Cleveland Clinic

Cleveland Clinic Laboratories

Syphilis Serological Testing

Background & Clinical Indications

Syphilis testing can be divided into two categories: Treponemal (specific) assays detect antibodies that directly react with the syphilis-causing organism *T. pallidum subsp. pallidum*, while non-tremponemal assays, such as RPR (and VDRL) test, detect antibodies against non-specific antigens, including cardiolipin, lecithin, and cholesterol, released during treponemal infections. In the traditional testing algorithm for diagnosing syphilis, patient serum was initially tested with a non-treponemal test, followed by confirmation with a specific/confirmatory treponemal test.

This algorithm has been abandoned by most laboratories because the RPR test does not recognize treponemal-specific antibodies and that a number of infectious and non-infectious clinical situations could result in false-positive RPR (or VDRL) results, including autoimmune diseases (1) acute or chronic viral infections, recent immunizations, pregnancy, or endocarditis (2–3). Most importantly, because RPR reactivity is a feature of active syphilis infection, the test could give false negative results in late latent or tertiary syphilis. It may also test non-reactive during primary syphilis, potentially leading to mother-to-child transmission. Therefore testing pregnant women using the traditional algorithm may culminate in catastrophic consequences. The preferred testing algorithm – the reverse sequence testing algorithm – (a misnomer) is the one in which serum is initially tested using a specific treponemal test and confirmed with a non-treponemal test (e.g., RPR) (4).

The algorithm shown here represents the current Cleveland Clinic's screening for syphilis serology testing. It is the only option available for ordering and it is all-encompassing. For individuals with a previous history of syphilis serology, that testing is done for monitoring of treatment success, providers may only pay attention to the automatically-added and performed RPR result; that being said, in patients in whom adequate therapy starts very early after infection and seroconversion, and also in the immunocompromised, treponemal antibody response may also wane over time to negativity; therefore another seroconversion along with at least a 4-fold rise in RPR titer strongly suggests re-infection. The specific treponemal screen assay is a total antibody (IgM+IgG) assay against recombinant antigens of *T. pallidum*. They appear 2–3 weeks post infection before non-treponemal (RPR) antibodies appear. Since the screen test may rarely test false positive (typically in a low-risk setting), where RPR is non-reactive, another specific Treponemal assay is added to confirm the initial screen result. An overall interpretation is provided with all results.

Limitations

- Infants up to 18 months (anitibodies usually disappear within 6 month after birth) may have a reactive syphilis total/screen test result. This may also be seen with RPR as antibodies detected by RPR may belong to both IgG and IgM classes, the former also crosses the placenta; however,

Current Algorithm for Syphilis Serology Testing

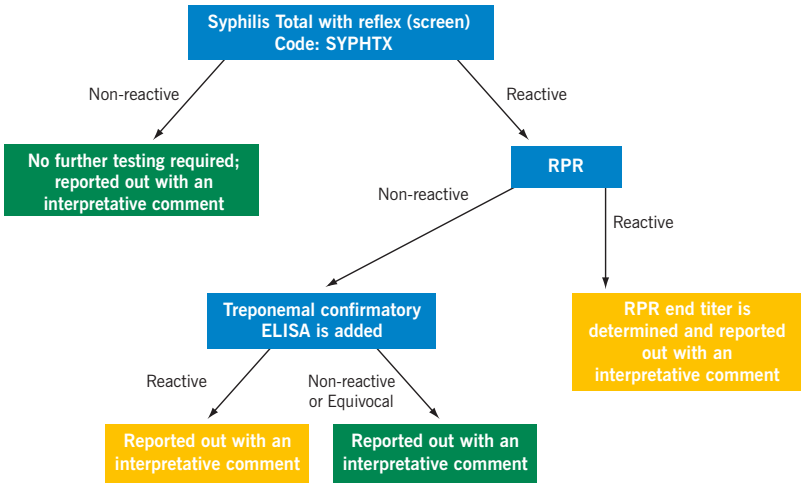

Figure legend: Syphilis Total with reflex methodology is Chemiluminescent microparticle immunoassay (CMIA) to detect IgM+IgG antibodies against specific recombinant Treponemal antigens. RPR is based on agglutination method and where reactive it will be tittered out automatically. The enzyme immunoassay method (aka ELISA) using specific recombinant Treponemal antigens is done as the confirmatory assay where needed per algorithm.

RPR antibodies shows faster clearance kinetics and usually disappears in 4–6 weeks postnatally, if only there is no congenital syphilis. The best approach would be to compare maternal and neonatal RPR titers collected at the same time, and where there is at least 4-fold higher titer seen in the neonate, it should significantly raise suspicion for congenital syphilis. It is still important to order SYPHTX to ensure the reactive RPR results are in the context of the reactive Treponemal result.

- Sera with very high RPR antibody concentrations may produce false negative results for the RPR test due to the prozone effect. This has always remained a concern among clinicians, however, in the lab, the so-called "rough" RPR results are blindly diluted to rule out prozone phenomenon. Therefore such possibility remains exceedingly rare.
- Most patients become seronegative for non-treponemal tests following adequate treatment; however, some patients have a low RPR titer for extended periods when they present with late latent or tertiary disease, despite being adequately treated in the past (5). These patients are referred to as being "serofast." VDRL testing is used for diagnosis of neurosyphilis, otosyphilis, and ocular syphilis using CSF specimens. It is not included in this algorithm and must be ordered separately where clinically warranted.

References

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2. Harris A, Brown L, Portnoy J, Price EV. Narcotic addiction and BFP reactions in tests for syphilis. *Public Health Rep.* 1962;77:537.
3. Kaufman RE, Weiss S, Moore JD. Biologic false positive serological tests for syphilis among drug addicts. *Brit J Vener Dis.* 1974;50:350.
4. Pope, V., Use of Syphilis Test to Screen for Syphilis. *Infect Med.* 2004;21(8):399-404.
5. Pettit DE, Larsen SA, Harbec PS. Toluidine red unheated serum test, a non-treponemal test for syphilis. *J Clin Micro.* 1983;18:1141.
6. CDC, MMWR, Vol. 60 (5):133-140, 2011.

Test Overview

Test Name	Syphilis Total with reflex
Ordering Mnemonic	SYPHTX
Reference Range	Nonreactive
Patient Preparation	None
Specimen Requirements	1.0 mL serum
Test Ordering Information	SYPHTX
Reflex Information	If Syphilis Total (screen) is Reactive, RPR and/or ELISA may be ordered and billed, depending on the algorithm.
CPT Codes	86780

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