Interpretation of Serological Tests for Syphilis

Proper evaluation of serological test results in syphilis is often a confusing matter. Physicians are rarely confronted with problems in this regard. The following comments are provided as a matter of review. More extensive discussion may be found in the references listed at the end of this article. Several tests are in use. Most common of these are the **Rapid Plasma Reagin (RPR) Test**, the **Veneral Disease Research Laboratory (VDRL) Test**, the **Treponema Pallidum-Particle Agglutination (TP-PA) Test**, and the **Fluorescent Treponemal Antibody Absorption (FTA-ABS) Test.**

Nontreponemal Antigen Tests

Nontreponemal antigen tests, including the RPR and VDRL tests, are designed to test serum for reagin, a heterogeneous group of antibodies which combine with a cardiolipin-lecithin antigen. This antigen, usually derived from beef heart, is a normal component of human tissue. The RPR and VDRL tests are sometimes described as serologic tests for syphilis (STS).

The RPR test is quick, inexpensive, and easy to use; it is exceptionally useful in screening. Like its older nontreponemal counterpart, the VDRL, it may be quantitated. These tests are very sensitive; i.e., there are few false negatives, except as discussed below.

RPR quantitative tests are reported as "nonreactive" or "reactive" at dilutions of 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, etc. While considerable confidence may be placed in the RPR or VDRL tests, remember that patients with active infectious primary syphilis frequently have nonreactive VDRL and RPR tests. This is a result of the patient's immune system lagging behind the disease process. At the time of appearance of the primary lesion, the chancre, only about 25% of the cases will have a reactive VDRL or RPR test. After the chancre has been present for one week, 50% will have a reactive test; after two weeks, the ratio rises to 75%. Almost all will have reactive serologies 3-4 weeks after appearance of the primary lesion; and virtually 100% of the cases will have a reactive serology by the time secondary syphilis develops. Although the development of secondary syphilis may take as long as six months, the rash or other signs may appear even before the primary lesion has "healed." Treatment with penicillin and other antibiotics will interrupt the development of subsequent stages of the disease if the dosage is adequate. If the dosage is **not** adequate, the development of clinical signs may be disrupted to some extent or delayed temporarily; the development of seroreactivity to the RPR or VDRL tests may also be masked or delayed by inadequate treatment.

Prior to treatment of any patient for syphilis, a quantitative VDRL or RPR should be performed. The test should be repeated at specified intervals after therapy (see table page 4).

Individuals who are recent sexual contacts (within 90 days) of patients with infectious syphilis (primary, secondary, and early latent stages) and who have negative RPR or VDRL findings still must receive prophylactic treatment for syphilis as an epidemiologic control measure.

In untreated **primary syphilis**, the seroreactivity usually reaches a titer of at least 1:4. Following treatment of primary syphilis, the reactivity may continue to rise for a few weeks but should revert to non reactivity within 6-12 months following treatment. Ninety-seven percent of patients will be nonreactive within two years.

In **secondary** or **early latent syphilis**, the VDRL and/or RPR tests are invariably reactive, usually with a titer of 1:32 or higher. While the titer may continue to rise immediately after successful treatment, the reactivity should gradually revert to non reactivity within 18 months following the completion of successful treatment. After 2 years, over 75% will be nonreactive. Twenty-five percent will have positive titers that have stabilized at or below the four-fold decrease needed to document adequate treatment. The majority of these titers will have a low reactive titer 1:4 or less. If the patient with secondary syphilis develops a VERY strong reactivity, the VDRL or RPR test could be read spuriously as nonreactive, due to the prozone phenomenon. The laboratory should, therefore, be asked to dilute the "nonreactive" serum and continue the titration in all cases wherein suspicious lesions or clinical findings are present.

Late syphilis (syphilis of more than one year's duration) may be symptomatic or asymptomatic. A patient may have late syphilis, either acquired or congenital, and have a nonreactive VDRL or RPR test. Further evaluation by means of a test such as the TP-PA is necessary in such persons suspected of having late stage manifestations of syphilis. Cerebrospinal fluid (CSF) studies are recommended to rule out neurosyphilis in these cases. Late syphilis must be adequately treated.

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After treatment for primary and secondary syphilis, failure of the highest titer achieved to decrease four-fold within six months suggests a treatment failure and warrants reevaluation of the case, (the highest titer may be reached a week or two after treatment is instituted). Following treatment for latent syphilis, patients with normal CSF examination should be re-treated if a) titers increase fourfold b) an initially high titer (> 1:32) fails to decrease to at least fourfold (i.e., two dilutions) within 12 – 24 months of therapy or c) signs or symptoms attributable to syphilis develop.

In cases where the epidemiological and clinical information fails to support serological findings, the diagnosis of syphilis should be questioned. A "biologic false positive" basis for seropositivity should be sought.

Biologic false positivity (BFP), meaning a nonsyphilitic basis for reactive RPR or VDRL tests, must be established by the use of treponemal antigen test such as the TP-PA. The RPR and the VDRL are two among many nontreponemal antigen tests. Among the nontreponemal tests, "false positivity" or "false reactivity" occurs in at least 1% of persons tested. Barring laboratory error, the treponemal antigen tests are seldom falsely positive. They are more complicated and expensive to perform, and there are relatively more false negative test results. Treponemal antigen tests, such as the TP-PA and FTAABS, are inappropriate for use as screening tests. They also are not quantitative tests and cannot be used for following titers in response to therapy or progression of disease.

While most persons infected with HIV will have typical antibody responses to syphilis infections, some may not. All persons suspected of having syphilis should be referred for HIV counseling and testing. If clinical findings are suggestive of syphilis in a person known to be HIV-infected, but serological tests are nonreactive or the interpretation is unclear, alternative tests (biopsy of a lesion, darkfield examination, or direct fluorescent antibody staining of lesion material) may be useful for diagnosis.

Treponemal Antigen Tests

Treponemal tests detect an antibody that is directed toward pathogenic members of the genus *Treponema*.

The Bureau of Laboratories, Texas Department of Health, uses the **Treponema Pallidum-Particle Agglutination (TP-PA)** test for routine confirmatory testing for syphilis. The specificity of the TP-PA test is as good as, or better than, the FTA-ABS test. Usually, a nonreactive result on an TP-PA test will establish the "biologic false positive" diagnosis of a "positive" nontreponemal antigen test (RPR or VDRL). Transient (acute) false reactivity of the RPR or VDRL tests occurs in some patients due to intercurrent viral and bacterial infections, when the serum titer of heterophile antibodies is high. Serum controls used in the laboratory identify this heterophile activity when it occurs in the TP-PA, permitting further evaluation with the FTA-ABS test. Infectious mononucleosis (Epstein-Barr viral infections) and viral hepatitis, as well as herpes simplex infections, chancroid, and lymphogranuloma venereum, may be accompanied by biologic false positive serological tests for syphilis. Long term (chronic) biologic false positivity may be present in Hansen's disease and collagen diseases, such as systemic lupus erythematosus and rheumatoid arthritis, as well as in narcotics addiction (especially methamphetamines) and in some forms of neoplasms. A determination that a patient has a biologic false positive RPR or VDRL mandates a search for the etiology of the positivity.

The Bureau of Laboratories will perform the FTA-ABS test, but only under the following circumstances:

1. In suspected cases of primary syphilis in which two nontreponemal tests performed five days apart have shown a static reactive titer and in which the TP-PA test performed on the second specimen was nonreactive. This must be documented when the specimen is sent for FTA-ABS testing.
2. In diagnostic problems arising from conflicts between the overall clinical impression and results from both treponemal and nontreponemal tests. Such conflicts sometimes occur in cases of late syphilis. A brief written description of the diagnostic problem must accompany specimens sent for FTA-ABS testing.

The FTA-ABS Test is performed on Tuesday and Thursday. The RPR test is performed daily. The TP-PA Test is performed on Monday, Wednesday, and Friday but only on specimens that are reactive by our RPR Test, or in cases where the results of nontreponemal tests are equivocal and in which information describing this situation accompanies the specimen. Additionally, since it is recognized that the RPR test can be nonreactive in active tertiary syphilis, the TP-PA will be performed ***if this justification is indicated***.

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Unlike the nontreponemal antigen tests, the TP-PA and the FTA-ABS do not revert to nonreactivity after successful treatment of syphilis. Once reactive, they almost always stay reactive. Therefore, ordering repeated TP-PA tests to check on a patient's progress is not warranted. In these situations, the desired information would be obtainable through repetition of the RPR or VDRL quantitative test. Sequential serologic tests in individual patients should be performed by using the same testing method, preferably by the same laboratory. The RPR and VDRL are equally valid assays, but quantitative results from the two tests cannot be compared directly because RPR titers are often slightly higher than VDRL titers.

Patients treated for **other** sexually transmitted diseases should receive a serological test for syphilis because such persons are at relatively high risk for exposure to syphilis.

A reactive STS determined on serum raises the question as to whether testing of cerebrospinal fluid (CSF) is indicated. A lumbar puncture is generally not indicated during the primary and secondary stages of this infection, unless syphilitic meningitis is suspected or unexplained neurological findings occur. Following proper penicillin therapy for syphilis, a favorable blood serologic response (fourfold titer drop) generally indicates that NO testing of the CSF is required. Final judgment must be based on careful evaluation of the serologic response to treatment while following the serum titer for no less than 12 months, or reversion to nonreactivity (see table page 4).

Tests of the CSF (VDRL quantitative) **should** be performed in:

1. **all** cases of congenital syphilis
2. **any** syphilis patients with
    - neurologic or ophthalmic signs or symptoms,
    - evidence of active tertiary syphilis (e.g., aortitis, gumma, and iritis),
    - treatment failure
    - HIV infection with late latent or syphilis of unknown duration, or
    - patient preference (in immunocompetent patient).

Some specialists recommend performing CSF examination on all patients who have latent syphilis and a nontreponemal serologic test of > 1:32. A reactive VDRL performed on a sample of spinal fluid should be considered neurosyphilis until proven otherwise. A diagnosis of central nervous system syphilis is supported by CSF findings of lymphocytic pleocytosis (often <100 wbc/mm3 and normal to elevated protein). In general, interpretation of CSF results may be difficult. Invasion of CSF by *T. Pallidum* accompanied by CSF abnormalities is common among adults who have primary or secondary syphilis. However, neurosyphilis develops in only a limited number of patients after treatment with the penicillin regimens recommended for primary and secondary syphilis. Therefore, unless clinical signs or symptoms of neurologic or ophthalmic involvement are present, CSF analysis is not recommended for routine evaluation of patients who have primary or secondary syphilis.

All infants born to mothers who have reactive nontreponemal and treponemal test results should be evaluated with a quantitative nontreponemal serologic test (RPR or VDRL) performed on infant serum, because umbilical cord blood can become contaminated with maternal blood and could yield a false positive result. Refer to the Centers for Disease Control and Prevention, Sexually Transmitted Diseases Treatment Guidelines 2002 for evaluation and treatment of infants for congenital syphilis.

All seroreactive infants (or infants whose mothers were seroreactive at delivery) should receive careful follow-up examinations and serologic testing (i.e., a nontreponemal test) every 2-3 months until the test becomes nonreactive or the titer has decreased fourfold. Nontreponemal antibody titers should decline by 3 months of age and should be nonreactive by 6 months of age if the infant was not infected (i.e., if the reactive test result was caused by passive transfer of maternal IgG antibody) or was infected but adequately treated.

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References

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TABLE - Summary of Recommended Follow-up

		Follow-up Post-treatment	
Condition	Treatment	Serology	Discharge
Sexual contact of Primary or Secondary within 90 days of exposure (with negative RPR or VDRL)	Yes	6 months and 12 months	End of 6 months[1]
Late Syphilis of more than 1 year duration, cardio-vascular syphilis, gummas (Late benign syphilis)	Yes	6 months and 12 months	End of 2 years
Syphilis in pregnancy[2]	Yes	3rd trimester and at delivery[3]	6 months to 1 year
Subsequent pregnancies. No change in titer[2]	No	Initial visit and delivery[3]	
Congenital[1] – Seropositive untreated infants (suspect maternal transfer)	N/A – Only if RPR is positive at 3 months	Every 2-3 months	15 months
Congenital (treated infants[4])	Yes	Every 2-3 months	End of 6 months[1]
Neurosyphilis[5]	Yes	6, 12, 18, and 24 months	2 years
Syphilis in the HIV infected	Yes	3, 6, 9, 12, and 24 months	End of 2 years[1]
Latent syphilis in the HIV infected	Yes	6, 12, 18, and 24 months	End of 2 years[1]

1 - Discharged patients should have either negative serologic tests for syphilis or fixed low titers. If a positive titer is found, the patient may warrant further follow-up.

2 - Retreatment is indicated if there is any doubt concerning adequacy of previous treatment.

3 - Monthly serologies until delivery should be done if at increased risk for re-infection and/or in geographic area with high prevalence.

4 - An appropriate medical specialist should be consulted regarding treatment of complications of congenital syphilis.

5 - Neurosyphilis patients should have a spinal fluid examination at the 6-month follow-up visit. If a positive titer is found in the CSF, the patient may warrant further follow-up.

For HIV/STD testing locations in Texas, call: 2-1-1

For other HIV/STD questions, call:
1 (800) CDC-INFO (English/Español)
1 (888) 232-6348 (TTY)

For more information, go to: www.dshs.state.tx.us/hivstd

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