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Syphilis testing algorithms: A review

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Abstract

The methods and strategies used to screen for syphilis and to confirm initially reactive results can vary significantly across clinical laboratories. While the performance characteristics of these different approaches have been evaluated by multiple studies, there is not, as of yet, a single, universally recommended algorithm for syphilis testing. To clarify the currently available options for syphilis testing, this update will summarize the clinical challenges to diagnosis, review the specific performance characteristics of treponemal and non-treponemal tests, and finally, summarize select studies published over the past decade which have evaluated these approaches. Specifically, this review will discuss the traditional and reverse sequence syphilis screening algorithms commonly used in the United States, alongside a discussion of the European Centre for Disease Prevention and Control syphilis algorithm. Ultimately, in the United States, the decision of which algorithm to use is largely dependent on laboratory resources, the local incidence of syphilis and patient demographics.

Key words: Syphilis; Treponemal infection; Immunoassay; Reverse sequence screening; Rapid plasma regain; *Treponema pallidum* particle agglutination test; Automation; Algorithm; Primary infection; Late latent infection

Core tip: Many laboratories have adapted automated immunoassay methods for syphilis screening in the past decade. Since measurement of antibodies to *Treponema pallidum* (treponemal) antigens cannot readily distinguish current from past infection, additional tests, including traditional non-treponemal tests, are required to further clarify the disease state. As the incidence of syphilis and population demographics influence test performance, and due to local differences in the way clinical follow-up is offered, there is no single approach to syphilis testing that is universally applicable.

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INTRODUCTION

Over the past decade, the strategies and algorithms used by clinical laboratories to screen for and confirm syphilis have changed significantly. Among the three algorithms currently used for syphilis testing, there is not one that is universally recommended by medical or public health agencies. Two of these approaches, the traditional and reverse syphilis screening algorithms, were detailed in a point/counterpoint commentary in a recent issue of the *Journal of Clinical Microbiology*[1].

Readers lacking familiarity with syphilis testing might be surprised to learn that assays with suboptimal sensitivity and specificity are used to confirm considerably more sensitive and specific tests. These readers might also be surprised to hear that for the reverse screening algorithm, initial syphilis screening assays are often fully automated, whereas confirmatory tests are performed manually and can be subjective to interpret. To understand the options available for syphilis testing today, this update will summarize the clinical challenges to diagnosis and the specific performance characteristics of treponemal and non-treponemal tests. Additionally, select studies evaluating the various approaches to syphilis screening over the past decade will be summarized.

CLINICAL CHARACTERISTICS OF SYPHILIS INFECTION

Primary syphilis infections, caused by the spirochete *Treponema pallidum* (*T. pallidum*), can be identified visually by the presence of a characteristic sore with a raised boarder, referred to as a chancre, typically observed on the genitalia. While lacking in sensitivity due to the painless nature of the lesion, visual diagnosis offers good specificity. This is particularly true in resource poor nations where syphilis continues to be commonly encountered and readily recognizable by local physicians, with reported prevalence rates approaching 10% in some countries[2,3]. In industrialized nations, widespread treatment with potent antibiotics has had a dramatic effect on the prevalence of syphilis, as have safe sex initiatives and the availability of barrier protection methods. At one time there was hope that syphilis would be completely eliminated in industrialized countries, however, the opposite has occurred and syphilis has been on the rise for the past two decades, particularly among men having sex with men[4]. Primary syphilis is uncommonly encountered by physicians who practice in resource rich nations, and therefore the infection may not be included in the differential diagnosis for genital lesions or other, more chronic disease manifestations.

Without treatment of primary syphilitic infections, patients may progress to secondary syphilis, which can manifest with a wide range of systemic symptoms, including maculopapular rash, characteristically affecting the palms and soles in addition to the torso and extremities, fever, malaise and diffuse lymphadenopathy. Notably, these symptoms are significantly more severe among immunocompromised individuals, particularly human immunodeficiency virus (HIV) positive patients[5]. Due to the low prevalence of syphilis in some regions, and the lack of medical care in others, secondary syphilis infections may be misdiagnosed or not diagnosed at all, in both cases leading to a lack of curative treatment.

Following secondary syphilis (several months to a year after infection), patients enter a latent stage of infection that is often entirely asymptomatic. Many years later, 15%-40% of infected individuals may develop late or tertiary syphilis[3], which can present with a variety of serious health problems, including central nervous system, cardiovascular or ocular involvement.

PERFORMANCE OF TREPONEMAL AND NON-TREPONEMAL ASSAYS FOR DETECTION OF SYPHILIS

Currently, two different types of serologic tests are available to screen for syphilis and both approaches measure antibodies in serum or plasma. Non-treponemal assays, including the rapid plasma reagin (RPR) and Venereal Disease Research Laboratories (VDRL) tests, measure antibodies to lipoidal products (*i.e.*, cardiolipin), which are released from host cell membranes following injury or infection with *T. pallidum.* These assays have been used for over a century for syphilis screening[2,3,6]. Since the 1960's, methods that measure antibodies specific to *T. pallidum* proteins (*e.g.*, R17, R15, R44.5 and R47), referred to as treponemal tests, have been available and are increasingly used as laboratories migrate from microscopic testing methods to fully automated immunoassay systems[7].

Over the past decade multiple new treponemal tests have been developed. These methods incorporate highly purified, recombinant *T. pallidum* protein antigens, as well as polyvalent conjugates able to detect both IgM- and IgG-class antibodies to syphilis and are marketed as "total syphilis antibody" assays. Comparison studies between these methods, when used for screening, generally demonstrate very high concordance and excellent specificity (generally > 99%)[8-12].

Both non-treponemal and treponemal methods will fail to detect some cases of primary syphilis, since the antibody response to infection is not immediate[13]. Humoral antibodies generally appear and become detectable within 4 wk following chancre formation[2]. There is some evidence that treponemal assays are more sensitive in cases of primary syphilis. In a large study, 9137 patients who were initially positive by an enzyme immunoassay (EIA) and RPR negative were recalled 8 wk after initial screening; 0.59% of these patients seroconverted to RPR positive at the second draw date, consistent with a diagnosis of primary

syphilis[14].

Another challenge to detection of early syphilis, regardless of the method, is the host's immunostatus. Immunosuppressed individuals, whether due to infection (*e.g.*, HIV) or immunomodulatory therapy can have a delayed or suppressed serological response[15]. In general, the sensitivity of the common non-treponemal tests (*e.g.,* RPR and VDRL) during primary syphilis infections ranges from 74% to 100%, depending on the time of sample collection compared to the initial exposure. A similar sensitivity, ranging between 70% and 100%, has been documented for the traditional treponemal assays, including the *T. pallidum* particle agglutination test (TPPA) and the *T. pallidum* microhemagglutination assay[2]. Importantly, the sensitivity of both treponemal and non-treponemal assays improves to nearly 100% during secondary and latent infection. However, while treponemal assays will remain highly reactive during tertiary manifestation of disease, non-treponemal assays loose some sensitivity during this stage (range 71% to 73%).

Multiple factors can influence the specificity of both treponemal and non-treponemal tests. While false positive results are generally less common with treponemal assays, certain host conditions have been associated with inaccurate results, including systemic lupus erythematosus, the presence of high levels of heterophile antibody in patients with infectious mononucleosis and in some individuals with leprosy, collagen disease and drug addiction[16]. Regarding non-treponemal assays, the most common cause of non-specific reactions is due to biological interference. The lipoidal products released during syphilis infection are also produced in many other infectious and non-infectious conditions, including Epstein-Barr, hepatitis, varicella, measles, tuberculosis, malaria, lymphoma and certain autoimmune diseases[7]. Notably, none of these diseases produce false positive results by treponemal methods, thus making treponemal assays significantly more specific for syphilis. Additionally, infections caused by other *Treponema* species, including *T. pertenue* the causative agent of yaws, can lead to false positive results by either method[17]. Finally, due to an increase in immunological activity and maternal antibody production, pregnancy can lead to inaccurate results by either method, posing an interpretive challenge to syphilis testing results.

Patients tested by non-treponemal methods may sometimes yield falsely negative results when extremely high levels of antibody are present, a result of the "prozone effect". Best practice requires that patient sera are analyzed at two distinct dilutions to identify this potentially confounding reaction. The sensitivity of non-treponemal tests is also suboptimal in latent disease, as the destructive activity of the bacteria at this stage is minimal leading to the absence of circulating lipoidal antigens. In contrast, treponemal antibodies continue to circulate and are detectable for decades. Furthermore sera from patients who are successfully treated for syphilis will demonstrate decreased non-treponemal antibody titers, and 12-24 mo post-infection may be negative by these assays, while treponemal antibodies will continue to be positive[2,6]. Patients who are re-infected with syphilis, years after successful treatment for their initial infection, will seroconvert from RPR negative to RPR positive status[18].

Separate testing for IgM and IgG antibodies to *T. pallidum* using treponemal assays, is currently performed in many countries. IgM assays are considered less specific than IgG tests and concordance between the different available IgM tests is low, however a positive IgM result may be useful to provide supplemental information when an IgG screening assay is reactive[11]. In the United States, tests detecting only IgM-class antibodies to syphilis are not routinely available and no assay supporting separate measurement of IgM has been FDA-cleared in the last twenty years.

THE SYPHILIS TESTING ALGORITHMS AND ASSOCIATED CLINICAL DILEMMAS

The traditional and reverse sequence syphilis screening algorithms

The reverse sequence syphilis screening (RSSS) algorithm first uses a treponemal assay as a screen, followed by confirmatory testing of reactive samples with a non-treponemal test. The term "reverse" is appropriate, because for many decades the traditional syphilis screening algorithm involved initial screening using a non-treponemal test, followed by confirmation of reactive results using a treponemal test. A distinct advantage of the RSSS is that initial screening with a fully automated, treponemal test solves the workflow challenge faced by hospital and reference laboratories performing thousands of tests every month. However, as with any assay, when a treponemal test is used as the screen in an environment where the prevalence of syphilis is low, many of the reactive results will be false. The advantage of the RSSS for laboratories however, is that the number of required manual confirmatory tests will be significantly decreased, particularly in industrialized countries where reactive rates of treponemal assays range from 1%-3%.

Since non-treponemal tests generally become non-reactive 12-24 mo after treatment of early disease, a negative non-treponemal confirmatory test does not definitively indicate that the original treponemal result was falsely reactive. A treponemal reactive/non-treponemal non-reactive result suggests that the patient is unlikely to have active disease, however this result does not rule out acute infection or late/latent disease. To conclusively determine whether the initial treponemal result was falsely reactive, the RSSS algorithm requires that a second treponemal assay,

different from the initial screening treponemal assay, be performed. If this assay is reactive, the initial screening result is confirmed indicating that the patient has been exposed to syphilis at some time in the past and further clinical evaluation is required to stage the infection. If the second treponemal assay is non-reactive, this suggests that the initial treponemal test was likely a false screening result and the patient is unlikely to have been exposed to syphilis[19-21].

Many different treponemal assays can be used as the "third test" for the RSSS, including the TPPA test, the Fluorescent Treponemal Antibody Absorption test (FTA-ABS), multi-parameter line immunoassays and western blots. However, the ability of different treponemal tests to detect late untreated syphilis has not been evaluated in great detail and some studies suggest that there can be discrepancies[22-24]. The prevalence of syphilis in specific populations (such as in prisoners) may also affect the development of an algorithm for retesting results when treponemal and non-treponemal results do not agree[25].

The 2008 Center for Disease Control and Prevention guidance on syphilis[26] proposed that if a patient has "not been previously treated, patients with reactive results from treponemal tests and nonreactive results from non-treponemal tests should be treated for late latent syphilis". Hence the next intervention may be a tense conversation between doctor and patient. However, because medical records are often incomplete and because patients can be forgetful or less than fully truthful, determining whether a patient was previously treated for syphilis is not straightforward[27,28]. Additionally, in an era when exposure to β-lactam antibiotics, tetracyclines, and macrolides for indications other than syphilis is widespread, there may be no practical way to identify a history of "non-observed treated" syphilis[27]. For a physician, given discordant syphilis results, treatment with an inexpensive and highly effective drug may be a straightforward option; it is a low-risk and inexpensive choice that essentially eliminates the risk of complications from a serious infection. However, treatment in some cases will be unnecessary and possibly disturbing to the patient and the patient's family.

Finally, in many regions of the world, treatment is only possible when the patient returns to the clinic. While a confirmatory test on a new specimen may increase laboratory confidence in the result, patients may not return for a second visit. Losing patients to follow up can be a significant concern in testing centers and geographic areas where syphilis is commonly encountered[29].

The European Centre for Disease Prevention and Control syphilis algorithm

The 2008 European Guidelines on the Management of Syphilis [European Centre for Disease Prevention and Control (ECDC) guidelines] took a large step away from the traditional syphilis algorithm[30]. The authors recommended a treponemal test for screening, with an added IgM test when primary syphilis is suspected, followed by the use of a different treponemal test, preferably performed on second specimen, for confirmation. A treponemal EIA, TPPA or FTA-ABS assay (or multiple tests) may be used for confirmation following completion of the two treponemal tests. An IgG immunoblot is recommended as a supplementary confirmatory test if the first two antibody-based tests provide discrepant results. The ECDC guidance document recommends the use of RPR testing to monitor the response to therapy and greatly limits the situations where it may be used for screening. This algorithm offers two clear advantages compared to RSSS: Only two methods will be required to perform routine screening and confirmation, instead of three in cases of discordant results, and both of these methods can be fully automated.

Regarding treatment, since the ECDC does not mandate a non-treponemal test, patients tested according to this guideline are likely to be treated, or retreated, unless there are records to support prior therapy[30].

Other international guidelines published since 2008 maintain a role for non-treponemal testing. A proposed Eastern European guideline called for treponemal and non-treponemal testing, but did not specify the order in which they should be performed[31]. With regards to other international countries, most recently a guideline from Canada proposed the use of either the traditional or RSSS algorithm depending on laboratory needs and word flow restrictions[32].

The three most common syphilis testing algorithms are illustrated in Figure 1.

COMPARISON OF THE THREE SYPHILIS SCREENING ALGORITHMS

Since the introduction of the RSSS and ECDC algorithms, numerous studies have compared these new methods to the traditional testing algorithm. Performing such comparison studies however is hampered by several significant challenges, including: (1) in countries and regions where syphilis is uncommon, a large number of sera must be tested to identify clinically positive patients; (2) blood bank laboratories perform a high volume of tests for syphilis, however any patient with syphilis, has been removed from the donor and therefore, the usage of stored blood bank samples is unlikely to be helpful; (3) in regions with a high incidence of syphilis, the likelihood of a true positive is high and therefore, results from assay evaluation in these regions may not be directly applicable to regions where syphilis is not as prevalent; (4) patients may not be available for clinical follow-up, or may not recall prior treatment for syphilis. As mentioned above, syphilis may have been treated inadvertently,

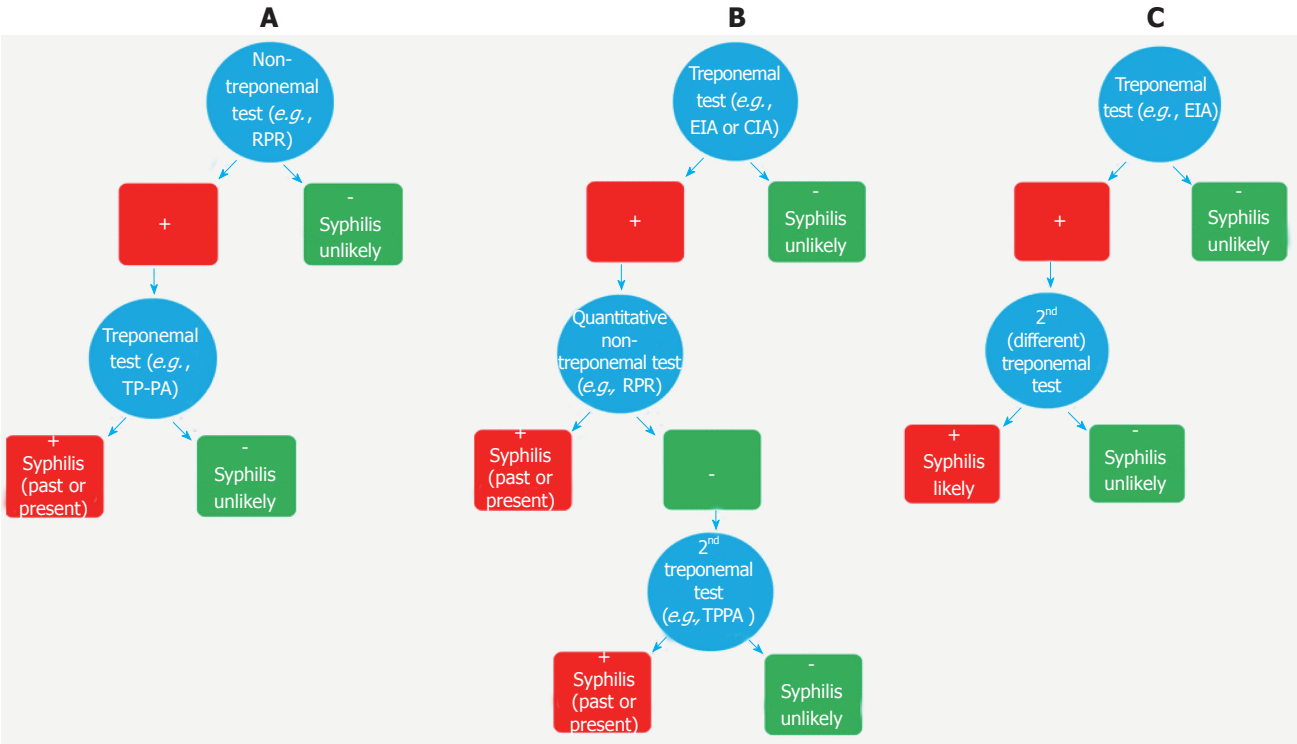

Figure 1 Comparison of the three common syphilis testing algorithms. A: Traditional algorithm; B: RSSS algorithm; C: ECDC algorithm. +: Positive result; -: Negative result; RPR: Rapid plasma reagin; TPPA: *Treponema pallidum particle* agglutination test; EIA: Enzyme immunoassay; CIA: Chemiluminescence immunoassay.

Table 1 Selected studies evaluating the reverse sequence syphilis screening and European Centre for Disease Prevention and control algorithms in comparison to the traditional algorithm for syphilis testing

Screening method	Confirmatory method	Third method	No. of samples screened	% reactive results in initial screen	% of screening results confirmed	% of discrepant results confirmed by third method	Ref.
EIA/CIA	RPR	TPPA, FTA-ABS	12774	14.5%	49%	86%	CDC[19]
TPPA	CIA	N/A	24124	11.4%	99%	N/A	Tong *et al*[28]
TPPA	RPR	CIA	24124	11.4%	76%	99%	Tong *et al*[28]
Multiple EIA methods	RPR	TPPA/FTA-ABS	116822	5.6%	44%	83%	CDC *et al*[26]
CIA	RPR	TPPA, FTA-ABS	28261	4.1%	31%	89%	Hunter *et al*[21]
EIA/CIA	RPR	TPPA, FTA-ABS	127402	2.3%	39%	59%	CDC[19]
CIA	RPR	TPPA	21623	2.2%	42%	78%	Park *et al*[33]
EIA	RPR	TPPA, FTA-ABS, TPHA	1037025	2.0%	30%	84%	Mishra *et al*[15]
CIA	RPR	TPPA	15713	1.7%	82%	82%	Lee *et al*[24]

RPR: Rapid plasma reagin; TPPA: *Treponema pallidum* particle agglutination test; EIA: Enzyme immunoassay; CIA: Chemiluminescence immunoassay; FTA-ABS: Fluorescent Treponemal Antibody Absorption test; CDC: Centers for Disease Control and Prevention.

without the patient's knowledge. Researchers are often unable to retrieve medical records for many patients. All of these factors make estimates of biological and analytical false positives increasingly difficult; (5) large prospective comparison studies are expensive and costly because two or more methods must be run in parallel for an extended period; and (6) while the cost of "over diagnosis" and "overtreatment" can be roughly quantified, it is much harder to determine the cost of a "missed diagnosis" and "under treatment". As a result, meaningful financial analyses are difficult to perform unless follow-up is available for patients who have negative screens.

Table 1 summarizes results from large studies (> 10000 patients) that were performed with sera from patients evaluated at a hospital or clinic. Each study compared the RSSS or ECDC algorithm to the traditional approach. The studies are sorted by the observed positivity rate in the initial screen. Some important and valuable studies have been omitted because the authors did not identify the number of positive results obtain in their initial screen[34,35] or used multiple confirmatory

methods at the same time[36], rather than following the approaches proposed in the new confirmatory algorithms discussed above, or reported on a much smaller patient cohort[37,38]. In the listed studies which evaluated the RSSS, it is clear that the confirmation rate, using a non-treponemal test, such as the RPR, varied widely, reflecting differences in the populations screened. However, the confirmation rate of an initially reactive treponemal assay by a second treponemal tests was more consistent, typically > 80% in most reports.

Three other review articles have included analyses of the utility of the new algorithms. Binnicker[39] described studies comparing the traditional and reverse algorithm published by 2011. Soreng *et al*[40] focused on the workflow advantages associated with a fully automated technology in the initial screen. The authors also mention that the discrepancies between TPPA and other treponemal methods are often associated with patients who were co-infected with HIV. The problem of inconsistent serological results in immunocompromised patients has been previously documented[41] and is well known; it likely plays a significant role in comparison studies of syphilis results. An extended review by Morshed *et al*[42] includes discussion of the new algorithms as well as the current status of neurosyphilis, prenatal and congenital syphilis screening.

CONCLUSION

The CDC's 2008 discussion of the RSSS proposed that "the best algorithm would depend on the population studied"[26]. In patient cohorts with little syphilis positivity, the traditional algorithm will lead to less follow up testing, but at an increased risk of missing early and late/latent syphilis. Laboratories that see very little syphilis may choose a strategy that minimizes the expense of screening. While the reagent costs of immunoassays are generally higher than RPR cards for screening, the semi-automated ELISA methods used in the previous decade have now been widely replaced by fully automated systems, which provide labor savings that offset the difference in reagent costs.

In the urban sexually transmitted disease clinic, where higher rates of syphilis are observed, or in developing countries where access to testing is limited, the likelihood of encountering both early syphilis and late/latent syphilis (LSS) increases. The RSSS algorithm is likely to identify more cases of untreated disease in these environments, but cases of treated disease will also be detected, with potential follow-up costs and a requirement to manage the social impact associated with identification of past infection.

These observations summarized here reflect what has been learned from a decade of experience with the reverse algorithm. Today the physician should evaluate any patient with a confirmed reactive immunoassay screen and a discrepant RPR result as a possible case of latent syphilis or an undiagnosed syphilis infection that has been successfully, but unintentionally, treated[27,43]. Determining the need for additional treatment will remain a decision to be made by the physician on a case by case basis[44]. Finally, for patients with HIV and other immunosuppressive diseases, repeat testing (or periodic testing) is essential due to the possibility of a delayed serological response (with a negative screen) or re-infection (with a positive treponemal screen).

The ECDC recommended algorithm, which was published several years ago, has not received much attention in the published literature. While it will not identify re-infection in sexually active individuals, it does offer a fully automated and more reproducible workflow than the RSSS and eliminates the positioning of a subjective test in a confirmatory role. It may be suitable in testing situations where patient information will be available to help a physician clarify the disease history.

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