are required to better estimate the NAAT performance. Sensitivities of the NAAT on secondary syphilis lesion biopsies vary between 26% and 75%. These studies are limited by different sample collection methods and reference standards, including a combination of clinical, IHC, or serologic findings (*155,163,165,166*); the highest sensitivity was reported using unfixed tissue frozen immediately after collection.

Among 24 MSM, the Hologic TMA demonstrated a sensitivity for rectal and pharyngeal swabs of 41.6% and 29.5% compared with a NAAT targeting *tpp47* that was 37.5% and 12.5% sensitive for rectal and pharyngeal swabs, respectively (*76*). Although target sequences for *T. pallidum* NAATs are specific to the organism (*180*) and minimal cross-reactivity with commensal *Treponema* spp. suggests they can be used on oral lesions, more research on target specificity is required to be conclusive. In addition, the *tpp47* and *polA* NAATs tests detect all three pathogenic *T. pallidum* subsp. (*pallidum, pertenue, endemicum*). A NAAT that distinguishes among these three subspecies has been described but has not been validated with syphilis specimens (*181*).

NAAT sensitivity using whole blood or its components (serum/plasma) or CSF from adults varies considerably and is limited by small sample sizes; additional studies are needed before these sample

types can be considered for clinical testing (*78,157,178*). Compared with the rabbit infectivity test, sensitivity of NAATs looks promising for amniotic fluid (75% versus 100%), neonatal CSF (60% versus 75%), and neonatal whole blood or serum (67% versus 94%) in congenital syphilis (*160,161,182-184*). The CDC 2021 STI Treatment Guidelines suggest that examination of the placenta, umbilical cord, suspicious lesions, nasal discharge, or other body fluids with a CLIA-validated NAAT could be considered in aiding the diagnosis of congenital syphilis (*24*).

NAATs amplifying the *tpp47* gene are highly specific (98%–100%) and have been performed on different specimen types, including lesion exudates of primary and secondary syphilis; lesion biopsies of secondary syphilis; CSF from neurosyphilis cases; and whole blood, serum, and plasma from primary, secondary, and latent syphilis cases. Assays targeting the *polA* gene demonstrate similar specificity (98%–100%) and have been performed on lesion exudates of primary and secondary syphilis as well as CSF from neurosyphilis cases. (*77,78,157,171,172,178,179*). NAATs with an open platform, regardless of target, are more susceptible than other direct detection tests to false-positive results caused by sample contamination if strict "clean" quality control procedures are not used.

Based on limited data, laboratory-developed NAATs can be used primarily for primary or possible secondary syphilis lesions (e.g., moist lesions, including oral lesions [mucous patches]) in seronegative patients provided that laboratories establish performance specifications to satisfy CMS regulations for CLIA compliance. NAATs might offer more timely diagnosis of primary syphilis compared with serologic testing but have limited additional benefit over serology for secondary syphilis. NAATs can be considered as an adjunct test in amniotic fluid, neonatal CSF, or neonatal blood in cases of suspected congenital infection. While positive NAAT results are helpful in establishing a diagnosis, a negative result in any of these specimens does not rule out infection because of limited sensitivity. NAATs are not recommended for whole blood or blood fractions because of low sensitivity, and data are insufficient

to recommend CSF NAAT testing in adults with symptoms suggestive of neurosyphilis. There are also insufficient data to recommend their use on ocular fluid or tissue from gummas or other tertiary syphilis lesions.

Point-of-Care (POC) Serologic Testing

Because the syphilis algorithm might require confirmatory or other reflex testing, laboratory-based serologic testing for syphilis might take 3–5 days and might require patients to return to the clinic for follow-up or treatment. An accurate POC serologic antibody test for syphilis could shorten the time to treatment because the patient could be identified at the time of the visit or encounter. Studies evaluating the performance of POC syphilis serologic tests include traditional or reverse algorithms that use nontreponemal (lipoidal antigen) and treponemal laboratory-based serologic tests as reference standards (Table 3). There are several POC syphilis serologic tests or dual POC serologic tests for HIV and syphilis that are available and used internationally (https://www.who.int/reproductivehealth/topics/rtis/Diagnostic-Landscape-for-STIs-2019.pdf), but only the Syphilis Health Check (Trinity Biotech, Ireland) and Dual Path Platform (DPP) HIV-Syphilis assay (Chembio Diagnostics, Inc, New York) are FDA-cleared for the detection of *T. pallidum* antibodies. There is a paucity of published information related to their real-world use and test performance in the United States. The Syphilis Health Check, which detects antibodies to *T. pallidum* recombinant treponemal antigens, is the only CLIA-waived rapid POC syphilis test currently marketed in the United States (Table 1). Physician office laboratories and public health field-based screening programs that offer CLIA-waived tests are required to have and maintain a CLIA certificate of waiver that requires that these tests are quality assured and operated by trained personnel according to manufacturer instructions (www.cdc.gov/labquality/waived-tests). The DPP HIV-Syphilis assay is a multiplex, single-use test read

on the DPP Micro Reader optical analyzer; it is formatted as a POC test but classified as moderately complex as of September 1, 2022 (Table 1).

Syphilis Health Check. In two prospective studies with 202 and 562 participants, the sensitivity and specificity of the Syphilis Health Check ranged from 50.0% to 71.4% and 91.5% to 95.9%, respectively, when compared with the Trep-Sure EIA as the reference standard (*185*,*186*) (Table 3). When compared with a reference standard of RPR and TPPA in two other studies with 965 and 690 participants, the Syphilis Health Check had a sensitivity of 76.9% and 90.0% and a specificity of 98.5% and 99.0% (*187*,*188*). In the study with 965 participants, the sensitivity of the Syphilis Health Check was 50.0% and specificity was 99.4%, compared with TPPA alone (*188*). The goal of POC testing is to reach populations who might not seek care and might otherwise go undetected and untreated. Conducted in an outreach setting and emergency departments, the results of the two latter studies suggest that this test might be successful in reaching populations who do not to seek routine health care, and therefore, might be more likely to go undetected and untreated. A 2018 CDC retrospective study used 1,406 archived sera from U.S. commercial and public health labs to evaluate the performance of Syphilis Health Check against treponemal serologic tests only (TPPA, EIA, and CIA) and both treponemal and nontreponemal (lipoidal antigen) (RPR) serologic tests in a laboratory setting (*189*). The overall analysis showed that the sensitivity and specificity of the Syphilis Health Check were 88.7% and 93.1%, respectively, when compared with treponemal serologic tests alone; comparison with both treponemal and nontreponemal (lipoidal antigen) serologic tests showed 95.7% sensitivity and 93.2% specificity. This one study demonstrates that the performance of Syphilis Health Check might be comparable to the current treponemal antibody tests used in clinical settings but does not provide performance data on the populations who might have inconsistent health care seeking. In addition, syphilis history and treatment status data were not available for the patients in this retrospective study.

DPP HIV-Syphilis assay. In two studies with 150 and 450 participants that used the FDA-cleared version of the DPP HIV-Syphilis assay with the DPP Micro Reader, sensitivity and specificity of the DPP HIV-Syphilis assay for syphilis were 95.3% and 100% and 98.7% and 100%, respectively, when compared with TPPA (*190,191*). A CLIA-waived, rapid POC version of this test is needed for populations who do not seek routine health care to benefit from the use of this test outside a primary care or sexual health clinic setting.

While accurate, low-cost rapid tests have the potential to expand testing to populations who otherwise would not be tested in a timely manner, there are insufficient data to recommend when and where to use these tests. Further data on the costs and predictive value of POC serologic tests are needed to assess the implementation of tests in settings that serve populations without regular medical care and those with and without a history of treated syphilis. Costs of testing and timely treatment of those with untreated syphilis in established syphilis screening programs need to be compared with the costs of reaching, testing, and treating populations in outreach settings, emergency departments, or delivery rooms.

Syphilis Laboratory Test Reporting

Reporting to public health departments. Syphilis has significant public health implications, and cases are required to be reported to state or local health departments by the health care provider, laboratory, or both, depending on the state public health reporting statutes.

Because clinical information might be unavailable to the laboratory, all positive syphilis direct detection tests, along with specimen site and positive syphilis serologic tests, should be reported to state and local health departments. State laws detail which syphilis test results to report and timeframes for reporting laboratory results.

Both probable and confirmed cases of syphilis should be reported by health care providers to the local or state health department. Clinical criteria used to stage patients with syphilis might differ from public health surveillance case definitions. Current case definitions are available at: https://ndc.services.cdc.gov/case-definitions/syphilis-2018/. For surveillance purposes, probable cases are defined as the patient presenting with signs or symptoms consistent with the stage of syphilis and having supportive laboratory test results, such as serology, that detect an immune response to the pathogen (*192*). A confirmed case is similar except that the presence of the organism is verified by a direct detection method, either with darkfield microscopy or specific NAAT for *T. pallidum*.

Reporting to health care providers. When reporting results to health care providers, laboratories should list all tests used, report each result with an interpretation, and document the syphilis algorithm applied to render the interpretation, when appropriate (*193*). Any changes in the test algorithm should be communicated to the submitter and include information about differences in interpretation depending on the test algorithm. Preliminary results released to the submitter should list tests that are pending. All the tests and results should be listed in the final report, even if one or more tests, such as the nontreponemal (lipoidal antigen) serologic tests or TPPA, was sent to an outside laboratory.

Opportunities for Additional Research to Inform the Laboratory Detection of *T. pallidum* Infection

Serology and CSF antibody tests. Serologic antibody tests for syphilis have been the mainstay for syphilis testing in the United States for decades; however, despite advancements in automation, additional research in several areas would enhance the utility of current serologic tests:

- Studies of test performance are needed to estimate the sensitivity of nontreponemal serologic tests for primary syphilis against a reference standard of darkfield microscopy or well-characterized NAATs on anogenital lesions. Additional data are needed on serologic test performance in cases of latent syphilis (stratified by duration of infection: early latent, late latent, and latent of unknown duration), late-stage syphilis, symptomatic neurosyphilis, ocular and otic syphilis. To conduct these studies, specimen banks of sera that are well characterized by syphilis stage are essential.
- Test performance studies of dried blood spot testing compared with laboratory-based treponemal serologic tests would allow assessment of its potential as a serologic diagnostic tool.
- Establishing cutoff values for signal strength of immunoassays that are likely to be confirmed as true positives for syphilis should be a priority. More studies are needed to determine if such information would aid in clinical decision-making.
- Continued research on the performance of the two different serologic testing algorithms in populations with low, medium, and high prevalence of syphilis and the development of a cost-benefit analysis tool would aid in laboratory decision-making when selecting the best approach for their setting.
- Evaluation of the CSF TPPA in studies with larger sample sizes and in populations with and without syphilis is needed to better assess specificity of the assay. To better determine the test performance characteristics of the CSF antibody tests, head-to-head studies of CSF nontreponemal and treponemal antibody tests would be conducted with larger samples, using comparable, high quality, agreed-upon reference standards, and in more populations with well-characterized symptom status,

Direct detection. Direct detection of *T. pallidum* has been based on microscopy but is being modernized with molecular methods for detection. There are no FDA-cleared molecular tests marketed in the United States, although some laboratories offer such testing using in-house laboratory developed and validated tests. Molecular tests that are FDA cleared for *T. pallidum* would facilitate their uptake in laboratories; however, additional research is needed in the following areas:

- determining optimal specimen types, including genital and extragenital specimens stratified by stage of syphilis, specimen transport and storage, and specimen adequacy;
- identifying molecular markers that could be used to monitor for the emergence of antimicrobial resistance and strain typing to better inform epidemiological investigations;
- evaluating the sensitivity of NAATs on whole blood or its components (serum and plasma); and
- assessing the cross-reactivity of NAATs with commensal *Treponema* spp.

POC tests. Despite years of study internationally, non-laboratory based POC tests for syphilis are in their infancy in the United States, with only two FDA-cleared tests, and only one CLIA-waived test. There is a clear need for additional CLIA-waived POC tests and data to increase understanding of their performance in clinical and outreach settings. Additional areas needed for research include:

- Well-designed prospective studies are needed on POC test performance in the context of screening algorithms, special patient populations, linkage to treatment and care, and cost-benefits so that recommendations can be made regarding performance and use in the United States.
- Studies comparing POC tests with FDA-cleared laboratory-based treponemal serologic tests, followed by programmatic recommendations for implementation to guide their appropriate use in syphilis testing algorithms.

Future revisions to these recommendations will be based upon new research or technologic advancements for syphilis clinical laboratory science.

TABLE 1. Serologic antibody tests for syphilis testing in the United States

Assay Name and Manufacturer		Technical Specifications
Nontreponemal (lipoidal antigen) Serologic Assays		
AIX1000 **Gold Standard Diagnostics** **2851 Spafford St** **Davis, CA 95618** www.gsdx.us/aix-1000	**System overview**	Automated nontreponemal macroscopic flocculation test. Instrumentation can only be used for syphilis nontreponemal serology.
	Reagent storage conditions and shelf life	Some components stored at temperatures between 2°C–8°C and others at room temperature. The shelf life was not indicated in product literature, but all reagents must be used prior to expiration.
	Specimen type and storage conditions	Serum: May be collected using standard sampling tubes or tubes containing separating gel. The use of tubes containing clot activators has not been evaluated. Store for up to 7 days at 2°C–8°C. If longer storage is required, specimens should be stored frozen at –20°C or below for 14 days. A maximum of two freeze thaw cycles can be used if necessary. Collection: Footnote *, †.
	Volume of specimen required	300µl
	Target antigens	Cardiolipin, cholesterol, phosphatidylcholine.
	Antibody isotype detected	Both IgM and IgG are detected but not differentiated.
	Specimen processing	192 specimens can be tested per 90 minutes.
	Results	Qualitative: Reactive and nonreactive of undiluted specimen. Quantitative: Titer range 1:2–1:256.

		Reflex testing of specimens using a manual RPR test for an endpoint titer might be required if the initial titer is >1:256 or <1:2.
	Dimensions	25.2” wide x 17.7” long x 22.4” high. Weight: 61.7 lbs. Requires bench space for sample preparation prior to loading onto the system.
	Additional comments	Reflex testing of specimens using a manual RPR test for an endpoint titer might be required if the initial titer is >1:256. Footnote §, ¶, **, ††, §§, ***.
Arlington Scientific RPR Card Test **Arlington Scientific** **1840 N Technology Dr** **Springville, UT 84663** www.arlingtonscientific.com	**System overview**	Macroscopic manual nontreponemal flocculation card test.
	Reagent storage conditions and shelf life	Store all reagents at temperatures between 2°C–8°C and others at room temperature. The shelf life was not indicated in product literature, but all reagents must be used prior to expiration.
	Specimen type and storage conditions	Serum: If testing is delayed more than a few hours, remove serum from clotted cellular material. Store at temperatures between 2°C–8°C and test within 5 days of collection. If longer storage is required, specimens should be stored frozen at –20°C or lower. Plasma: Store at temperatures between 2°C–8°C and test within 5 days hours. Do not store plasma beyond 5 days. Collection: Footnote *, †, ¶¶
	Volume of specimen required	50µl
	Target antigens	Cardiolipin, cholesterol, phosphatidylcholine.
	Antibody isotype detected	Both IgM and IgG are detected but not differentiated.
	Specimen processing	Processing time will vary based on the number of specimens and titer range. Agglutination is determined after 8 minutes.
	Results	Qualitative: Reactive and nonreactive of undiluted specimen.

		Quantitative: The highest dilution that results in agglutination is reported as the titer.
	Dimensions	Requires bench space for sample preparation, pipetting, and rotator.
	Additional comments	Footnote §, **, ††, §§, ***, †††
ASI Evolution **Arlington Scientific** **1840 N Technology Dr** **Springville, UT 84663** www.arlingtonscientific.com	**System overview**	Automated nontreponemal flocculation test. Instrumentation can only be used for syphilis nontreponemal serology.
	Reagent storage conditions and shelf life	Some components stored at temperatures between 2°C–8°C and others at room temperature. Shelf life up to 2 years from date of manufacture.
	Specimen type and storage conditions	Serum: May be collected using standard sampling tubes or tubes containing separating gel. The use of tubes containing clot activators has not been evaluated. Store at temperatures between 2°C–8°C and test within 5 days of collection. If longer storage is required, specimens should be stored frozen at –20°C or lower. Plasma: May be collected in tubes containing sodium citrate. Store at temperatures between 2°–8°C and test within 5 days of collection. Cannot be stored longer than 5 days. Collection: Footnote *, †, ¶¶
	Volume of specimen required	110µl
	Target antigens	Cardiolipin, cholesterol, phosphatidylcholine.
	Antibody isotype detected	Both IgM and IgG are detected but not differentiated.
	Specimen processing	190 specimens can be tested per 60 minutes.
	Results	Qualitative: Reactive and nonreactive of undiluted specimen. Quantitative: Titer range 1:2–1:2048.

		Reflex testing of specimens using a manual RPR test for an endpoint titer might be required if the initial titer is >1:2048 or <1:2.
	Dimensions	20” wide x 20” long x 16” high. Weight 78 lbs. Requires bench space for sample preparation prior to loading onto the system.
	Additional comments	Reflex testing of specimens using a manual RPR test for an endpoint titer might be required if the initial titer is >1:2048. Footnote §, **, ††, §§, ***
BBL VDRL Antigen **Becton Dickinson and Company** **1 Becton Dr** **Franklin Lakes, NJ 07417** www.bd.com/en-us	**System overview**	Manual slide microagglutination nontreponemal test.
	Reagent storage conditions and shelf life	Some components stored at temperatures between 2˚C–8˚C and others at room temperature. The shelf life was not indicated in product literature, but all reagents must be used prior to expiration.
	Specimen type and storage conditions	Serum: If testing is delayed more than a few hours, remove serum from clotted cellular material. Store at temperatures between 2˚C–8˚C and test within 5 days of collection. If longer storage is required, specimens should be stored frozen at –20˚C or lower. Collection: Footnote *, †, ¶¶
	Volume of specimen required	50µl
	Target antigens	Cardiolipin, cholesterol, phosphatidylcholine.
	Antibody isotype detected	Both IgM and IgG are detected but not differentiated.
	Specimen processing	Processing time will vary based on the number of specimens and titer range. Agglutination is determined after 4 minutes.
	Results	Qualitative: Reactive and nonreactive of undiluted specimen. Quantitative: The highest dilution that results in agglutination is reported as the titer.

	Dimensions	Requires bench space for sample preparation, pipetting, rotator, and microscope.
	Additional comments	Footnote §, **, ††, §§, ***, †††
Becton Dickinson Macro-Vue RPR **Becton Dickinson and Company** **1 Becton Dr** **Franklin Lakes, NJ 07417** www.bd.com/en-us	**System overview**	Macroscopic manual nontreponemal flocculation card test.
	Reagent storage conditions and shelf life	Some components stored at temperatures between 2°C–8°C and others at room temperature. The shelf life was not indicated in product literature, but all reagents must be used prior to expiration.
	Specimen type and storage conditions	Serum: If testing is delayed more than a few hours, remove serum from clotted cellular material. Store at temperatures between 2°C–8°C and test within 5 days of collection. If longer storage is required, specimens should be stored frozen at –20°C or lower. Plasma: Refer to product literature regarding appropriate collection tubes. Store at temperatures between 2°C–8°C and test within 48 hours. Do not store plasma beyond 24 hours. Collection: Footnote *, †, ¶¶
	Volume of specimen required	50µl
	Target antigens	Cardiolipin, cholesterol, phosphatidylcholine.
	Antibody isotype detected	Both IgM and IgG are detected but not differentiated.
	Specimen processing	Processing time will vary based on the number of specimens and titer range. Agglutination is determined after 8 minutes.
	Results	Qualitative: Reactive and nonreactive of undiluted specimen. Quantitative: The highest dilution that results in agglutination is reported as the titer.
	Dimensions	Requires bench space for sample preparation, pipetting, and rotator.
	Additional comments	Footnote §, **, ††, §§, ***, †††

Toluidine Red Unheated Serum Test (TRUST) **New Horizons Diagnostics Corp** **9110 Red Rd** **Columbia, MD 21045** https://nhdiag.com	**System overview**	Macroscopic manual nontreponemal flocculation card test.
	Reagent storage conditions and shelf life	Some components stored at temperatures between 2°C–8°C and others at room temperature. The shelf life was not indicated in product literature, but all reagents must be used prior to expiration.
	Specimen type and storage conditions	Serum: If testing is delayed more than a few hours, remove serum from clotted cellular material. Store at temperatures between 2°C–8°C and test within 5 days of collection. If longer storage is required, specimens should be stored frozen at –20°C or lower. Plasma: Store at temperatures between 2°C–8°C and test within 48 hours. Do not store plasma beyond 48 hours. Collection: Footnote *, †, h
	Volume of specimen required	50µl
	Target antigens	Cardiolipin, cholesterol, phosphatidylcholine.
	Antibody isotype detected	Both IgM and IgG are detected but not differentiated.
	Specimen processing	Processing time will vary based on the number of specimens and titer range. Agglutination is determined after 8 minutes.
	Results	Qualitative: Reactive and nonreactive of undiluted specimen. Quantitative: The highest dilution that results in agglutination is reported as the titer.
	Dimensions	Requires bench space for sample preparation, pipetting, and rotator.
	Additional comments	Footnote §, **, ††, §§, ***, †††
Stanbio Quicktest RPR	**System overview**	Macroscopic manual nontreponemal flocculation card test.

EKA Diagnostics USA **1261 N Main St** **Boerne, TX 78006** www.ekfusa.com	**Reagent storage conditions and shelf life**	Some components stored at temperatures between 2°C–8°C and others at room temperature. The shelf life was not indicated in product literature, but all reagents must be used prior to expiration.
	Specimen type and storage conditions	Serum: If testing is delayed more than a few hours, remove serum from clotted cellular material. Store at temperatures between 2°C–8°C and test within 5 days of collection. If longer storage is required, specimens should be stored frozen at –20°C or lower. Plasma: Refer to product literature regarding appropriate collection tubes. Store at temperatures between 2°C–8°C and test within 48 hours. Do not store plasma beyond 24 hours. Collection: Footnote *, †, ¶¶
	Volume of specimen required	50µl
	Target antigens	Cardiolipin, cholesterol, phosphatidylcholine.
	Antibody isotype detected	Both IgM and IgG are detected but not differentiated.
	Specimen processing	Processing time will vary based on the number of specimens and titer range. Agglutination is determined after 8 minutes.
	Results	Qualitative: Reactive and nonreactive of undiluted specimen. Quantitative: The highest dilution that results in agglutination is reported as the titer.
	Dimensions	Requires bench space for sample preparation, pipetting, and rotator.
	Additional comments	Footnote §, **, ††, §§, ***, †††
Teco Diagnostics RPR **Teco Diagnostics** **1268 N Lakeview Ave** **Anaheim, CA 92807**	**System overview**	Macroscopic manual nontreponemal flocculation card test.
	Reagent storage conditions and shelf life	Some components stored at temperatures between 2°C–8°C and others at room temperature. The shelf life was not

www.tecodiagnostics.com		indicated in product literature, but all reagents must be used prior to expiration.
	Specimen type and storage conditions	Serum: If testing is delayed more than a few hours, remove serum from clotted cellular material. Store at temperatures between 2°C–8°C and test within 5 days of collection. If longer storage is required, specimens should be stored frozen at –20°C or lower. Plasma: Refer to product literature regarding appropriate collection tubes. Store at temperatures between 2°C–8°C and test within 48 hours. Do not store plasma beyond 24 hours. Collection: Footnote *, †, ¶¶
	Volume of specimen required	50µl
	Target antigens	Cardiolipin, cholesterol, phosphatidylcholine.
	Antibody isotype detected	Both IgM and IgG are detected but not differentiated.
	Specimen processing	Processing time will vary based on the number of specimens and titer range. Agglutination is determined after 8 minutes.
	Results	Qualitative: Reactive and nonreactive of undiluted specimen. Quantitative: The highest dilution that results in agglutination is reported as the titer.
	Dimensions	Requires bench space for sample preparation, pipetting, and rotator.
	Additional comments	Footnote §, **, ††, §§, ***, †††
Treponemal Serologic Assays		
ADVIA Centaur **Siemens Medical Solutions USA, Inc** **40 Liberty Blvd** **Malvern, PA 19355**	**System overview**	Automated direct sandwich chemiluminescence treponemal specific immunoassay with random access or batch processing for a wide range test menu, including hematologic diseases, metabolic diseases, and various cancers and infectious diseases.

www.siemens-healthineers.com/en-us		
	Reagent storage conditions and shelf life	Some components stored at temperatures between 2°C–8°C and others at room temperature. The shelf life was not indicated in product literature, but all reagents must be used prior to expiration.
	Specimen type and storage conditions	Serum: May be collected using standard sampling tubes or tubes containing separating gel. The use of tubes containing clot activators has not been evaluated. Store at temperatures between 2°C–8°C and test within 5 days of collection. Long-term storage requires serum to be separated from cells and frozen at temperatures of –20°C or lower. Plasma: May be collected in tubes containing sodium or lithium heparin, EDTA, or sodium citrate. Store at temperatures between 2°C–8°C and test within 48 hours. Do not store plasma beyond 48 hours. Collection: Footnote *, †, ¶¶
	Volume of specimen required	100µl
	Target antigens	*T. pallidum* recombinant antigens TpN15 and TpN17.
	Antibody isotype detected	Both IgM and IgG are detected but not differentiated.
	Specimen processing	ADVIA Centaur CP: 240 tests per 60 minutes; first result in 29 minutes. ADVIA Centaur XP: 180 tests per 60 minutes; first result in 29 minutes. ADVIA Centaur XPT: 240 tests per 60 minutes; first result in 29 minutes.
	Results	Qualitative: The optical density (OD) is determined by the system and results reported as nonreactive (OD = ≤0.9), equivocal (OD = ≥0.9 to <1) or reactive (OD = ≥1.1). Specimens with equivocal results should be retested. If the retest result remains equivocal, an alternative serologic test should be performed, or a new specimen collected and tested.

		Not quantitative.
	Dimensions	ADVIA Centaur CP: 43” wide x 29” long x 32” high. Weight 366 lbs. ADVIA Centaur XP: 72.4” wide x 41.0” long x 51.5” high. Weight: 80 lbs. ADVIA Centaur XPT: 196” wide x 104” long x 167” high. Weight: 84 lbs. All systems require bench space for sample preparation prior to loading onto the system.
	Additional comments	Footnote §, ¶, **, ††, §§§
Architect Syphilis TP **Abbott Laboratories** **100 Abbott Park Rd** **Abbott Park, IL 60064** www.corelaboratory.abbott/us/en	**System overview**	Random access automated immunoassay with a wide range test menu, including hematologic diseases, metabolic diseases, and various cancers and infectious diseases. Two-step chemiluminescent microparticle treponemal immunoassay.
	Reagent storage conditions and shelf life	Some components stored at temperatures between 2°C–8°C and others at room temperature. The shelf life was not indicated in product literature, but all reagents must be used prior to expiration.
	Specimen type and storage conditions	Serum: May be collected using standard sampling tubes or tubes containing separating gel. The use of tubes containing clot activators has not been evaluated. Stored for up to 24 hours at room temperature or up to 7 days between 2°C–8°C. Serum must be removed for cells and stored frozen if testing is delayed by more than 7 days. Avoid repeated freezing and thawing. Plasma: May be collected in tubes containing potassium EDTA, lithium heparin, sodium heparin, sodium citrate, or citrate-phosphate-dextrose. Stored for up to 24 hours at room temperature or up to 7 days at temperatures between 2°C–8°C. Plasma must be removed for cells and stored frozen if testing is delayed by more than 7 days. Avoid repeated freezing and thawing.

		Collection: Footnote *, †, ¶¶
	Volume of specimen required	100µl
	Target antigens	*T. pallidum* recombinant antigens TpN15, TpN17, TpN47.
	Antibody isotype detected	Both IgM and IgG are detected but not differentiated.
	Specimen processing	Architect *i*1000SR: 100 tests per 60 minutes. Architect *i*2000SR: 200 tests per 60 minutes. Architect *i*4000SR: 400 tests per 60 minutes.
	Results	Qualitative: The OD is determined by the system and results reported as nonreactive (OD = <1.0) or reactive (OD = ≥1.0). Not quantitative.
	Dimensions	Architect *i*1000SR: 59” wide x 30” long x 49” high. Weight: 636 lbs. Architect *i*2000SR: 62” wide x 49” long x 48” high. Weight 1,081 lbs. Architect *i*4000SR: 127” wide x 49” long x 48” high. Weight 2,162 lbs. All systems require bench space for sample preparation prior to loading onto the system.
	Additional comments	Footnote §, **, ††, §§§, ¶¶¶.
AtheNA Multi-Lyte *T. pallidum* IgG Plus Test System, ZEUS Scientific **199 & 200 Evans Way** **Branchburg, NJ 08876** www.zeusscientific.com	**System overview**	Automated direct sandwich chemiluminescence treponemal specific immunoassay with random access or batch processing for a wide range test menu, including hematologic diseases, metabolic diseases, and various cancers and infectious diseases.
	Reagent storage conditions and shelf life	Some components stored at temperatures between 2°C–8°C and others at room temperature. The shelf life was not indicated in product literature, but all reagents must be used prior to expiration.

	Specimen type and storage conditions	Serum: May be collected using standard sampling tubes or tubes containing separating gel. The use of tubes containing clot activators has not been evaluated. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, serum must be removed from cells and be stored at temperatures between 2°C–8°C for no longer than 48 hours. If a delay in testing is anticipated, store test serum at a temperature of –20°C or lower. Avoid repeated freezing and thawing. Collection: Footnote *, †
	Volume of specimen required	10µl
	Target antigens	*T. pallidum* recombinant antigen TpN17.
	Antibody isotype detected	IgG
	Specimen processing	100 tests per 60 minutes.
	Results	Qualitative: An absorption unit per ml (AU/ml) is determined by the system and results reported as nonreactive (AU/ml = <100), equivocal (AU/ml = 100–120) or reactive (AU/ml = >120). Specimens with equivocal results should be retested. If the retest result remains equivocal, an alternative serologic test should be performed, or a new specimen collected and tested. Not quantitative.
	Dimensions	Footprint and weight not reported in product literature. Requires bench space for sample preparation prior to loading onto the system.
	Additional comments	Footnote §, **, ††, §§§, ¶¶¶.
CAPTIA Syphilis IgG EIA **Trinity Biotech USA Inc**	**System Overview**	Manual treponemal enzyme-linked immunoassay. 96-well microtiter plate format.

2823 Girts Rd **Jamestown, NY 14701** www.trinitybiotech.com	**Reagent storage conditions and shelf life**	Some components stored at temperatures between 2˚C–8˚C and others at room temperature. The shelf life was not indicated in product literature, but all reagents must be used prior to expiration.
	Specimen type and storage conditions	Serum: May be collected using standard sampling tubes or tubes containing separating gel. The use of tubes containing clot activators has not been evaluated. Store at temperatures between 2˚C–8˚C for up to 5 days. Serum must be removed for cells and stored frozen if testing is delayed by more than 7 days. Avoid repeated freezing and thawing. Plasma: May be collected in tubes containing potassium EDTA or sodium citrate. Stored for up to 48 hours at temperatures between 2˚C–8˚C. Plasma should not be stored frozen. Collection: Footnote *, †, ¶¶
	Volume of specimen required	50µl
	Target antigens	Antigens from sonicated *T. pallidum* cells.
	Antibody isotype detected	IgG
	Specimen processing	Up to 93 specimens can be tested per 96-well microtiter plate. Each test run should include a duplicate of the low titer control, which is included with the test kit, and an independent low titer control, such as well-characterized serum. Throughput will depend on the number of specimens tested that require manual pipetting. Plates are incubated for 30 minutes before the optical density is read.
	Results	Qualitative; reported as nonreactive (OD = ≤0.9), equivocal (OD = ≥0.9 to <1) or reactive (OD = ≥1.1). Specimens with equivocal results should be retested. If the retest result remains equivocal, an alternative serologic test should be performed, or a new specimen collected and tested. Not quantitative.

	Dimensions	Requires bench space for sample preparation, pipetting, and a microplate reader. The dimensions of the plate reader will vary by manufacturer.
	Additional comments	Footnote §, **, ††, §§§, ¶¶¶
Elecsys Syphilis **RocheDiagnostics** **9115 Hague Rd** **Indianapolis, IN 46256** www.diagnostics.roche.com/us/en	**System overview**	Automated one-step double-antigen sandwich treponemal specific immunoassay with random access or batch processing for a wide range test menu, including hematologic diseases, metabolic diseases, and various cancers and infectious diseases.
	Reagent storage conditions and shelf life	Some components stored at temperatures between 2°C–8°C and others at room temperature. The shelf life was not indicated in product literature, but all reagents must be used prior to expiration.
	Specimen type and storage conditions	Serum: Can be collected using standard sampling tubes or tubes containing separating gel. The use of tubes containing clot activators has not been evaluated. Store serum at room temperature (25°C) for up to 5 days, or at temperatures between 2°C–8°C for 14 days, or frozen at –20°C for up to 12 months. The samples may undergo a maximum of 5 freeze /thaw cycles. Plasma: May be collected in tubes containing potassium EDTA, lithium heparin, sodium citrate, citrate-phosphate-dextrose, and potassium EDTA with separating gel. Store plasma at room temperature (25°C) for up to 5 days, or at temperatures between 2°C–8°C for 14 days, or frozen at –20°C for up to 12 months. The samples may undergo a maximum of 5 freeze /thaw cycles. Collection: Footnote *, †, ¶¶
	Volume of specimen required	cobas e 411, 601, 602 modules: 10 µL cobas e 801: 6 µL
	Target antigens	*T. pallidum* recombinant antigens TpN15, TpN17, TpN47.

	Antibody isotype detected	Both IgM and IgG are detected but not differentiated.
	Specimen processing	cobas e 411 analyzer: Up to 86 specimens per 60 minutes; first result in 60 minutes. cobas e 601 and 602 module: Up to 170 specimens per 60 minutes; first result in 60 minutes. cobas e 801 module: Up to 300 specimens per 60 minutes; first result in 60 minutes.
	Results	Qualitative and quantitative: The analyzer automatically calculates the cutoff value based on the measurements from the calibrators. Each specimen will be given as reactive or nonreactive and with the cutoff index (COI; signal sample/cutoff). Results are reported as nonreactive when the COI is <1.00 and reactive when the COI is ≥1.00. All initially reactive samples should be repeated. Not quantitative.
	Dimensions	cobas e 411 analyzer alone or with modules 601, 602, or 801: 67’ wide x 37.4” long x 43” high. Weight 397 lbs. All systems require bench space for sample preparation prior to loading onto the system.
	Additional comments	Footnote §, **, ††, §§§, ¶¶¶
Enzy-Well Syphilis IgG **Diesse Diagnostica Senese** **Ingresso 6 Monteriggioni** **53035 Siena, Italy** www.diesse.it/en	**System overview**	Manual treponemal enzyme-linked immunoassay. 96-well microtiter plate format.
	Reagent storage conditions and shelf life	Some components stored at temperatures between 2°C–8°C and others at room temperature. Shelf life for up to 15 months from date of manufacture.
	Specimen type and storage conditions	Serum: May be collected using standard sampling tubes or tubes containing separating gel. The use of tubes containing clot activators has not been evaluated. Stored for up to 7 days between 2°C–8°C. Serum can be stored for up to 3 years at a temperature of –20°C or lower. Plasma: Can be collected in tubes containing potassium EDTA, lithium heparin, sodium heparin, sodium citrate, or

		citrate-phosphate-dextrose. Stored for up to 7 days between 2°C–8°C. Plasma can be stored for up to 3 years at a temperature of –20°C or lower. Collection: Footnote *, †, ¶¶
	Volume of specimen required	30µl
	Target antigens	Recombinant *T. pallidum* antigens. The exact antigens were not specified in the product insert.
	Antibody isotype detected	IgG
	Specimen processing	Up to 92 specimens can be tested per 96-well microtiter plate. Each test run should include duplicates of the positive and negative control, both of which are included with the test kit. Throughput will depend on the number of specimens tested that require manual pipetting. Plates are incubated for 30 minutes before the optical density is read.
	Results	Qualitative: A positive/negative cutoff OD value must be calculated with each test run. The calculation is cutoff = (OD negative control + OD positive control) / 3. Specimens that have test results above and below the cutoff are reported as positive and negative, respectively. Not quantitative.
	Dimensions	Requires bench space for sample preparation, pipetting, and a microplate reader. The dimensions of the plate reader will vary by manufacturer.
	Additional comments	IgM antibodies might react with the target antigen and be detected but not differentiated from IgG antibodies in the assay. Footnote §, **, ††, †††, §§§.
Immulite 2000 Syphilis Screen **Siemens Medical Solutions USA, Inc** **40 Liberty Blvd**	**System overview**	Automated solid-phase, one-step chemiluminescent treponemal specific immunoassay with random access or batch processing for a wide range test menu, including

Malvern, PA 19355 www.siemens-healthineers.com/en-us		hematologic diseases, metabolic diseases, and various cancers and infectious diseases.
	Reagent storage conditions and shelf life	Some components stored at temperatures between 2°C–8°C and others at room temperature. The shelf life was not indicated in product literature, but all reagents must be used prior to expiration.
	Specimen type and storage conditions	Serum: May be collected using standard sampling tubes or tubes containing separating gel. The use of tubes containing clot activators has not been evaluated. Store at temperatures between 2°C–8°C for up to 5 days. Serum must be removed for cells and stored frozen at –20°C or lower if testing is delayed by more than 7 days. Avoid repeated freezing and thawing. Plasma: May be collected in tubes containing lithium heparin or sodium heparin. Though acceptable, heparinized plasma might increase values at or near the cutoff level. Tubes containing EDTA are not recommended for collection. Stored for up to 48 hours at temperatures between 2°C–8°C. Plasma should not be stored frozen. Collection: Footnote *, †, ¶¶
	Volume of specimen required	100µl
	Target antigens	*T. pallidum* recombinant antigen TpN17.
	Antibody isotype detected	Both IgM and IgG are detected but not differentiated.
	Specimen processing	Up to 200 tests per 60 minutes; first result in 60 minutes.
	Results	Qualitative: The OD is determined by the system and results reported as nonreactive (OD = ≤0.9), equivocal (OD = ≥0.9 to <1) or reactive (OD = ≥1.1). Specimens with equivocal results should be retested. If the retest result remains equivocal, an alternative serologic test should be performed, or a new specimen collected and tested.

		Not quantitative.
	Dimensions	93” wide x 45” long x 65” high. Weight 800 lbs.
	Additional comments	Footnote §, **, ††, §§§, ¶¶¶
LIAISON **DiaSorin Molecular LLC** **11331 Valley View St** **Cypress, CA 90630** www.diasorin.com/en	**System overview**	Automated solid-phase, one-step sandwich chemiluminescent treponemal specific immunoassay with random access or batch processing for a wide range test menu, including hematologic diseases, metabolic diseases, and various cancers, and infectious diseases.
	Reagent storage conditions and shelf life	Some components stored at temperatures between 2°C–8°C and others at room temperature. The shelf life was not indicated in product literature, but all reagents must be used prior to expiration.
	Specimen type and storage conditions	Serum: May be collected using standard sampling tubes or tubes containing separating gel. The use of tubes containing clot activators has not been evaluated. Store at temperatures between 2°C–8°C for up to 7 days. Serum stored frozen at –20°C or lower if testing is delayed by more than 7 days. Avoid repeated freezing and thawing. Collection: Footnote *, †
	Volume of specimen required	220µl
	Target antigens	*T. pallidum* recombinant antigen TpN17.
	Antibody isotype detected	Both IgM and IgG are detected but not differentiated.
	Specimen processing	Liaison XS: 85 tests per 60 minutes; first test result in 17 minutes. Liaison XL: 180 tests per 60 minutes; first test result in 17 minutes. Liaison: 180 tests per 60 minutes; first test result in 17 minutes.
	Results	Qualitative: The system determines the relative light unit and automatically calculates an index value based on the

		measurements from the controls. Results are reported as nonreactive when the index value is < 0.9, equivocal when the index value is 0.9–1.1, and reactive when the index value is >1.1. Specimens with equivocal results should be retested. Specimens are considered positive if the retest result is positive and negative if the retest result is negative. A second specimen should be collected and tested no less than 1 week later when the result is repeatedly equivocal. Not quantitative.
	Dimensions	Liaison XS: 50” wide x 26” long x 27” high Liaison XL: 59” wide x 36” long x 59” high Liaison: 54” wide x 26” long x 25” high All systems require bench space for sample preparation prior to loading onto the system.
	Additional comments	Footnote §, **, ††, §§§, ¶¶¶
Lumipulse G TP-N **Fujirebio US, Inc** **205 Great Valley Pkwy** **Malvern, PA 19355** www.fujirebio.com	**System overview**	Automated chemiluminescent treponemal specific immunoassay with random access or batch processing for a wide range test menu, including hematologic diseases, metabolic diseases, and infectious diseases.
	Reagent storage conditions and shelf life	Some components stored at temperatures between 2°C–8°C and others at room temperature. The shelf life was not indicated in product literature, but all reagents must be used prior to expiration.
	Specimen type and storage conditions	Serum: May be collected using standard sampling tubes or tubes containing separating gel. The use of tubes containing clot activators has not been evaluated. Store at temperatures between 2°C–8°C for up to 5 days. Serum must be removed for cells and stored frozen if testing is delayed by more than 7 days. Avoid repeated freezing and thawing. Plasma: May be collected in tubes containing potassium EDTA or sodium citrate. Stored for up to 48 hours at

		temperatures between 2°C–8°C. Plasma should not be stored frozen. Collection: Footnote *, †, ¶¶
	Volume of specimen required	60µl
	Target antigens	*T. pallidum* recombinant antigens TpN15, TpN17 and TpN47.
	Antibody isotype detected	Both IgM and IgG are detected but not differentiated.
	Specimen processing	120 tests per 60 minutes; first results in 30 minutes.
	Results	Qualitative: Results are reported as cutoff index values with a range of 0.1–100 and interpreted as nonreactive (<1.0) or reactive (≥1.0). Not quantitative.
	Dimensions	Dimensions not reported in product literature.
	Additional comments	Footnote §, **, ††, §§§, ¶¶¶
MarDx Syphilis FTA-ABS Test System **Trinity Biotech USA Inc** **2823 Girts Rd** **Jamestown, NY 14701** www.trinitybiotech.com	**System overview**	Manual indirect fluorescent treponemal antibody test.
	Reagent storage conditions and shelf life	Some components stored at temperatures between 2°C–8°C. The shelf life was not indicated in product literature, but all reagents must be used prior to expiration.
	Specimen type and storage conditions	Serum: If testing is delayed more than a few hours, remove serum from clotted cellular material. Store at temperatures between 2°C–8°C and test within 5 days of collection. If longer storage is required, specimens should be stored frozen at –20°C or lower. Collection: Footnote *, †
	Volume of specimen required	50µl
	Target antigens	*T. pallidum* fixed to glass slide.
	Antibody isotype detected	IgG
	Specimen processing	Read slides within 1 hour after adding the fluorescently labeled anti-human antibody. Slides may be read within 24

		hours if stored refrigerated in a moist chamber. Allow slides to warm to room temperature before reading.
	Results	Qualitative: The degree of fluorescence of patient serum is visually compared against a minimally reactive control serum. Results are reported as reactive when the fluorescence is comparable to or greater than the minimally reactive control serum. Nonreactive results are reported when the fluorescence is lower than the minimally reactive control serum. Not quantitative.
	Dimensions	Requires bench space for sample preparation, pipetting, and fluorescent microscope. Slides must be read in a darkened room.
	Additional comments	The test interpretation is subjective and requires excellent quality control reagents and technical experience. Footnote §, ††, §§§
Serodia *Treponema pallidum* Particle (TPPA) **205 Great Valley Pkwy** **Malvern, PA 19355** www.fujirebio.com/en-us	**System overview**	Manual microtiter plate agglutination test.
	Reagent storage conditions and shelf life	Some components stored at temperatures between 2°C–8°C and others at room temperature. Shelf life will vary by manufacturer.
	Specimen type and storage conditions	Serum: Refer to product literature regarding appropriate collection tubes. If testing is delayed more than a few hours, remove serum from clotted cellular material. Store at temperatures between 2°C–8°C and test within 5 days of collection. Long-term storage requires serum to be separated from cells and frozen at temperatures of –20°C or lower. Plasma: Refer to product literature regarding appropriate collection tubes. Store at temperatures between 2°C–8°C and test within 48 hours. Do not store plasma beyond 48 hours. Collection: Footnote *, †, ¶¶

	Volume of specimen required	25µl
	Target antigens	Antigens from sonicated *T. pallidum* cells.
	Antibody isotype detected	Both IgM and IgG are detected but not differentiated.
	Specimen processing	Processing time will vary based on the number of specimens and titer range. Plates are incubated at room temperature for at least 2 hours prior to being read.
	Results	Qualitative: Read the settling patters of the sensitized and unsensitized gelatin particles. Report as reactive if the gelatin particles spread out at the bottom of the well or form a large ring with a rough multiform outer margin. Indeterminate results appear as gelatin particles concentrated in the shape of a compact ring with a smooth, round outer margin. Nonreactive results are characterized by gelatin particles that are concentrated in the shape of a button in the center of the well with a smooth, round outer margin. Quantitative: Same as qualitative, except the highest reactive dilution is reported as the titer.
	Dimensions	Requires bench space for sample preparation and pipetting.
	Additional comments	Footnote §, ††, †††, §§§
Trep-Sure **Trinity Biotech USA Inc** **2823 Girts Rd** **Jamestown, NY 14701** www.trinitybiotech.com	**System overview**	Manual treponemal enzyme-linked immunoassay. 96-well microtiter plate format.
	Reagent storage conditions and shelf life	Some components stored at temperatures between 2°C–8°C and others at room temperature. The shelf life was not indicated in product literature, but all reagents must be used prior to expiration.
	Specimen type and storage conditions	Serum: May be collected using standard sampling tubes or tubes containing separating gel. The use of tubes containing clot activators has not been evaluated. Serum samples that are left on the “clot” and kept at ambient temperatures (up

		to 40°C) should be tested within 5 days of collection. If storage exceeding 5 days is necessary, serum should be removed from the clot and stored at temperatures between 2°C–8°C. Store separated serum at temperatures between 2°C–8°C within 8 hours. If assays are not completed within 48 hours, or the separated serum is to be stored beyond 48 hours; serum should be frozen at or below –20°C. Avoid repeated freezing and thawing. Plasma: Can be collected in tubes containing potassium EDTA, sodium citrate, or citrate-phosphate-dextrose. Plasma should be stored at temperatures between 2°C–8°C within 8 hours. If assays are not completed within 48 hours, plasma should be frozen at or below –20°C. Avoid repeated freezing and thawing. Do not inactivate plasma and use within 48 hours. Collection: Footnote *, †, ¶¶
	Volume of specimen required	100µL
	Target antigens	Recombinant *T. pallidum* antigens. The exact antigens were not specified in the product insert.
	Antibody isotype detected	Both IgM and IgG are detected but not differentiated.
	Specimen processing	Up to 88 specimens can be tested per 96-well microtiter plate. Each test run should include one well for a blank, two wells for the negative control, two wells for the positive control, and three wells for the cutoff calibrator. Throughput will depend on the number of specimens tested that require manual pipetting. Plates are incubated for 105 minutes before the optical density is read.
	Results	Qualitative: The OD of patient serum is compared with the OD of the cutoff calibrator. Results are reported as negative or positive if the patient serum OD is 20% below or 20% above the mean cutoff calibrator OD, respectively.

		Equivocal results are reported when the patient serum OD is within 20% of the mean cutoff calibrator. Samples with equivocal range or positive results should be retested. If the sample remains equivocal on retest, the patient should be considered suspect for disease because a low level of antibody is detected. A new sample should be obtained and retested. If the patient remains equivocal, a second sample should be collected and tested 2–4 weeks later. An equivocal result indicates that a low level of antibody is detected, and the patient should be monitored for antibody status. Not quantitative.
	Dimensions	Requires bench space for sample preparation, pipetting, and a microplate reader. The dimensions of the plate reader will vary by manufacturer.
	Additional comments	Footnote §, **, ††, §§§, ¶¶¶
Virgo FTA-ABS IgG **Hemagen Diagnostics Inc** **9033 Red Branch Rd** **Columbia, MD 21045** www.hemagen.com	**System overview**	Manual indirect fluorescent treponemal antibody test.
	Reagent storage conditions and shelf life	Some components stored at temperatures between 2°C–8°C. The shelf life was not indicated in product literature, but all reagents must be used prior to expiration.
	Specimen type and storage conditions	Serum: If testing is delayed more than a few hours, remove serum from clotted cellular material. Store at temperatures between 2°C–8°C and test within 3 days of collection. If longer storage is required, specimens should be stored frozen at –20°C or lower. Collection: Footnote *, †
	Volume of specimen required	50µl
	Target antigens	*T. pallidum* fixed to glass slide.
	Antibody isotype detected	IgG
	Specimen processing	Read slides within 1 hour after adding the fluorescently labeled anti-human antibody. Slides may be read within 24

		hours if stored refrigerated in a moist chamber. Allow slides to warm to room temperature before reading.
	Results	Qualitative: The degree of fluorescence of patient serum is visually compared against a minimally reactive control serum. Results are reported as reactive when the fluorescence is comparable to or greater than the minimally reactive control serum. Nonreactive results are reported when the fluorescence is lower than the minimally reactive control serum. Not quantitative.
	Dimensions	Requires bench space for sample preparation, pipetting, and fluorescent microscope. Slides must be read in a darkened room.
	Additional comments	The test interpretation is subjective and requires excellent quality control reagents and technical experience. Footnote §, ††, §§§
Zeus Scientific *T pallidum* IgG Test System **ZEUS Scientific** **199 & 200 Evans Way** **Branchburg, NJ 08876** www.zeusscientific.com	**System overview**	Manual treponemal enzyme-linked immunoassay. 96-well microtiter plate format.
	Reagent storage conditions and shelf life	Some components stored at temperatures between 2°C–8°C and others at room temperature. The shelf life was not indicated in product literature, but all reagents must be used prior to expiration.
	Specimen type and storage conditions	Serum: May be collected using standard sampling tubes or tubes containing separating gel. The use of tubes containing clot activators has not been evaluated. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, serum may be stored between 2°C–8°C for no longer than 48 hours. If a delay in testing is anticipated, store test serum at –20°C or lower. Avoid repeated freezing and thawing, which could cause loss of antibody activity and give erroneous results. Collection: Footnote *, †

Volume of specimen required	10µL
Target Antigens	*T. pallidum* recombinant antigen TpN17.
Antibody isotype detected	IgG
Specimen processing	Up to 90 specimens can be tested per 96-well microtiter plate. Each test run should include one blank, one negative control, one positive control, and a calibrator run in triplicate. The controls and calibrator are included in the test kit. Throughput will depend on the number of specimens tested that require manual pipetting. Plates are incubated for 30 minutes before the optical density is read.
Results	Qualitative: A positive and negative cutoff optical density value must be calculated with each test run. The calculation is cutoff = correction factor x mean OD of the calibrator. The correction factor is provided with each lot of test kits. Nonreactive specimens have an OD ratio ≤0.90, equivocal specimens have an OD ratio between 0.91 and 1.09, and reactive tests results have an OD ratio ≥1.10. Specimens with equivocal results should be retested in duplicate. Report any two of the three results which agree. Evaluate repeatedly equivocal specimens using an alternate serological method or re-evaluate by drawing another sample 1–3 weeks later.
Dimensions	Requires bench space for sample preparation, pipetting, and a microplate reader. The dimensions of the plate reader will vary by the manufacturer.
Additional comments	Footnote §, **, ††, §§§, ¶¶¶

Combined Nontreponemal and Treponemal Serologic Assays		
BioPlex 2200 Syphilis Total & RPR **Biorad, 2000 Alfred Nobel Dr** **Hercules, CA 94547** www.bio-rad.com	**System overview**	Automated dual treponemal/nontreponemal multiplex flow immunoassay.
	Reagent storage conditions and shelf life	Some components stored at temperatures between 2°C–8°C and others at room temperature. The shelf life was not indicated in product literature, but all reagents must be used prior to expiration.
	Specimen type and storage conditions	Serum: May be collected using standard sampling tubes or tubes containing separating gel. The use of tubes containing clot activators has not been evaluated. Stored for up to 7 days between 2°C–8°C. Beyond 7 days, serum should be stored at a temperature of –20°C or lower. Plasma: Can be collected in tubes containing potassium EDTA, lithium heparin, or sodium heparin. Stored for up to 7 days between 2°C–8°C. Beyond 7 days, plasma should be stored at a temperature of –20°C or lower. Collection: Footnote *, †, ¶¶
	Volume of specimen required	10µL
	Target antigens	Nontreponemal antigen targets: Cardiolipin, cholesterol, phosphatidylcholine. Treponemal antigen targets: *T. pallidum* recombinant antigens TpN17 and TpN47.
	Antibody isotype detected	Both IgM and IgG are detected but not differentiated.
	Specimen processing	Up to 200 specimens per 60 minutes; time to first test result is not specified in the product literature.
	Results	Qualitative (Nontreponemal): RPR assay results in undiluted specimens are reported as nonreactive (<1.0 antibody index [AI]) or reactive (≥1.0 AI). Quantitative: Titer range 1:4–1:64. Qualitative results are measured against an AI determined by internal calibration. Qualitative (Treponemal): Syphilis Total treponemal assay

		results are reported as nonreactive (≤ 0.8 AI), equivocal (0.9, 1.0 AI) or reactive (≥ 1.1 AI).
	Dimensions	51” wide x 34” long x 53” high. Weight: 1032 lbs.
	Additional comments	Reflex testing of specimens using a manual RPR test for an endpoint titer might be required if the initial titer is >1:64. Footnote §, **, ††, ***, §§§, ¶¶¶
Combined HIV and Treponemal Point-of-Care Serologic Assays		
DPP HIV-Syphilis Assay, Chembio Diagnostic Systems, Inc **555 Wireless Blvd** **Hauppauge, NY, 11788** www.chembio.com	**System overview**	Manual single-use rapid HIV-1/2 and treponemal immunoassay.
	Reagent storage conditions and shelf life	Unopened pouches can be stored at temperatures between 2°C–30°C. Do not freeze and do not open the pouch until ready for use. The running buffer and DPP sample trainer bottles should be stored at temperatures between 2°C–30°C in their original containers. The shelf life was not indicated in product literature, but all reagents must be used prior to expiration.
	Specimen type and storage conditions	Whole blood: Finger stick collection and test immediately. Whole blood collected in potassium EDTA tubes may be stored at 2°C–8°C and tested within 3 days of collection. Plasma: Collected in tubes containing potassium EDTA. Stored for up to 3 days at 2°C–8°C. Beyond 3 days, plasma should be stored at a temperature of –20°C or lower. Collection: Footnote *, ¶¶
	Volume of specimen required	One drop collected using the sample loop for whole blood fingerstick or 10µL if using venous collected blood.
	Target antigens	HIV and *T. pallidum* recombinant antigens not reported.
	Antibody isotype detected	Both IgM and IgG are detected but not differentiated.
	Specimen processing	Single use lateral flow cassette read using the DPP Micro Reader within 10–25 minutes.

	Results	Qualitative: Reactive, nonreactive, or invalid for antibodies to HIV-1/2 and/or *T. pallidum*. Invalid results should be retested with a new device. Customer service should be contacted if the repeat test is invalid. Not quantitative.
	Dimensions	Requires bench space for sample preparation and a DPP Micro Reader.
	Additional comments	Moderately complex CLIA classification. Footnote **, ††
Point-of-Care Treponemal Serologic Assays		
Syphilis Health Check Treponemal Antibody Test, Diagnostics Direct LLC **359 9th St, Suite 303** **Stone Harbor, NJ 08247** www.diagnosticsdirect2u.com	**System overview**	Manual single-use rapid treponemal immunochromatographic assay.
	Reagent storage conditions and shelf life	Can be stored at temperatures between 4°C–30°C. The shelf life was not indicated in product literature, but all reagents must be used prior to expiration.
	Specimen type and storage conditions	Whole blood: Finger stick collection and test immediately. Whole blood collected in potassium EDTA tubes may be stored at 2°C–8°C and tested within 8 hours of collection. Serum: Stored for up to 5 days between 2°C–8°C. Beyond 5 days, serum should be stored at a temperature of –20°C or lower. Plasma: Collected in tubes containing potassium EDTA. Stored for up to 5 days between 2°C–8°C. Beyond 5 days, plasma should be stored at a temperature of –20°C or lower. Collection: Footnote *, †
	Volume of specimen required	Two drops collected using the sample loop for whole blood fingerstick or 50µL if using venous collected blood; 25µL if using serum or plasma.
	Target antigens	*T. pallidum* recombinant antigens not reported.
	Antibody isotype detected	Both IgM and IgG are detected but not differentiated.
	Specimen processing	Single-use lateral flow cassette read within 15 minutes.

	Results	Qualitative: Reactive, nonreactive, or invalid for antibodies to *T. pallidum*. Invalid results should be retested with a new device. Customer service should be contacted if the repeat test is invalid.
	Dimensions	Requires bench space for sample preparation.
	Additional comments	CLIA waived. Footnote **, ††

Abbreviations: IgM = immunoglobulin M; IgG = immunoglobulin G; RPR = rapid plasma reagin; EDTA = ethylenediaminetetraacetic acid; OD = optical density; AU/ml = absorption unit per ml; COI = cutoff index; CLIA = Clinical Laboratory Improvement Amendments

*Collect ≥2ml of whole blood by venipuncture.

†Blood collection tube for serum: Following collection, gently invert the tube 5–10 times to activate clot formation. Avoid hemolysis when inverting the tube. Allow cellular components of blood to clot at room temperature for at least 30 minutes. Refrigeration will slow or prevent clot formation and should be avoided. Serum should be removed from clotted blood if the tube cannot be transported to the laboratory within 2 hours of collection. Once clotted, the tube should be centrifuged at 1,000–2,000 xg for 10 minutes and the supernatant serum pipetted into a clean serum storage tube.

§Specimens should be free of gross hemolysis (i.e., able to read printed material through the specimen), icterus, bacterial contamination, and lipemia.

¶Serum must not be heat inactivated.

**Refer to https://www.fda.gov/vaccines-blood-biologics/complete-list-donor-screening-assays-infectious-agents-and-hiv-diagnostic-assays for specific assays that are FDA-cleared for use in screening blood or plasma donors.

††May be reactive with serum from patients with yaws (*T. pallidum* subsp. *pertenue*), pinta (*T. carateum*), bejel (*T. pallidum* subsp. *endemicum*), or other treponemal diseases.

§§A prozone reaction can occur. In a prozone reaction, reactivity with an undiluted sample is inhibited because of high antibody concentrations. A prozone may be suspected when an undiluted specimen produces only a weakly reactive result. Therefore, all undiluted specimens producing weakly reactive results should be diluted and titered to determine an endpoint. In addition, a specimen should be tested for the prozone when the clinician suspects syphilis, but the undiluted nontreponemal test result is nonreactive.

¶¶Blood collection tube for plasma: Following collection, gently invert the tube 8–10 times to allow for mixing of the anticoagulant. Avoid hemolysis when inverting the tube. Plasma should be removed from the cells if the tube cannot be transported to the laboratory within 2 hours of collection. The tube should be centrifuged at 1,000–2,000 xg for 10 minutes and the supernatant plasma pipetted into a clean plasma storage tube.

***Sequential serologic tests in individual patients should be performed using the same testing method, preferably by the same laboratory. Titers between two different nontreponemal (lipoidal antigen) tests (e.g., RPR and VDRL) are not interchangeable.

†††Heat inactivation of serum at 56°C for 30 minutes will not affect the result but is unnecessary.

§§§Detection of treponemal antibodies could indicate recent, past, or successfully treated syphilis infections; therefore, the test cannot be used to differentiate between active and cured cases.

¶¶¶The effect of heat inactivation on serum was not reported in the product insert.

TABLE 2. Comparison of traditional and reverse algorithms for syphilis screening by serology

Parameter	Traditional algorithm with a nontreponemal test as the initial test	Reverse algorithm with a treponemal test as the initial test
Reagent cost	Rapid and inexpensive reagents	Higher reagent cost per specimen Automated treponemal serologic tests widely available with high throughput and lower human labor costs
Specimen throughput	Good for small-throughput laboratories Less suitable for high-throughput laboratories because of labor and resources needed and occupational hazard of pipetting of individual specimens	Possible batching of samples that could delay test result turnaround time
Performance characteristics	Results of nontreponemal (lipoidal antigen) serologic tests can be subjective, and there is laboratory variability in titers Possible prozone reaction that might be falsely interpreted as negative unless the serum sample is diluted Biologic false positive resulting from nonspecific reactivity resulting from conditions other than syphilis Might be less sensitive for detecting early and late/latent syphilis	Treponemal serologic tests produce objective results No prozone reaction Specific for *T. pallidum* antigens Might have increased detection of patients with early syphilis

Screening applications	Good for populations with a high likelihood of prior syphilis	If algorithm is used in populations with a high likelihood of prior syphilis, an increased number of primary screening tests could be false positives*

*** False positives are defined as being reactive serum specimen during the initial treponemal serologic test that is nonreactive when reflex tested by a nontreponemal serologic test and a second treponemal serologic test.**

TABLE 3. Performance characteristics of serologic tests used for the diagnosis of syphilis*

Assay	Study summary and reference standard	Performance characteristics†	Reference
	Nontreponemal serologic tests		
AIX1000	Retrospective cross-sectional clinical trial study for submission to FDA Reference standard: ASI RPR card Clinically characterized samples: Primary syphilis: genital lesion, positive for spirochetes on darkfield microscopy (if performed), and reactive treponemal serologic test Secondary syphilis: rash or mucous patches or condyloma lata with reactive treponemal serologic test Latent syphilis reactive treponemal and nontreponemal serologic test with a nonreactive nontreponemal serologic test for more than a year or unknown duration	Prospective serum samples (N = 765) PPA: 95.5% (95% CI: 77.2%–99.9%) PNA: 99.9% (95% CI: 99.3%–100%) Retrospective serum from patients referred for syphilis testing (N = 2,246) PPA: 97.2% (95% CI: 95.5%–98.4%) PNA: 99.1% (95% CI: 98.5%–99.5%) Samples from HIV+ patients (n = 250 non-treponemal test negative; n = 30 nontreponemal test positive) PPA: 100% (95% CI: 90.5%–100%) PNA: 100% (95% CI: 98.8%–100%) Clinically characterized samples: All samples positive on AIX1000 and comparator; 100% sensitive at all stages. Primary treated (n = 13): 100% agreement (95% CI: 79.4%–100%)	(*194*)§

		Primary untreated (n = 12): 100% agreement (95% CI: 77.9% –100%) Secondary treated (n = 25): 100% agreement (95% CI: 88.7%–100%) Secondary untreated (n = 25): 100% agreement (95% CI: 88.7%–100%) Latent treated (n = 25): 100% agreement (95% CI: 88.7%–100%) Latent untreated (n = 25): 100% agreement (95% CI: 88.7%–100%)	
ASI Evolution	Prospective and retrospective cross-sectional clinical trial study for submission to FDA Prospective serum samples: 1,068 Retrospective serum samples: 10 Retrospective plasma samples: 1003 Clinically diagnosed syphilis patients: 143 Pregnant women: 250 Reference standard: ASI RPR card Clinical characteristics not defined beyond the stage of syphilis being diagnosed by a licensed physician	Prospective serum samples (N = 1,068) PPA: 99.1% (95% CI: 95.2%–99.9%) PNA: 99.9% (95% CI: 99.4%–100%) Retrospective serum samples (N = 10) PPA: 100% (95% CI: 59%–100%) PNA: 100% (95% CI: 29.2%–100%) Retrospective plasma samples (N = 1,003) PPA: 100% (95% CI: 69.2%–100%) PNA: 100% (95% CI: 99.6%–100%) Clinically diagnosed syphilis patients (N = 143) Primary treated (n = 25): 100% agreement (95% CI: 81.5%–100%) Primary untreated (n = 18): 100% agreement (95% CI: 86.3%–100%) Secondary treated (n = 25): 100% agreement (95% CI: 86.3%–100%) Secondary untreated (n = 25): 100% agreement (95% CI: 86.3%–100%) Latent treated (n = 25): 100% agreement (95% CI: 86.3%–100%)	(*195*)[§]

		Latent untreated (n = 25): 100% agreement (95% CI: 86.3%–100%) All phases treated (n = 75): 100% agreement (95% CI: 95.1%–100%) All phases untreated (n = 25): 100% agreement (95% CI: 94.7%–100%) Pregnant women (N = 250) PPA: 100% (95% CI: 88.7%–100%) PNA: 100% (95% CI: 98.5%–100%)	
Rapid Plasma Reagin (RPR)	Retrospective cross-sectional study Patients with primary syphilis: 106 Reference standard: Darkfield positive chancre and no signs of secondary syphilis (signs and symptoms not reported in the paper)	Primary syphilis (n = 106) Sensitivity: 72.5%	(*63*)
	Cross-sectional study Patients with primary syphilis: 109 Reference standard: Darkfield positive chancre and no signs of secondary syphilis	Primary syphilis (n = 109) Sensitivity: 92.7%	(*70*)
	Retrospective cross-sectional study based on stored serum from clinically classified patients Patients with primary syphilis: 119 Patients with secondary syphilis: 98	Primary syphilis (n = 119) Sensitivity: 72.3% Secondary syphilis (n = 98) Sensitivity: 100%	(*64*)

	Reference standard: Darkfield positive lesions consistent with primary and secondary syphilis (signs and symptoms not reported in the paper)		
	Cross-sectional study Patients with primary syphilis: 111 Patients with secondary syphilis: 56 Reference standard: (1) Primary syphilis—darkfield positive chancre and no signs of secondary syphilis; (2) secondary syphilis—darkfield positive secondary lesions or at least two symptoms of secondary syphilis, such as condylomata lata, alopecia, and lymphadenopathy	Primary syphilis (n = 111) Sensitivity: 64.8% Secondary syphilis (n = 56) Sensitivity: 100%	(*65*)
	Cross-sectional study Patients with primary syphilis: 80 Patients with secondary syphilis: 29 Reference standard: (1) Primary syphilis—darkfield positive chancre and no signs of secondary syphilis; (2) secondary syphilis—darkfield positive secondary lesions or at least two symptoms of secondary syphilis, such as condylomata lata, alopecia, and lymphadenopathy	Primary syphilis (n = 80) Sensitivity: 62.5% Secondary syphilis (n = 29) Sensitivity: 100%	(*66*)
	Cross-sectional study Patients with primary syphilis: 134 Patients with secondary syphilis: 217	Primary syphilis (n = 134) Sensitivity: 76.1% Secondary syphilis (n = 217) Sensitivity: 91.2%	(*67*)

Reference standard: Darkfield positive lesions consistent with primary and secondary syphilis (signs and symptoms not reported in the paper)		
Cross-sectional study Patients with primary syphilis: 21 Reference standard: Darkfield positive chancre and no signs of secondary syphilis	Primary syphilis (n = 21) Sensitivity: 71%	(*68*)
Retrospective cross-sectional study Patients with primary syphilis: 76 Patients with secondary syphilis: 100 Reference standard: Darkfield positive lesions consistent with primary and secondary syphilis (signs and symptoms not reported in the paper)	Primary syphilis (n = 76) Sensitivity: 48.7% Secondary syphilis (n = 100) Sensitivity: 91%	(*69*)
Prospective cross-sectional study Patients with secondary syphilis: 23 Reference standard: Positive FTA-ABS serology plus clinical findings	Secondary syphilis (n = 23) Sensitivity: 100%	(*96*)
Cross-sectional study Patients with secondary syphilis: 31 Reference standard: Positive VDRL plus clinical findings	Secondary syphilis (n = 31) Sensitivity: 100%	(*98*)

	Retrospective case series Patients with late latent syphilis: 1,303 Reference standard: Positive FTS-ABS or MHA-TP serologic tests plus a diagnosis of late latent syphilis	Late latent syphilis (n = 1,303) Sensitivity: 63.6%	(*102*)
	Retrospective cross-sectional study Patients with neurosyphilis: 25 (24 patients were considered to have neurosyphilis, from which 8 had symptomatic neurosyphilis [disease meningovascular = 6; meningitis = 1; cranial neuritis =1], 16 asymptomatic neurosyphilis [no neurologic symptoms or signs], and 1 patient with all clinical and laboratory criteria of neurosyphilis, except increased proteins; all 25 were living with HIV) Syphilis positive control patients: 163 patients with syphilis based on serology and no signs of neurosyphilis Syphilis negative control patients with other neurologic disorders: 126 Reference standard: Reactive FTA-ABS, increased CSF protein ≥45 mg/dL and CSF pleocytosis ≥10 cell/mm^3	Combined data from asymptomatic and symptomatic neurosyphilis patients (n = 25) Sensitivity: 75% Specificity: 99.3% Asymptomatic neurosyphilis patients (n = 16) Sensitivity: 68.8% Symptomatic neurosyphilis patients (n = 8) Sensitivity: 100%	(*120*)
Unheated Serum Reagin (USR)	Retrospective cross-sectional study based on stored serum from clinically classified patients Patients with primary syphilis: 119 Patients with secondary syphilis: 98	Primary syphilis (n = 119) Sensitivity: 71.4% Secondary syphilis (n = 98) Sensitivity: 100%	(*64*)

	Reference standard: Darkfield positive lesions consistent with primary and secondary syphilis (signs and symptoms not reported in the paper)		
Venereal Disease Research Laboratory (VDRL)	Retrospective cross-sectional study Patients with primary syphilis: 106 Reference standard: Darkfield positive chancre and no signs of secondary syphilis (signs and symptoms not reported in the paper)	Primary syphilis (n = 106) Sensitivity: 72.6%	(*63*)
	Cross-sectional study Patients with primary syphilis: 109 Reference standard: Darkfield microscopy	Primary syphilis (n = 109) Sensitivity: 72.5%	(*70*)
	Retrospective cross-sectional study based on stored serum from clinically classified patients Patients with primary syphilis: 119 Patients with secondary syphilis: 98 Reference standard: Darkfield positive lesions consistent with primary and secondary syphilis (signs and symptoms not reported in the paper)	Primary syphilis (n = 119) Sensitivity: 66.4% Secondary syphilis (n = 98) Sensitivity: 100%	(*64*)
	Cross-sectional study Patients with primary syphilis: 111 Patients with secondary syphilis: 56	Primary syphilis (n = 111) Sensitivity: 63.1% Secondary syphilis (n = 56) Sensitivity: 100%	(*65*)

	Reference standard: (1) Primary syphilis—darkfield positive chancre and no signs of secondary syphilis; (2) secondary syphilis—darkfield positive secondary lesions or at least two symptoms of secondary syphilis, such as condylomatalata, alopecia, and lymphadenopathy		
	Cross-sectional study Patients with primary syphilis: 80 Patients with secondary syphilis: 29 Reference standard: (1) Primary syphilis - darkfield positive chancre and no signs of secondary syphilis; (2) Secondary syphilis - darkfield positive secondary lesions or at least two symptoms of secondary syphilis such as condylomata lata, alopecia, and lymphadenopathy	Primary syphilis (n = 80) Sensitivity: 62.5% Secondary syphilis (n = 29) Sensitivity: 100%	(*66*)
	Cross-sectional study Patients with primary syphilis: 134 Patients with secondary syphilis: 217 Reference standard: Darkfield positive lesions consistent with primary and secondary syphilis (signs and symptoms not reported in the paper)	Primary syphilis (n = 134) Sensitivity: 78.4% Secondary syphilis (n = 217) Sensitivity: 100%	(*67*)
	Cross-sectional study Patients with primary syphilis: 63 Patients with secondary syphilis: 23	Primary syphilis (n = 63) Sensitivity: 76.2% Secondary syphilis (n = 23) Sensitivity: 100%	(*71*)

	Reference standard: (1) Primary syphilis—darkfield positive chancre and no signs of secondary syphilis; (2) secondary syphilis—darkfield positive secondary lesions or at least two symptoms of secondary syphilis, such as condylomata lata, alopecia, and lymphadenopathy		
	Cross-sectional study Patients with primary syphilis: 130 Reference standard: Darkfield positive chancre and no signs of secondary syphilis	Primary syphilis (n = 130) Sensitivity: 68.5%	(*72*)
	Cross-sectional study Patients with primary syphilis: 13 Patients with secondary syphilis: 16 Reference standard: Darkfield positive lesions consistent with primary and secondary syphilis (signs and symptoms not reported in the paper)	Primary syphilis (n = 13) Sensitivity: 76.9% Secondary syphilis (n =16) Sensitivity: 100%	(*73*)
	Cross-sectional study Patients with primary syphilis: 62 Reference standard: Darkfield positive chancre and no signs of secondary syphilis (signs and symptoms not reported in the paper)	Primary syphilis (n = 62) Sensitivity: 63%	(*74*)
	Retrospective cross-sectional study Patients with primary syphilis: 322	Primary syphilis (n = 322) Sensitivity: 73.3%	(*75*)

Reference standard: Darkfield positive chancre and no signs of secondary syphilis (signs and symptoms not reported in the paper)		
Retrospective cross-sectional study Patients with primary syphilis: 76 Patients with secondary syphilis: 100 Reference standard: Darkfield positive lesions consistent with primary and secondary syphilis (signs and symptoms not reported in the paper)	Primary syphilis (n = 76) Sensitivity: 50% Secondary syphilis (n = 100) Sensitivity: 100%	(*69*)
Retrospective cross-sectional study Patients with early latent syphilis: 6 Patients with late latent syphilis: 12 Reference standard: Reactive TPPA, FTA-ABS tests and Western blot plus a diagnosis of syphilis (signs and symptoms not reported in the paper)	Early latent syphilis (n = 6) Sensitivity: 100% Late latent syphilis (n = 12) Sensitivity: 75%	(*196*)
Retrospective cross-sectional study Patients with early latent syphilis: 23 Patients with late latent syphilis: 44 Reference standard: Reactive FTA-ABS, TPHA, and VDRL serologic tests plus a diagnosis of syphilis (signs and symptoms not reported in the paper). Early latent was defined as <1 year and late latent syphilis defined as >1 year	Early latent syphilis (n = 23) Sensitivity: 82.1% Late latent syphilis (n = 12) Sensitivity: 65.9%	(*101*)

Cross-sectional study Patients with recent secondary syphilis: 17 Patients with recurrent secondary syphilis: 44 Patients with early latent syphilis: 34 Patients with late latent syphilis: 44 Reference standard: Positive FTA-ABS, TPHA, and CAPTIA Syphilis M serologic tests plus clinical findings consistent with secondary syphilis	Recent secondary syphilis (n = 17) Sensitivity: 100% Recurrent secondary syphilis (n = 44) Sensitivity: 100% Early latent syphilis (n = 34) Sensitivity: 100% Late latent syphilis (n = 44) Sensitivity: 63.6%	(*97*)
Prospective study Patients with secondary syphilis: 68 Patients with early latent syphilis: 72 Reference standard: (1) Secondary syphilis—based on clinical features consistent with secondary syphilis (lab confirmation and clinical features not reported in the paper); (2) early latent syphilis—reactive antitreponemal EIA, TPPA, or antitreponemal IgM EIA in the absence of clinical signs of infection in patients who had had nonreactive serology within the preceding 2 years or were known to have had recent sexual contact with an individual infected with syphilis.	Secondary syphilis (n = 68) Sensitivity: 100% Early latent syphilis (n = 72) Sensitivity: 100%	(*99*)

	Treponemal serologic tests		
ADVIA Centaur	Prospective cross-sectional study Patients with primary syphilis: 55 Patients with secondary syphilis: 98 Patients with early latent syphilis: 41 Patients with late latent syphilis: 68 Reference standard for primary syphilis: Presence of a lesion or chancre with visible spirochetes on darkfield microscopy or the absence of spirochetes on darkfield microscopy plus reactive treponemal and nontreponemal serologic tests Reference standard for secondary syphilis: Mucocutaneous lesions with reactive treponemal (EIA or TPPA) and nontreponemal (RPR) serologic tests Reference standard for early latent syphilis: Absence of symptoms plus reactive treponemal and nontreponemal serologic tests or two reactive treponemal serologic tests and no history of prior syphilis or prior sexual contact with an individual with early syphilis within the past 12 months or prior nonreactive serology within the past 12 months Reference standard for late latent syphilis: Absence of symptoms plus reactive treponemal (EIA or TPPA) and nontreponemal (RPR) serologic tests or two reactive treponemal serologic tests, no history of prior syphilis, no serologic test results on the past 12 months, and no	Overall sensitivity (N = 262): 97.3% (95% CI: 94.6%– 98.9%) Overall specificity (N = 403): 95.5% (95% CI: 93%–97.3%) Primary syphilis (n = 55) Sensitivity: 94.5% (95% CI: 84.9%–98.9%) Secondary syphilis (n = 98) Sensitivity: 100% (95% CI: 96.2%–100%) Early latent syphilis (n = 41) Sensitivity: 100% (95% CI: 90.7%–100%) Late latent syphilis (n = 68) Sensitivity: 94.1% (95% CI: 85.6%–98.4%)	(*83*)

	sexual contact with an individual with early latent syphilis in the past 12 months		
Architect Syphilis TP	Prospective and retrospective cross-sectional clinical trial study for submission to FDA Patient samples collected from intended use population: 1145 Preselected patient samples reactive in treponemal serologic tests: 406 (including 20 pregnant women) Apparently healthy individuals: 480 Patients with primary treated syphilis: 44 Patients with primary untreated syphilis: 25 Patients with secondary treated syphilis: 29 Patients with secondary untreated syphilis: 27 Patients with latent treated syphilis: 25 Patients with latent untreated syphilis: 29 Reference standard: Chemiluminescent immunoassay, RPR, and TPPA. Two out of three tests must be reactive for a sample to be considered reactive Stage of syphilis determined by a licensed physician based on the clinical symptoms, medical history, and laboratory test results at the time of diagnosis	Samples from intended use population (N = 1145) PPA: 96.2% (95% CI: 92%–98.3%) PNA: 99% (95% CI: 98.1%–99.4%) Preselected patient samples (N = 406) Patients with reactive serology for syphilis (n = 386) PPA: 98.9% (95% CI: 97.2%–99.6%) PNA: 92.3% (95% CI: 75.9%–97.9%) Pregnant women with reactive serology for syphilis (n = 20) PPA: 100% (95% CI: 83.9%–100%) PNA: Not applicable Clinically diagnosed syphilis patients (N = 179) Primary treated (n = 44): 75% agreement Primary untreated (n = 25): 100% agreement Secondary treated (n = 29): 100% agreement Secondary untreated (n = 27): 100% agreement Latent treated (n = 25): 100% agreement Latent untreated (n = 25): 100% agreement All phases treated (n = 29): 100% agreement	(*197*)[§]
AtheNA Multi-Lyte *T. pallidum* IgG Plus Test System	Retrospective cross-sectional clinical trial study for submission to the FDA Patient serum samples: 280 Previously characterized serum samples by syphilis stage	Patient serum samples (N = 280) PPA: 96.3% (95% CI: 81%–99.9%) PNA: 96% (95% CI: 92.8%–98.1%) Primary treated (n = 11): 90.9% agreement (95% CI: 58.7%–99.8%)	(*198*)[§]

	Primary treated syphilis: 11 Secondary treated syphilis: 39 Secondary untreated syphilis: 43 Latent treated syphilis: 52 Latent untreated syphilis: 11 Congenital syphilis: 3 Reference standard for patient serum samples: Reactive RPR and TPPA Reference standard for clinically characterized serum sample: CDC specimen bank	Secondary treated (n = 39): 100% agreement (95% CI: 92.6%–100%) Secondary untreated (n = 43): 93% agreement (95% CI: 80.8%–98.5%) Latent treated (n = 52): 86.5% agreement (95% CI: 74.2%–94.4%) Latent untreated (n = 11): 54.5% agreement (95% CI: 23.4%–83.3%) Congenital syphilis (n = 3): 66.7% agreement (95% CI: 9.4%–99.2%)	
Bioplex 2200 Syphilis IgG	Prospective cross-sectional study Patients with primary syphilis: 55 Patients with secondary syphilis: 98 Patients with early latent syphilis: 41 Patients with late latent syphilis: 68 Reference standard for primary syphilis: Presence of a lesion or chancre with visible spirochetes on darkfield microscopy or the absence of spirochetes on darkfield microscopy plus reactive treponemal and nontreponemal serologic tests Reference standard for secondary syphilis: Mucocutaneous lesions with reactive treponemal and nontreponemal serologic tests Reference standard for early latent syphilis: Absence of symptoms plus reactive treponemal and nontreponemal	Overall sensitivity (N = 262): 96.9% (95% CI: 94.1%–98.7%) Overall specificity (N = 403): 96.7% (95% CI: 94.4%–98.2%) Primary syphilis (n = 55) Sensitivity: 96.4% (95% CI: 94.5%–98.2%) Secondary syphilis (n = 98) Sensitivity: 100% (95% CI: 96.2%–100%) Early latent syphilis (n = 41) Sensitivity: 95.1% (95% CI: 83.8%–99.4%) Late latent syphilis (n = 68) Sensitivity: 94.1% (95% CI: 85.6%–98.4%)	(*83*)

	serologic tests or two reactive treponemal serologic tests and no history of prior syphilis or prior sexual contact with an individual with early syphilis within the past 12 months or prior nonreactive serology within the past 12 months Reference standard for late latent syphilis: Absence of symptoms plus reactive treponemal and nontreponemal serologic tests or two reactive treponemal serologic tests, no history of prior syphilis, no serologic test results on the past 12 months, and no sexual contact with an individual with early latent syphilis in the past 12 months		
CAPTIA Syphilis-G Assay	Cross-sectional study Unselected screening specimens: 1,617 Known specimen panel: 114 Reference standard: VDRL reactive	Unselected screening specimens (N = 1,617) Sensitivity: 92.1% Specificity: 99.2% Retesting of unselected screening specimens Sensitivity: 92.1% Specificity: 99.2% Primary treated (n = 8): 100% agreement Primary untreated (n = 6): 100% agreement Secondary treated (n = 23): 95.7% agreement Secondary untreated (n = 3): 100% agreement Early latent treated (n = 11): 90.9% agreement Early latent untreated (n = 4): 100% agreement Late latent treated (n = 19): 94.7% agreement Late latent untreated (n = 13): 92.3% agreement Neurosyphilis treated (n = 5): 100% agreement Neurosyphilis untreated (n = 5): 100% agreement Cardiovascular syphilis treated (n = 1): 100% agreement	(*87*)

	Congenital syphilis treated (n = 1): 100% agreement Unknown syphilis stage treated (n = 2): 100% agreement Unknown treatment status (n = 13): 84.6% agreement	
Cross-sectional study Unselected screening specimens: 1,184 Known specimen panel: 101 (89 were classified as primary, secondary, early latent, or late latent) Unselected screening serum samples reference standard: ICE Syphilis immunoassay (DiaSorin Molecular LLC), CDRL, TPHA, and FTA-ABS Clinical stage reference standard: Medical diagnosis and syphilis serology. Early latent and late latent cutoff was at two years, not one year	Unselected screening specimens (N = 1,184) Sensitivity: 91.4% Retesting of unselected screening specimens Sensitivity: 92.4% Known specimen panel classified as primary, secondary, early latent, and late latent (N = 89) Primary treated (n = 17): 88.2% agreement Primary untreated (n = 7): 100% agreement Secondary treated (n = 21): 90.5% agreement Secondary untreated (n = 2): 100% agreement Early latent treated (n = 9): 88.9% agreement Early latent untreated (n = 2): 100% agreement Late latent treated (n = 19): 100% agreement Late latent untreated (n = 12): 91.7% agreement	(*88*)
Retrospective cross-sectional study Patients with untreated syphilis: 96 Patients with old syphilis: 63 Neonatal serum samples from mothers treated for syphilis: 10 Reference standard: Reactive MHA-TA, FTA-ABS, and chart review for clinical characterization	Patient serum samples (N = 169) Primary syphilis (n = 17) Sensitivity: 82.3% Secondary syphilis (n = 13) Sensitivity: 100% Early latent syphilis (n = 14) Sensitivity: 100%	(*104*)

		Late latent syphilis (n = 33) Sensitivity: 100% Neurosyphilis (n = 3) Sensitivity: 100% Congenital syphilis (n = 1) Sensitivity: 100% Reinfection (n = 15) Sensitivity: 100% Patients with old syphilis (n = 63) Sensitivity: 100% Neonatal serum from mothers treated for syphilis (n = 10) Sensitivity: 100%	
Elecsys Syphilis	Prospective and retrospective cross-sectional clinical trial study for submission to FDA Patient samples collected from intended use population: 2,282 (including 1,524 routine syphilis, 457 patients living with HIV, and 301 pregnant women) Preselected patient samples reactive in treponemal serologic tests: 169 (including 15 pregnant women) Apparently healthy individuals: 209 Patients with primary treated syphilis: 29 Patients with primary untreated syphilis: 25 Patients with secondary treated syphilis: 25 Patients with secondary untreated syphilis: 25	Samples from intended use population (N = 2,282) Overall PPA: 100% (95% CI: 98.4%–100%) Overall PNA: 99.2% (95% CI: 98.7%–99.5%) Routine syphilis (N = 1,524) PPA: 100% (95% CI: 94.6%–100%) PNA: 99.8% (95% CI: 99.4%–100%) Patients living with HIV (N = 457) PPA: 100% (95% CI: 97.8%–100%) PNA: 95.6% (95% CI: 92.6%–97.6%) Pregnant women (N = 301) PPA: Not applicable	(*199*)[§]

	Patients with latent treated syphilis: 25 Patients with latent untreated syphilis: 25 Reference standard: Chemiluminescent immunoassay, RPR, and TPPA. Two out of three tests must be reactive for a sample to be considered reactive Stage of syphilis determined by a licensed physician based on clinical symptoms, medical history, and laboratory test results at the time of diagnosis	PNA: 100% (95% CI: 98.8%–100%) Preselected patient samples (N =169) PPA: 98.7% (95% CI: 95.5%–99.9%) PNA: 100% (95% CI: 73.5%–99.6%) Clinically diagnosed syphilis patients (N = 154) Primary treated (n = 29): 55.2% agreement Primary untreated (n = 25): 100% agreement Secondary treated (n = 25): 96% agreement Secondary untreated (n = 25): 100% agreement Latent treated (n = 25): 100% agreement Latent untreated (n = 25): 100% agreement	
Fluorescent Treponemal Antibody-Absorption Test (FTA-ABS)	Prospective cross-sectional study Patients with primary syphilis: 55 Patients with secondary syphilis: 98 Patients with early latent syphilis: 41 Patients with late latent syphilis: 68 Reference standard for primary syphilis: Presence of a lesion or chancre with visible spirochetes on darkfield microscopy or the absence of spirochetes on darkfield microscopy (or if darkfield microscopy is not performed) plus reactive treponemal and nontreponemal serologic tests Reference standard for secondary syphilis: Mucocutaneous lesions with reactive treponemal (EIA or TPPA) and nontreponemal (RPR) serologic tests	Overall sensitivity (N = 262): 90.8% (95% CI: 86.7%–94%) Overall specificity (N = 403): 98% (95% CI: 96.1%–99.1%) Primary syphilis (n = 55) Sensitivity: 78.2% (95% CI: 65%–88.2%) Secondary syphilis (n = 98) Sensitivity: 92.8% (95% CI: 85.7%–97%) Early latent syphilis (n = 41) Sensitivity: 100% (95% CI: 90.7%–100%) Late latent syphilis (n = 68) Sensitivity: 92.6% (95% CI: 83.7%–97.6%)	(*83*)

Reference standard for early latent syphilis: Absence of symptoms plus reactive treponemal (EIA or TPPA) and nontreponemal (RPR) serologic tests or two reactive treponemal serologic tests and no history of prior syphilis or prior sexual contact with an individual with early syphilis within the past 12 months or prior nonreactive serology within the past 12 months Reference standard for late latent syphilis: Absence of symptoms plus reactive treponemal (EIA or TPPA) and nontreponemal (RPR) serologic tests or two reactive treponemal serologic tests, no history of prior syphilis, no serologic test results on the past 12 months, and no sexual contact with an individual with early latent syphilis in the past 12 months Reference standard for specificity (no syphilis): No diagnosis of syphilis on the day of testing or in the 6 months after the day of specimen collection, no syphilis in the past medical history, no reactive prior syphilis serology (all available lab records reviewed), and at least 4 out of 7 treponemal serologic tests were negative (after testing by CDC reference laboratory)		
Retrospective cross-sectional study Patients with primary syphilis: 50 Patients with secondary syphilis: 43 Patients with latent syphilis: 47 Patients with neurosyphilis: 11 Reference standard for primary syphilis: Presence of a lesion or chancre plus presence of spirochetes in lesion	Primary syphilis (n = 50) Sensitivity: 90% Secondary syphilis (n = 43) Sensitivity: 100% Latent syphilis (n = 47) Sensitivity: 100% Results for neurosyphilis presented in Table 4	(*85*)

or lymph node (method to visualize spirochetes was not described) and/or reactive serologic tests Reference standard for secondary syphilis: Presence of spirochetes in generalized skin lesions or lymph node (method to visualize spirochetes was not described) and/or reactive serologic tests Reference standard for latent syphilis: Absence of symptoms or a history of syphilis plus reactive serologic tests Reference standard for neurosyphilis: Reactive FTA or TPHA plus reactive CSF VDRL or mononuclear cell count of >5 cell per µl of CSF		
Retrospective cross-sectional study Patients with primary syphilis: 55 Patients with secondary syphilis: 39 Patients with latent syphilis: 54 Patients with yaws: 15 Reference standard for new and old syphilis: Prior clinical diagnosis of syphilis	Primary syphilis (n = 55) Sensitivity: 84% Secondary syphilis (n = 39) Sensitivity: 100% Latent syphilis (n = 54) Sensitivity: 100% Yaws (n = 15) Sensitivity: 93%	(*90*)
Prospective cross-sectional study Patients with primary syphilis: 63 Patients with secondary syphilis: 3	Primary and secondary syphilis combined (n = 66) Sensitivity: 93% Specificity: 87%	(*109*)

	Reference standard for new and old syphilis: Presence of a lesion or chancre with visible spirochetes on darkfield microscopy and/or reactive serologic tests or a four-fold increase in a quantitative RPR		
Immulite 2000 Syphilis Screen	Prospective cross-sectional clinical trial study for submission to FDA Patient samples collected from intended use population: 1,286 (including 281 from patients medically diagnosed with syphilis of unknown stage, 420 patients living with HIV, and 924 samples submitted to laboratories for routine syphilis testing; some samples might overlap categories) Reference standard: Results compared with a commercially available assay	Retrospective serum samples (N = 1,286) Medically diagnosed syphilis of unknown stage (n = 281) PPA: 99.3% (95% CI: 97.4%–99.9%) PNA: 75% (95% CI: 34.9%–96.8%) Patients living with HIV (N = 420) PPA: 99.6% (95% CI: 97.9%–100%) PNA: 95.6% (95% CI: 91.1%–98.2%) Routine syphilis testing (N = 924) PPA: 99.4% (95% CI: 98%–99.9%) PNA: 99.1% (95% CI: 97.9%–99.7%)	(*200*)[§]
LIAISON	Prospective cross-sectional study Patients with primary syphilis: 55 Patients with secondary syphilis: 98 Patients with early latent syphilis: 41 Patients with late latent syphilis: 68 Reference standard for primary syphilis: Presence of a lesion or chancre with visible spirochetes on darkfield microscopy or the absence of spirochetes on darkfield microscopy plus reactive treponemal and nontreponemal serologic tests	Overall sensitivity (N = 262): 96.9% (95% CI: 94.1%– 98.7%) Overall specificity (N = 403): 94.5% (95% CI: 91.8%–96.5%) Primary syphilis (n = 55) Sensitivity: 96.4% (95% CI: 94.5%–98.2%) Secondary syphilis (n = 98) Sensitivity: 100% (95% CI: 96.2%–100%) Early latent syphilis (n = 41)	(*83*)

		Sensitivity: 97.6% (95% CI: 87.4%–99.9%)	
	Reference standard for secondary syphilis: Mucocutaneous lesions with reactive treponemal and nontreponemal serologic tests	Late latent syphilis (n = 68) Sensitivity: 96.2% (95% CI: 83.7%–97.6%)	
	Reference standard for early latent syphilis: Absence of symptoms plus reactive treponemal and nontreponemal serologic tests or two reactive treponemal serologic tests and no history of prior syphilis or prior sexual contact with an individual with early syphilis within the past 12 months or prior nonreactive serology within the past 12 months		
	Reference standard for late latent syphilis: Absence of symptoms plus reactive treponemal and nontreponemal serologic tests or two reactive treponemal serologic tests, no history of prior syphilis, no serologic test results on the past 12 months, and no sexual contact with an individual with early latent syphilis in the past 12 months		
	Reference standard for specificity (no syphilis): No diagnosis of syphilis on the day of testing or in the 6 months after the day of specimen collection, no syphilis in the past medical history, no reactive prior syphilis serology (all available lab records reviewed), and at least 4 out of 7 treponemal serologic tests were negative (after testing by CDC reference laboratory)		
Lumipulse G TP-N	Prospective and retrospective cross-sectional clinical trial study for submission to FDA Patient samples collected from intended use population: 1,290	Samples from intended use population (N = 1,290) PPA: 92.7% (95% CI: 88.6%–95.4%) PNA: 99.6% (95% CI: 99%–99.9%)	(*201*)[§]

	Retrospective samples: 1,472 (including 379 pregnant women, 520 patients living with HIV, 130 samples known to be reactive in treponemal serologic tests, 68 samples from a research facility from patients clinically diagnosed with syphilis, and 375 samples submitted to laboratories for routine syphilis testing) Apparently healthy individuals: 474 Patients with primary treated syphilis: 2 Patients with primary untreated syphilis: 27 Patients with secondary treated syphilis: 25 Patients with secondary untreated syphilis: 30 Patients with latent treated syphilis: 5 Patients with latent untreated syphilis: 200 Reference standard: Treponemal EIA, RPR, and TPPA. Two out of three tests must be reactive for a sample to be considered reactive Stage of syphilis determined by a licensed physician based on clinical symptoms, medical history, and laboratory test results at the time of diagnosis	Retrospective serum samples (N = 1,472) Pregnant women (N = 379) PPA: 96.8% (95% CI: 91.1%–98.9%) PNA: 96.8% (95% CI: 94.1%–98.3%) Patients living with HIV (N = 520) PPA: 90.3% (95% CI: 85.9%–93.4%) PNA: 97.5% (95% CI: 95%–98.8%) Reactive by previous laboratory testing (n = 130) PPA: 99.2% (95% CI: 94.6%–99.8%) PNA: 100% (95% CI: 67.6%–100%) Routine syphilis (N = 375) PPA: 91.2% (95% CI: 77%–97%) PNA: 99.7% (95% CI: 98.4%–99.9%) Medically diagnosed syphilis of unknown stage (N = 68) PPA: 98.2% (95% CI: 90.6%–99.7%) PNA: 91.7% (95% CI: 64.6%–98.5%) Clinically diagnosed syphilis patients (N = 289) Primary treated (n = 2): 100% agreement Primary untreated (n = 27): 100% agreement Secondary treated (n = 25): 100% agreement Secondary untreated (n = 30): 100% agreement Latent treated (n = 5): 100% agreement Latent untreated (n = 200): 91.5% agreement	
Microhemagglutination Assay for Antibodies	Cross-sectional study Patients with primary syphilis: 109	Sensitivity: 72.5%	(*70*)

to *Treponema pallidum* (MHA-TP)	Reference standard: Darkfield microscopy		
	Prospective cross-sectional study Patient serum samples: 510 (including 128 from patients with primary syphilis, 243 with secondary syphilis, and 139 with early latent syphilis) Reference standard: Darkfield microscopy, RPR, FTA-ABS	Primary syphilis (n = 128) Sensitivity: 88.6% Secondary syphilis (n = 243) Sensitivity: 98.8% Early latent syphilis (n = 139) Sensitivity: 100%	(*81*)
	Retrospective cross-sectional study Serum from patients with syphilis: 328 (including 78 from patients with primary syphilis, 89 with secondary syphilis, 103 with early latent syphilis, 10 from neurosyphilis, 21 from cardiovascular syphilis, and 25 from patients with old syphilis) Reference standard: Hemagglutination treponemal test for syphilis, MHA-TP, FTA-ABS, and VDRL. Darkfield microscopy.	Primary syphilis (n = 78) Sensitivity: 88.6% Secondary syphilis (n = 89) Sensitivity: 100% Early latent syphilis (n = 103) Sensitivity: 99% Cardiovascular syphilis (n = 21) Sensitivity: 89.5% Old syphilis (n = 25) Sensitivity: 100% Results for neurosyphilis presented in Table 4	(*82*)
	Retrospective cross-sectional study Serum from patients with syphilis: 75 (including 24 from patients with primary syphilis, 20 with secondary	Primary syphilis (n = 24) Sensitivity: 45.9% Secondary syphilis (n = 20)	(*91*)

	syphilis, 27 with latent syphilis, 3 from neurosyphilis, and 1 from cardiovascular syphilis) Serum from patients without syphilis: 222 Reference standard: FTA-ABS	Sensitivity: 90% Latent syphilis (n = 31) Sensitivity: 90.3% Cardiovascular syphilis (n = 1) Sensitivity: 100% Results for neurosyphilis presented in Table 4	
	Retrospective cross-sectional study Serum from patients with syphilis based on clinical history and laboratory findings: 312 (including 63 from patients with primary syphilis, 43 with secondary syphilis, 53 with early latent syphilis, 87 with late latent syphilis, and 66 from late symptomatic syphilis) Reference standard: VDRL, FTA-ABS, MHA-TP, and *T. pallidum* immobilization (TPI) test	Primary syphilis (n = 63) Percent reactive: MHA-TP 64%, VDRL 73%, FTA-ABS 82%, and TPI 67% Secondary syphilis (n = 43) Percent reactive: MHA-TP 96%, VDRL 100%, FTA-ABS 100%, and TPI 100% Early latent syphilis (n = 53) Percent reactive: MHA-TP 96%, VDRL 100%, FTA-ABS 98%, and TPI 96% Late latent syphilis (n = 87) Percent reactive: MHA-TP 97%, VDRL 93%, FTA-ABS 98%, and TPI 97% Early symptomatic syphilis (n = 66) Percent reactive: MHA-TP 98%, VDRL 94%, FTA-ABS 100%, and TPI 98%	(*86*)
Treponema pallidum Passive Particle	Prospective cross-sectional study Patients with primary syphilis: 55 Patients with secondary syphilis: 98	Overall sensitivity (N = 262): 95.4% (95% CI: 92.1%–97.6%) Overall specificity (N = 403): 100% (95% CI: 99%–100%)	(*83*)

Agglutination (TPPA)	Patients with early latent syphilis: 41 Patients with late latent syphilis: 68 Reference standard for primary syphilis: Presence of a lesion or chancre with visible spirochetes on darkfield microscopy or the absence of spirochetes on darkfield microscopy plus reactive treponemal and nontreponemal serologic tests Reference standard for secondary syphilis: Mucocutaneous lesions with reactive treponemal and nontreponemal serologic tests Reference standard for early latent syphilis: Absence of symptoms plus reactive treponemal and nontreponemal serologic tests or two reactive treponemal serologic tests and no history of prior syphilis or prior sexual contact with an individual with early syphilis within the past 12 months or prior nonreactive serology within the past 12 months Reference standard for late latent syphilis: Absence of symptoms plus reactive treponemal and nontreponemal serologic tests or two reactive treponemal serologic tests, no history of prior syphilis, no serologic test results on the past 12 months, and no sexual contact with an individual with early syphilis in the past 12 months Reference standard for specificity (no syphilis): No diagnosis of syphilis on the day of testing or in the 6 months after the day of specimen collection, no syphilis in the past medical history, no reactive prior syphilis serology (all available lab records reviewed),	Primary syphilis (n = 55) Sensitivity: 94.5% (95% CI: 84.9%–98.9%) Secondary syphilis (n = 98) Sensitivity: 100% (95% CI: 96.2%–100%) Early latent syphilis (n = 41) Sensitivity: 100% (95% CI: 90.7%–100%) Late latent syphilis (n = 68) Sensitivity: 86.8% (95% CI: 76.4%–93.8%)

	and at least 4 out of 7 treponemal serologic tests were negative (after testing by CDC reference laboratory)		
	Prospective observational study Patients with primary syphilis: 50 Patients with secondary syphilis: 26 Patients with early latent syphilis: 8 Patients with late latent syphilis: 21 Reference standard for primary syphilis: Presence of a lesion or chancre with visible spirochetes and reactive serologic tests Reference standard for secondary syphilis: Mucocutaneous lesions and reactive serologic tests Reference standard for early latent syphilis: Reactive serologic tests and nonreactive serologic test in the past 2 years Reference standard for late latent syphilis: Reactive serologic tests and nonreactive serologic test in the past 2 years or no serologic tests within the past 2 years	Primary syphilis (n = 50) Sensitivity: 96% Secondary syphilis (n = 26) Sensitivity: 100% Early latent syphilis (n = 8) Sensitivity: 100% Late latent syphilis (n = 21) Sensitivity: 100%	(*84*)

	Prospective cross-sectional study Patients with primary syphilis: 39 Patients with secondary syphilis: 20 Patients with early latent syphilis: 18 Patients with late latent syphilis: 58 Reference standard for primary syphilis: Presence of a lesion or chancre and reactive serologic tests Reference standard for secondary syphilis: Mucocutaneous lesions and reactive serologic tests Reference standard for early latent syphilis: no symptoms or signs together with reactive syphilis serology results and nonreactive syphilis serology results within past 12 months Reference standard for late latent syphilis: no symptoms or signs together with reactive syphilis serology results and no nonreactive syphilis serology results within the past 12 months.	Primary syphilis (n = 39) TPPA sensitivity: 94.9% (95% CI: 83.1%–98.6%) FTA-ABS sensitivity: 84.6% (95% CI: 70.3%–92.8%) Secondary syphilis (n = 20) TPPA sensitivity: 100% (95% CI: 83.9%–100%) FTA-ABS sensitivity: 95% (95% CI: 76.4%–99.1%) Early latent syphilis (n = 18) TPPA sensitivity: 94.4% (95% CI: 74.2%–99.0%) FTA-ABS sensitivity: 94.4% (95% CI: 74.2%–99.0%) Late latent syphilis (n = 58) TPPA sensitivity: 91.4% (95% CI: 81.4%–96.3%) FTA-ABS sensitivity: 84.5% (95% CI: 73.1%–91.6%) Specificity: 100% (95% CI: 91.8%–100%) for all tests	(*92*)
Trep-Sure	Prospective cross-sectional study Patients with primary syphilis: 55 Patients with secondary syphilis: 98 Patients with early latent syphilis: 41 Patients with late latent syphilis: 68	Overall sensitivity (N = 262): 98.5% (95% CI: 96.1%–99.6%) Overall specificity (N = 403): 82.6% (95% CI: 78.4%–86.1%) Primary syphilis (n = 55) Sensitivity: 94.5% (95% CI: 84.9%–98.9%)	(*83*)

	Reference standard for primary syphilis: Presence of a lesion or chancre with visible spirochetes on darkfield microscopy or the absence of spirochetes on darkfield microscopy plus reactive treponemal and nontreponemal serologic tests Reference standard for secondary syphilis: Mucocutaneous lesions with reactive treponemal and nontreponemal serologic tests Reference standard for early latent syphilis: Absence of symptoms plus reactive treponemal and nontreponemal serologic tests or two reactive treponemal serologic tests and no history of prior syphilis or prior sexual contact with an individual with early syphilis within the past 12 months or prior nonreactive serology within the past 12 months Reference standard for late latent syphilis: Absence of symptoms plus reactive treponemal and nontreponemal serologic tests or two reactive treponemal serologic tests, no history of prior syphilis, no serologic test results on the past 12 months, and no sexual contact with an individual with early syphilis in the past 12 months	Secondary syphilis (n = 98) Sensitivity: 100% (95% CI: 96.2%–100%) Early latent syphilis (n = 41) Sensitivity: 100% (95% CI: 90.7%–100%) Late latent syphilis (n = 68) Sensitivity: 98.5% (95% CI: 92.1%–99.9%)	
	Retrospective cross-sectional study Patients with primary syphilis: 52 Reference standard for primary syphilis: Presence of a lesion or chancre, reactive serologic tests, and no reported history of syphilis	Primary syphilis (n = 52) Trep-Sure sensitivity: 53.8% (95% CI: 39.5%–67.8%) RPR sensitivity: 76.9% (95% CI: 63.2%–87.5%)	(*89*)

Zeus Scientific *T. pallidum* IgG Test System	Prospective and retrospective cross-sectional clinical trial study for submission to FDA Specimens submitted for routine syphilis testing: 500 Specimens from pregnant women submitted for routine syphilis testing: 500 Unselected specimens from hospitalized patients: 1,000 Retrospective specimens from patients living with HIV: 223 Retrospective specimens known to be reactive to RPR and TPPA: 280 Retrospective specimens from pregnant persons known to have been previously tested by RPR and TPPA: 250 nonreactive both tests and 27 reactive both tests CDC specimen panel: 157 (clinically staged) Reference standard: Phoenix Bio-Tech Syphilis Trep-Check Test	Specimens submitted for routine syphilis testing (N = 500) PPA: 80% (95% CI: 28.4%–99.5%) PNA: 99.2% (95% CI: 97.9%–99.8%) Specimens from pregnant women submitted for routine syphilis testing (N = 500) PPA: 75% (95% CI: 19.4%–99.4%) PNA: 100% (95% CI: 99.4%–100%) Unselected specimens from hospitalized patients (N = 1,000) PPA: 61.9% (95% CI: 38.4%–81.9%) PNA: 97.1% (95% CI: 95.9%–98.1%) Retrospective specimens from patients living with HIV (N = 223) PPA: 85.4% (95% CI: 72.2%–93.9%) PNA: 99.4% (95% CI: 96.9%–100%) Retrospective specimens known to be reactive to RPR and TPPA (N = 280) PPA: 98.5% (95% CI: 96.2%–99.6%) PNA: 70.6% (95% CI: 46.9%–98.7%) Retrospective specimens from pregnant persons known to have been previously tested by RPR and TPPA (n = 250 nonreactive both tests and N=27 reactive both tests) PPA: 92.9% (95% CI: 76.5%–99.1%) PNA: 99.6% (95% CI: 97.8%–100%)	(*202*)[§]

		CDC specimen panel (N = 157) Primary treated (n = 11): 100% agreement (95% CI: 76.2%–100%) Secondary treated (n = 39): 100% agreement (95% CI: 92.6%–100%) Secondary untreated (n = 43): 95.3% agreement (95% CI: 84.2%–99.4%) Latent treated (n = 50): 96% agreement (95% CI: 86.3%–99.5%) Latent untreated (n = 11): 54.5% agreement (95% CI: 23.4%–83.3%) Congenital syphilis (n = 3): 33.3% agreement (95% CI: 0.84%–90.6%) Late latent untreated (n = 12): 91.7% agreement	
Combined nontreponemal and treponemal serologic assays			
BioPlex 2200 Syphilis Total & RPR	Prospective and retrospective cross-sectional clinical trial study for submission to FDA Prospective samples: 1,001 (including 401 samples submitted for syphilis testing, 295 from pregnant women, and 305 patients living with HIV) Retrospective samples: 546 (including 412 reactive by RPR and treponemal serologic test, 32 syphilis-positive pregnant women, 45 pregnant women with a history of STD infection, and 57 HIV/syphilis dual-positive patients) Apparently healthy individuals: 301 Clinically diagnosed patients: 156	BioPlex Total testing of prospective samples compared two of three tests being reactive (N = 1,001) PPA: 92.5% (95% CI: 87.3%–95.6%) PNA: 97.9% (95% CI: 96.7%–98.6%) BioPlex RPR component testing of prospective samples compared with BD Macro-Vue RPR Card Tests (N = 1,001) PPA: 81.5% (95% CI: 72.4%–88.1%) PNA: 96.5% (95% CI: 95.1%–97.5%) BioPlex Total testing of retrospective samples compared two of three tests being reactive (n = 546) PPA: 99.6% (95% CI: 98.5%–99.9%)	(*203*)[§]

	Reference standard: Treponemal IgG/IgM assay, a nontreponemal serologic test, and TPPA. Two out of three tests must be reactive for a sample to be considered reactive. Bioplex 2200 RPR results compared with BD Macro-Vue RPR card Tests. Stage of syphilis determined by a licensed physician based on clinical symptoms, medical history, and laboratory test results at the time of diagnosis	PNA: 100% (95% CI: 93.6%–100%) BioPlex RPR component testing of retrospective samples compared with BD Macro-Vue RPR Card Tests (n = 546) PPA: 98.1% (95% CI: 96.4%–99.1%) PNA: 80.7% (95% CI: 72.5%–86.9%) BioPlex Total testing of samples pregnant women compared two of three tests being reactive (n = 372) PPA: 100% (95% CI: 89.3%–100%) PNA: 98.8% (95% CI: 97%–99.5%) BioPlex RPR component testing of samples pregnant women compared with BD Macro-Vue RPR Card Tests (n = 372) PPA: 100% (95% CI: 86.7%–100%) PNA: 98.3% (95% CI: 96.3%–99.2%) BioPlex Total testing of samples from patients living with HIV compared two of three tests being reactive (n = 362) PPA: 93.3% (95% CI: 88.2%–96.3%) PNA: 93.9% (95% CI: 89.8%–96.4%) BioPlex RPR component testing of samples from patients living with HIV compared with BD Macro-Vue RPR Card Tests (N=362) PPA: 85.7% (95% CI: 72.2%–93.3%) PNA: 90.6% (95% CI: 86.9%–93.4%)

		BioPlex Total reactivity compared two of three tests being reactive in medically diagnosed syphilis patients (n = 156) Primary treated (n = 29): BioPlex Total reactivity 86.2%; comparator algorithm reactivity 86.2% Primary untreated (n = 26): BioPlex Total reactivity 96.2%; comparator algorithm reactivity 100% Secondary treated (n = 26): BioPlex Total reactivity 100%; comparator algorithm reactivity 100% Secondary untreated (n = 25): BioPlex Total reactivity 100%; comparator algorithm reactivity 100% Latent treated (n = 27): BioPlex Total reactivity 100%; comparator algorithm reactivity 100% Latent untreated (n = 23): BioPlex Total reactivity 100%; comparator algorithm reactivity 100% All phases treated (n = 82): BioPlex Total reactivity 95.1%; comparator algorithm reactivity 95.1% All phases untreated (n = 74): BioPlex Total reactivity 98.6%; comparator algorithm reactivity 100% BioPlex Total testing of samples from apparently healthy individuals compared two of three tests being reactive (n = 301) PPA: 75% (95% CI: 30.1%–95.5%) PNA: 99% (95% CI: 97.1%–95.7%)

		BioPlex RPR component testing of samples from apparently healthy individuals compared with BD Macro-Vue RPR Card Tests (N = 301) PPA: 0% (95% CI: 0%–49%) PNA: 98% (95% CI: 95.7%–99.1%) BioPlex RPR reactivity compared with BD Macro-Vue RPR Card Tests in medically diagnosed syphilis patients (N = 156) Primary treated (n =29): BioPlex RPR reactivity 65.5%; RPR card reactivity 75.9% Primary untreated (n = 26): BioPlex RPR reactivity 92.3%; RPR card reactivity 88.5% Secondary treated (n = 26): BioPlex RPR reactivity 88.5%; RPR card reactivity 80.8% Secondary untreated (n = 25): BioPlex RPR reactivity 100%; RPR card reactivity 100% Latent treated (n = 27): BioPlex RPR reactivity 66.7%; RPR card reactivity 66.7% Latent untreated (n = 23): BioPlex RPR reactivity 95.7%; RPR card reactivity 95.7% All phases treated (n = 82): BioPlex RPR reactivity 73.2%; RPR card reactivity 74.4% All phases untreated (n = 74): BioPlex RPR reactivity 95.9%; RPR card reactivity 95%	
Point-of-care syphilis tests			
Syphilis Health Check Treponemal Antibody Test	Prospective cross-sectional study Patients enrolled: 562 Specimens tested with Syphilis Health Check: fingerstick whole blood and serum	Reactive by RPR and Trep-Sure: 7 Reactive by Trep-Sure: 16 Reactive by Syphilis Health Check using fingerstick whole blood: 31 Reactive by Syphilis Health Check using serum: 18	(*185*)

Stage of syphilis was not determined Reference standard: RPR and Trep-Sure EIA	Syphilis Health Check (fingerstick whole blood) versus RPR and Trep-Sure (N = 562) Sensitivity: 100% (95% CI 59.0%–100%) Specificity: 95.7% (95% CI 93.6%–97.2%) Syphilis Health Check (fingerstick whole blood) versus Trep-Sure (N = 562) Sensitivity: 50.0% (95% CI 24.7%–75.4%) Specificity: 95.9% (95% CI 93.8%–97.4%) Syphilis Health Check (serum) versus RPR and Trep-Sure (N = 562) Sensitivity: 100% (95% CI 59.0%–100%) Specificity: 98.0% (95% CI 96.5%–99.2%) Syphilis Health Check (serum) versus Trep-Sure (N = 562) Sensitivity: 43.8% (95% CI 19.8%–70.1%) Specificity: 98.0% (95% CI 96.4%–98.9%)	
Prospective cross-sectional study Patients enrolled: 202 Stage of syphilis was determined for 6 patients Reference standard: Trep-Sure EIA RPR performed but not included as a comparator test	Nonreactive by all tests: 171 Reactive by RPR: 10 Reactive by Trep-Sure: 10 Reactive by Syphilis Health Check: 26 Primary syphilis: 1 Secondary syphilis: 3 Early latent syphilis: 1 Previously treated syphilis: 1 Syphilis Health Check versus Trep-Sure (N = 202)	(*186*)

	Sensitivity: 71.4% (95% CI 41.9%–95.1%) Specificity: 91.5% (95% CI 87.5%–95.5%)	
Observational study Patients enrolled: 690 Stage of syphilis was determined for 10 patients Clinical data, including the stage of syphilis, was extracted from the medical record. The criteria used to stage syphilis was not reported in the paper. Reference standard: TPPA and RPR	Nonreactive by all tests: 671 Reactive by TPPA and RPR: 10 Reactive by Syphilis Health Check: 9 Primary syphilis: 0 Secondary syphilis: 1 Early latent syphilis: 2 Late latent syphilis: 3 Neurosyphilis: 2 Unspecified stage: 1 Previously treated syphilis: 1 Syphilis Health Check versus TPPA and RPR (N = 690) Sensitivity: 90.0% (95% CI 55.5%–99.8%) Specificity: 98.5% (95% CI 97.3%–99.3%)	(*187*)
Prospective cross-sectional study Patients enrolled: 965 Stage of syphilis was not determined Reference standard: TPPA and RPR	Syphilis Health Check versus TPPA and RPR (N = 965) Sensitivity: 76.9% (95% CI 46.2%–95.0%) Specificity: 99.0% (95% CI 98.1%–99.5%) Syphilis Health Check versus TPPA (N = 962; 3 patients excluded from the initial 965 because of a nonreactive RPR and indeterminate TPPA) Sensitivity: 50.0% (95% CI 29.9%–70.1%) Specificity: 99.4% (95% CI 98.6%–99.8%)	(*188*)
Retrospective study Patients enrolled: 1,406	Syphilis Health Check versus TPPA, EIA, CIA and, RPR (n = 1,237) Sensitivity: 95.7% (95% CI 93.6%–97.2%) Specificity: 93.2% (95% CI 91.0%–95.1%)	(*189*)

	Stage of syphilis was not determined Reference standard: TPPA, EIA, CIA, and RPR	Syphilis Health Check versus TPPA, EIA, and CIA (N = 1,406) Sensitivity: 88.7% (95% CI 86.2%–90.9%) Specificity: 93.1% (95% CI 91.0%–94.9%)	
DPP HIV-Syphilis Assay	Retrospective study Patients enrolled: 150 Stage of syphilis was not determined Reference standard: TPPA	DPP HIV-Syphilis Assay versus TPPA (N = 150) Sensitivity: 95.3% (95% CI 87.9%–98.5%) Specificity: 100% (95% CI 92.9%–100%)	(*190*)
	Retrospective study Patients enrolled: 450 Stage of syphilis was not determined Reference standard: TPPA	DPP HIV-Syphilis Assay versus TPPA (N = 450) Sensitivity: 100% (95% CI 97.6%–100%) Specificity: 98.7% (95% CI 96.6%–99.6%)	(*191*)

Abbreviations: FDA = Food and Drug Administration; PPA = percent positive agreement; PPN = percent negative agreement; CI = confidence interval; FTA-ABS = fluorescent treponemal antibody-absorption; VDRL = Venereal Disease Research Laboratory; MHA-TP = microhemagglutination assay for antibodies to *T. pallidum*; CSF = cerebral spinal fluid; TPPA = *T. pallidum* particle agglutination; TPHA = *T. pallidum* hemagglutination assay; EIA = enzyme immunoassay; RPR = rapid plasma reagin; IgG = immunoglobulin G; IgM = immunoglobulin M

*Information presented is a summary of studies used for these recommendations. They do not represent a compendium of all studies reviewed during the formulation of these recommendations. Additional tables of evidence detailing studies reviewed during the APHL meeting in 2017 can be viewed at (https://www.cdc.gov/std/syphilis/lab/testing/lab-recs-for-testing.htm).

[†]Performance characteristics are stratified by syphilis stage if available. Otherwise, the performance characteristics are derived from data that did not specify the stage of syphilis.

[§]Unpublished data from the FDA 510(k) Substantial Equivalence Determination Decision Summary.

TABLE 4. Performance characteristics of tests used to detect syphilis reactive antibodies in the cerebral spinal fluid*

Assay	Study summary and reference standard	Performance characteristics†	Reference
	Nontreponemal tests used to detect specific antibodies in the CSF		
Rapid Plasma Reagin (RPR)	Retrospective cross-sectional study Patients with neurosyphilis: 25 (24 patients were considered to have neurosyphilis, from which 8 had symptomatic neurosyphilis [disease meningovascular = 6; meningitis = 1; cranial neuritis = 1], 16 asymptomatic neurosyphilis [no neurologic symptoms or signs], and 1 patient with all clinical and laboratory criteria of neurosyphilis, except increased proteins; all 25 were living with HIV) Syphilis-positive control patients: 163 patients with syphilis based on serology and no signs of neurosyphilis Syphilis-negative control patients with other neurologic disorders: 126 Reference standard: Reactive FTA-ABS, increased CSF protein ≥45 mg/dL, and CSF pleocytosis ≥10 cell/mm^3	Combined data from asymptomatic and symptomatic neurosyphilis patients (N = 25) CSF RPR sensitivity: 75% CSF RPR specificity: 99.3% Asymptomatic neurosyphilis patients (n = 16) CSF RPR sensitivity: 68.8% Symptomatic neurosyphilis patients (n = 8) CSF RPR sensitivity: 100%	(*120*)
	Prospective cross-sectional study Patients with asymptomatic neurosyphilis: 56	Combined data from asymptomatic and symptomatic neurosyphilis patients (N = 210)	(*119*)

Patients with symptomatic neurosyphilis: 154 Asymptomatic neurosyphilis reference standard: ≥10 white blood cells in the CSF and reactive CSF TPPA with no blood contamination Symptomatic neurosyphilis reference standard: Reactive CSF TPPA with no blood contamination and with clinical signs and symptoms	CSF RPR sensitivity: 76.2% (95% CI: 70.2%–82.2%) CSF RPR specificity: 93.4% (95% CI: 91.4%–95.4%) CSF RPR-V† sensitivity: 79.2% (95% CI: 73.5%–85.5%) CSF RPR-V† specificity: 92.7% (95% CI: 90.7%–94.7%) Asymptomatic neurosyphilis patients (n = 56) CSF RPR sensitivity: 60.7% (95% CI: 50.7%–70.7%) CSF RPR specificity: 82.6% (95% CI: 80.6%–84.6%) CSF RPR-V† sensitivity: 69.6% (95% CI: 59.6%–79.6%) CSF RPR-V† specificity: 87.8% (95% CI: 79.8%–83.8%) Symptomatic neurosyphilis patients (n = 154) CSF RPR sensitivity: 81.8% (95% CI: 75.8%–87.8%) CSF RPR specificity: 90.2% (95% CI: 88.2%–92.2%) CSF RPR-V† sensitivity: 83.1% (95% CI: 77.1%–89.1%) CSF RPR-V† specificity: 89.1% (95% CI: 87.1%–91.1%)

	Retrospective cross-sectional study Patients with neurosyphilis: 149 Patients with symptomatic neurosyphilis: 33 Neurosyphilis reference standard: Reactive CSF FTA-ABS and >20 white blood cells in the CSF Symptomatic neurosyphilis reference standard: Vision or hearing loss with clinical or serologic evidence of neurosyphilis	Neurosyphilis patients (N = 149) CSF RPR sensitivity: 56.4% (95% CI: 40.8%–72%) CSF RPR specificity: 100% (95% CI: 100%–100%) CSF RPR-V† sensitivity: 59% (95% CI: 43.6%–74.4%) CSF RPR-V† specificity: 98.4% (95% CI: 95%–100%) Symptomatic neurosyphilis patients (n = 33) CSF RPR sensitivity: 51.5% (95% CI: 34.4%–68.6%) CSF RPR specificity: 89.7% (95% CI: 84.2%–95.2%) CSF RPR-V† sensitivity: 57.6% (95% CI: 40.7%–74.5%) CSF RPR-V† specificity: 84.5% (95% CI: 77.9%–91.1%)	(*118*)
Toluidine Red Unheated Serum Test (TRUST)	Prospective cross-sectional study Patients with asymptomatic neurosyphilis: 56 Patients with symptomatic neurosyphilis: 154 Asymptomatic neurosyphilis reference standard: ≥10 white blood cells in the CSF and reactive CSF TPPA with no blood contamination	Combined data from asymptomatic and symptomatic neurosyphilis patients (N = 210) CSF TRUST sensitivity: 76.2% (95% CI: 70.2%–82.2%) CSF TRUST specificity: 93.1% (95% CI: 91.1%–95.1%) Asymptomatic neurosyphilis patients (n = 56) CSF TRUST sensitivity: 58.9% (95% CI: 48.9%–68.9%)	(*119*)

	Case classification: Symptomatic neurosyphilis reference standard: Reactive CSF TPPA with no blood contamination and with clinical signs and symptoms	CSF TRUST specificity: 82.1% (95% CI: 80.1%–84.1%) Symptomatic neurosyphilis patients (n = 154) CSF TRUST sensitivity: 82.5% (95% CI: 76.5%–88.5%) CSF TRUST specificity: 90.1% (95% CI: 76.5%–88.5%)	
Venereal Disease Research Laboratory (VDRL)	Retrospective cross-sectional study Patients with neurosyphilis: 25 (24 patients were considered to have neurosyphilis, from which 8 had symptomatic neurosyphilis [disease meningovascular = 6; meningitis = 1; cranial neuritis =1], 16 asymptomatic neurosyphilis [no neurologic symptoms or signs], and 1 patient with all clinical and laboratory criteria of neurosyphilis, except increased proteins; all 25 were living with HIV) Syphilis positive control patients: 163 patients with syphilis based on serology and no signs of neurosyphilis Syphilis negative control patients with other neurologic disorders: 126 Reference standard: Reactive FTA-ABS, increased CSF protein ≥45 mg/dL, and CSF pleocytosis ≥10 cell/mm^3	Combined data from asymptomatic and symptomatic neurosyphilis patients (N = 25) CSF VDRL sensitivity: 70.8% CSF VDRL specificity: 99% Asymptomatic neurosyphilis patients (n = 16) CSF VDRL sensitivity: 62.5% Symptomatic neurosyphilis patients (n = 8) CSF VDRL sensitivity: 87.5%	(*120*)
	Prospective cross-sectional study	Combined data from asymptomatic and symptomatic neurosyphilis patients (N = 210)	(*119*)

	Patients with asymptomatic neurosyphilis: 56 Patients with symptomatic neurosyphilis: 154 Asymptomatic neurosyphilis reference standard: ≥10 white blood cells in the CSF and reactive CSF TPPA with no blood contamination Symptomatic neurosyphilis reference standard: Reactive CSF TPPA with no blood contamination and with clinical signs and symptoms	CSF VDRL sensitivity: 81.4% (95% CI: 75.4%–87.4%) CSF VDRL specificity: 90.3% (95% CI: 88.3%–92.3%) Asymptomatic neurosyphilis patients (n = 56) CSF VDRL sensitivity: 69.6% (95% CI: 59.6%–79.6%) CSF VDRL specificity: 79.4% (95% CI: 77.4%–81.4%) Symptomatic neurosyphilis patients (n = 154) CSF VDRL sensitivity: 85.7% (95% CI: 79.7%–91.7%) CSF VDRL specificity: 86.7% (95% CI: 84.7%–88.7%)	
	Retrospective cross-sectional study Patients with neurosyphilis: 149 Patients with symptomatic neurosyphilis: 33 Neurosyphilis reference standard: Reactive CSF FTA-ABS and >20 white blood cells in the CSF Symptomatic neurosyphilis reference standard: Vision or hearing loss with clinical or serologic evidence of neurosyphilis	Neurosyphilis patients (n = 149) CSF VDRL sensitivity: 71.8% (95% CI: 57.7%–85.9%) CSF VDRL specificity: 98.3% (95% CI: 95%–100%) Symptomatic neurosyphilis patients (n = 33) CSF VDRL sensitivity: 66.7% (95% CI: 50.6%–82.8%) CSF VDRL specificity: 80.2% (95% CI: 72.9%–87.5%)	(*118*)
Treponemal tests used to detect specific antibodies in the CSF			
Fluorescent Treponemal	Retrospective cross-sectional study	Neurosyphilis (n = 11) CSF FTA-ABS sensitivity: 100%	(*85*)

Antibody-Absorption Test (FTA-ABS)	Patients with primary syphilis: 50 Patients with secondary syphilis: 43 Patients with latent syphilis: 47 Patients with neurosyphilis: 11 Reference standard for primary syphilis: Presence of a lesion or chancre plus presence of spirochetes in lesion or lymph node (method to visualize spirochetes was not described) and/or reactive serologic tests Reference standard for secondary syphilis: Presence of spirochetes in generalized skin lesions or lymph node (method to visualize spirochetes was not described) and/or reactive serologic tests Reference standard for latent syphilis: Absence of symptoms or a history of syphilis plus reactive serologic tests Reference standard for neurosyphilis: Reactive FTA-ABS or TPHA plus reactive CSF VDRL or mononuclear cell count of >5 cell per µl of CSF	Results for syphilis other than neurosyphilis presented in Table 3	
Microhemagglutination Assay for Antibodies to *Treponema pallidum* (MHA-TP)	Retrospective cross-sectional study Serum from patients with syphilis: 75 (including 24 from patients with primary syphilis, 20 with secondary syphilis, 27 with latent syphilis, 3 with neurosyphilis, and 1 with cardiovascular syphilis) Serum from patients without syphilis: 222 Reference standard: CSF FTA-ABS	Neurosyphilis (n = 3) CSF MHA-TP sensitivity: 66.7% Results for syphilis other than neurosyphilis presented in Table 3	(*91*)

Treponema pallidum Passive Particle Agglutination (TPPA)	Prospective cross-sectional study Two data sets Training data set (CSF samples from individuals enrolled in a study of CSF abnormalities in syphilis; n = 191), including 45 with *T. pallidum* detected in CSF by NAAT and 40 with symptoms Validation data set (study participants enrolled after the last training sample was collected; n = 380), including 41 with *T. pallidum* detected in CSF by NAAT and 95 with symptoms Reference standard: CSF VDRL positive or *T. pallidum* detected in CSF or new vision or hearing loss with clinical or serologic evidence of syphilis	Training dataset compared with *T. pallidum* detected in CSF by NAAT CSF TPPA sensitivity: 75.6% (95% CI: 63.0%–88.1%) CSF TPPA specificity with a titer ≥1:160: 63.0% (95% CI: 55.2%–70.8%) CSF TPPA specificity with a titer ≥1:320: 73.3% (95% CI: 66.1%–80.5%) CSF TPPA specificity with a titer ≥1:640: 81.5% (95% CI: 75.2%–87.8%) CSF FTA-ABS sensitivity: 66.7% (95% CI: 52.9%–80.4%) CSF VDRL sensitivity: 58.9% (95% CI: 34.3%–63.5%) Training dataset compared with new vision or hearing loss CSF TPPA sensitivity: 77.5% (95% CI: 64.6%–90.4%) CSF TPPA specificity with a titer ≥1:160: 63.4% (95% CI: 55.5%–71.3%) CSF TPPA specificity with a titer ≥1:320: 75.4% (95% CI: 68.3%–82.5%) CSF TPPA specificity with a titer ≥1:640: 85.2% (95% CI: 79.4%–91.0%) CSF FTA-ABS sensitivity: 77.5% (95% CI: 64.6%–90.4%) CSF VDRL sensitivity: 67.5% (95% CI: 53.0%–82.0%)	(*130*)

	Training dataset compared with reactive CSF VDRL CSF TPPA sensitivity: 95.0% (95% CI: 89.5%–100%) CSF TPPA specificity with a titer ≥1:160: 75.6% (95% CI: 68.2%–83.0%) CSF TPPA specificity with a titer ≥1:320: 86.3% (95% CI: 80.4%–92.2%) CSF TPPA specificity with a titer ≥1:640: 93.9% (95% CI: 89.8%–98.0%) CSF FTA-ABS sensitivity: 98.3% (95% CI: 95.0%–100%) Validation dataset compared with *T. pallidum* detected in CSF by NAAT CSF TPPA specificity with a titer ≥1:640: 93.8% (95% CI: 91.2%–96.4%) CSF VDRL specificity: 91.2% (95% CI: 88.1%–94.2%) Validation dataset compared with new vision or hearing loss CSF TPPA specificity with a titer ≥1:640: 93.3% (95% CI: 90.4%–96.2%) CSF VDRL specificity: 90.2% (95% CI: 86.7%–93.6%) Validation dataset compared with reactive CSF VDRL

		CSF TPPA specificity with a titer ≥1:640: 97.0% (95% CI: 95.2%–98.8%) No difference in sensitivity or specificity based on HIV status

Abbreviations: CSF = cerebral spinal fluid; RPR = rapid plasma reagin; FTA-ABS = fluorescent treponemal antibody-absorption; CI = confidence interval; TPPA = *T. pallidum* particle agglutination; TRUST = Toluidine Red Unheated Serum Test; VDRL = Venereal Disease Research Laboratory; TPHA = *T. pallidum* hemagglutination assay; MHA-TP = microhemaggluntination assay for antibodies to *T. pallidum*; NAAT = nucleic acid amplification test

*Information presented is a summary of studies used for these recommendations. They do not represent a compendium of all studies reviewed during the formulation of these recommendations. Additional tables of evidence detailing studies reviewed during the APHL meeting in 2017 can be viewed at (be https://www.cdc.gov/std/syphilis/lab/testing/lab-recs-for-testing.htm).

†CSF RPR-V is a modified RPR by diluting it 1:2 in 10% saline to account for the lower concentration of immunoglobulin in CSF compared with serum.

TABLE 5. Specimen types, storage, and transport for direct detection tests for *T. pallidum*

Direct detection test	Specimen types	Specimen storage and transport
Darkfield microscopy	Serous exudate of moist lesions (except oral lesions) should be collected directly on a microscope slide or using a sterile bacteriological loop; avoid red blood cells	Fresh, room temperature (20°C–26°C)
Immunofluorescent antibody test staining	Smear from suspected lesion(s)	Fresh, room temperature (20°C–26°C)
Immunohistochemistry staining	Formalin-fixed and paraffin-embedded tissue sections of brain, placenta, umbilical cord, or skin lesions from secondary or tertiary syphilis	Room temperature (20°C–26°C)

Silver stain	Formalin fixed and paraffin embedded tissue sections of brain, placenta, umbilical cord, or skin lesions from secondary, tertiary, or congenital syphilis	Room temperature (20°C–26°C)
Nucleic Acid Amplification Test	Primary syphilis: Serous exudate of moist lesions should be collected with a sterile Dacron swab and placed in a commercial transport medium. Secondary syphilis: Mucous patches, condyloma lata; specimen should be collected with a sterile Dacron swab and placed in a commercial transport medium. Fresh frozen tissue biopsy or formalin-fixed and paraffin-embedded tissue. Neonatal whole blood or serum; whole blood should be collected in an EDTA (purple top) tube.	Frozen (–20°C to –80°C), frozen ice packs or dry ice

TABLE 6. Performance characteristics of tests for the direct detection of *T. pallidum**

Direct Detection Test	Study Summary and Reference Standard	Performance Characteristics	Reference
Darkfield microscopy	Prospective cross-sectional study Patients with primary syphilis: 63 Patients with secondary syphilis: 3 Patients without syphilis: 62 Syphilitic patients with genital lesion(s): 63 Syphilitic patients with anogenital lesion(s): 3	Patients with primary or secondary syphilis (n = 66) Positive by darkfield microscopy: 78.8% Positive by direct fluorescence microscopy: 72.7% Non-syphilitic patients with genital or anogenital lesions (n = 62) Positive by darkfield microscopy: 0%	(*109*)

Non-syphilitic patients with genital lesion(s): 59 Non-syphilitic patients with anogenital lesion(s): 3 Specimen type for darkfield microscopy: Lesion exudate Tests performed: Darkfield microscopy, direct fluorescence microscopy using H9-1 monoclonal antibody to 47-58kDa tp protein, RPR serology Syphilis diagnosis: Clinical presentation and RPR serology	Positive by direct fluorescence microscopy: 0% Results were not grouped by stage of syphilis or anatomic site of lesion	
Prospective cross-sectional study Patients with secondary syphilis: 12 Patients with non-syphilitic lesions: 24 Specimen types: Lesion exudate and biopsy Tests performed: Darkfield microscopy, PCR tppa47 (amplicons detected by Southern blot for 25bp region and sequenced), IHC on FFPE using avidin-biotin peroxidase complex technique with polyclonal antibodies (BioCare) Syphilis diagnosis: Clinical presentation, RPR, and TPHA serology	Patients with secondary syphilis (n = 12) Positive by darkfield microscopy: 58% Positive by PCR: 75% Positive by IHC: 91.7% Patients without syphilis (n = 24) Positive by darkfield microscopy: 0% Positive by PCR: 0% Positive by IHC: 0%	(*155*)

Prospective cross-sectional study Two studies with only study A relevant to darkfield microscopy Study A Patients with skin lesion(s): 350 Stage of syphilis not defined Specimen type for darkfield microscopy: Lesion exudate Tests performed: Darkfield microscopy, PCR tppa47 (amplicons detected by Southern blot for 25bp region and sequenced), immunohistochemistry on FFPE using avidin-biotin peroxidase complex technique with rabbit polyclonal antibodies Syphilis diagnosis: Clinical presentation, VDRL, and FTA-ABS serology Sensitivity and specificity based on clinical diagnosis of syphilis	Patients with skin lesions (n = 350) Sensitivity of darkfield microscopy: 73.8% Specificity of darkfield microscopy: 97.4%	(*156*)
Prospective cross-sectional study Patients with primary syphilis: 87 (specimens from 65 patients used to assess darkfield microscopy)	Patients with primary syphilis assessed by darkfield microscopy (n = 65) Positive by darkfield microscopy: 75.4%	(*157*)

Patients with secondary syphilis: 103 (specimens from 44 patients used to assess darkfield microscopy) Patients without syphilis: 35 (specimens from 12 patients used to assess darkfield microscopy) Primary syphilis patients with genital lesions: 35 Primary syphilis patients with anal lesions: 6 Primary syphilis patients with oral lesions: 4 Primary syphilis patients with cutaneous lesions: 2 Primary syphilis patients with lesions from unknown anatomic site: 18 Secondary syphilis patients with genital lesions: 22 Secondary syphilis patients with anal lesions: 3 Secondary syphilis patients with oral lesions: 5 Secondary syphilis patients with cutaneous lesions: 10 Secondary syphilis patients with lesions from unknown anatomic site: 4 Non-syphilitic patients with genital lesions: 8 Non-syphilitic patients with anal lesions: 2	Patients with primary syphilis and genital lesions (n = 35) Positive by darkfield microscopy: 88.6% Patients with primary syphilis and anal lesions (n = 6) Positive by darkfield microscopy:66.7% Patients with primary syphilis and oral lesions (n = 4) Positive by darkfield microscopy: 75% Patients with primary syphilis and cutaneous lesions (n = 2) Positive by darkfield microscopy:100% Patients with primary syphilis and lesions from unknown anatomic site (n = 18) Positive by darkfield microscopy: 50% Patients with secondary syphilis and assessed by darkfield microscopy (n = 44) Positive by darkfield microscopy: 70.5% Patients with secondary syphilis and genital lesions (n = 22) Positive by darkfield microscopy: 63.6% Patients with secondary syphilis and anal lesions (n = 3) Positive by darkfield microscopy: 66.7%

Non-syphilitic patients with oral lesions: 0 Non-syphilitic patients with cutaneous lesions: 0 Non-syphilitic patients with lesions from unknown anatomic site: 2 Specimen type for darkfield microscopy: Lesion exudate Tests performed: Darkfield microscopy, PCR tppa47 Syphilis diagnosis: Clinical presentation, nontreponemal and treponemal serology (test types not stated)	Patients with secondary syphilis and oral lesions (n = 5) Positive by darkfield microscopy: 100% Patients with secondary syphilis and cutaneous lesions (n = 10) Positive by darkfield microscopy: 80% Patients with secondary syphilis and lesions from unknown anatomic site (n = 4) Positive by darkfield microscopy: 50% Non-syphilitic patients assessed by darkfield microscopy (n = 12) Positive by darkfield microscopy: 0% Non-syphilitic patients with genital lesions (n = 8) Positive by darkfield microscopy: 0% Non-syphilitic patients with anal lesions (n = 2) Positive by darkfield microscopy: 0%	
Prospective cross-sectional study Primary syphilis patients: 22 Secondary syphilis patients: 8 Of the 30 patients with syphilis, 24 had genital lesions, 5 had anal lesions and 1 had cutaneous lesions	Patients with primary or secondary syphilis (N = 30) Positive by darkfield microscopy: 96.7% Non-syphilitic patients (n = 31) Positive by darkfield microscopy: 6.5%	(*158*)

	Non-syphilitic patients: 31 Of the 30 patients without syphilis, 20 had genital lesions, 6 had anal lesions and 5 had oral lesions Specimen type for darkfield microscopy: Lesion exudate Tests performed: Darkfield microscopy and direct fluorescence microscopy using H9-1 monoclonal antibody to 47-58kDa tp protein Syphilis diagnosis: Clinical presentation, nontreponemal (VDRL) and treponemal serology (FTA-ABS)		
	Retrospective cross-sectional study Patients with syphilis: 30 Specimens from patients with primary syphilis: 5 (3 specimens used to assess darkfield microscopy) Specimens from patients with secondary syphilis: 31 (14 specimens used to assess darkfield microscopy) Note: More than one specimen was obtained from a patient, but the number of specimens per patient was not defined	Patients with primary syphilis assessed by darkfield microscopy (n = 3) Positive by darkfield microscopy: 100% Patients with secondary syphilis assessed by darkfield microscopy (n = 14) Positive by darkfield microscopy: 64.3%	(*159*)

Specimen type for darkfield microscopy: Lesion exudate Tests performed: Darkfield microscopy, avidin-biotin-peroxidase complex, indirect immunoperoxidase, and FTA-ABS Complement Syphilis diagnosis: Clinical presentation, nontreponemal (VDRL) and treponemal serology (FTA-ABS, TPHA)		
Prospective cross-sectional study Pregnant women with syphilis: 11 (included in darkfield microscopy assessment) Neonates with probable or suspected congenital syphilis: 20 (not included in darkfield microscopy assessment) Pregnant women with primary syphilis: 4 Pregnant women with secondary syphilis: 3 Pregnant women with early latent syphilis: 4 Specimen type for darkfield microscopy: Amniotic fluid Tests performed: Darkfield microscopy, rabbit infectivity test, PCR for Tpp47 gene with Southern blot confirmation	Amniotic fluid from pregnant women with primary syphilis (n = 4) Positive by darkfield microscopy: 25% Amniotic fluid from pregnant women with secondary syphilis (n = 3) Positive by darkfield microscopy: 33.3% Amniotic fluid from pregnant women with early latent syphilis (n = 4) Positive by darkfield microscopy: 100%	(*160*)

	Syphilis diagnosis: Clinical presentation and nontreponemal (VDRL) serology		
	Prospective cross-sectional study Pregnant women with primary syphilis: 6 Pregnant women with secondary syphilis: 12 Pregnant women with early latent syphilis: 6 Specimen type for darkfield microscopy: Amniotic fluid Tests performed: Darkfield microscopy, rabbit infectivity test, PCR for Tpp47 gene with Southern blot confirmation Syphilis diagnosis: Clinical presentation, nontreponemal (VDRL), and treponemal (MHA-TP) serology	Amniotic fluid from pregnant women with primary syphilis (n = 6) Positive by darkfield microscopy: 16.7% Amniotic fluid from pregnant women with secondary syphilis and assessed by darkfield microscopy (n = 20) Positive by darkfield microscopy: 20% Amniotic fluid from pregnant women with early latent syphilis and assessed by darkfield microscopy (n = 5) Positive by darkfield microscopy: 60%	(*161*)
Immunofluorescent antibody test staining	Prospective cross-sectional study Two studies with both study A and B relevant to immunofluorescent antibody test staining Study A Patients with skin lesion(s): 350 Study B	Patients with skin lesions (n = 445) Sensitivity of immunofluorescent antibody test stain: 85.9% Specificity of immunofluorescent antibody test stain: 100%	(*156*)

Patients with skin lesion(s): 95 Stage of syphilis not defined in both studies Specimen type for immunofluorescent antibody test staining (both studies): Lesion exudate Syphilis diagnosis (both studies): Clinical presentation, VDRL, and FTA-ABS serology Sensitivity and specificity based on clinical diagnosis of syphilis in both studies		
Prospective cross-sectional study Primary syphilis patients: 22 Secondary syphilis patients: 8 Of the 30 patients with syphilis, 24 had genital lesions, 5 had anal lesions and 1 had cutaneous lesions Non-syphilitic patients: 31 Of the 30 patients without syphilis, 20 had genital lesions, 6 had anal lesions and 5 had oral lesions Specimen type for immunofluorescent antibody test staining: Lesion exudate Tests performed: Darkfield microscopy and direct fluorescence microscopy using H9-1	Patients with primary or secondary syphilis patients (n = 30) Positive by immunofluorescent antibody test stain: 100% Non-syphilitic patients (n = 31) Positive by immunofluorescent antibody test stain: 0%	(*158*)

	monoclonal antibody to 47-58kDa tp protein Syphilis diagnosis: Clinical presentation, nontreponemal (VDRL) and treponemal serology (FTA-ABS)		
Immunohistochemistry staining	Prospective cross-sectional study Patients with secondary syphilis: 12 Patients with non-syphilitic lesions: 24 Specimen types: Lesion exudate and biopsy Tests performed: Darkfield microscopy, PCR tppa47 (amplicons detected by Southern blot for 25bp region and sequenced), immunohistochemistry staining on FFPE using avidin-biotin peroxidase complex technique with polyclonal antibodies (BioCare) Syphilis diagnosis: Clinical presentation, RPR, and TPHA serology	Patients with secondary syphilis (n = 12) Positive by immunohistochemistry stain: 91.7% Non-syphilitic patients (n = 24) Positive by immunohistochemistry stain: 0%	(*155*)
	Retrospective cross-sectional study Patient with syphilis: 30	Patients with primary syphilis patients (n = 5) Positive by avidin-biotin-peroxidase complex staining: 100% Positive by indirect immunoperoxidase stain: 100%	(*159*)

Specimens from patients with primary syphilis to assess immunohistochemistry staining: 5 Specimens from patients with secondary syphilis immunohistochemistry staining: 31 Note: More than one specimen was obtained from a patient, but the number of specimens per patient was not defined Specimen type for immunohistochemistry staining: cutaneous lesion that was FFPE Tests performed: Darkfield microscopy, immunohistochemistry using avidin-biotin-peroxidase complex, indirect immunoperoxidase immunohistochemistry, FTA-ABS, and complement fixation Syphilis diagnosis: Clinical presentation, nontreponemal (VDRL) and treponemal serology (FTA-ABS, TPHA)	Patients with secondary syphilis (n = 31) Positive by avidin-biotin-peroxidase complex staining: 90.3% Positive by indirect immunoperoxidase stain: 87.1%	
Retrospective cross-sectional study Secondary syphilis patients: 36 (33 confirmed by serology and 3 not serologically tested) Specimen type for immunohistochemistry staining: cutaneous lesion that was FFPE	Patients with secondary syphilis (n = 35) Positive by indirect immunohistochemistry stain: 48.6%	(*163*)

Tests performed: Immunohistochemistry using rabbit polyclonal antibodies, Dieterle silver stain, nested PCR (Tp1; 228 bp) and semi-nested (Tp2; 125 bp) PCR for DNA polymerase I Syphilis diagnosis: Clinical presentation and, in 33/36 patients, syphilis serology (undefined)		
Retrospective cross-sectional study Secondary syphilis patients: 17 Biopsies from patients without syphilis: 14 (similar histologic pattern to secondary syphilis, including 2 with lichen planus, 3 with psoriasis, 3 with psoriasiform dermatitis, 2 with pityriasis lichenoides et varioliformis acuta, 1 with erythema annulare centrifugum, 2 with acne keloidalis, and 1 with folliculitis decalvans Specimen type for immunohistochemistry staining: cutaneous lesion that was FFPE Tests performed: Immunohistochemistry using avidin-biotin-peroxidase complex and Steiner silver stain	Patients with secondary syphilis (n = 17) Positive by avidin-biotin-peroxidase complex immunohistochemistry stain: 70.6% Non-syphilitic patients (n = 14) Positive by avidin-biotin-peroxidase complex immunohistochemistry stain: 0%	(*164*)

	Syphilis diagnosis: Clinical presentation, nontreponemal (RPR or VDRL), and treponemal (TPPA or FTA-ABS) serology		
Silver stain	Retrospective cross-sectional study Secondary syphilis patients: 36 (33 confirmed by serology and 3 not serologically tested) Specimen type for Dieterle silver staining: cutaneous lesion that was FFPE Tests performed: Immunohistochemistry using rabbit polyclonal antibodies, Dieterle silver stain, nested PCR (Tp1; 228 bp) and semi-nested (Tp2; 125 bp) PCR for DNA polymerase I Syphilis diagnosis: Clinical presentation and, in 33/36 patients, syphilis serology (undefined)	Patients with secondary syphilis (n = 35) Positive by Dieterle silver stain: 25.7%	(*163*)
	Retrospective cross-sectional study Secondary syphilis patients: 17 Biopsies from patients without syphilis: 14 (similar histologic pattern to secondary syphilis, including 2 with lichen planus, 3 with psoriasis, 3 with psoriasiform	Patients with secondary syphilis (n = 17) Positive by Steiner silver stain: 41.2% Non-syphilitic patients (n = 14) Positive by Steiner silver stain: 0%	(*164*)

dermatitis, 2 with pityriasis lichenoides et varioliformis acuta, 1 with erythema annulare centrifugum, 2 with acne keloidalis, and 1 with folliculitis decalvans Specimen type for Steiner silver staining: cutaneous lesion that was FFPE Tests performed: Immunohistochemistry using avidin-biotin-peroxidase complex and Steiner silver stain Syphilis diagnosis: Clinical presentation, nontreponemal (RPR or VDRL), and treponemal (TPPA or FTA-ABS) serology		
Prospective cross-sectional study Secondary syphilis patients: 57 (only 11 lesion biopsies were microscopically examined after Warthin-Starry silver staining) Specimen type for Warthin-Starry silver staining: cutaneous lesion that was FFPE Tests performed: Warthin-Starry silver stain, nested PCR (Tp1; 228 bp), and RT-PCR for Tp *polA*	Patients with secondary syphilis (n = 11) Positive by Warthin-Starry silver stain: 9.1%	(*165*)

	Syphilis diagnosis: Clinical presentation, nontreponemal (RPR), and treponemal (FTA-ABS) serology		
	Retrospective cross-sectional study Secondary syphilis patients: 6 Tertiary syphilis patients: 7 Non-syphilitic patients: 5 Specimen type for Warthin-Starry silver staining: cutaneous lesion that was FFPE Tests performed: Warthin-Starry silver stain, nested PCR (Tp1; 228 bp), and nested PCR for Tp47 Syphilis diagnosis: Clinical presentation and treponemal (TPHA and FTA-ABS) serology	Patients with secondary or tertiary syphilis (n = 13) Positive by Warthin-Starry silver stain: 0% Non-syphilitic patients (n = 5) Positive by Warthin-Starry silver stain: 0%	(*166*)
NAATs	Prospective cross-sectional study Patients with suspected primary syphilis: 716 Patients with suspected secondary syphilis: 133 Specimen type for RT-PCR: dry swab from anogenital lesion or cutaneous lesion	Patients with suspected primary syphilis (n = 716) Positive by RT-PCR: 13% Patients with suspected secondary syphilis (n = 133) Positive by RT-PCR: 25.6%	(*77*)

Tests performed: Darkfield microscopy on all anogenital lesions and RT-PCR for polA on all anogenital and cutaneous lesions Primary syphilis diagnosis standard 1: Darkfield microscopy positive Primary syphilis diagnosis standard 2: Clinical presentation, darkfield microscopy positive, and syphilis serology (not defined) Primary syphilis diagnosis standard 3: Patients with a positive TPPA result (irrespective of the RPR test result) without a history of syphilis or in patients with an RPR titer of ≥1:8 and a history of syphilis Clinical presentation, darkfield microscopy, and syphilis serology (not defined) Secondary syphilis diagnosis: Clinical presentation with cutaneous or mucosal lesions characteristic of secondary syphilis and RPR titer of ≥1:8	Patients with primary syphilis defined by clinical standard 1 involving darkfield microscopy (n = 716) RT-PCR sensitivity: 87% RT-PCR specificity 93.1% Patients with primary syphilis defined by clinical standard 2 involving clinical history, darkfield microscopy, and serology (n = 716) RT-PCR sensitivity: 72.8% RT-PCR specificity: 98.8% Patients with primary syphilis clinical standard 3 involving clinical history and serology (n = 716) RT-PCR sensitivity: 74.5% RT-PCR specificity: 97.2% Patients with secondary syphilis (n = 133) RT-PCR sensitivity: 42.9% RT-PCR specificity: 98.2%	
Prospective cross-sectional study Case-control nested in prospective cohort Primary syphilis patients: 26 (10 HIV positive and 16 HIV negative) Secondary syphilis patients: 40 (19 HIV positive and 21 HIV negative)	Patients with primary syphilis (n = 26) RT-PCR sensitivity: 65.4% (95% CI: 44%–83%) Patients with secondary syphilis (n = 40) RT-PCR sensitivity: 52.5% (95% CI: 36%–68%)	(*78*)

Latent syphilis patients: 8 Case control for primary syphilis: 7 patients with genital or oral lesion Case control for secondary syphilis: 5 patients with cutaneous rash Case control for latent syphilis: 3 patients without symptoms Specimen types for RT-PCR from primary syphilis patients: 8 dry lesion swab, 18 whole blood, 11 serum, and 7 urine Specimen types for RT-PCR from secondary syphilis patients: 5 dry lesion swab, 31 whole blood, 15 serum, 2 plasma, 6 CSF, and 9 urine Specimen types for RT-PCR from latent syphilis patients: 6 whole blood, 2 serum, 2 CSF, and 2 urine Tests performed: Darkfield microscopy on all anogenital lesions and RT-PCR for tpp47 Syphilis diagnosis: Clinical presentation, nontreponemal (VDRL), and treponemal (TPHA) serology to determine stage	Patients with latent syphilis (n = 8) RT-PCR sensitivity: 0% No difference in performance based on HIV status Lesion swab specimens tested from patients with primary syphilis (n = 10) RT-PCR sensitivity: 80% (95% CI: 44%– 97%) Whole blood tested from patients with primary syphilis (n = 18) RT-PCR sensitivity: 28% (95% CI: 10%–53%) Serum tested from patients with primary syphilis (n = 11) RT-PCR sensitivity: 55% (95% CI 23% - 83%) Urine tested from patients with primary syphilis (n = 7) RT-PCR sensitivity: 29% (95% CI: 4%– 71%) All controls negative Lesion swab specimens tested from patients with secondary syphilis (n = 5) RT-PCR sensitivity: 20% (95% CI: 0.5%–72%) Whole blood tested from patients with primary syphilis (n = 31) RT-PCR sensitivity: 36% (95% CI: 19%–55%)

	Serum tested from patients with primary syphilis (n = 15) RT-PCR sensitivity: 47% (95% CI: 21%–73%) Plasma tested from patients with primary syphilis (n = 2) RT-PCR sensitivity 100% (95% CI: 16%–100%) CSF tested from patients with primary syphilis (n = 6) RT-PCR sensitivity: 50% (95% CI: 12%–88%) Urine tested from patients with primary syphilis (n = 7) RT-PCR sensitivity: 29% (95% CI: 4%–71%) All controls negative	
Prospective cross-sectional study Patients with secondary syphilis: 12 Patients with non-syphilitic lesions: 24 Specimen types: Lesion exudate and biopsy Tests performed: Darkfield microscopy, PCR tppa47 (amplicons detected by Southern blot for 25bp region and sequenced), immunohistochemistry on FFPE tissue using avidin-biotin peroxidase	Patients with secondary syphilis (n = 12) Positive by PCR: 75% PCR limit of detection: 1ng of DNA	(*155*)

complex technique with polyclonal antibodies (BioCare) Syphilis diagnosis: Clinical presentation, RPR, and TPHA serology		
Prospective cross-sectional study Study A Patients with primary syphilis: 87 (specimens from 65 patients used to assess PCR) Patients with secondary syphilis: 103 (specimens from 44 patients used to assess PCR) Patients without syphilis: 35 (specimens from 12 patients used to assess PCR) Primary syphilis patients with genital lesions: 35 Primary syphilis patients with anal lesions: 6 Primary syphilis patients with oral lesions: 2 Primary syphilis patients with cutaneous lesions: 2 Primary syphilis patients with lesions from unknown anatomic site: 18 Secondary syphilis patients with genital lesions: 22	Study A Patients with primary syphilis (n = 65) Positive by PCR: 80% Patients with primary syphilis and genital lesions (n = 35) Positive by PCR: 82.9% Patients with primary syphilis and anal lesions (n = 6) Positive by PCR: 83.3% Patients with primary syphilis and oral lesions (n = 4) Positive by PCR: 50% Patients with primary syphilis and cutaneous lesions (n = 2) Positive by PCR: 100% Patients with primary syphilis and lesions from unknown anatomic site (n = 18) Positive by PCR: 77.8% Patients with secondary syphilis (n = 44) Positive by PCR: 86.4%	(*157*)

Primary syphilis patients with anal lesions: 3
Primary syphilis patients with oral lesions: 5
Primary syphilis patients with cutaneous lesions: 10
Primary syphilis patients with lesions from unknown anatomic site: 4

Non-syphilitic patients with genital lesions: 8
Non-syphilitic patients with anal lesions: 2
Non-syphilitic patients with oral lesions: 0
Non-syphilitic patients with cutaneous lesions: 0
Non-syphilitic patients with lesions from unknown anatomic site: 2

Study B
Primary syphilis patients: 81 (not all tested specimen types tested for all patients)
Secondary syphilis patients: 97 (not all tested specimen types tested for all patients)
Latent syphilis patients: 40 (not all tested specimen types tested for all patients)

Specimen types for PCR (both studies): Lesion exudate, whole blood, serum, plasma, and peripheral blood mononuclear cells

Patients with secondary syphilis and genital lesions (n = 22)
Positive by PCR: 86.4%

Patients with secondary syphilis and anal lesions (n = 3)
Positive by PCR: 66.7%

Patients with secondary syphilis and oral lesions (n = 5)
Positive by PCR: 80%

Patients with secondary syphilis and cutaneous lesions (n = 10)
Positive by PCR: 100%

Patients with secondary syphilis and lesions from unknown anatomic site (n = 4)
Positive by PCR: 75%

Non-syphilitic patients (n = 12)
Positive by PCR: 0%

Non-syphilitic patients with genital lesions (n = 8)
Positive by PCR: 0%

Non-syphilitic patients with anal lesions (n = 2)
Positive by PCR: 0%

Tests performed: Darkfield microscopy, PCR tppa47 (study A), and PCR tppa47 (study B) Syphilis diagnosis (both studies): Clinical presentation, nontreponemal, and treponemal serology (test types not stated)	Study B Whole blood tested from patients with primary syphilis (n = 61) Positive by PCR: 13.1% Serum tested from patients with primary syphilis (n = 63) Positive by PCR: 19% Plasma tested from patients with primary syphilis (n = 67) Positive by PCR: 11.9% Peripheral blood mononuclear cells tested from patients with primary syphilis (n = 72) Positive by PCR: 31.9% Whole blood tested from patients with secondary syphilis (n = 69) Positive by PCR: 37.7% Serum tested from patients with secondary syphilis (n = 65) Positive by PCR: 15.4% Plasma tested from patients with secondary syphilis (n = 66) Positive by PCR: 28.8% Peripheral blood mononuclear cells tested from patients with secondary syphilis (n = 83) Positive by PCR: 31.3%

	Whole blood tested from patients with latent syphilis (n = 28) Positive by PCR: 14.3% Serum tested from patients with latent syphilis (n = 28) Positive by PCR: 3.6% Plasma tested from patients with latent syphilis (n = 29) Positive by PCR: 10.3% Peripheral blood mononuclear cells tested from patients with latent syphilis (n = 31) Positive by PCR: 16.1% Specimens for patients without syphilis were all negative PCR limit of detection: 20 organisms/mL	
Retrospective cross-sectional study Secondary syphilis patients: 36 (33 confirmed by serology and 3 were not serologically tested) Specimen type for PCR: cutaneous lesion that was FFPE	Patients with secondary syphilis (n = 36) Positive by nested PCR: 19.4% Positive by semi-nested PCR: 38.9%	(*163*)

Tests performed: Immunohistochemistry using rabbit polyclonal antibodies, Dieterle silver stain, nested PCR (Tp1; 228 bp), and semi-nested (Tp2; 125 bp) PCR for DNA polymerase I Syphilis diagnosis: Clinical presentation and, in 33/36 patients, syphilis serology (undefined)		
Prospective cross-sectional study Secondary syphilis patients: 57 (only 12 lesion biopsies were tested by PCR and whole blood tested from 26 patients) Specimen type for PCR: cutaneous lesion that was FFPE and whole blood Tests performed: Warthin-Starry silver stain, nested PCR (Tp1; 228 bp), and RT-PCR for Tp polA Syphilis diagnosis: Clinical presentation, nontreponemal (RPR), and treponemal (FTA-ABS) serology	Lesion biopsy from patients with secondary syphilis (n = 12) Positive by PCR: 66.7% Whole blood from patients with secondary syphilis (n = 23) Positive by PCR: 46.2% Limit of detection by PCR: 12–150 spirochetes/mL (one log higher if specimens stored at 4°C for 26h versus room temperature for 1h)	(*165*)
Retrospective cross-sectional study Secondary syphilis patients: 6 Tertiary syphilis patients: 7 Non-syphilitic patients: 5	Patients with secondary syphilis (n = 6) Positive by PCR: 66.7% Patients with tertiary syphilis (n = 7)	(*166*)

	Positive by PCR: 14.3% (the positive specimen was from a gumma) Non-syphilitic patients (n = 5) Positive by PCR: 0%	
Specimen type for PCR: cutaneous lesion that was FFPE Tests performed: Warthin-Starry silver stain, nested PCR (Tp1; 228 bp), and nested PCR for Tp47 Syphilis diagnosis: Clinical presentation and treponemal (TPHA and FTA-ABS) serology		
Prospective cross-sectional study Number of patients evaluated: 298 Specimen type for PCR: Genital lesion exudate Tests performed: Darkfield microscopy and multiplex PCR for *T. pallidum* tpp47, HSV, and *Haemoplilus ducreyi* Syphilis diagnosis: Clinical presentation, darkfield microscopy, and nontreponemal (RPR or VDRL) serology	Patients with syphilis and tested by multiplex PCR and darkfield microscopy (n = 295) Positive by multiplex PCR and darkfield microscopy: 19.7% Positive by multiplex PCR and negative by darkfield microscopy: 5.8% Negative by multiplex PCR and positive by darkfield microscopy: 2.4% Negative by multiplex PCR and darkfield microscopy: 72.2% Patients with syphilis and tested by multiplex PCR and serology (n = 296) Positive by multiplex PCR and syphilis serology: 21.7% Positive by multiplex PCR and negative by syphilis serology: 3.7% Negative by multiplex PCR and positive by syphilis serology: 8.1% Negative by multiplex PCR and syphilis serology: 66.6%	(*171*)

Prospective cross-sectional study Primary syphilis patients: 19 (4 from anal lesions, 6 from oral lesions, 13 from penial lesions, 1 from a rectal lesion, and 2 lesions from unspecified anatomic site) Secondary syphilis patients: 10 (2 from anal lesions, 6 from oral lesions, 5 from penial lesions, and 1 from a vulval lesion) Patients with HSV: 17 (2 from anal lesions, 9 from penial lesions, 4 from vulval lesions, and 3 lesions from unspecified anatomic site) Non-syphilitic patients: 48 (9 from anal lesions, 11 from oral lesions, 19 from penial lesions, 2 from rectal lesions, 7 from vulval lesions and 1 lesion from unspecified anatomic site) Non-syphilitic patients but with history of syphilis: 6 (2 from anal lesions and 4 from penial lesions) Specimen type for PCR: Dry swab or swab from lesion placed in viral or chlamydia suitable transport medium Tests performed: PCR for *T. pallidum* tpp47	Patients with primary syphilis (n = 19) Positive by PCR: 94.7% (anatomic site not specified) Patients with secondary syphilis (n = 10) Positive by PCR: 80% (anatomic site not specified) Patients with HSV (n = 17) Positive by PCR: 0% Non-syphilitic patients with lesions (n = 48) Positive by PCR: 2.1% (anatomic site not specified) Non-syphilitic patients but with history of syphilis (n = 6) Positive by PCR: 0% PCR limit of detection: 1pg *T. pallidum* DNA	(*172*)

Syphilis diagnosis: Clinical presentation, darkfield microscopy (34 specimens), nontreponemal (RPR), and treponemal (TPHA or IgM/IgG EIA) serology		
Prospective cross-sectional study Primary syphilis patients: 19 Secondary syphilis patients: 9 Latent syphilis patients: 10 Congenital syphilis patients: 3 Non-syphilitic patients: 27 Specimen type for PCR: Swab from ulcer or cutaneous lesion placed in viral or chlamydia-suitable transport medium, whole blood collected in tube containing EDTA, serum, or CSF Tests performed: Nested PCR for *T. pallidum* bmp, and tpp47 nPCR for bmp and tpp47, and PCR for tpp47 Primary syphilis diagnosis: (1) The identification of *T. pallidum* by darkfield microscopy, fluorescent antibody, or equivalent examination of material from a chancre or a regional lymph node; or (2) the presence of one or more typical lesions (chancres) and reactive treponemal	Patients with primary syphilis (n = 19) Positive by PCR: 47.4% (9 swab specimens positive, 3 swab specimens negative (β-globin control also negative), and 7 blood specimens negative) Patients with secondary syphilis (n = 9) Positive by PCR: 44.4% (1 swab specimen positive, 2 tissue specimens positive, 4 blood specimens positive, 4 blood specimens negative, and 1 CSF specimen negative [β-globin control also negative]) Patients with congenital syphilis (n = 3) Positive by PCR: 33.3% (1 blood specimen positive and 2 blood specimens negative) Patients with latent syphilis (n = 10) Positive by PCR: 0% Non-syphilitic patients (n = 27) Positive by PCR: 0%	(*178*)

serology, regardless of nontreponemal test reactivity, in individuals with no previous history of syphilis; or (3) the presence of one or more typical lesions (chancres) and at least a fourfold increase in the titer over that of the last known nontreponemal test in individuals with a past history of syphilis treatment

Secondary syphilis diagnosis: (1) The identification of *T. pallidum* by microscopy, as in primary syphilis, or equivalent examination of mucocutaneous lesions, condylomata lata, and reactive serology (nontreponemal and treponemal); or (2) the presence of typical mucocutaneous lesions, alopecia, loss of eyelashes and the lateral third of eyebrows, iritis, generalized lymphadenopathy, fever, malaise or splenomegaly, and either a reactive serology (nontreponemal and treponemal) or at least a fourfold increase in titer over that of the last known nontreponemal test

Early latent syphilis diagnosis: Asymptomatic patient with reactive serology (nontreponemal and treponemal) who within the past 12 months had one of the following: nonreactive serology or symptoms suggestive of primary or secondary syphilis or exposure to a sexual

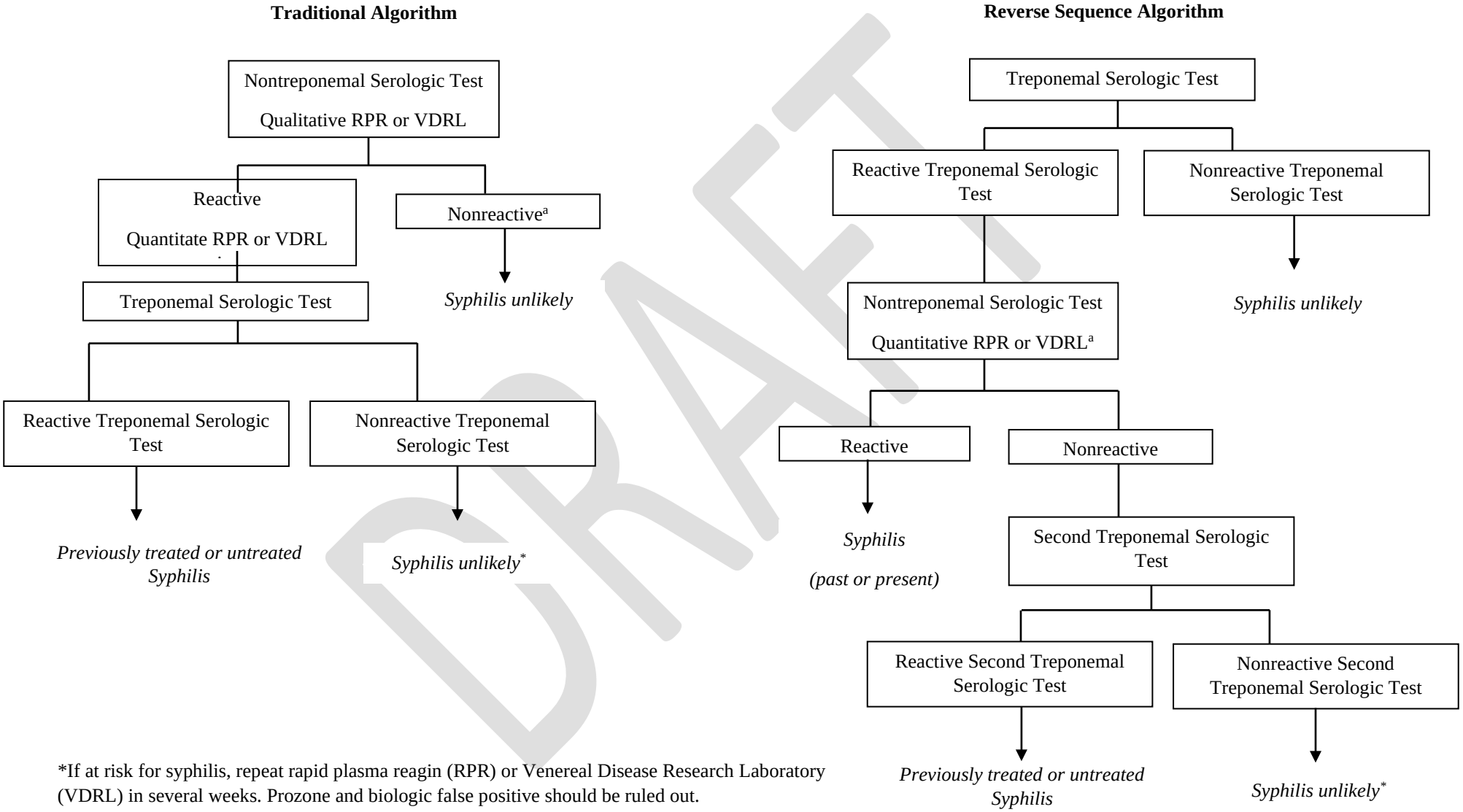
partner with primary, secondary, or early latent syphilis		
Late latent syphilis diagnosis: Asymptomatic patient with persistently reactive treponemal serology (regardless of nontreponemal serology reactivity) who does not meet the criteria for early latent disease and who has not been previously treated for syphilis		
Prospective cross-sectional study Patient population: Male (N = 267); 90.6% of whom were living with HIV Primary syphilis patients: 38 (17 had oral lesions) Secondary syphilis patients: 76 (0 had oral lesions) Early latent syphilis patients: 125 (0 had oral lesions) Late latent syphilis patients: 5 (0 had oral lesions) Congenital syphilis patients: 3 Non-syphilitic patients: 27 Specimen type for PCR: Oral swab from lesion (if present) or upper and lower gingiva, tonsils, hard palate, and soft palate in the absence of a lesion	Oral swabs tested from patient population (N = 267) Positive by PCR: 42.3% Oral swabs tested from patients with primary syphilis and oral lesions (n = 17) Positive: 100% Oral swabs tested from patients with primary syphilis without oral lesions (n= 21) Positive by PCR: 61.9% Patients with secondary syphilis (n = 76) Positive PCR: 64.5% Patients with early latent syphilis (n = 125) Positive by PCR: 28% Patients with late latent syphilis (n = 5) Positive by PCR: 40%	(*179*)

	Tests performed: PCR for *T. pallidum* polA and typing using arp, tpr, and tp0548 Syphilis diagnosis and staging: According to the CDC Sexually Transmitted Treatment Guidelines (no additional information provided)

Abbreviations: kDa = kilodaltons; RPR = rapid plasma reagin; PCR = polymerase chain reaction; bp = base pairs; IHC = immunohistochemistry; FFPE = formalin fixed and paraffin embedded tissue; TPHA = *T. pallidum* hemagglutination assay; VDRL = Venereal Disease Research Laboratory; FTA-ABS = fluorescent treponemal antibody-absorption; MHA-TP = microhemaggluntination assay for antibodies to *T. pallidum*; DNA = deoxyribonucleic acid; TPPA = *T. pallidum* particle agglutination; NAAT = nucleic acid amplification test; CI = confidence interval; CSF = cerebral spinal fluid; HSV = herpes simplex virus; IgG = immunoglobulin G; IgM = immunoglobulin M; EIA = enzyme immunoassay; EDTA = ethylenediaminetetraacetic acid

*Information presented is a summary of studies used for these recommendations. They do not represent a compendium of all studies reviewed during the formulation of these recommendations. Additional tables of evidence detailing studies reviewed during the APHL meeting in 2017 can be viewed at (https://www.cdc.gov/std/syphilis/lab/testing/lab-recs-for-testing.htm).

FIGURE 1. Algorithms that can be applied to screening for syphilis with serologic tests



*If at risk for syphilis, repeat rapid plasma reagin (RPR) or Venereal Disease Research Laboratory (VDRL) in several weeks. Prozone and biologic false positive should be ruled out.

Appendix 1. APHL meeting attendees, conflict of interest disclosures, and key questions

APHL Attendees: Laura Bachmann, MD, MPH, Wake Forest School of Medicine, Winston-Salem, North Carolina; William Becker, DO, MPH, Quest Diagnostics Laboratory, Lenexa, Kansas; Eric Blank, DrPH, APHL, Silver Spring, Maryland; Marc Couturier, PhD, D(ABMM), ARUP Laboratories/University of Utah, Salt Lake City, Utah; Marilyn Freeman, PhD, M(ASCP), Virginia Division of Consolidated Laboratory Services, Richmond, Virginia; Anne Gaynor, PhD, APHL, Silver Spring, Maryland; Laura Gillim-Ross, PhD, HCLD (ABB), LabCorp Englewood, Colorado; William A. Glover II, PhD, Washington Public Health Laboratories, Seattle, Washington; Edward Hook, MD, University of Alabama at Birmingham, Birmingham, Alabama; Jeffrey Klausner, MD, MPH, University of California Los Angeles, Los Angeles, California; Michael Loeffelholz, PhD, University of Texas Medical Branch, Galveston, Texas; Ruth Lynfield, MD, Minnesota Department of Health, St. Paul, Minnesota; William C. Miller, MD, PhD, The Ohio State University, Columbus, Ohio; Daniel Ortiz, PhD, University of Texas Medical Branch, Galveston, Texas; Susan Philip, MD, MPH, San Francisco Department of Public Health, San Francisco, California; Arlene C Seña, MD, MPH, University of North Carolina, Chapel Hill, North Carolina; Jeanne Sheffield, MD, Johns Hopkins University, Baltimore, Maryland; Marty Soehnlen, PhD, MPH, Michigan Public Health Laboratory, Lansing, Michigan; Elitza Theel, PhD, Mayo Clinic, Rochester, Minnesota; Anthony Tran, DrPH, MPH, District of Columbia Public Health Laboratory, Washington, DC; Susan Tuddenham, MD, MPH, Johns Hopkins University, Baltimore, Maryland; George Wendel, PhD, American Board of Obstetrics and Gynecology, Dallas, Texas; Kelly Wroblewski, MPH, APHL, Silver Spring, Maryland.

Meeting Facilitators: Joan Jarret and Paul Marquardt, PhD, AlignOrg Solutions, Shawnee, Kansas.

CDC Attendees: Sevgi Aral, PhD; Roxanne Barrow, MD, MPH; Gail Bolan, MD; Cheng Chen, PhD; Yetunde Fakile, PhD; Joseph Kang, PhD; Samantha Katz, PhD; Ellen Kersh, PhD; Sarah Kidd, MD; Jonathan Mermin, MD, MPH; S. Michele Owen, PhD; Ina Park, MD, MS; Lara Pereira, PhD; Tom Peterman, MD; Allan Pillay, PhD; Raul Romaguera, MPH, DMD; Mayur Shukla, PhD; Benedict Truman, MD; Kimberly Workowski, MD, National Center for HIV, Viral Hepatitis, STD, and TB Prevention, CDC.

Non-CDC Federal Employee Attendees: Carolyn Deal, PhD, National Institutes of Health, Rockville, Maryland; Tamara Feldblyum, MS, PhD, U.S. Food and Drug Administration, Silver Spring, Maryland; Delmyra Turpin, RN, MPH, National Institutes of Health, Rockville, Maryland.

Conflict of Interest Disclosures: Laura Bachmann, research funds awarded directly to Wake Forest University Health Sciences Medical School from Becton-Dickenson, Cepheid, Atlas, National Institutes of Health, CDC; William Becker, CLIA Lab Director, Columbus Public Health; Jeffrey Klausner, Laboratory Director at AIDS Healthcare Foundation, received donated test kits for research from Hologic and Cepheid; Michael Loeffelholz, member CDC Office of Infectious Diseases Board of Scientific Counselors, has previously received grant funding from Fujirebio Inc; Ruth Lynfield, Committee of Infectious Diseases for the American Academy of Pediatrics; Ina Park, Medical Consultant, CDC Division of STD Prevention (Intergovernmental Personnel Act contractor).

Key Question: What are the performance characteristics of each direct detection test for *Treponema pallidum* and what are the optimal specimen types for each test (darkfield microscopy, direct fluorescent antibody, PCR and immunohistochemical, or silver staining of tissue)?

Key Question: What options are available for molecular epidemiology and what should be considered for specimen collection and preservation?

APHL Workgroup Reviewer: Elitza Theel

Literature Search Terms: (syphilis OR Treponema pallidum) AND (genital ulcer disease OR primary syphilis OR secondary syphilis OR tertiary syphilis OR congenital syphilis OR ocular syphilis) AND (diagnosis OR lesions OR polymerase chain reaction OR PCR OR nucleic acid amplification test OR NAAT OR multiplex test OR silver stain OR silver staining OR immunohistochemistry OR IHC OR rabbit infectivity testing OR RIT OR direct detection OR dark field microscopy OR darkfield microscopy OR dark-field microscopy OR direct fluorescent antibody OR DFA OR direct fluorescent antibody for *T. pallidum* OR DFA-TP OR direct fluorescent antibody tissue test for *T. pallidum* OR DFAT-TP). Solely-based international studies were excluded from the literature search.

Key Question: What are the performance characteristics, stratified by the stage of syphilis, for non-treponemal serologic tests?

APHL Work Group Reviewers: Khalil Ghanem, MD, PhD and Susan Tuddenham, MD, MPH

Literature Search Terms: (syphilis (mesh) OR syphilis (tiab) OR maternal syphilis (tiab) OR syphilis in pregnancy (tiab) OR neurosyphilis (tiab)) AND (syphilis serodiagnosis (mesh) OR serofast (tiab) OR nontreponemal (tiab) OR non-treponemal (tiab) OR VDRL (tiab) OR venereal disease research laboratory (tiab) OR RPR (tiab) OR rapid plasma reagin (tiab) OR Toluidine Red Unheated Serum Test" (tiab)) NOT (review (publication type)) AND (1960/01/01 (PDat): 3000/12/31(PDat)) AND (English (lang)). Solely-based international studies were excluded from the literature search.

Key Question: What are the performance characteristics, stratified by the stage of syphilis, for treponemal serologic tests? (*T. pallidum* particle agglutination, fluorescent treponemal antibody-absorption, enzyme immunoassay, chemiluminescence assay, multiplex bead-based immunoassay)

APHL Work Group Reviewers: Ina Park, MD, MS and Anthony Tran, DrPH, MPH

Literature Search Terms: ((Treponema pallidum OR neurosyphilis OR syphilis) AND (sero-diagnos* OR serodiagnos* OR (serolog* AND (test* OR exam* OR assay* OR screen* OR lab* OR diagnos* OR nontreponemal OR treponemal OR algorithm* OR antibody titer)) OR serofast) NOT exp animals/ not exp humans/. Solely-based international studies were excluded from the literature search.

Key Question: Do laboratory tests perform differently when applied to special populations such as HIV positive individuals or pregnant women? What tests should be used in cases of suspected congenital syphilis?

APHL Work Group Reviewers: Jeanne Sheffield, MD and Ahizechukwu Eke, MD

Literature Search Terms: ((Treponema pallidum OR neurosyphilis OR syphilis) AND (sero-diagnos* OR serodiagnos* OR (serolog* AND (test* OR exam* OR assay* OR screen* OR lab* OR diagnos* OR nontreponemal OR treponemal OR algorithm* OR antibody titer)) OR serofast OR trimester OR rapid test*) NOT exp animals/ not exp humans/. Solely-based international studies were excluded from the literature search.

Key Question: What considerations (i.e., diagnostics and cost-effective implications) should be taken into account when screening for syphilis using either the traditional and reverse algorithm?

APHL Work Group Reviewers: Daniel Ortiz, PhD and Michael Loeffelholz, PhD

Literature Search Terms: ((Treponema pallidum OR neurosyphilis OR syphilis) AND (sero-diagnos* OR serodiagnos* OR (serolog* AND (test* OR exam* OR assay* OR screen* OR lab* OR diagnos* OR nontreponemal OR treponemal OR algorithm* OR antibody titer)) OR serofast) NOT exp animals/ not exp humans/. Solely-based international studies were excluded from the literature search.

Key Question: What serologic-based point-of-care (POC) tests are available to support a syphilis diagnosis, including single syphilis POC tests and combination syphilis/HIV and nontreponemal/treponemal POC tests, and what are the performance characteristics?

APHL Work Group Reviewer: Anthony Tran, DrPH, MPH

Literature Search Terms: (syphilis OR Treponema pallidum) AND (Syphilis Health Check OR rapid test OR point-of-care test OR point of care test OR POC test OR rapid point-of-care test OR rapid point of care test OR RPOC test OR diagnostic test OR combination test OR dual test OR multiplex test OR ASSURED OR rapid syphilis test OR RST OR saliva test OR immunochromatographic test OR finger-stick test). Solely-based international studies were excluded from the literature search.

Appendix 2. Peer Review Panel

Megan Crumpler, PhD, HCLD
Laboratory Director
Orange County Public Health Laboratory, Santa Ana, California

Sheila Lukehart, PhD
Professor Global Health, Associate Dean in the School of Medicine
University of Washington, Seattle, Washington

Beth M. Marlowe, PhD, D(ABMM), SM(ASCP)
Senior Scientific Director, Head R&D, Infectious Disease & Immunology
Quest Diagnostic Infectious Disease
Quest Diagnostics, San Juan Capistrano, California

Arlene C. Seña, MD, MPH
Professor of Medicine
Institute for Global Health and Infectious Diseases
Adjunct Professor of Epidemiology
Gillings School of Public Health
University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

Charge to Peer Reviewers: We request your review of the body of literature used to develop "Recommendations for Tests to Detect *Treponema pallidum*, the Causative Agent of Syphilis." As you review the Background, Methods, and Results sections, we would appreciate your thoughts as to whether any key studies have been left out or, in your opinion, misinterpreted as well as comments on the appropriateness of the conclusions. Above all, we are interested in your thoughts about the determinations regarding the quality of the evidence and the strength of the recommendations that were drawn. The questions below will serve as a template to collect and organize your responses. Once you complete your review, please send the review back to the CDC. After the Division of STD Prevention (DSTDP) reviews your comments, they will be posted without attribution along with our responses on the DSTDP.

Template of specific questions:

1. Are there omissions of information or key studies that are critical for the intended audience of clinical laboratory scientists, clinicians, and community health workers? If so, what should be included?

2. Have we included inappropriate information? If so, what should be removed?

3. Does the current scientific understanding of the biology of *T. pallidum* align with the terms "nontreponemal tests" and "treponemal tests" as discussed under the section Syphilis Serologic Laboratory Testing Terminology? Should new terms for nontreponemal tests and treponemal tests be adopted if scientifically appropriate? Would updating these terms add to confusion in the

literature? Do you foresee any regulatory implications regarding product insert literature if new terms are proposed? Please explain.

4. Are the recommendations appropriately drawn from the evidence presented? Please explain.
5. Is this document clear and comprehensible? If not, which sections should be revised?
6. Are the recommendations practical and achievable? For example, are resources available for laboratories interested in establishing darkfield microscopy? If not, do you have any suggestions regarding capacity building to ensure the recommendations are practical and achievable.
7. Other comments you might have?

Appendix 3. Updating Syphilis Serologic Laboratory Terminology

Syphilis serologic tests were developed at the beginning of the 20th century and used by medical personnel to diagnose syphilis. The first test described, known as the Wassermann test, was a complement fixation test that used liver extracts, initially from fetuses and subsequently from the heart tissue of patients with syphilis (*204*). The assay was further standardized to improve reproducibility by laboratories following the publication of a method to isolate cardiolipin and lecithin (phosphorylcholine) from beef heart and combine them with cholesterol as the antigens for these tests (*205*). Subsequent tests involving immobilization of *T. pallidum*, agglutination, or flocculation were based on the same principle of detecting serum that reacted to *T. pallidum* itself (*T. pallidum* immobilization [TPI] test) or to antigens found in the membranes of *T. pallidum* (cardiolipin [diphosphatidylglycerol], phosphorylcholine, and cholesterol) used in the VDRL and RPR tests. The World Health Organization (WHO) convened an expert committee on treponematoses in 1954 and made recommendations regarding antigen preparation, standardization of tests, and terminology (*206*). The terminology was based on the understanding of the contemporaneous scientific findings and became the basis for which to describe the serologic testing concepts for syphilis that are still used today (*207*). The use of these should be based on current scientific evidence related to the immunobiology of *T. pallidum*.

Immunobiology

T. pallidum are obligate microaerophilic spirochete bacteria with a flexuous, flat-wave morphology that range from 5 to 20 µm in length and 0.1 to 0.4 µm in diameter (*208*). The protoplasm is enclosed by a cell wall composed of a cytoplasmic membrane, a thin peptidoglycan layer, and a simple lipid bilayer outer membrane (*209,210*). The bacterial structure is like other Gram-negative bacteria in that a periplasmic space separates the cytoplasmic and outer membranes. However, in contrast to most other Gram-negative bacteria, the outer membrane of *T. pallidum* is extremely fragile, lacks a lipopolysaccharide outer layer, the peptidoglycan layer is above the cytoplasmic membrane rather than beneath the outer membrane, and there is approximately a 100-fold lower density of proteins that span the membrane (*2,211-216*). The organism exhibits corkscrew-like motility, rotating around its longitudinal axis that is provided by endoflagella located in the periplasmic space and are wrapped around the cell body (*217-219*). The relatively few integral membrane proteins, exposed lipoproteins, and phospholipids likely comprise the bacterial surface and contribute to its relative lack of surface antigenicity (*210,220*).

Following entry through the mucosa or microabrasions in the skin, *T. pallidum* replicates locally and quickly spreads throughout the body, including the central nervous system, through the cardiovascular and lymphatic systems (*180*). The dearth of pathogen-associated molecular patterns on the cell surface of *T. pallidum* contribute to the inability of the innate immune system to clear the organism during primary infection and subsequent dissemination (*221*). Activation of the innate immune system might be downregulated by a treponemal phospholipid found in the outer membrane (*222*). However, dendritic cells phagocytize *T. pallidum* early during infection, and most migrate to draining lymph nodes where they present processed treponemal antigens (mostly protein antigens) to B and T cells to initiate adaptive immune responses (*223*).

Antigens that are processed and presented by phagocytic cells during *T. pallidum* infection are either unique to the organism or common to both organism and/or host cells. Cardiolipin, diphosphatidylglycerol, is an integral mitochondrial cell membrane phospholipid required for proper mitochondrial function (*224*). B1 cells, a subset of B cells, secrete antibodies of low to moderate affinity in the absence of activation by prior infection (*225*). The B1 secreted antibodies are referred to as natural antibodies, and they can bind to cardiolipin and other phospholipids such as cholesterol and phosphatidylcholine. However, other infections or conditions, in addition to syphilis and autoimmune diseases, can cause a transient increase in natural antibodies against cardiolipin (*226*). The cytoplasmic membrane of *T. pallidum* contains cardiolipin and other phospholipids that can contribute to immune stimulation during infection (*227,228*). Cholesterol and phosphatidylcholine are host phospholipids that are also constituent macromolecules in the *T. pallidum* cytoplasmic membrane (*227*). Phosphorylcholine can be a target for protective immunity as demonstrated by the bactericidal effect of a monoclonal antibody binding to this antigen on the surface of *T. pallidum* (*229*). Antibodies to both cholesterol and phosphatidylcholine are elevated during some stages of infection with *T. pallidum* (*25*) and are detected by rapid plasma reagin (RPR) and Venereal Disease Research Laboratory (VDRL) tests.

Syphilis Serologic Laboratory Testing Terminology

Nontreponemal tests. Antibodies that reacted to the lipoidal antigens used in the Wassermann and subsequent agglutination or flocculation tests were either an indication of a concomitant *T. pallidum* infection or another condition related to host tissue damage and release of lipoidal antigens. The term “nontreponemal test” was first used in the literature in 1960 to differentiate tests based on *T. pallidum* specific antigens (TPI, FTA-ABS, MHA-TP, TPHA, TPPA) from tests based on antigens (cardiolipin, phosphatidylcholine, cholesterol) found in normal animal tissues and other organisms in addition to *T.*

pallidum and used in VDRL and RPR tests. The lipid composition of *T. pallidum* was first described in 1979 when Matthews and colleagues reported that the organism contained all the phospholipids used in nontreponemal tests (*227*). Genomic analysis of *T. pallidum* further revealed the lack of some enzymes for biosynthetic pathways necessary for these cytoplasmic and outer membrane phospholipids, indicating an inherent requirement for phospholipids from the host (*230*).

It is now recognized that the increase in antibodies to cardiolipin, phosphatidylcholine, and cholesterol during *T. pallidum* infection is likely the result of a combination of antigens from both the bacteria and the host, not just from host tissue damage. In a rabbit model, *T. pallidum* cardiolipin induced a high antibody titer during active infection (*228*). Inoculating rabbits with inactivated *T. pallidum* resulted in a lower anti-cardiolipin titer, suggesting the increased response observed during active infection was attributable to immune stimulation from a combination of cardiolipin released from *T. pallidum* and damaged host cells (*228*). Because the antigens used in nontreponemal tests are found in *T. pallidum* membranes and host membranes, it is a misnomer to refer to these tests as nontreponemal. A 2019 study published demonstrated that 11% of 526,540 reactive nontreponemal tests were not associated with syphilis, and in those cases, the tests were detecting antibodies to nontreponemal antigens generated by host tissue damage from other diseases (*231*). However, 89% of the reactive tests were associated with syphilis, implying that most “nontreponemal” tests detect antibodies triggered by *T. pallidum* phospholipid antigens during infection. So-called “nontreponemal” tests should more accurately be called “lipoidal” antigen tests.

Treponemal tests. Although the term “treponemal” tests was introduced in 1960 along with nontreponemal tests (*232*), it remains an accurate description of a test that an antibody response to *T. pallidum* specific antigens.

Nonspecific antibody. The term “nonspecific antibodies” has been used in the syphilis literature to characterize antibodies that are not specific to *T. pallidum* but are detected in nontreponemal tests. All antibodies bind to specific epitopes on an antigen and are specific to that antigen. However, they might not be specific for the detection of the disease or condition for which the test is ordered, and thus, their presence impacts the test specificity. Antibody specificity and the effect on test specificity should be reported rather than using the blanket term “nonspecific antibody.”

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