Choosing a Testing Algorithm for Syphilis

After verging on elimination, the overall primary and secondary rates of syphilis in the US are increasing steadily, with the greatest proliferation in the past several years occurring among men who have sex with men (MSM). In 2013, the CDC reported an overall rate of 5.3 cases of primary and secondary syphilis per 100,000, a more than twofold increase from 2000’s incidence of 2.1 cases per 100,000.

In addition to escalating incidence, the option to use a reverse sequence screening algorithm has left many in a quandary regarding whether to employ this or the traditional screening algorithm. Issues at stake include ease of use, automated versus manual screening, and the potential for enhanced disease detection versus the risk of overdiagnosis, overtreatment, and higher costs. As a result, laboratories are challenged to offer cost-effective diagnostic testing for syphilis that is sufficiently sensitive and specific to provide an accurate diagnosis and clear interpretation for clinicians in an expedient manner.

Diagnosing Syphilis

Syphilis, caused by the spirochete Treponema pallidum, can be difficult to diagnose because T. pallidum cannot be cultivated in the laboratory on standard culture media, and diagnostic testing results may change throughout the course of infection, depending on the stage in which a patient presents.

Historically, the definitive method for diagnosing syphilis was to visualize the spirochete directly via darkfield microscopy. This technique rarely is used today because it is technically difficult and requires special equipment and expertise. In addition, such direct detection methods are less sensitive than today’s tests, failing to detect up to 30% of primary cases.

Diagnosis typically is made using serologic tests that detect one or more of the antibodies provoked by the infection. Host antibodies are of two known types: 1. nontreponemal antibodies, or reagin, which reacts with lipid antigens; and 2. treponemal antibodies, which react with T. pallidum. No ideal single test is available at present.

Nontreponemal Tests

In the course of certain diseases, including syphilis, a substance known as reagin, which has the properties of an antibody, appears in the serum of affected patients. Reagin possesses the ability to combine with colloidal suspensions of lipids extracted from animal tissue, which then clump together to form visible masses, a process known as flocculation. Examples of nontreponemal tests include venereal disease research laboratory (VDRL) tests, rapid plasma reagin (RPR) tests, and toluidine red unheated serum tests (TRUST).

Although these tests are simple, inexpensive, and frequently used for screening, they are not specific for syphilis, can produce false-positive results, and, alone, are insufficient for diagnosis. Patients receiving a positive nontreponemal test result should subsequently undergo a treponemal test to confirm a syphilis diagnosis. This sequence of testing (ie, nontreponemal followed by treponemal) comprises the classic testing algorithm (see FIGURE 1).

Treponemal Tests

Treponemal antibody specific tests (eg, fluorescent treponemal antibody absorbed [FTA-ABS], microhemaglutination for T. pallidum [MHA-TP], T. pallidum particle agglutination [TP-PA], various enzyme immunoassays [EIA], and chemiluminescence immunoassays [CIA]) detect antibodies that are specific for syphilis. Treponemal antibodies appear earlier in the course of illness than nontreponemal antibodies and usually are detectable for life, even after successful treatment (see SIDEBAR). Patients who test positive on a treponemal screen should then receive a nontreponemal test with titer to confirm the diagnosis and guide treatment. This sequence of testing (ie, treponemal followed by nontreponemal) is known as the reverse sequence testing algorithm.

Testing Algorithms

Serologic diagnosis of syphilis always requires detection of two types of antibodies. Laboratories must decide which antibody to screen for first, thereby following either the traditional testing algorithm or the reverse sequence testing algorithm. Often, testing volume dictates which test to perform first; laboratories performing more than 100 or so tests per month should consider automated methods and the reverse screening algorithm, while those with lower test volume may find the traditional screening algorithm preferable. The laboratory at Beebe Healthcare, which performs slightly more than 100 tests per month, follows the reverse screening algorithm, starting with an automated platform ELISA, followed by an RPR test. Another deciding factor for Beebe’s lab was that management wanted testing to be consistent with that of our reference laboratory.
The traditional algorithm, which is designed to detect active infection, starts with a nontreponemal screening test, such as an RPR, while the reverse algorithm starts with a treponemal specific antibody test, typically an EIA or CIA. Nontreponemal antibodies typically rise early and rapidly in the course of the disease, making these tests highly sensitive to active infection. However, nontreponemal antibodies are not specific for syphilis; they can be elevated in the presence of a variety of diseases and conditions, including malaria, various autoimmune diseases, pregnancy, and after certain immunizations. Thus, nontreponemal tests have a high rate of false-positive results and require confirmation with a treponemal test. In addition, nontreponemal tests may be negative in early primary syphilis, treated cases, and late-stage disease. Nontreponemal tests also are subject to the prozone phenomenon, a state of high-antibody concentration where the agglutination reaction does not occur in the undiluted screening test, but occurs as the concentration of antibody is diluted out. Because most specimens are screened undiluted, specimens with high-antibody concentrations will yield a false-negative result. Nontreponemal antibodies also decline in response to treatment and eventually are undetectable in treated patients, making these tests good indicators of treatment response. Although the low cost of nontreponemal tests makes them a reasonable choice for screening, their manual, subjective, and labor-intensive nature makes them less well suited for laboratories with high-volume testing and automation.

The reverse algorithm starts with a treponemal specific antibody test, typically an EIA or a CIA. Both types of tests are automated easily and provide objective and specific results. Also, they entail no manual pipetting, and so offer less of an occupational hazard than traditional methods. Some detect IgM antibodies, which potentially is useful for diagnosis of early disease. Downsides are that their reliability tends to vary with prevalence of disease in the population, and they have difficulty distinguishing between active and past disease. Thus, a positive screening result on these tests may indicate active infection, past treated infection, or may be the result of a false-positive reaction. Positive screening results are followed by a nontreponemal antibody test to confirm whether the infection is active. If the nontreponemal antibody test is positive, the patient has active syphilis infection. A negative nontreponemal antibody test following a positive treponemal specific antibody test, however, poses a diagnostic challenge for clinicians. Such discordant results indicate either a past treated syphilis infection, very early or very late syphilis infection, or no syphilis infection (ie, a false-positive EIA/CIA reaction). To resolve the discordance, experts recommend that such specimens be tested further using a second treponemal specific test, such as a TP-PA. A positive result on the second treponemal test supports a diagnosis of past treated or latent syphilis; a negative result suggests that the initial screening test was falsely positive and that syphilis infection is unlikely.

Making a Choice
Traditionally, the CDC recommends that syphilis screening begin with a nontreponemal test, followed by confirmation using a treponemal test. However, a growing number of laboratories are using a reverse screening algorithm for syphilis because it lends itself well to automation and high throughput, and is a cost-effective, efficient means for screening large numbers of specimens. Automated tests provide the added advantage of interfacing with various computer systems in the laboratory, which minimizes data entry and consequent errors. Such interfacing means that scanning bar coded specimens can positively identify patients. The ability to automate and interface results to avoid errors and ensure that patient and result are matched correctly were two of the main reasons Beebe Healthcare chose to adopt the reverse screening algorithm.

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Syphilis Immunological Markers: What to Expect at Each Stage of Illness

Primary Syphilis
- IgM Antibody: Expect to be positive 2 to 3 weeks after infection
- IgG Antibody: Expect to be positive 4 to 5 weeks after infection
- Direct Darkfield Microscopy: Should be positive and is diagnostic
- RPR: @86% sensitive in primary syphilis
- FTA: @84% sensitive in primary syphilis

Secondary Syphilis
- IgM Antibody: Should still be positive early in stage 2, but may start to decrease
- IgG Antibody: Expect to be positive at high levels
- Direct Darkfield Microscopy: Should be positive and is diagnostic
- All nontreponemal and treponemal tests should be positive during this stage (ie, RPR, FTA, TP-PA, MHA-TP, VDRL, etc)

Latent Syphilis
- IgM Antibody: Should be negative
- IgG Antibody: Expect to be positive
- RPR: Expect to be negative or weakly positive
- FTA: Expect to be positive
- CSF VDRL: Should be positive in patients with central nervous system involvement

Congenital Syphilis
- IgM Antibody: Should be positive from the infant
- IgG Antibody: Should be positive, most often because of passive antibody transfer from mother. Infant does not begin producing his/her own IgG until about 2 to 3 months of age.
- Direct darkfield microscopy from the umbilical cord, placenta, nasal discharge, or skin lesion material should be positive.
- Congenital syphilis can be ruled out in a baby if a treponemal test is non-reactive after 8 months, or if a nontreponemal test is non-reactive at 3 months.

In addition to workflow advantages, adopting the reverse algorithm may result in increased detection of syphilis cases, particularly those involving early, latent, or late-stage disease. The downside is that the reverse screening method may create an increase in false-positive results, resulting in unnecessary patient follow-up, overdiagnosis, overtreatment, and higher costs. To offset this risk, patients with positive treponemal screening results subsequently should be tested using a nontreponemal assay to determine active infection, and any discordant results should be tested with a second treponemal assay to confirm true syphilis infection (either past treated or latent infection). Regardless of which algorithm laboratories choose to adopt, it is essential that the laboratory educate clinicians on how to interpret the results of the syphilis testing cascade to ensure accurate diagnoses. Such education may take the form of memos to physicians or periodic facility newsletter features. Just as physicians are obliged to know what tests their laboratory offers, laboratory workers are responsible for educating clinicians about the test menu and interpretation of results.

References

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