referring to these tests as nontreponemal is a misnomer. A 2019 study demonstrated that 11% of 526,540 reactive nontreponemal tests were not associated with syphilis, and in those cases, the tests were detecting antibodies to nontreponemal antigens generated by host tissue damage from other diseases (*53*). However, 89% of the reactive tests were associated with syphilis, implying that most nontreponemal tests detect antibodies triggered by *T. pallidum* phospholipid antigens during infection. Purported nontreponemal tests could more accurately be called lipoidal antigen tests. Hereafter in this report, these tests will be referred to as nontreponemal (lipoidal antigen) tests.

Treponemal Test

The term treponemal test was introduced in 1960 along with nontreponemal tests (*54*). Treponemal test remains an accurate description of a test that detects an antibody response to antigens specific to *T. pallidum*.

Nonspecific Antibodies

The term nonspecific antibodies has been used in the syphilis literature to characterize antibodies that are not specific to *T. pallidum* but are detected in nontreponemal tests. All antibodies bind to specific epitopes on an antigen and are specific to that antigen. However, the antibodies might not be specific for the detection of the disease or condition for which the test is ordered; thus, their presence affects the test specificity. Reporting antibody specificity and the effect on test specificity rather than using the blanket term nonspecific antibodies would be more accurate.

Principles for Syphilis Diagnosis

Indications for syphilis testing include identification of individual, population, or community risk factors for exposure to *T. pallidum;* signs and symptoms suggestive of syphilis; or a known sexual contact of someone who has syphilis. The selection of laboratory tests and interpretation of results vary by stage of syphilis and previous treatment history. After diagnosis and staging has occurred, benzathine penicillin G is the recommended therapy for clinical resolution of infection and avoidance of sequelae (*55*). Patients with a history of penicillin allergy should be managed according to CDC's *Sexually Transmitted Infections Treatment Guidelines, 2021* (*55*).

Testing for syphilis is based on the detection of reactive antibodies (typically in serum or cerebrospinal fluid [CSF]), suggestive of exposure to *T. pallidum;* direct observation of the organism by darkfield or fluorescent microscopy of lesion fluids or exudate; or histologic assessment of infected tissues or amplification of *T. pallidum*-specific nucleic acid sequences in fluids, exudate, or tissue biopsy material. Conventional microscopy used to examine Gram-stained smears is insufficient to visualize *T. pallidum* because of the bacterium's slender morphology and poor uptake of aniline dyes (*51*). No available nucleic acid amplification tests (NAATs) are cleared by FDA for marketing in the United States, and culture for *T. pallidum* is cumbersome and is available only in selected research laboratories. Nontreponemal (lipoidal antigen) tests are most suitable for screening or diagnosis in conjunction with a medical history and physical examination when antibody titers are important to determine recent exposure to infection, a presumptive diagnosis in persons with signs or symptoms suggestive of syphilis, or to determine response to treatment.

Treponemal tests target specific *T. pallidum* antigens, either intact or sonicated *T. pallidum* or defined recombinant proteins; these tests were traditionally used to confirm that a reactive nontreponemal (lipoidal antigen) test is the result of *T. pallidum* infection (*51*). Treponemal antibodies generally persist after treatment and cannot be used to distinguish between a current infection or a previously treated infection. None of the nontreponemal (lipoidal antigen) or treponemal tests can distinguish infections caused by other *T. pallidum* subspecies. Multiple capillary whole blood immunoassays for which the specimen is collected by skin puncture have been developed as rapid tests and might offer diagnostic utility in clinical, public health, or nonclinical settings. Direct detection tests of *T. pallidum* are limited to darkfield microscopic examination of lesion fluids, staining of lesion fluid or exudate smears or tissue sections obtained by biopsy for treponemal spirochetes, or amplification of specific nucleic acid sequences by validated laboratory-developed tests.

Recommendations for Syphilis Testing in the United States

Nontreponemal (Lipoidal Antigen) Tests

Nontreponemal (lipoidal antigen) tests typically have been used as a screening test for syphilis, as a diagnostic test when patients have signs or symptoms suggestive of syphilis or have a known sexual contact, when assessing possible reinfections, and when monitoring treatment outcome (Figure 1). RPR and VDRL tests are still the primary screening methods used in public health laboratories in the United States (*56*); other FDA-cleared nontreponemal (lipoidal antigen) tests (e.g., the toluidine red unheated serum test [TRUST] and unheated serum reagin test [USR]) are available but are less commonly used in the United States. Regardless of which test method is applied, serum antibody titers from RPR, VDRL, and