

Improved Syphilis Reverse Screening Cascade

Earlier Detection with Fewer Ambiguous Results

Introduction

Syphilis is a complex systemic illness with protean clinical manifestations caused by the spirochete *Treponema pallidum*.¹ Primarily transmitted by sexual contact, it can also be transmitted by passage through the placenta (congenital syphilis), other direct contacts with the infected person, or by blood transfusion.¹

In the United States, the rate of primary and secondary syphilis decreased during the 1990s, with the rate reported in 2000 being the lowest since reporting began in 1941.² The rate of primary and secondary syphilis, however, has subsequently increased each year since 2001,³ with increases noted particularly among men who have sex with men (MSM).² In 2016, the national primary and secondary syphilis rate was 9 cases per 100,000 population, an 18% increase from 2015. There were more than 600 reported cases of congenital syphilis.³

The Centers for Disease Control and Prevention (CDC) recommends that all sexually active MSM be tested at least annually for syphilis and that all pregnant women be screened for syphilis during the early stages of pregnancy.³ According to the US Preventive Services Task Force (USPSTF), for women at high risk many recommend repeated serologic testing in the third trimester and at delivery.⁴

Groups at increased risk for syphilis infection include⁴:

- Uninsured women
- Women living in poverty
- Sex workers
- Illicit drug users
- Those with other sexually transmitted diseases (STD)
- Other women living in communities with high syphilis morbidity

The course of untreated syphilis includes incubation period (usually 9 – 90 days), primary (up to six months), secondary and early latent syphilis (up to two years), late latent and tertiary syphilis (lasting up to a lifetime).⁵ A patient is most infectious early in the disease. On average, an infected person has contact

with three different partners who are at risk of contracting the illness, and approximately half of these contacts become infected.¹

Laboratory Diagnosis of Syphilis

Serological testing has been the method of choice for syphilis screening — except for neurosyphilis, which cannot be diagnosed serologically. For the diagnosis of neurosyphilis, several CSF tests are recommended, eg, CSF VDRL.⁶

Methods for the direct detection of *Treponema pallidum* include polymerase chain reaction (PCR), dark field microscopy (DF) or microscopy following immunostaining (DFA), and the rabbit infectivity test (RIT). For a variety of reasons, however, these methods have not been commonly employed.⁶

Serologic diagnosis of syphilis relies on testing for nontreponemal and treponemal antibodies. Nontreponemal tests detect antibodies to putative nonspecific antigens (primary cardiolipin) produced by the host in response to syphilis infection.⁶ They include complement fixation tests (Wasserman reaction) and flocculation tests (such as rapid plasma reagin [RPR] and Venereal Disease Research Laboratory [VDRL] tests), which are prone to yield false-positive results.⁶

Treponemal tests detect antibodies to specific antigenic components of *Treponema pallidum*.⁶ These antibodies differ markedly with respect to antigenic reactivities and kinetics during the course of disease.⁶ These tests include the *Treponema pallidum* immobilization (TPI) assay, fluorescent treponemal antibody absorption (FTA-ABS), *Treponema pallidum* hemagglutination assay (TPHA), *Treponema pallidum* passive particle agglutination assay (TPPA), enzyme immunoassay (EIA), Western blot (WB) and pseudoblots, chemiluminescence and microsphere immunoassays, and chromatographic point-of-care tests.⁶

Serologic tests (nontreponemal and treponemal) have been shown to yield false results in the presence of several conditions, eg, (1) autoimmune disorders (2) HIV infection, and (3) other

spirochetal diseases.⁷ Traditionally, treponemal tests employed antigens purified from experimentally infected animals (cell lysates) and contamination with animal tissue components may be partially responsible for nonspecific results. Recently, the use of recombinant antigens in immunoassays has increased the specificity of these tests.⁷

At least nine *Treponema pallidum* polypeptides have been identified as major immunogens, and at least five (Tp15, Tp17, Tp37, Tp47, and TmpA) have proved to be of diagnostic relevance.⁷ Tp47 was reported to trigger early humoral response, three to six days after infection, and anti-Tp47 IgM has been detected in patients with congenital syphilis.⁸

In order to improve sensitivity and specificity of treponemal tests, it is critical to use recombinant antigens of the highest diagnostic value. Below are recent recommendations of the Association of Public Health Laboratories and the Centers for Disease Control and Prevention that point out the need for treponemal assays to be used as first-line screening tests for high-risk populations.⁶

- Treponemal tests that are currently FDA-approved
- EIAs that detect both IgG and IgM should be more sensitive in early disease than those that detect only IgG
- Benefit of having multiple recombinant antigens rather than antigens obtained from whole cell lysates
- New and/or improved treponemal tests should be brought to market

All of these recommendations are characteristic of the treponemal EIA test described here.

Treponemal EIA

The treponemal EIA test is cleared by the FDA as an initial screening test for syphilis. Its performance has been documented by several studies. Specificity of the test was reported to be 98.8%¹⁰ and the sensitivity was 100% for all stages of syphilis.¹⁰ It uses recombinant antigens Tp47, Tp17, and Tp15.¹⁰⁻¹²

Improved Syphilis Screening

The traditional syphilis screening approach that employs a nontreponemal first-line assay (such as RPR)² and, when positive, a second-line confirmatory test (such as *Treponema pallidum* passive particle agglutination assay) was developed many years ago when treponemal tests lacked the necessary sensitivity but delivered acceptable specificity.

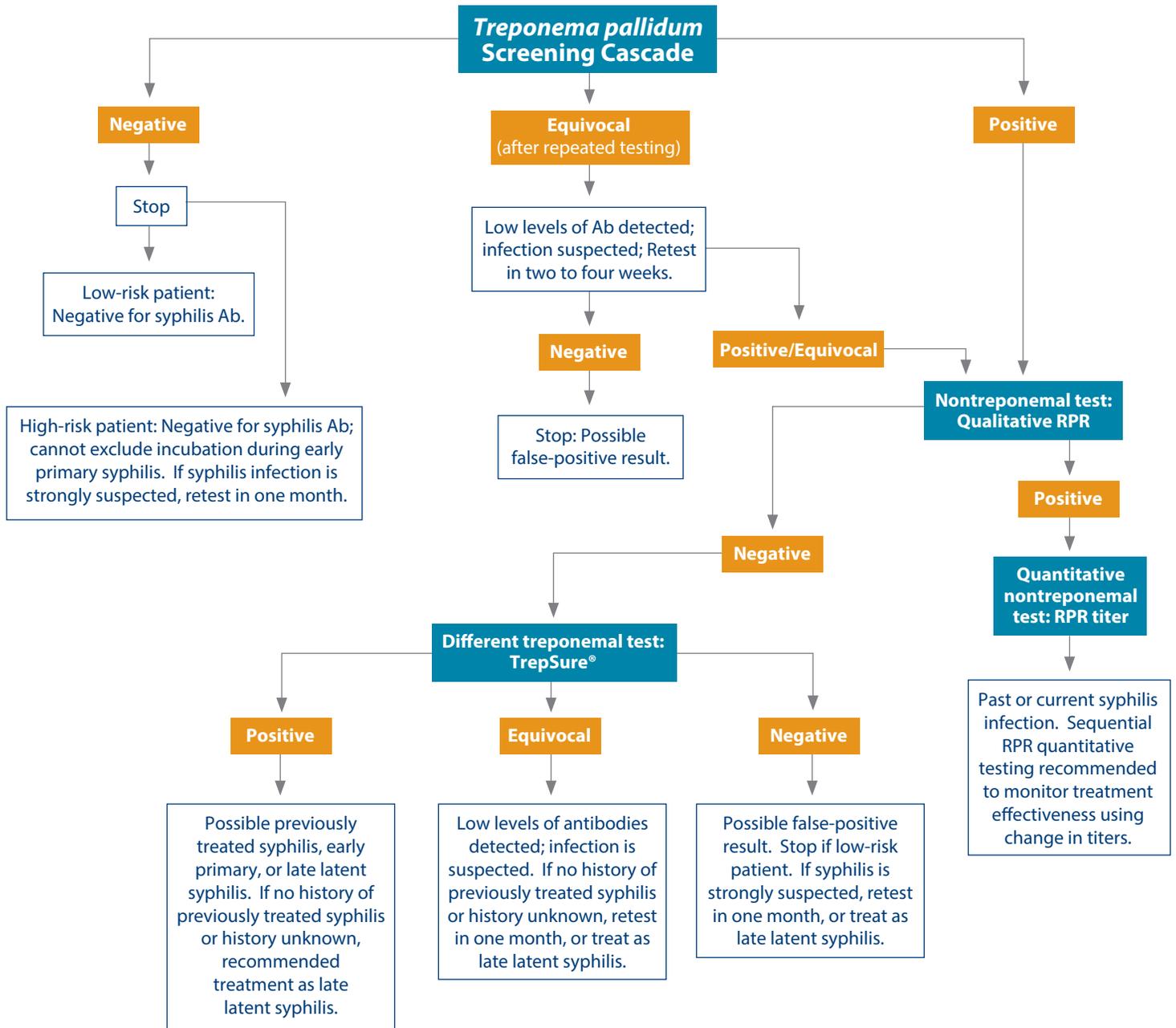
In the early 1990s, the CDC published guidelines that recommended the traditional approach for screening. Since that time, a number of new treponemal immunoassays have been introduced. Recently, CDC scientists recognized the need for a change in the syphilis screening approach in order to respond to the rising prevalence of syphilis.¹³ In 2008, the CDC issued a report that describes the reverse syphilis screening algorithm in which a treponemal test is used as a first-line test and, if positive, reflexes to the nontreponemal test. This report demonstrated that a number of syphilis infections could be missed using the traditional algorithm, and a number of false-positive results could occur.¹⁴

RPR testing is prone to false-positive results due to the lack of specificity that is associated with a large number of conditions commonly seen in the population. The CDC report points out that false-positive nontreponemal tests occur in as many as 2% of the US population.¹⁴ The sensitivity of the RPR test is under question as well. A recent study shows that among confirmed primary syphilis cases, 26% were found to be nonreactive by RPR, and among confirmed late latent cases, 39% were found to be nonreactive by RPR.¹⁵ Simple calculations show that at the current prevalence rate of syphilis, more false-positive results than true-positive results could be obtained when screening with RPR.

The implementation of the new algorithm that employs the more sensitive and specific treponemal assay allows for measurable improvement in the early detection of syphilis infection.¹² Consequently, more effective treatment and a decrease in the spread of syphilis could be expected. This new approach is now successfully used by laboratories in New York¹⁴ and Stanford University,¹⁶ among others.

The improved LabCorp syphilis reverse screening approach is embodied in the ***Treponema pallidum* Screening Cascade (082345)**, which includes a detailed interpretation of results with recommendations on patient management based on the individual patient's situation. The cascade is illustrated in figure 1 (page 3).

082345 *Treponema pallidum* (Syphilis) Screening Cascade



Legend

Test Names	Test Results	Actions
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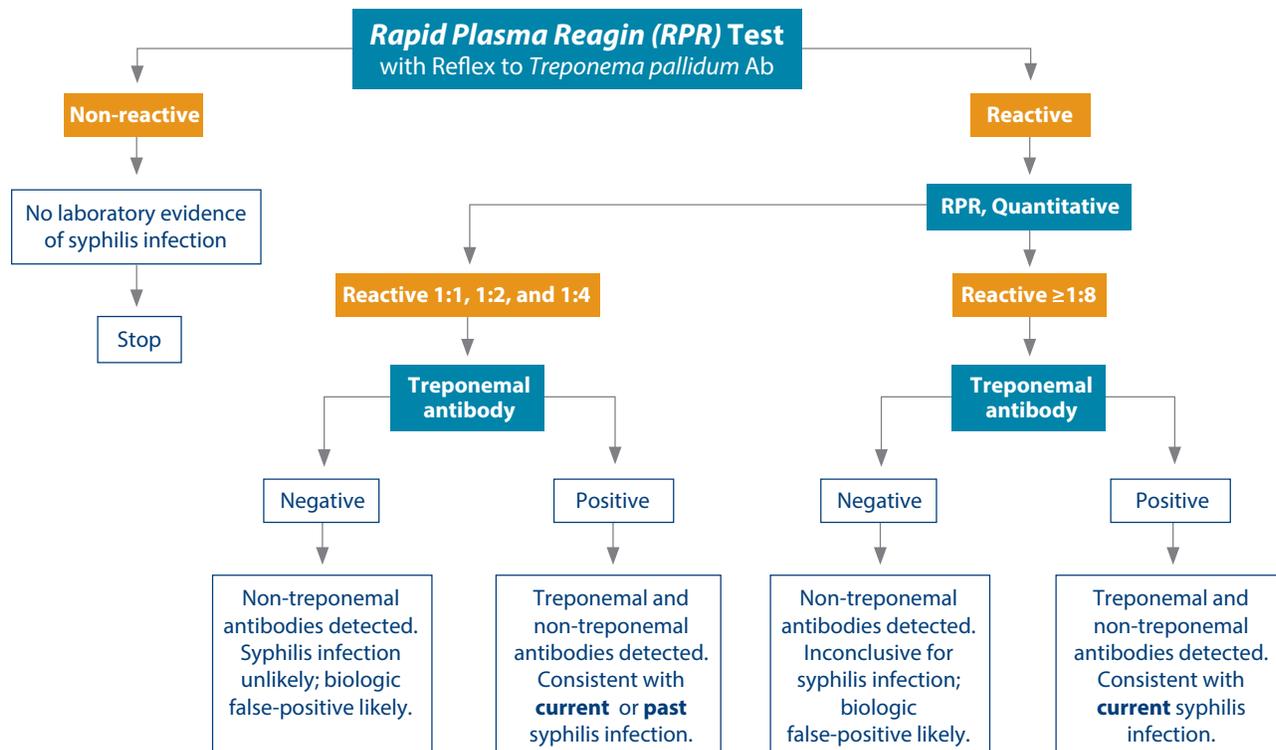
Figure 1 - Improved Syphilis Reverse Screening Cascade

Relevant Assays*

Test Name	Test N ^o
<i>Treponema pallidum</i> (Syphilis) Screening Cascade [§]	082345
<i>Treponema pallidum</i> Antibodies	082370
VDRL, Cerebrospinal Fluid	006445
Rapid Plasma Reagin (RPR), Quantitation	006460
Rapid Plasma Reagin (RPR) Test with Reflex to Quantitative RPR and Confirmatory <i>Treponema pallidum</i> Antibodies	012005

*For the most current information regarding test options, including specimen requirements and CPT codes, please consult the online Test Menu at www.LabCorp.com.

§If further cascade testing is required, additional CPT code(s) and concomitant charges may apply.



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