

Newer laboratory testing algorithms for syphilis begin with EIA

By Kenneth Katz, MD, MSc, MSCE

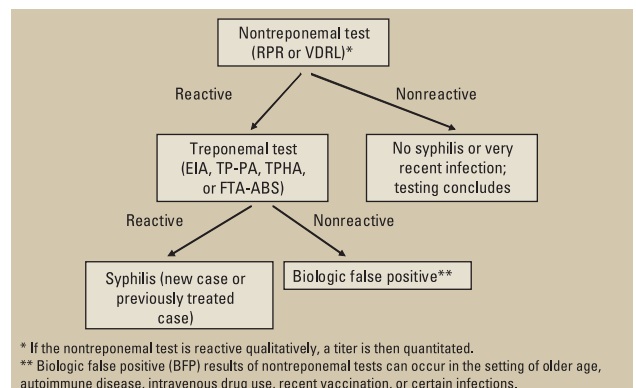
Syphilis is a sexually transmitted disease caused by the spirochetal bacterium *Treponema pallidum* variant *pallidum*. After reaching a 60-year nadir in 2000, cases of primary and secondary syphilis in the United States have increased yearly, including an 18% rise between 2007 (N=11,466) and 2008 (N=13,500). As incidence of syphilis increases, syphilis testing remains a cornerstone of prevention and control efforts. What has begun to change, however, is the availability of new types of syphilis tests and laboratory testing algorithms. This article describes types of serologic tests for syphilis that are performed on sera, discusses traditional and newer algorithms for use of those tests, and summarizes data on use of newer algorithms.

Two types of tests

The two types of serologic tests for syphilis are non-treponemal tests and treponemal tests. Commonly used nontreponemal tests include the rapid plasma reagin (RPR) test and the Venereal Disease Research Laboratory (VDRL) test. Both of these tests detect antibodies to cardiolipin, which are non-specific markers for syphilis. Non-treponemal tests typically become reactive within several weeks following infection, remain reactive without treatment, and can be persistently reactive for months to years following treatment. Importantly, biologic false positive (BFP) results of non-treponemal tests can occur in the setting of older age, autoimmune disease, intravenous drug use, recent vaccination, or certain infections. In addition to qualitative results, non-treponemal tests can be quantitated by serially diluting the serum until the test is no longer reactive. The ratio of serum to diluent of the most dilute serum at which the test is still reactive is called the titer. Non-treponemal test titers correlate with, and are used as a marker of, disease activity and response to treatment.⁴

As clinical laboratories continue to use or to switch over to newer algorithms for syphilis testing, laboratorians and clinicians will have to become more comfortable with interpreting test results produced when starting with a treponemal EIA test.

Treponemal tests, by contrast, detect antibodies specific to *T pallidum* and other pathogenic treponemal species.³ Commonly used treponemal assays include non-enzyme immunoassays, such as the *T pallidum* particle agglutination (TP-PA) test, the *T pallidum* hemagglutinin assay (TPHA) and the fluorescent treponemal antibody-absorption (FTA-ABS) test, and treponemal enzyme immunoassays and immunochemoluminescence



RPR: Rapid plasma reagin; VDRL: Venereal Disease Research Laboratory; EIA: enzyme immunoassay or immunochemoluminescence; TP-PA: *Treponema pallidum* particle agglutination; TPHA: *Treponema pallidum* hemagglutination; FTA-ABS: fluorescent treponemal antibody-absorption.

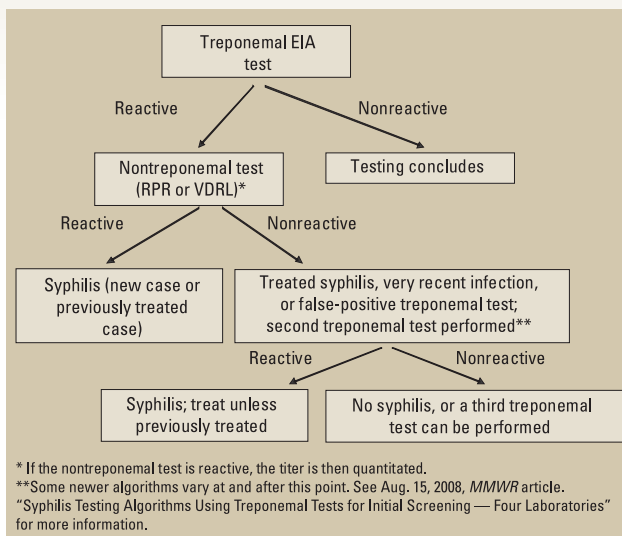
Figure 1. Traditional laboratory testing algorithm for syphilis.

(EIA) tests (hereafter collectively referred to as treponemal EIA tests). Treponemal tests typically become reactive within several weeks following infection (sometimes preceding reactivity of non-treponemal tests) and, in most cases, remain reactive for life. Unlike non-treponemal test titers, treponemal test titers do not correlate with disease activity or response to treatment.⁴

The syphilis-testing algorithm in the United States has traditionally started with a non-treponemal test, because those tests have historically been less expensive for laboratories to perform than treponemal tests.^{2,6} According to the traditional algorithm (see Figure 1), a non-reactive non-treponemal ends the testing process (except when very recent infection is suspected, in which case clinicians sometimes request that the laboratory perform a treponemal test, which can become reactive before a non-treponemal test does). A reactive non-treponemal test leads to titer quantitation and then to confirmatory testing with a treponemal test. A reactive treponemal test confirms an old or new diagnosis of syphilis (or other treponemal disease, which is rare in the United States). A non-reactive treponemal test suggests that the reactive non-treponemal test was a BFP.

Testing algorithms change

Newer syphilis testing algorithms, by contrast, reverse the order of use of non-treponemal and treponemal tests (see Figure 2). The newer algorithms capitalize on the fact that treponemal EIA tests can be automated, making it economically more efficient in settings of high testing volumes to start the testing algorithm



EIA: enzyme immunoassay or immunochromoluminescence; RPR: Rapid plasma reagin; VDRL: Venereal Disease Research Laboratory.

Figure 2. Newer laboratory testing algorithm for syphilis.

with a treponemal EIA test, rather than a non-treponemal test. In the newer algorithm, testing concludes if the treponemal EIA test is non-reactive. A reactive treponemal EIA test leads to testing with a non-treponemal test and titer quantitation which, in some newer algorithms, leads to further testing if the non-treponemal test is non-reactive.

Importantly, all of the newer algorithms can produce a combination of results (i.e., a reactive treponemal test and a non-reactive non-treponemal result) that is not produced with the traditional algorithm (unless a clinician specifically requests a TPPA despite a nonreactive non-treponemal test). Variations of laboratory testing and reporting practices as well as limited clinical experience with patients with results not typically seen with the traditional algorithm can complicate interpretation of results produced by newer algorithms.⁷

A study published in the Centers for Disease Control and Prevention's *MMWR* on Aug. 15, 2008, investigated use of the newer screening algorithm and highlighted some of the variations in laboratory practice and complications of interpretation that occur in some cases.⁷ The study investigators reviewed data on a convenience sample of 116,822 specimens tested during Oct. 1, 2005 to Dec. 1, 2006, at four New York City laboratories that used syphilis screening algorithms that started with a treponemal EIA. (Specifics of the algorithm used by each laboratory after a reactive treponemal EIA test are available in the *MMWR* article.) Composite study results and their recommended interpretations were as follows:

- Of all 116,822 specimens tested, 6% were reactive to a treponemal EIA test, of which 99% were then tested with the RPR test. Further testing was not performed on the 94% of specimens initially nonreactive to the treponemal EIA test. Patients whose specimens were non-reactive to the treponemal EIA test either do not have syphilis or have very recent infections.
- Of all 6,548 specimens reactive to the treponemal EIA test and then tested using an RPR test, 44% were reactive and 56% (3% of the overall sample) non-reactive with an RPR test. Patients whose specimens were reactive to both

treponemal EIA and RPR tests should be considered to have an old or new case of syphilis. If previous treatment can be documented and the titer has not increased at least fourfold, those patients require no further management. Retreatment should be provided if a previously treated patient has a fourfold or greater increase in titer. Patients whose specimens are reactive to a treponemal EIA test but non-reactive to a non-treponemal test have old, previously treated syphilis; very recently acquired syphilis; or a false-positive treponemal EIA test result. For those patients, performing a different treponemal test (e.g., TP-PA, TP-PA, or FTA-ABS) can help assess whether the treponemal EIA test result was a false positive.

- Of all 3,664 specimens reactive to the treponemal EIA test and non-reactive with the RPR test, 69% were tested with a different treponemal test. Of those specimens tested with a different treponemal test, 83% were reactive with the different treponemal test. Patients with those test results should be staged and treated, unless previous treatment can be documented. The other 17% of patients were non-reactive (e.g., potentially had false-positive results) with a different treponemal test. For those patients, clinicians may decide to forego treatment, or a third treponemal test can be used to help resolve the discrepancy. Clinicians should also incorporate individual patients' histories and physical findings in interpreting syphilis testing results.

As clinical laboratories continue to use or to switch over to newer algorithms for syphilis testing, laboratorians and clinicians will have to become more comfortable with interpreting test results produced when starting with a treponemal EIA test. Additional clinical experience with patients diagnosed and treated using the newer testing algorithms should contribute to that goal — and to the overall aim of improved syphilis prevention and control. □

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