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Serology in the Laboratory Diagnosis of Syphilis

Syphilis is caused by the spirochete *Treponema pallidum (T. pallidum)*. The organism is usually transmitted by direct, typically sexual, contact. Later stages of the disease can affect multiple organs, including heart, aorta and brain, as well as fetuses in utero (congenital syphilis) causing diverse clinical manifestations and difficulty in clinical diagnosis. Despite the introduction of antibiotics, it remains a problem in Western countries, with the majority of new cases occurring in men who have sex with men and in intravenous drug abusers. There is also frequent co-infection with HIV.

Syphilis typically proceeds through a series of stages, from **primary syphilis** or chancre, in which one or more skin lesions are present at the site of organism entry into the body (10-90 days after infection), to **secondary syphilis**, characterized by non-itching skin rashes (six weeks to six months after infection), to **latent syphilis**, to **tertiary syphilis**, in which life threatening damage to organs such as the heart, arteries, and brain occur (years after infection). The laboratory diagnosis of early syphilis, neurosyphilis, congenital syphilis and syphilis in intravenous drug users and persons co-infected with serologically cross-reacting spirochetes and/or HIV can be challenging.

T. pallidum cannot be cultured. Diagnostic methods include detection of the organism by direct microscopic examination of fluid or smears, or histological examination of tissues from primary or secondary lesions (chancres and skin lesions), which are typically rich in organisms, but may not always be available. Nucleic acid amplification methods such as polymerase chain reaction (PCR) can also be used to directly test for the organism. However, the mainstay of diagnosis in most cases is serological testing. Serological tests fall into two categories: **nontreponemal** tests typically used for screening, and **treponemal** tests typically used for confirmation.

Nontreponemal tests detect IgG and IgM antibodies produced during infection against phospholipid material from the damaged host cells and cell surfaces of the organism. These antibodies are not specifically directed against the organism itself. **Treponemal** tests, by contrast, detect antibodies that are specifically directed against *T. pallidum*. There is no single serological test that is diagnostic of active, untreated infection; rather positive results must be present in both types of tests to confirm active syphilis, for reasons given below.

Nontreponemal tests include rapid plasma reagin (RPR) and Venereal Disease Research Laboratory (VDRL). The latter is the test of choice when the specimen is cerebrospinal fluid. These tests are sensitive beginning 20-90 days after infection. However, they can be falsely negative in very early stages of the disease and falsely positive in certain other diseases. Conditions that can cause false positive results in nontreponemal tests include pregnancy, auto-immune diseases (i.e., systemic lupus erythematosus), and infections such as Lyme disease, malaria, and tuberculosis. **Therefore, a positive non-treponemal antibody test must be confirmed with a more specific treponemal antibody test directed against the spirochete itself.**

Treponemal antibody tests are directed against antigens of *T. pallidum*; hence they are more specific for syphilis than the nontreponemal tests. Two commonly used tests include fluorescent treponemal antibody absorption (FTA-ABS), and *T. pallidum* particle agglutination assay (TP-PA). These tests become positive 3-4 weeks after infection, but they remain positive for the lifetime of the individual, even after effective treatment, so a positive test result does not necessarily indicate current active disease. By comparison, nontreponemal antibodies typically disappear in an adequately treated person after about three years. **Therefore, a positive treponemal antibody result must be confirmed by a nontreponemal test (such as RPR) to help differentiate between an inactive, treated infection and one that is active.** A confirmed serological test result does not indicate the stage of disease.

Table 1. The application and limitations of diagnostic tests in different stages of syphilis[1]

Stage	Recommended tests (comments)
Primary	Direct microscopic exam (and/or PCR), non-treponemal, treponemal (in the first 2-3 weeks, serology may not be positive in many cases)
Secondary	Direct microscopic exam (and/or PCR), non-treponemal, treponemal (serology tests nearly 100% sensitive)
Latent	Non-treponemal, treponemal (serology tests' sensitivity declines with time; both non-treponemal and treponemal should be positive)
Tertiary	Non-treponemal, treponemal (treponemal tests almost always reactive; nontreponemal tests may be negative in up to 30% of cases)

Viracor-IBT offers both screening and confirmatory tests for syphilis. As part of Viracor-IBT's continued commitment to providing you the highest quality of laboratory services, our serology lab began using the FDA-cleared ASiManager-AT™ reader for RPR testing in December 2014. The ASiManager-AT received FDA clearance in early 2014 for the qualitative detection of reagin antibodies to *T. pallidum* in living donors.[2] It is the first non-treponemal (RPR) analyzer cleared for blood donor screening, and brings state-of-the-art digital technology to laboratory analysis, interpretation and data management of ASI serology agglutination tests.[3]

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Future Readings and References

1. Ratnam S. The laboratory diagnosis of syphilis. Can J Infect Dis Med Microbiol. 2005 16(1): 45–51.
2. http://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/LicensedProductsBLAs/BloodDonorScreening/InfectiousDisease/UCM080466#anti_Tpallidum_Assays.
3. http://www.arlingtonscientific.com/assets/cber-press-release.pdf.