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Advantages and limitations of current diagnostic laboratory approaches in syphilis and congenital syphilis

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Abstract

Introduction: The reemergence of syphilis, especially congenital syphilis, presents a significant public health threat. Accurate diagnosis of syphilis depends on recognition of a constellation of symptoms, review of medical and sexual history, and multiple laboratory tests. While reliable, current tests for syphilis can be difficult to interpret, which can lead to delays in treatment.

Area covered: This review summarizes the major advantages and limitations of available diagnostic laboratory methods for syphilis, provides an update on recent advances in laboratory tools, and highlights the urgent need for coordinated efforts to create new tools to halt the resurgence of syphilis.

Expert opinion: In syphilis, the wide variety of short-lived signs and symptoms followed by periods of latency create diagnostic challenges. Currently available laboratory tests, when positive, require additional information to interpret (prior testing, treatment, and sexual history). Point-of-care tests that can rapidly and accurately detect both treponemal and non-treponemal antibodies would be a huge step toward reducing test turnaround time and time to treatment. Incorporating biological insights and technology innovations to advance the development of direct detection assays is urgently needed. A comprehensive coordinated effort is critical to stem the tide of rising syphilis in the United States and globally.

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Keywords

Congenital syphilis; molecular detection; nontreponemal test; serologic test; syphilis; syphilis screening; treponema pallidum; treponemal test

1. Introduction

Syphilis is a sexually transmitted infection (STI) caused by the spirochete *Treponema pallidum*, subspecies *pallidum* (*T. pallidum*). Within this genus, three other organisms cause nonvenereal or endemic treponematoses including yaws (*Treponema pallidum* subsp *pertenue*), bejel (*Treponema pallidum* subsp *endemicum*), and pinta (*Treponema carateum*) [1,2]. *T. pallidum* is an obligate microaerophilic spirochete with fragile outer membrane that lacks a lipopolysaccharide outer layer, which contains fewer proteins (approximately 100-fold lower protein density) compared to other gram-negative bacteria [3–6]. Fewer integral membrane proteins, exposed lipoproteins, and phospholipids on the spirochete's surface may contribute to the host's immune system's inability to effectively detect *T. pallidum* and prevent systemic infection [7,8].

1.1. Signs and symptoms of syphilis

Without treatment, syphilis infection may progress through a wide range of symptoms that are grouped into stages, including primary, secondary, latent, and tertiary [9,10]. During the primary stage, which starts three weeks after exposure (range 10–90 days), a single, often painless chancre appears at the site of *T. pallidum* exposure. The chancre spontaneously resolves within a few weeks (range 3–6 weeks). Occasionally, multiple or painful chancres and lymphadenopathy near the site of infection may occur, increasing the likelihood of detection. The secondary stage has variable symptoms with various morphologies of skin rash, patchy alopecia, mucous membrane lesions (e.g. mucous patches in the mouth, vagina, or anus) and/or condylomata lata in intertriginous areas. These signs and symptoms typically occur two to eight weeks after the primary stage chancre resolves, but in some the chancre may still be present. Primary and secondary lesions (i.e. chancres, mucous patches, and condylomata lata) have large concentrations of spirochetes and are highly infectious [11]. Secondary syphilis symptoms usually resolve over 3–12 weeks without treatment. During the latent stage, no visible signs or symptoms of syphilis are observed. Latent stages can occur between the primary and secondary stages, or only after the secondary stage; and if syphilis remains untreated, may last for years. Early latent syphilis (a latent stage within one year of initial infection), and late latent syphilis (a latent stage one or more years after initial infection), are distinguished by careful review of symptoms and treatment, prior serology and sexual history. Tertiary syphilis can appear years or even decades after the initial infection and generally presents with gummatous lesions, cardiovascular syphilis, psychiatric symptoms (e.g. memory loss or personality changes) or late neurosyphilis (e.g. tabes dorsalis and paresis). Individuals living with human immunodeficiency virus (HIV) or who are immunocompromised may progress more rapidly. Neurosyphilis can occur at any stage of syphilis and is characterized by cranial nerve dysfunction, meningitis, stroke, loss of vibration sense, auditory or ophthalmic abnormalities, or altered mental status. Ocular

and otosyphilis can result in permanent vision and hearing loss. Importantly, at any point in pregnancy, *T. pallidum* can cross the placenta, causing congenital syphilis.

1.2. Diagnosis and treatment of syphilis

Beyond recognizing signs and symptoms, diagnosing syphilis relies on laboratory testing, which is especially critical in asymptomatic cases. Some laboratory tests directly detect *T. pallidum* spirochetes, including microscopic examination for bacteria using darkfield microscopy (DFM), antigen detection by direct florescent antibody (DFA) and immunohistochemistry (IHC) staining, and nucleic acid detection by molecular methods. Nucleic acid amplification tests (NAATs) are molecular tests which use polymerase chain reaction (PCR) or other technologies to amplify *T. pallidum* nucleic acid for detection. Serologic tests detect antibodies produced by the host immune system in response to *T. pallidum* infection. Two categories of serologic tests, nontreponemal and treponemal tests, have been used to diagnose syphilis. Briefly, nontreponemal tests detect anti-lipoidal antibodies that appear about 6 weeks post-infection. A qualitative nontreponemal test is often used to screen for antibodies. If positive, the test is repeated as a quantitative test with results reported as dilutional titers, which can reflect the rise and fall of antibodies over the natural disease course. Antibody levels, and their corresponding titer, typically also fall with appropriate treatment, thus titers may be used to monitor treatment response. Typically, a four-fold or two dilutions drop in titer (e.g. from 1:16 to 1:4) signals an appropriate response to treatment. Titer increases after treatment may indicate re-infection and any four-fold or greater increase should be thoroughly investigated. In some individuals infected for several years, the nontreponemal test may remain reactive at low titer, even after appropriate treatment; and there is not seroreversion to negative (Figure 1), a condition referred to as ‘serofast.’

Treponemal tests detect specific anti-*T. pallidum* antibodies. Treponemal antibodies generally appear slightly earlier than nontreponemal antibodies. Treponemal IgM antibodies appear as early as 2–3 weeks after infection, followed in a few weeks by treponemal IgG antibodies. While IgM antibodies become undetectable after about a year, treponemal IgG antibodies typically persist for life. Thus, treponemal tests indicate infection at some prior point, but without the additional nontreponemal titer test and other medical history, no additional clinical determination of infectiousness or the need for treatment can be made. Both tests are antibody-dependent and therefore may be negative in some patients (14–46%) with primary syphilis, before the host’s immune system has responded [12]. Therefore, a non reactive test result does not rule out an incubating infection, and persons with known exposure to an infected partner should be considered for presumptive treatment. Both nontreponemal and treponemal serologic tests are reactive in secondary and early latent syphilis. In late latent and tertiary syphilis, nontreponemal tests may be nonreactive (Figure 1). Syphilis serologic screening is recommended for persons with signs or symptoms of infection, persons with an infected sex partner, pregnant persons, sexually active men who have sex with men, sexually active HIV-infected persons, and other persons at increased risk [13]. Long-acting benzathine penicillin G (e.g. Bicillin L-A®) is the recommended treatment for all stages of syphilis and aqueous crystalline penicillin G is recommended when neurologic, otic or ocular involvement is present [13]. One dose (2.4 million units)

for primary, secondary, or early latent (less than 1 year) and three doses at weekly intervals for late latent or unknown duration syphilis are recommended [13]. If syphilis is diagnosed during pregnancy, treatment with benzathine penicillin G should be started as soon as possible and at least 30 days before delivery to prevent congenital syphilis.

1.3. Resurgence of syphilis

Despite availability of an effective treatment, syphilis is reemerging as a significant public health threat. Since 2000, the national rate of syphilis in the United States (U.S.) has increased almost every year. In 2017, there were 101,590 total cases of syphilis (all stages and congenital syphilis), including 30,644 cases of primary and secondary (P&S) syphilis. In 2021, 176,713 cases of syphilis were reported with 53,767 cases of P&S syphilis, a 74% increase from 2017 [13]. Syphilis rates have increased in both males and females, in all age groups, and in all regions of the U.S. Men who have sex with men (MSM) are disproportionately impacted by syphilis and accounted for 46.5% of all male P&S syphilis cases in 2021. Most alarming is the skyrocketing rate of congenital syphilis in the U.S., reaching a national rate of 77.9 cases per 100,000 live births in 2021 which represents a 219.3% increase relative to 2017. Based on 2018 U.S. data, the total estimated medical cost of syphilis was $174 M [14]. Globally, the World Health Organization (WHO) estimated that 7.1 million adults between 15–49 years old acquired syphilis in 2020 [15]. Various strategies and calls to action have been issued by national and international health organizations to stem the tide of rising syphilis [16,17]. The Department of Health and Human Services (HHS) issued the Sexually Transmitted Infection (STI) National Strategic Plan 2021–2025 setting forth its specific vision, goals, objectives and strategies to prevent and control STIs, including syphilis, in the U.S [18].

A wide variety of factors including changing social and behavioral trends, social media, ease of travel, systematic barriers to comprehensive sexual healthcare and the aforementioned diagnostic difficulties are all plausible factors contributing to the reemergence of syphilis [19,20]. Thus, a multi-dimensional approach is urgently needed with interventions from clinical, public health, and biomedical fields, with the active inclusion of impacted and at-risk populations. Vaccine development is unlikely in the near term. Efforts to develop better diagnostic tests could play a critical role in easing the burden of disease by detecting and facilitating the treatment of more infections, thus lead to a significant reduction of syphilis. One of the goals emphasized by the STI National Strategic Plan is to accelerate progress in STI research, technology, and innovation [18]. This review will focus on current and emerging testing technologies in the diagnosis of syphilis in adults and newborns and discuss the advantages and limitations of those diagnostic approaches, as well as provide updates on current syphilis screening program recommendations to reduce or eliminate transmission.

2. Direct detection methods support a diagnosis of syphilis

2.1. Darkfield microscopy (DFM) and methods to directly visualize *T. pallidum*

The beginning of laboratory diagnosis of syphilis started in 1906 when American immunologist Karl Landsteiner introduced darkfield microscopy (DFM) [21]. DFM uses

an opaque disc to create oblique light rays on a dark background, leading to enhanced contrast of thin objects, such as spirochetes, which are below the resolution of conventional light microscopes. Correct identification of live *T. pallidum* requires both morphology (e.g. brightly illuminated helically coiled corkscrew) and characteristic motility (e.g. rapid rotation with occasionally flexing or bending). Less commonly used today, DFM is performed at the point of care (POC) on clear serous exudate freshly collected from suspected primary or secondary syphilis lesions. Motile spirochetes are required for accurate interpretation. When available, it can provide an immediate diagnosis and support the provision of treatment before the patient leaves the clinic, which is especially critical in the most infectious stages. The accuracy of results depends on technician experience and the ability to exclude other spirochetal organisms. For primary syphilis, DFM offers rapid, definitive evidence of infection with 75–100% sensitivity and 94–100% specificity for specimens from non-oral exudative lesions. For secondary syphilis, DFM sensitivity ranges from 58% to 71% and specificity is up to 100% [12,22]. The sensitivity of DFM decreases as syphilis infection progresses.

DFM was listed on the test menu for syphilis diagnosis from 8 public health laboratories in the U.S. (Table 1) but may be used more widely at STD clinics. DFM limitations include how quickly it must be performed, lack of microscopes with dark-field capabilities and microbiological technical expertise to identify the characteristic motion of *T. pallidum* to differentiate it from other spirochetes that may be normal flora. Having robust darkfield microscopy experience is increasingly rare among laboratory professionals. There are DFM for syphilis diagnosis training courses available (Course List – STD/HIV Prevention Training Center at Johns Hopkins (stdprevention-training.com)) and *T. pallidum* spirochetes stocks can be obtained from CDC's Division of STD Prevention for training on the use of DFM (Spirochete Request For Training (cdc.gov)). However, currently no Centers for Medicare and Medicaid Services (CMS)-approved proficiency testing programs are available in the U.S. to measure the quality of DFM testing. DFM is also not included in the list of moderately complex, provider-performed microscopy (PPM) techniques under Clinical Laboratory Improvement Amendments (CLIA) PPM certificates which would allow providers to perform the test themselves. Correctly collecting specimens adds additional complication. To obtain a high yield of motile bacteria, the scab or crust, if present, should first be removed, and pressure applied at the base of the lesions to express exudate. Dry skin lesions usually do not contain sufficient live organisms for DFM testing. The specimen slide needs to be examined immediately, ideally within 20 minutes of specimen collection, to retain the motility of any live *T. pallidum* for accurate results. This requires near-bedside laboratory facilities available to evaluate specimens immediately after collection. Due to the presence of commensal spirochetes (e.g. *T. denticola*), DFM is inappropriate for detection of *T. pallidum* from oral lesions [23]. Despite these limitations, given the rising rate of P&S syphilis, DFM could still be a useful diagnostic assay in high-incidence regions. A recent study by Lejarraga-Canas et al. demonstrated that, among 806 syphilis suspected patients with genital ulcer diseases, 53.2% (429) patients were positive for DFM, and 48% of these patients had negative serologic test results [24].

Immunostaining detection of *T. pallidum* antigens using anti-*T. pallidum* antibodies include, but are not limited to, direct fluorescent antibody (DFA) and immunohistochemistry (IHC)

staining. DFA is primarily performed on body fluids and lesion exudate by fixing samples on slides, staining with direct fluorescent tagged antibodies and interpreting results using immunofluorescent microscopy. DFA may be used for formalin-fixed tissue biopsies in combination with a histological stain. Due to autofluorescence induced by formalin cross-link fixation, IHC using purified anti-*T. pallidum* antibodies followed by enzyme-conjugated or avidin-biotin complex (ABC) methods with biotinylated secondary antibodies is an alternative method for the detection of *T. pallidum* in formalin-fixed paraffin-embedded (FFPE) specimens. DFA and IHC methods require fixed samples and usually take 1–2 days for testing, thus cannot be used as POC tests for diagnosis. DFA was shown to have 73–100% sensitivity and 100% specificity in non-oral lesion exudate samples from patients with P&S syphilis using DFM as a reference test [25,26]. IHC was shown to have 49–92% sensitivity and 100% specificity using polyclonal anti-*T. pallidum* primary antibodies via ABC technology on secondary syphilis biopsy samples [27,28]. There are commercially available anti-*T. pallidum* antibodies which may be used clinically, but there are no US Food and Drug Administration (FDA)-cleared DFA and IHC diagnostic tests.

2.2. Nucleic acid amplification tests (NAATs)

In the mid-1980s, nucleic acid amplification techniques, including polymerase chain reaction (PCR) and transcription-based amplification, were developed [29,30]. Since 1992, real-time PCR has allowed monitoring of amplification changes with time and quantitation of target nucleic acid copies. Due to its improved sensitivity and specificity, NAAT has become the preferred method to detect many pathogens, especially if the agent is difficult to culture *in vitro*. While there are currently no FDA-cleared *T. pallidum* NAATs available for use in the U.S., NAATs are being explored using different specimen types from all syphilis stages. The two most widely used and evaluated genes are *T. pallidum* 47 Kda lipoprotein (tpp47) and the DNA polymerase I gene (polA). Both genes show excellent specificity (97–100%). However, sensitivity is highly dependent on the specimen source and disease stage; exudate from primary syphilis lesions is associated with highest overall test sensitivity (72–95%) [12]. For secondary lesions, NAATs demonstrate high specificity and 20–86% sensitivity compared to clinical diagnosis with serologic testing. The latent syphilis stage often has negative *T. pallidum* NAAT test results [31]. NAATs used with FFPE tissues from secondary syphilis showed moderate sensitivity (up to 67%), suggesting possible DNA degradation and inhibition due to the formalin fixation. Whole blood and blood fractions from P&S stages of syphilis can have up to 62% sensitivity as compared to standard syphilis diagnosis methods [32,33]. Therefore, *T. pallidum* NAATs could be a valuable tool in the detection of syphilis that might be missed by standard diagnostics, especially in P&S syphilis infections presenting with ulcers [34].

Limited numbers of laboratory-developed or commercial *T. pallidum* NAATs are currently available and in use, and the few available testing facilities are listed in Table 2. Laboratory developed real-time PCR assays specific for detection of *T. pallidum* are available from Quest Diagnostics and the University of Washington. These tests are approved for clinical use in the U.S. in compliance with CLIA. The Hologic transcription-mediated amplification (TMA) assay detects *T. pallidum* ribosomal RNA (rRNA) and is a research-use-only (RUO) assay.

Genital ulcers can also be the clinical manifestation of other sexually transmitted infections, most commonly herpes (causative agent *Herpes simplex* virus HSV-1 or HSV-2), and chancroid (*Haemophilus ducreyi*) in the U.S [35]. A multiplex PCR could allow simultaneous detection of single or multiple pathogens in the same sample. ARUP Laboratories, Medical Diagnostics Laboratories and CDC offer laboratory developed CLIA-compliant genital ulcer disease (GUD) tests for the detection of multiple STI pathogens including *T. pallidum*, HSV-1/2 and *H. ducreyi* [36]. Different specimen types were validated for the test offered by each laboratory as listed in Table 2. In addition, several GUD multiplex tests: PlexPCR VHS (SpeeDx Inc.), Allplex Genital Ulcer Assay (Seegene Inc.), and Vivalytic STI array (Randox Laboratories Ltd), obtained Conformité Européenne (CE) marking, which complies with the European *in-vitro* diagnostic devices directive and are legally commercialized in the European Union. A Fast Track Diagnostics developed Genital Ulcer assay, which is RUO (only in Germany), for the detection of HSV-1, HSV-2 and *T. pallidum* from Siemens Healthcare Diagnostics is also available for genital swabs, urine and rectal swab samples.

During the 2022 global outbreak of mpox, a non-variola Orthopoxvirus, many patients presented with skin lesions localized to the genital and perianal areas [37]. Multiple laboratory-developed mpox molecular tests received emergency use authorization (EUA) from the FDA, including a POC test from Cepheid (Nucleic Acid Based Tests | FDA, Xpert® Mpox (cepheid.com)). Emerging technology using novel protocols with integrated sample preparation, simpler and faster amplification and detection methods, and innovative microfluidic or biosensor platforms to support point-of-care testing are rapidly growing, especially since the emergence of COVID-19 pandemic. Such innovative assays that could rapidly and accurately detect active syphilis infection at the point-of-care are urgently needed. In 2022, CDC awarded two contracts to support the development and evaluation of such assays for direct detection of *T. pallidum*, ideally at all stages of syphilis. Such an assay could be very useful for early diagnosis of syphilis to allow timely treatment and prevent horizontal and vertical syphilis transmission. The possibility for syphilis assay development as part of a multiplex GUD assay including mpox could also be explored and validated for FDA clearance under EUA.

3. Serology laboratory and point-of-care tests

3.1. Nontreponemal tests

In the same year DFM was developed, bacteriologist August Paul von Wassermann introduced a nontreponemal serologic test for syphilis (Wassermann test) using complement fixation [21]. In the 1940s, cardiolipin was considered the/a major antigen in the Wassermann test and in the presence of lecithin and cholesterol, forms immune complexes with serum antibodies, called ‘reagin’ antibodies. But confusion remained as cardiolipin is found in many bacteria and human tissue and is thus not specific to *T. pallidum*. In the 1960s, these nontreponemal tests were distinguished from other treponemal tests (discussed below) that detect *T. pallidum* specific antigens. Since then, our understanding has evolved. *T. pallidum* contains cardiolipin and other bacterial phospholipids, along with host-derived cholesterol and phosphatidylcholine. Collectively, they are referred to

as lipoidal antigens [38,39]. The use of nontreponemal is somewhat of a misnomer since the antibodies recognize both host and treponemal lipoidal antigens, but it is the currently accepted nomenclature.

Flocculation, which results in immune complexes clumping together more quickly, was an important advancement and replaced the tedious complement fixation method in the Wassermann test. The commonly used Venereal Disease Research Laboratory (VDRL) slide test and Rapid Plasma Reagin (RPR) nontreponemal tests are descendants of the Wassermann test. The VDRL is a microflocculation test that uses heat-inactivated serum to flocculate a reagin suspension, visualized by microscopes. The RPR is a macroflocculation card test, using agglutination of reagin antibodies with charcoal-containing antigens, which can be visualized with the naked eye as a black clump against the white card background. The RPR test also uses a modified VDRL antigen suspension which contains choline chloride to eliminate the need for serum heat inactivation. The RPR test now largely supersedes VDRL as a nontreponemal test for syphilis serum diagnosis (see below). In the Toluidine Red Unheated Serum Nontreponemal (TRUST) test, particles of toluidine red are used in place of the charcoal particles of the RPR test.

All samples with a reactive qualitative nontreponemal test should be diluted quantitatively to determine the actual endpoint titer, which measures antibody levels in response to *T. pallidum* infection [13,40]. These titers usually correlate with disease and are monitored for treatment response, as titers typically decline after adequate treatment and increase in treatment failure or re-infection. Successful treatment is conventionally defined as ≥ 4-fold reduction in nontreponemal titer [13]. Rarely, titers might not decrease ≥ 4-fold (serological nonresponse) or low titers might persist without seroreversion to nonreactive results (serofast status) and patients may need longer periods of follow-up [41].

Nontreponemal antibody reactivity alone does not always indicate syphilis infection, and results should be interpreted in the context of the entire clinical presentation, sexual history, medical chart review and laboratory findings. Cardiolipin antigens are not exclusive to *T. pallidum*, and other medical conditions have been associated with the formation of nontreponemal or cardiolipin antibodies, including autoimmune and other infectious diseases, whether viral, bacterial, or parasitic [42–44]. A study conducted by Font et al. demonstrated that 24% of patients with varied autoimmune disorders were found to be positive for anticardiolipin antibodies [45]. In addition, pregnancy, IV drug use, and aging have also been reported to generate a positive nontreponemal test in the absence of syphilitic infection. RPR false reactivity was observed in some tests following COVID-19 vaccination [46]. Non-syphilitic positive nontreponemal tests are called biological false positives. In very rare situations, a nonreactive non-treponemal test with clinical evidence of syphilis can be seen in patients due to the interference of high antibody titer with the formation of antigen-antibody lattice, i.e. the ‘prozone’ phenomenon. If suspected, the specimen should be retested after dilution. The prevalence of this phenomenon is low (0.5–2%) and has been associated with primary syphilis, pregnancy, and neurosyphilis [47,48].

RPR and VDRL are equally valid assays. Serum RPR seems to be more sensitive and specific than serum VDRL tests [49]. However, the VDRL test shows more sensitivity and

specificity on CSF samples for the laboratory diagnosis of neurosyphilis. RPR titers are typically higher than VDRL titers for the same sample. Therefore, quantitative results from these two nontreponemal tests cannot be compared directly and sequential serologic tests for a patient or comparative serologic tests for a birthing parent and neonate should be performed by using the same serologic test, preferably from the same manufacturer to avoid variation in results.

Lastly, RPR and VDRL tests are labor intensive and require subjective interpretation of the results. Recently the FDA cleared three automated RPR systems, AIX 1000 (Gold Standard Diagnostics), ASI Evolution (Arlington Scientific), and BioPlex 2200 Syphilis Total & RPR assay (Bio-Rad Laboratories), which can facilitate high-volume testing and expedite turnaround time (up to 200 samples per 60 minutes). When compared to the manual RPR assay, automated RPR tests demonstrated good qualitative test performance. In addition, higher test reproducibility and better quantitative performance were recorded for the AIX 1000 compared to the other two automated RPR test systems [50]. In February 2022, BioRad issued a recall for the RPR portion of the Bioplex 2200 due to the concerns related to COVID-19 vaccine interference and false reactive RPR test results (Class 2 Device Recall BioPlex 2200 Syphilis Total & RPR (fda.gov). The automated RPR assays are only approved for a limited titer range. Manual titrations are needed to obtain endpoint titers. Unfortunately, when automated systems were initially introduced, some diagnostic laboratories did not perform the necessary manual titrations when titers reached the end of the approved spectrum and reported the highest or lowest titers from the automated instrument or used symbols (< or >). Our recent commentary on best laboratory practices for these new instruments emphasized that all reactive RPR results should report an actual endpoint titer [40].

3.2. Treponemal tests

Treponemal tests qualitatively detect specific antibodies to *T. pallidum* proteins or peptides [23]. Fluorescent antibody tests and hemagglutination assays were introduced from 1949 to 1966. The fluorescent treponemal antibody test absorption test (FTA-ABS) uses antigen from testes of infected rabbits and a fluorescein-labeled anti-human gamma globulin to detect *T. pallidum* specific antibodies in serum after absorption of nonpathogenic treponemal antigens. *T. pallidum* particle agglutination assay (TP-PA) and *T. pallidum* hemagglutination assay (TP-HA) are based on the agglutination of gel particles (TP-PA) or red blood cells (TP-HA) sensitized with *T. pallidum* (Nichols strain) antigens by antibodies found in syphilis patients. In a recent systematic literature review, FTA-ABS was found to be less sensitive and specific in P&S syphilis than TP-PA [51]. Many enzyme immunoassays (EIA) and chemiluminescence immunoassays (CIA) were developed later using various recombinant antigens and are commercially available for the detection of treponemal antibodies [51]. In these immunoassays, specific *T. pallidum* recombinant antigens are fixed to a solid phase. Antibodies in patients' sera or plasma bind to the immobilized antigen and the antigen-antibody complex is subsequently detected by enzyme labeled antibody (EIA) or chemiluminescent labeled antibody (CIA). In the multiplex flow immunoassay (MFIA), antigen is immobilized on paramagnetic microbeads and detected by fluorescence-conjugated antibody. Immunoassays can be automated for high-throughput screening and

to minimize inter-operator variations. A list of currently recommended syphilitic serologic antibody tests is available in Laboratory Recommendations for Syphilis Testing in the United States (Federal Register: Laboratory Recommendations for Syphilis Testing in the United States). Most are automated EIA and CIA assays. Based on a review of published data by Park et al., there is insufficient evidence to recommend any one assay over the 13 immunoassays available.

Most treponemal tests detect both IgG and IgM antibodies. However, some EIA assays were developed for the detection of IgM only, in order to aid the diagnosis of very early syphilis. Limited published data indicates that the usefulness of IgM test for early syphilis detection is debatable [52,53] and IgM antibodies fade about one year after infection. Upon infection, treponemal antibodies appear earlier than nontreponemal antibodies and typically persist lifelong, even with successful treatment. Thus, treponemal test reactivity could be useful for early syphilis diagnosis when nontreponemal antibodies have yet to form and tests might be negative. Reactive treponemal test results may indicate active infection, past treated or untreated infection and need to be used in combination with nontreponemal test results and clinical signs/symptoms for accurate syphilis diagnosis. Currently available treponemal tests are not subspecies specific and therefore cannot distinguish syphilis from other endemic treponemal diseases including yaws, bejel, and pinta [54,55].

3.3. Point-of-care tests (POCT)

Standard syphilis laboratory testing methods lead to delays in diagnosis and treatment because they require days or weeks to complete, thus patients must return for follow-up clinical care. Samples from remote areas regularly need to be transported to regional or central laboratories, leading to further diagnostic delays. Point-of-care tests (POCT), designed to be simple and performable with minimal training and no equipment, can provide test results while the patient is present and can meaningfully reduce time to treatment. Two FDA-cleared and CLIA-waived rapid tests are available for the detection of *T. pallidum* treponemal antibodies in the U.S. Syphilis Health Check (SHC, Trinity Biotech) is a qualitative, rapid treponemal membrane immunochromatographic assay for syphilis. It can be performed on fingerstick whole blood, venous whole blood, serum and plasma, with results in 10 minutes. The DPP HIV-Syphilis system (Chembio Diagnostics) is a dual rapid test for the detection of antibodies to HIV1/2 and *T. pallidum* (treponemal antibodies) in fingerstick whole blood, venous whole blood, or plasma, with results in 15 minutes [56,57]. Systematic reviews showed performance with 50–100% sensitivity and specificity for SHC. Performance of the DPP showed a 47.4–98.8% sensitivity and 97–100% specificity for syphilis [58]. Because current POCTs only detect treponemal antibodies, they can be used for initial screening, but not to diagnose syphilis alone. A new serologic rapid test that simultaneously detects treponemal and nontreponemal antibodies is urgently needed. An assay, DPP® Syphilis Screen & Confirm, meeting such requirements was developed by Chembio Diagnostics, has obtained CE Mark and is available in select countries, but not currently in the U.S., for clinical use. An evaluation study of DPP® Syphilis Screen & Confirm test by Vargas et al. demonstrated 96.9% sensitivity for samples with RPR titers of ≥ 1:8. However, only 54.0–58.5% sensitivity was reported for samples with RPR titers up to 1:4 which may result in significant underdiagnosis [59]. Chembio

Diagnostics is currently working to improve and clinically validate a rapid point-of-care combo treponemal/nontreponemal antibody test for active syphilis diagnosis [60]. It is vital to have better tools for diagnosing syphilis and determining response to treatment.

3.4. Syphilis serologic testing algorithms

The laboratory support of a diagnosis of syphilis requires both nontreponemal and treponemal tests. In U.S., there are two widely used approaches or algorithms for the serologic diagnosis evaluation of syphilis called the traditional or reverse algorithms (Figure 2) [61]. The traditional algorithm uses a 'qualitative' nontreponemal test (e.g. RPR and VDRL) to screen. If that is non-reactive, no laboratory evidence of syphilis exists, and results are reported as 'nonreactive.' If it is reactive, a quantitative nontreponemal test is conducted to determine the end-point titer and a treponemal test (e.g. TP-PA, CIA and EIA) is required to confirms *T. pallidum* infection. In contrast, the reverse algorithm begins with a treponemal test, most often in automated format (e.g. EIA or CIA). Non-reactive results indicate no laboratory evidence of syphilis. For treponemal reactive samples, a quantitative nontreponemal test with titer is performed. If both tests are reactive, results are suggestive of past or current syphilis. If the nontreponemal test is nonreactive, a second treponemal test utilizing platform and/or antigen different than the initial treponemal testing (e.g. TP-PA), should be used to adjudicate the results of the initial treponemal test. If the second treponemal test is reactive, it is consistent with syphilis and clinical correlation is necessary to guide further patient management. Importantly, it could be a case of early syphilis since treponemal antibodies arise before nontreponemal antibodies. If the second treponemal test is negative and review of clinical and other factors suggest that the probability for syphilis is low, further evaluation or treatment is not indicated [13]. Both testing algorithms involve interpretation of a sequence of tests to generate a result. It is important for healthcare providers to be familiar with what is available in their area. The Association of Public Health Laboratories (APHL) has published 'suggested reporting language for syphilis serology testing' to provide guidance for standardized language when reporting laboratory results to avoid misinterpretation, unnecessary additional testing and to expedite linkage to medical care for infected patients [62].

Traditional screening, with a nontreponemal test first, detects cases of active syphilis but may miss early primary and, very rarely, late latent syphilis cases as these may not have nontreponemal reactivity [63]. Reverse screening with a treponemal test first will likely identify a higher rate of treponemal reactive patients, including persons with early, untreated, incompletely treated, or previously treated syphilis. In such cases more confirmatory testing overall is needed. Both algorithms produce reliable results and were reported to generate equivalent rates of active infection cases [64]. CDC supports the use of either algorithm [13,65]. A 2015 survey demonstrated approximately 80% vs 20% of laboratories use the traditional vs reverse algorithm, respectively, when a single algorithm is offered in the laboratory [66]. Patient population and risk, syphilis prevalence, test cost, volume, laboratory staffing, workflow, and availability of equipment may factor into which algorithm is appropriate. The traditional algorithm may be well suited for low-volume laboratories and was shown more cost-effective in a low-prevalence setting [67]. The reverse algorithm may be more appropriate for high prevalence settings and high-risk populations, where

more primary syphilis patients are likely to present, and who may be missed by the traditional screening method [68,69]. High-volume laboratories often perform the reverse algorithm using automated treponemal assays. This increases throughput with fewer medical laboratory technicians, lower costs and provides an ergonomic benefit by eliminating the repetitive pipetting steps of manual assays. With the launch of automated RPR instruments, this trend might change. Regardless of the syphilis testing algorithm used, laboratory results should be correlated with patients' clinical symptoms, their sexual and medical history and risk for syphilis infection.

4. Diagnostics for special cases and populations

4.1. Neuro, ocular, and otosyphilis

Neurosyphilis, ocular, and otosyphilis can occur at any stage of syphilis. If clinical evidence of central nervous system involvement is present or suspected, including cognitive dysfunction or altered mental status, motor or sensory deficits (especially vibratory), cranial nerve palsies, or signs or symptoms of meningitis or stroke, obtaining CSF for further testing should be performed before treatment. Ophthalmic abnormalities may indicate ocular syphilis, (e.g. syphilitic uveitis, neuroretinitis, and optic neuritis). Ocular symptoms, hearing loss, and other otologic symptoms can be isolated abnormalities or may be associated with neurosyphilis. If ocular syphilis is suspected, cranial nerve evaluation and immediate referral to an ophthalmologist for full ocular examination is crucial. If ocular cranial nerve dysfunction is present, a CSF evaluation is needed prior to initiating treatment. Otosyphilis should be managed in collaboration with an otolaryngologist. HIV testing should be done for individuals diagnosed with any form of neurosyphilis.

No single test can diagnose neurosyphilis in all instances. Diagnosis relies on a combination of CSF tests [e.g. CSF cell count indicating pleocytosis (>5 cells/ml), protein, and CSF VDRL] along with reactive syphilis serologic tests and neurologic signs and symptoms. The CSF-VDRL is the only FDA-cleared test to support the diagnosis of neurosyphilis. It shows 74–100% specificity and a reactive CSF VDRL test with neurologic signs and symptoms is considered diagnostic of neurosyphilis [13,49]. However, the sensitivity of CSF VDRL is suboptimal (49–87.5%) and a negative test result should not rule out the neurosyphilis diagnosis.

Multiple studies evaluated the performance of treponemal tests, especially FTA-ABS and TP-PA, on CSF specimens for neurosyphilis diagnosis. Compared to CSF VDRL, FTA-ABS and TP-PA have shown 90.9–100% and 75.6–95.0% sensitivity, respectively, in patients with definitive neurosyphilis diagnosis. The reported specificity for CSF FTA-ABS is highly variable from 55% to 100% depending on diagnostic criteria and the test used as the standard for comparison [51]. Therefore, neurosyphilis is highly unlikely with a negative CSF FTA-ABS or TP-PA test result, especially among persons with nonspecific neurologic signs and symptoms. The specificity of a CSF TP-PA improves as its titer rises and when it is ≥ 1:640, it is comparable to CSF VDRL [70]. Performance evaluation of nontreponemal and treponemal tests using CSF for the diagnosis of ocular syphilis and otosyphilis are very limited.

Although CSF specimens typically have few bacteria, direct detection of *T. pallidum* in CSF using NAATs was evaluated as tool for neurosyphilis diagnosis. Sensitivity ranging from 40% to 70% and specificity from 60–100% of NAAT for CSF from patients diagnosed using national syphilis management guidelines has been reported [12]. The most commonly used PCR assay (targeting tpp47) had an overall sensitivity of 68% and specificity of 91.9% as demonstrated by 5 studies including 88 neurosyphilis patients [12,71]. The overall study sample sizes were small and additional studies are needed to evaluate the usefulness of CSF NAAT for the diagnosis of neurosyphilis.

4.2. Congenital syphilis (CS)

Syphilis rates are surging in the U.S., including among women of reproductive age. During 2020 to 2021, P&S syphilis rates increased 52.3% among women aged 15–44 years and cases of CS correspondingly increased 32.4%, (N = 2,157 in 2020 to N = 2,855 in 2021), with a 203.4% increase relative to 2017 (N = 941) [72]. Syphilis can be transmitted to the fetus at any time during pregnancy, with P&S syphilis being associated with increased rates of fetal infection and poorer outcomes. Among CS cases reported in 2021, 197 (6.9%) were reported as stillborn and 23 (0.8%) as an infant death. Screening and adequate treatment for syphilis during pregnancy can prevent neonatal infection. Because most newborns diagnosed with CS are asymptomatic at birth, maternal testing and screening is also critical in identifying neonates needing additional evaluation. Currently, CDC recommends that no mother or newborn infant should leave the hospital without maternal serologic status having been documented at least once during pregnancy [13].

Diagnosing CS in the neonate requires clinical evaluation of the newborn and careful review of maternal syphilis history to assess risk of transmission during pregnancy. This includes maternal testing (nontreponemal and treponemal) results, evaluation of treatment adequacy, and comparing maternal and neonatal nontreponemal titers. Neonatal evaluation for congenital syphilis includes, at a minimum, physical exam and infant non-treponemal testing; and may also include complete blood count (CBC) and differential; CSF for VDRL, cell count and protein; and long-bone radiographs, as well as other investigations as indicated by clinical presentation. These additional evaluations are predicated on clinical assessment of the likelihood of congenital syphilis infection using a standardized algorithm (Figure 3) [13]. Clinical evidence of congenital syphilis may include nonimmune hydrops; conjugated or direct hyperbilirubinemia, cholestatic jaundice or cholestasis; hepatosplenomegaly; rhinitis; various skin rashes; pseudoparalysis of an extremity; or osseous lesions in radiographs of the long bones. Stillborn infants may have direct detection methods performed on suspicious lesions or tissue, as well as undergo long bone radiographs to aid in the diagnosis of syphilitic stillbirth. According to CDC recommendations, any person who delivers a stillborn infant should be screened for syphilis if not previously identified to be infected [13]. More details on the full evaluation and treatment are available in the 2021 CDC STI Treatment Guidelines. The decision to treat a neonate is based upon the likelihood of congenital infection, and to aid diagnostic decision-making four scenarios are described (confirmed proven or highly probable, possible congenital syphilis, congenital syphilis less likely, and congenital syphilis unlikely) (Figure 3) [13]. Briefly, newborns with an abnormal physical examination consistent with CS; or laboratory evidence of infection

(neonatal nontreponemal titers four-fold or higher than maternal titers at delivery, or direct detection through positive DFM or PCR) would be considered ‘confirmed proven or highly probable’ for CS. DFM or PCR testing of lesions or bodily fluids (i.e. nasal discharge or bullous rash), suspicious placenta, or cord were evaluated using rabbit infectivity testing as a reference. DFM demonstrated 100% specificity with sensitivity ranging from 42–86% when using in amniotic fluid specimens [12]. PCR targeting tpp47 to detect *T. pallidum* in amniotic fluid, neonatal sera and CSF to diagnosis CS was evaluated by Grimprel et al. The PCR demonstrated 100% specificity for all clinical sample types tested. Higher sensitivity for amniotic fluids (100%) was observed than sera and CSF, which typically contain less *T. pallidum* [73]. When comparing maternal delivery and neonatal quantitative nontreponemal tests (RPR or VDRL), the same test, preferably conducted by the same laboratory, should be used [13]. For the remaining three scenarios, where newborns have a normal physical exam, negative direct detection testing (if performed), and neonatal nontreponemal titers equal to or lower than maternal titer at delivery, assessment of risk of neonatal infection is based on careful review of maternal syphilis history, including 1. timing of infection (before or during current pregnancy), 2. adequacy of treatment (a penicillin-based regimen appropriately dosed for the stage of maternal syphilis and started at least 30 days before delivery), and 3. lack of evidence of maternal reinfection or relapse (titers dropped or did not increase during pregnancy). Regardless of treatment, all neonates with positive nontreponemal tests should be retested every 2–3 months, with expectation that titers drop and reach nonreactive by 6 months of age. Neonates where neonatal nontreponemal serology was negative and maternal serology was seroreactive should be retested at age 3 months to rule out congenital syphilis infection that may have been incubating prior to delivery. Neonates with persistent nontreponemal titers beyond age 6 months should be thoroughly reevaluated. Neonatal treponemal tests are not recommended since maternal syphilis IgG antibodies are passively transferred across the placenta during pregnancy, and these maternal antibodies can persist for more than 15 months [13].

Maternal IgM does not cross the placenta and the fetus is capable of producing specific IgM antibody by 24 weeks of gestation [74]. Therefore, treponemal IgM fluorescent treponemal antibody absorption (FTA-ABS), IgM-ELISA (enzyme-linked immunosorbent assay) and IgM-western blot tests have been explored for the aid of congenital syphilis diagnosis. Although IgM FTA-ABS did not show good performance, laboratory developed IgM-ELISA and Western blot showed sensitivity of 83% for detection of 6 symptomatic congenital syphilis cases as reported by Schmitz et al. [75,76]. More data is needed to evaluate the IgM test as currently no commercial IgM test is recommended to use for the diagnosis of congenital syphilis in the US.

4.3. Syphilis screening

Screening is an important public health intervention to prevent syphilis and CS. CDC has previously analyzed missed prevention opportunities among mothers delivering infants with congenital syphilis in the U.S. From 2017 to 2021, 38% lacked timely prenatal care and timely syphilis testing [13]. CDC recommendations are that all pregnant persons should be screened serologically for syphilis at the first prenatal care visit. For those living in communities with high rates of syphilis or who are at high risk for syphilis during

pregnancy, screening should also be performed at 28 weeks gestation and at delivery [13]. All deliveries should have documentation of maternal syphilis testing, during pregnancy or at delivery, prior to maternal and newborn discharge. Pregnant persons suffering a fetal loss after 20 weeks' gestation should be tested for syphilis infection. Syphilis screening can follow either traditional or reverse sequence algorithm. The traditional and reverse screening algorithm was shown to have similar rates of screen positivity in pregnancy in a diverse U.S. region with high syphilis prevalence, as reported in study from 2012 to 2017 with more than 20,000 pregnant women [77]. However, if the reverse algorithm is used for screening in pregnant women, up to 65% of discordant results from initial treponemal immunoassay and nontreponemal tests could be false positive and confirmatory treponemal tests are necessary [78]. If reverse algorithm screening suggests false positive treponemal test results, CDC recommends the following: for those with low risk of syphilis, repeat serology testing within 4 weeks could be considered; for those for whom follow-up is unlikely and with no syphilis treatment history, treatment should be provided [13].

Based on 2021 surveillance data, most cases of syphilis in U.S. are among gay, bisexual, and other MSM. Almost half (46.5%) of all male P&S syphilis cases in 2021 occurred in MSM, although they only account for 4% of the U.S. male population. In addition, the number of syphilis cases among MSM who were reported as HIV positive increased 2.6% compared to 2020. The highest rate of reported P&S syphilis cases was among non-Hispanic American Indian or Alaska Native persons (46.7 per 100,000), followed by non-Hispanic Black or African American persons (41.9 per 100,000) in 2021. History of incarceration, sex work, and being a man younger than age 29 years are factors associated with a higher risk of contracting syphilis. Since 2016, the US Preventive Services Task Force (USPSTF) has recommended annual screening of asymptomatic, nonpregnant adolescents and adults who are at increased risk for syphilis infection [79]. This recommendation was reaffirmed in 2022 [80]. The American Academy of Family Physicians and the HIV Medicine Association recommend syphilis screening for increased risk populations including all patients with HIV infection [81,82]. CDC recommends at least annual screening, and more frequent screening if at increased risk, for sexually active MSM and persons with HIV infection. Screening for women, transgender, and gender-diverse people at increased risk is also recommended [83]. CDC additionally recommends opt-out syphilis screening in correctional facilities based on the local and institutional prevalence of syphilis, and screening at entry might be indicated in short-term facilities [84].

5. Conclusion

As cases rise, syphilis remains a public health threat. Biomedical challenges with the detection and elimination of syphilis include lack of widely available accurate tests to aid in the immediate diagnosis of syphilis. If a suspected spirochetal lesion is present, current direct detection tests, like DFM and immunostaining methods, are not widely available and no FDA-cleared NAATs yet exists. Current serologic tests are insensitive in the first few weeks of infection, leaving a critical gap during highly infectious early primary syphilis. Current treponemal tests, including those used for rapid testing, remain positive for life. This makes previously treated cases and re-infection indistinguishable based on treponemal testing alone, and interpretation reliant on additional clinical information,

which is sometimes difficult to obtain. In addition, vaccines to prevent infection are not available. Biomedical interventions to develop novel, sensitive, readily accessible, point-of-care tests are urgently needed to reduce the burden of syphilis, prevent complications, and eliminate mother-to-child transmission. Additionally, broadened and collaborative efforts from healthcare providers, public health agencies, and policy makers, informed by the communities impacted, are equally critical to combat the rising rates of syphilis.

6. Expert opinion

Like other areas of public health, investments in overcoming syphilis were made but as its elimination seemed imminent, ingenuity and innovation focused elsewhere. This led to uneven advancements and a lack of a cohesive approach to improve test diagnostics, where innovation can contribute most. Current mainstay serologic assays require both nontreponemal and treponemal tests, making them inherently challenging to interpret. Furthermore, to accurately diagnose syphilis, understanding recent sexual exposure, accessing prior medical information and laboratory results, and interpreting a wide range of clinical symptoms is required. Each aspect has the potential for misstep: many avoid disclosing sexual history and practices, previous laboratory results or treatment records are often incomplete or missing, and syphilis signs or symptoms may be overlooked. Diagnosing CS has additional complexities, illustrated by the four different probabilistic scenarios described in current guidelines. Aspects of the bacteria and disease course make creating a single test for all stages of syphilis difficult. Quantities of spirochetes sufficient for accurate testing are only obtainable during short windows when primary and moist secondary signs/symptoms are present. After these signs/symptoms resolve, the quantities of bacteria in blood and urine are not sufficient to produce reliable results with currently available tests. Additionally, the fastidious *T. pallidum* cannot routinely be grown on laboratory media, and thus the organism is not readily available, impeding further developments.

Nonetheless, development of a molecular direct detection test for *T. pallidum* that is sensitive and specific, which accurately diagnoses syphilis in all stages including during incubation, and which is validated on accessible specimen types would be ideal. Ever more sensitive molecular detection and amplification methods (CRISPR, next-generation sequencing, biosensing, isothermal amplification, etc.) in combination with signal transduction technologies (microfluidics, biosensor, smartphone, 3D printing, etc.) have some promise and need additional investment to reach their potential, as do protein-based sensitive detection methods [85]. Other approaches like identification and validation of biomarkers, including those in urine or related to specific immune responses or protein identification, have also not been fully explored. A much larger investment of resources and talent is needed to move toward better syphilis tests.

While the elimination of syphilis has challenges, syphilis is both preventable and treatable. Despite imperfect tests, screening and treatment are proven strategies for syphilis control and prevention [86,87]. Steps that lead toward reduced time between testing and treatment should be embraced. In addition to identifying better biomarkers, novel assays that enable testing and diagnosis of *T. pallidum* at or near patient care, thus enabling treatment at

the same visit, are vital to elimination efforts. STI POCTs were recognized by WHO's Global Health Sector Strategy for the control and prevention of STIs as an innovation that enables improvement in all steps of the STI services cascade and the ASSURED (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end users) criteria for POCTs was introduced in 2006 [88]. In 2023, the WHO further published target product profiles (TTP) for syphilis POCTs with potential to advance the field: tests clinical sensitivity and specificity are higher than 80%/90% for treponemal test and higher than 95%/90% for nontreponemal tests [89]. Currently available POCTs for syphilis only detect treponemal antibodies thus are unable to differentiate prior treated infections from current infections. A POCT serologic assay that can simultaneously detect treponemal and nontreponemal antibodies with targeted sensitivity and specificity is urgently needed. Attempts to simplify and integrate the sample preparations for molecular detection are still challenging. POCT molecular diagnostics have been transitioning from single pathogen detection toward multiplex molecular POCTs for infections that cause lesions in genital areas and in surrounding skin. Tests that could reliably identify and distinguish HSV1/2, syphilis, mpox, and potentially also LGV, VZV, and chancroid could advance the field. In addition, there is very limited availability of high-quality syphilis patient specimens that researchers and biotech companies can access, which further hinders the development/evaluation of serological and molecular diagnostic tests, and molecular surveillance of *T. pallidum*. The Division of STD Prevention at CDC, in collaboration with the Association of Public Health Laboratories (APHL), has developed a nationally available syphilis serum repository for research of FDA-cleared or investigational syphilis diagnostic assays in the U.S (APHL/CDC Syphilis Serum Bank). In 2021, the National Institutes of Health (NIH) awarded contracts to support the performance of a longitudinal clinical study to collect specimens from patients with syphilis and abstracted clinical data to deposit into an National Institute of Allergy and Infectious Diseases (NIAID) NIAID-supported repository Syphilis Specimen Collection 75N93021R00005 - GovTribe; Seña Awarded $1.9 Million to Advance Diagnostic Product Development for Syphilis | Institute for Global Health and Infectious Diseases (unc.edu)

The WHO International Advisory Group on STI POCT structured four main development phases for POCT development: basic research, translation, evaluation, and programmatic implementation [90]. More laboratory developed advanced methods are under evaluation for clinical implementation with the goal of bringing advances from research to impact real-world outcomes. Laboratory-developed tests may be validated and realistically implemented into clinical practice through research and clinical partnerships. Better syphilis testing tools are essential and building an accessible and collaborative healthcare system to support their development is equally important. This system should include equitable access to diagnostic testing and screening, as well as qualified health care providers to test patients for syphilis, treat immediately, treat partners, and quickly report all cases of syphilis so efforts can be tracked and adjusted as need varies. It is also essential that public health professionals make epidemiologic data available to clinicians for rapid local action, and to policymakers for use as the foundation for policy development and directing resources, while protecting patient privacy and confidentiality. CDC offers support, including the National Network of STD Clinical Prevention Training Centers, to aid in clinical development of skills, knowledge

and experience needed to address and prevent STDs. A combined effort between test developers and public health agencies in coordination with healthcare professionals and affected communities can improve screening, leading to more rapid detection and treatment and prevention of further transmission, halting the rapid spread of syphilis both nationally and globally.

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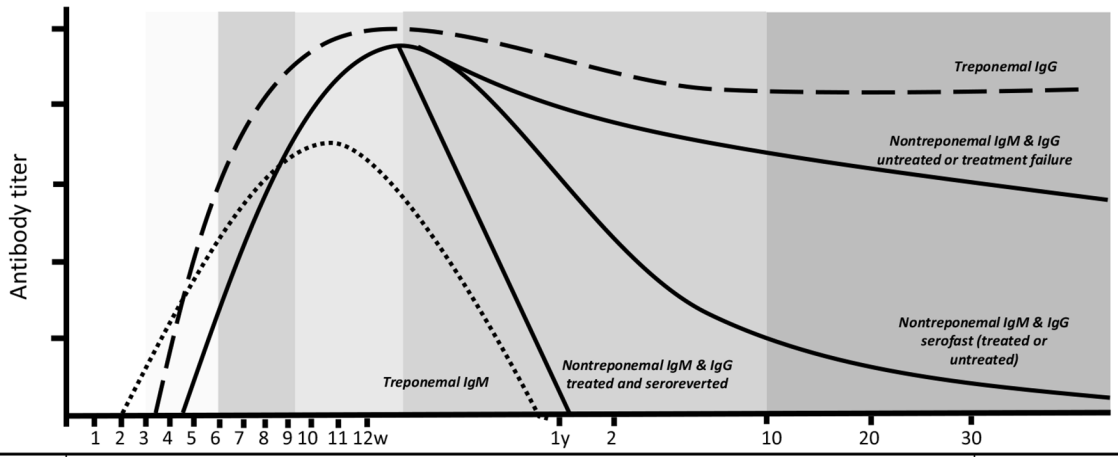
Article highlights

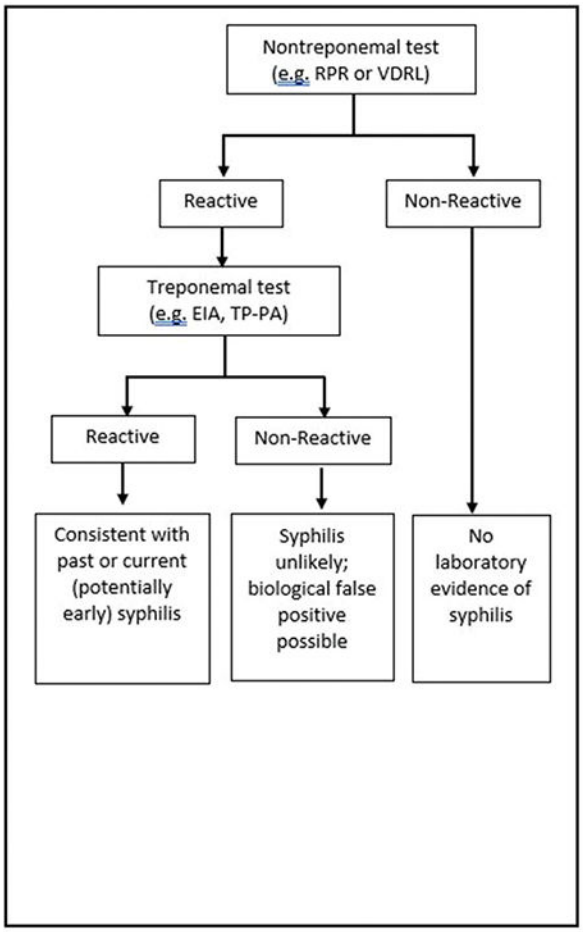
- The resurgence of syphilis poses a significant public health threat; current diagnostic laboratory approaches have limitations.
- The availability of direct detection methods including DFM and NAATs are limited. No FDA-cleared NAATs are available.
- Current serologic assays including rapid tests are insensitive in early infection and cannot distinguish newly acquired from previously treated syphilis infection.
- Biomedical interventions to develop novel, sensitive, readily accessible, point-of-care tests are urgently needed to reduce the burden of syphilis, prevent complications, and eliminate mother-to-child transmission.



	Time following exposure/inoculation							
	Early Syphilis (Up to 52 Weeks)					Syphilis (1 year or more)		Can happen at any stage
Stage	Incubation	Primary	Early Latent**	Secondary	Early Latent	Late Latent	Tertiary	Neurosyphilis
Ave. onset	0-3w	3w-6w	Variable	Variable	Variable, up to 1 year	Starts at 1 year and 1 day post inoculation, (important for treatment)	Variable	Can happen at any point of infection. Early neurosyphilis: usually within first few months or years of infection.
Range	10d-90d		2w-8w	3w-12w				
Sign/ Symptom	None	Chancre and lymphadenopathy	No visible signs or symptoms. In some, this stage may not occur	skin rashes, patchy alopecia, and/or mucous membrane lesions	no visible signs or symptoms	no visible signs or symptoms	affect multiple organ systems	Early: CNS involvement, including cognitive dysfunction or altered mental status, motor or sensory deficits (especially vibratory), cranial nerve palsies, or symptoms or signs of meningitis or stroke. Late neurologic manifestations (e.g., tabes dorsalis and general paresis) occur 10 to >30 years after infection
Tests Types*	None	Direct detection, specimens collected from lesions; serology likely but not 100%	Serology likely to be positive	Direct detection, using specimens from "moist lesions"; serology likely to be positive	Serology will be positive	Serology will be positive	Serology will be positive	Serology will be positive. CSF should be evaluated if neurosyphilis is suspected.
Treatment	Long-acting benzathine penicillin G (e.g., Bicillin L-A®) 2.4 million units IM, as a single dose					Long-acting benzathine penicillin G (e.g., Bicillin L-A®) 7.2 million units total, administered as 3 doses of 2.4 million units IM, at 1-week intervals		Aqueous crystalline penicillin G 18–24 million units per day, administered as 3–4 million units IV every 4 hours or continuous infusion for 10–14 days

*Test types includes **Direct Detection** (DFM, Silver staining, FA, IHC, and NAAT), **Serology** (nontreponemal and treponemal): CSF (VDRL)
** Most cases have several weeks between primary and secondary without symptoms.

Figure 1.
Serological responses and clinical manifestations & management of syphilis infection.



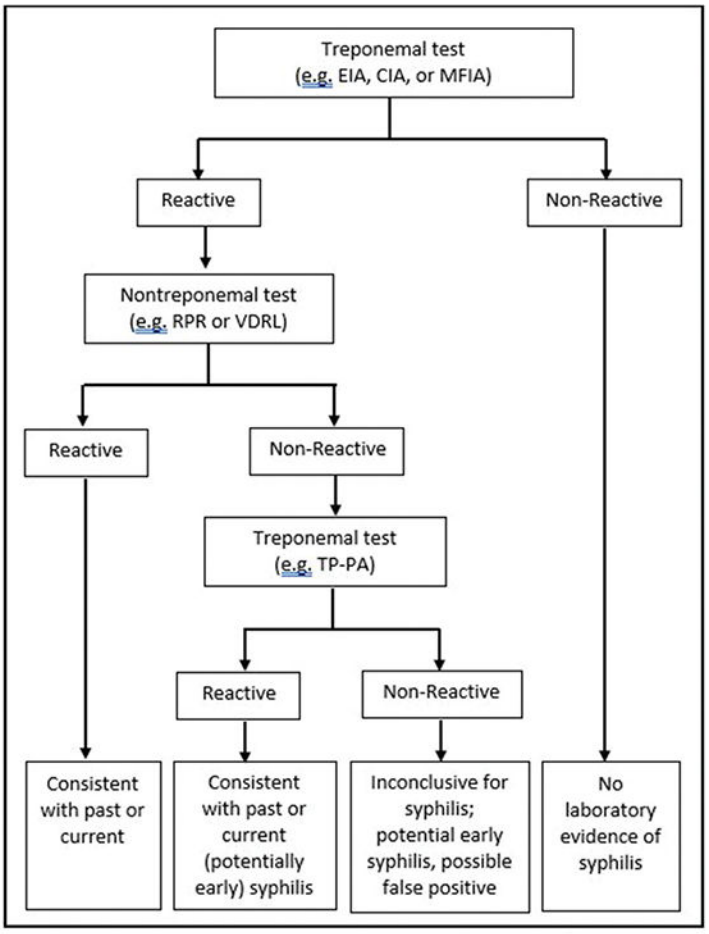


Figure 2.
Syphilis serology testing algorithm.

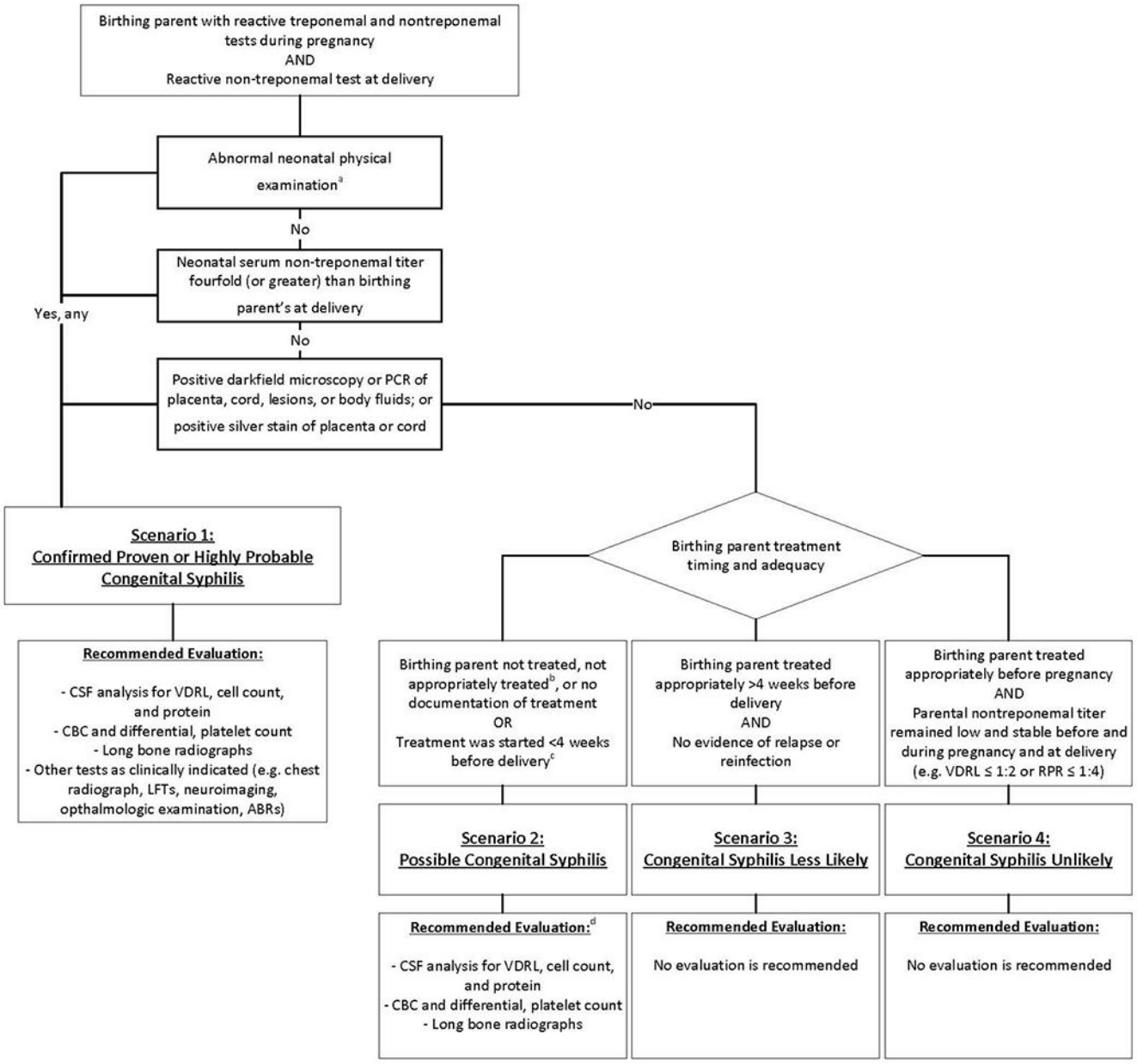


Figure 3.
Recommendations for evaluation and treatment of suspected congenital syphilis.

Table 1.

Select list of laboratories offering darkfield microscopy (DFM) and other methods to directly visualize *T. pallidum*.

Laboratory	Test	Specimen Type	Website
Orange County Health Care Agency	Syphilis Darkfield, Microscopic Exam	Serous fluid from genital lesion	Syphilis Darkfield, Microscopic Exam | Orange County California – Health Care Agency (ochealthinfo.com)
Dallas County Health and Human Services	Syphilis Darkfield, Microscopy	Serous fluid from genital lesion	DCHHS | Laboratory (dallascounty.org)
Nevada State Public health laboratory	Syphilis darkfield examination	Serous fluid from lesion prior to antimicrobial therapy	Syphilis, Darkfield Examination: Nevada State Public Health Laboratory: University of Nevada, Reno School of Medicine (unr.edu)
San Diego County Public health laboratory	Darkfield microscopy for *Treponema pallidum*	Penile lesion, rectal lesion, genital ulcer, any other epidermal surface (except oral)	Public Health Laboratory (sandiegocounty.gov)
Santa Clara County Public *Health Laboratory*	Syphilis Darkfield, Microscopic Exam		Public Health Laboratory – Public Health Providers – County of Santa Clara (sccgov.org)
City of Philadelphia Department of Public Health	Darkfield examinations	lesions	Philadelphia Department of Public Health – Laboratory Services – PDPH Health Information Portal
Ventura County Public Health Laboratory	Syphilis Darkfield	Serous fluid from genital lesion	Laboratory (vchca.org)
NC department of health and human Services	Syphilis Darkfield		N.C. DPH: State Lab > Microbiology (ncdhhs.gov)
Santa Clara County Public *Health Laboratory*	*Treponema pallidum* DFA	Vesicular fluid from genital lesion	Public Health Laboratory – Public Health Providers – County of Santa Clara (sccgov.org)
Michigan Department of Health & Human Services	Syphilis DFA-TP Assay	Smear from suspected lesions. Source of lesion (i.e. genital, oral, rectal)	A-Z Test Listing (michigan.gov)

*This is not intended to be an exhaustive list of available tests.

FITC: fluorescein isothiocyanate.

DFA: direct fluorescent antibody.

Table 2.

Select list of syphilis NAATs used in the U.S and internationallyx.

Company/Lab	Test name	FDA-cleared	Regulatory status	Pathogen/s	Specimen type	Website*
Laboratory-developed tests, cleared for clinical use in the U.S.						
ARUP Laboratories	GUD Panel by PCR	No	CLIA, LDT	*Haemophilus ducreyi* Herpes simplex virus type 1 Herpes simplex virus type 2 *Lymphogranuloma venereum* *Treponema pallidum*	Genital, anal, or rectal swabs	Laboratory Test Directory | ARUP Lab Test Directory
Medical Diagnostics Laboratories, LLC	GUD Panel	No	CLIA, LDT	*Haemophilus ducreyi* Herpes simplex virus type 1 Herpes simplex virus type 2 *Treponema pallidum*	OneSwab, ThinPrep,	Testing Menu | Medical Diagnostic Laboratories, L.L.C. (mdlab.com)
Centers for Disease Control and Prevention	GUD Molecular Detection	No	CLIA, LDT	*Haemophilus ducreyi* Herpes simplex virus type 1/type 2 *Treponema pallidum*	Anogenital lesion swabs	Test Order | Submitting Specimens to CDC | Infectious Diseases Laboratories | CDC
Quest Diagnostics	Treponema pallidum DNA, Qualitative Real-Time PCR	No	CLIA, LDT	*Treponema pallidum*	CSF, genital lesion swabs	Treponema pallidum DNA, Qualitative Real-Time PCR | Test Detail | Quest Diagnostics
University of Washington	Treponema pallidum DNA detection by NAAT	No	CLIA, LDT	*Treponema pallidum*	Tissue (Fresh or paraffin embedded), Swabs, Body fluids except whole blood	https://depts.washington.edu/molmicdx/mdx/available_tests.shtml
Tests used internationally but not currently cleared for clinical use in the U.S.						
SpeeDx Inc.	PlexPCR® VHS	No	CE-IVD, TGA cleared, IVDR Certified	Herpes simplex virus type 1 Herpes simplex virus type 2 *Treponema pallidum* Varicella-zoster virus	Genital, non-genital, anal/rectal, and oral swabs	SpeeDx | PlexPCR® VHS – Complete the Picture for Lesion Diagnostics
Seegene Inc.	Allplex™ Genital Ulcer Assay	No	CE-IVD	Cytomegalovirus *Haemophilus ducreyi* Herpes simplex virus type 1 Herpes simplex virus type 2 *Lymphogranuloma venereum* *Treponema pallidum* Varicella-zoster virus	Genital swab, urine, liquid based cytology (e.g. ThinPrep® and Surepath™)	Seegene Inc
Siemens Healthcare Diagnostics Inc	FTD Genital Ulcer (Distr. Only in GER)	No	RUO	Herpes simplex virus type 1 Herpes simplex virus type 2 *Treponema pallidum*	Genital swabs, urine, rectal swabs	Fast Track Diagnostics Multiplex Real-time PCR Assays (RUO) * - Siemens Healthineers (siemens-healthineers.com)
Randox Laboratories Ltd.	Vivalytic STI array	No	CE-IVD	*Chlamydia Trachomatis* *Haemophilus ducreyi* Herpes simplex virus type 1 Herpes simplex virus type 2 *Mycoplasma genitalium* *Mycoplasma hominis*	Swab or Urine	Vivalytic | STI Array | Randox Laboratories

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Company/Lab	Test name	FDA-cleared	Regulatory status	Pathogen/s	Specimen type	Website*
				Neisseria gonorrhoeae *Treponema pallidum* *Trichomonas vaginalis* *Ureaplasma urealyticum*		
Hologic Inc.	Aptima® *Treponema pallidum* Assay	No	RUO	*Treponema pallidum*	Swab from cutaneous and mucocutaneous epithelia, urine, whole blood, and serum	Lab Technologies | Hologic

*Information is current as of July 2023. This list may not be complete, provided by the authors' for general awareness of available syphilis NAATs.

CE-IVD: Approved for use in Europe Invitro DiagnosticCLIA: Clinical Laboratory Improvement Amendments.

CSF: Cerebrospinal fluidGUD: Genital Ulcer Disease.

IVDR: In Vitro Diagnostic RegulationLDT: Laboratory Developed Tests.

RUO: Research Use OnlySTI: Sexually Transmitted InfectionsTGA: Therapeutic Goods AdministrationTMA: Transcription Mediated Amplification.