

Have things changed? Really?



Source: The National Archives and Records, Washington.

Syphilis Testing a Review

Dr. Robert Notenboom

Historical perspective:

In their attempt to formulate a serological test for Syphilis, Wassermann and his colleagues encountered a major obstacle. Other than extracts from *T. pallidum* infected tissues there were no sources of antigen. And till today the organism cannot be cultivated in vitro.

As was later shown, the antigen that was extracted was a non-specific substance called cardiolipin and not a specific treponemal antigen(s). The results of the Wassermann Complement fixation test (utilizing cardiolipin) correlated reasonably well with exanthematous cases of Syphilis.

However, many other diseases and conditions can give rise to positive results as well. The use of cardiolipin restricted the sensitivity of the test in favour of specificity. Nonetheless, false positive test results were a frequent occurrence, which became even more obvious with the introduction of penicillin as an efficacious treatment for the disease.

Separating false positives from true positives became feasible when Nelson and Maier introduced the first treponemal test: Treponema Pallidum Immobilization test (TPI) in 1949. Less demanding treponemal tests were introduced in 1964 (FTA-ABS) and in 1965 (Hemagglutination). All along it was assumed that the screen test had an "adequate" sensitivity although a gold standard was never formulated.

In most jurisdictions the current syphilis-testing algorithm is still based on a non-specific test (VDRL, RPR etc) followed by confirmation with FTA-ABS or MHA-TP (TP-PA).

Such algorithms may not have the expected sensitivities, nor are they cost effective.

Limited sensitivities:

Studies carried out at CDC show that cardiolipin-based tests have a sensitivity ranging from 72 to 99% during primary infections; while the ranges for latent and late syphilis are 88-100% and 34-94%, respectively. As the sensitivities of confirmatory tests for primary infections range from 69 - 100%, the overall diagnostic efficiency during early disease may be as low as 50% (before taking into consideration the subjectivity of the assays.)

In addition, false negatives may be reported due to a phenomenon called: prozone, which can be the result of very high concentrations of antibody or failing to observe proper laboratory technique.

Cost effectiveness:

One of the major reasons that the current algorithm (non-specific test followed by treponemal antibody test) is still in place rests on the notion that current testing is inexpensive. If one considers a material cost of less than \$ 0.10 /test, it is hard to imagine a more cost effective way, until all activities and expenses (especially labour) are accounted for.

The following example may clarify this point.

If a technologist can perform 50,000 tests/year and earns \$35,000 (plus benefits) the cost/test will be \$0.95 (labour + \$ 0.10 material cost)

With a positivity rate of 5% by RPR (of which 50 % are false positive) we must add for repeat testing (> 2500x \$0.95), for various titrations (32,500 x \$ 0.95) and for confirmation by e.g. TP-PA (2500 x \$ 3.50) i.e. an additional \$ 42,000.

Total cost \$ 89,500 or **\$ 1.79** per submission.

Alternatively, with a sensitive and specific treponemal test (e.g. \$ 1.00/ EIA test) false positives can be eliminated, so that only the true positives are tested and titrated (\$ 16,812.50)

If confirmation by TP-PA is required (?) \$ 4,375.00 should be added for a total cost of \$71,187.50.

Labour cost of 0.25 FTE to operate equipment and carry out titrations \$ 10,500.

Total cost \$ 81,687.50 or \$ **1.63** per submission.

Recombinant treponemal antigens:

The expression of specific treponemal genes in e.g. E coli has made it possible to increase both sensitivity and specificity of tests as non-specific antigens have been eliminated. In the past, the presence of non-specific epitopes/antigens limited either the specificity (FTA-ABS) or sensitivity (MHA-TP) of treponemal tests. As a result, one may expect to find apparent discordant results that can be resolved by Western blot or immuno-blot assays.

Utilizing recombinant treponemal antigens in an EIA format will essentially identify all infectees (except for those with early incubating syphilis, prior to positive darkfields).

Such test:

- Allows for automation and greater flexibility in the laboratory,
- Provides objective reading of results, preventing false positives and negatives,
- Reduces ergonomic problems
- Provides the opportunity to tie in with an LIS.

Interpretation of results:

Positive or equivocal EIA results that are RPR and TP-PA negative:

The specificity of Trep-Chek™ is 99+ %, which means that the occurrence of false positives is less than 1%. However, many factors especially the condition of the sample may lead to an increase in this number. It may not be possible to determine the status of the patient without a follow up sample. In addition, in a small number of patients only antibody to one syphilitic antigen is present, which may give rise to a positive or equivocal EIA result. It is not clear yet whether this is due to a cross-reaction. Persons with these kinds of cross-reactions are generally not considered as infected with *T. pallidum*. The resolution of these "false positive" results may require a Western blot or line immuno assay with various treponemal antigens.

The sensitivities of TP-PA and Trep-Chek™ are comparable, however this does not mean that the agreement between the two tests is 100%. Without a more sensitive test (see above) it may not be possible to determine the true condition of the patient.

In the absence of a "reactive" RPR, a positive EIA result is most likely associated with an adequately treated old case (treatment could have been inadvertent); early primary Syphilis; late latent or late syphilis.

If, primary syphilis is suspected a second test, 7 to 10 days later, is indicated to establish seroconversion.

Positive treponemal tests during pregnancy are of concern as a non-reactive RPR does not necessary rule out in utero infections.

A positive EIA result together with a reactive RPR is most compatible with active Syphilis. Such instances require a detailed review of the patient's history to establish whether treatment is required or has been efficacious.

Adherence to the current algorithm suggests that the sensitivity of the Wassermann/VDRL/RPR is adequate to determine whether an active infection is (still) present and whether the patient should be treated. Treatment during the early stages of syphilis, normally result in non-reactive RPR result. However, particularly patients diagnosed during late latency may maintain low titers (this is referred to as serofast). Laboratory tests are only part of the assessment/diagnosis. Test results should be interpreted in light of the patient's history, life style and detailed clinical examination. Specific IgM tests have been used as a further aid in the diagnosis.

While there may be tests with very high sensitivities and specificities, absolute sensitivity and specificity in Syphilis tests are not likely achievable. Therefore, the limitations of the various tests and algorithms must be considered in evaluating a final report. For example, approximately 50% of the "reactive" test results obtained by cardiolipin-based tests (VDRL, RPR, TRUST etc) are not due to a treponemal infection. Approximately 1% of the general population when tested by FTA-ABS will give rise to false positive results. When this test is used to confirm "reactive" (non-specific) screen test results, the likelihood of reaching a false positive diagnosis is $50/100 \times 1/100 = 1$ in 200.

A low titer (by RPR etc), or even a "non-reactive" does not necessarily rule out active disease. Therefore all reactive sera are confirmed by a treponemal test. The overall yield of confirmed positives is limited by the sensitivities of both tests. While EIA's and TP-PA share high sensitivities the former are better suited for large volumes as the tests can be automated.

Author: Dr. Robert Notenboom, Phoenix Bio-Tech Corp., bnotenboom@phoenixbiotech.com
For any further information call 1-800-701-7450

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