The Laboratory Diagnosis of Venereal Diseases*
I. Serological tests for syphilis

by Dr. M. Jegathesan
A.M.N., P.J.K.,
M.B.B.S., D.T.M.& H., D.C.P., D.Path.
Institute for Medical Research
Kuala Lumpur.

EXAMINATION OF sera for the evidence of syphilitic infection forms a large part of the work of most routine serology laboratories.

For instance, in the serology division of the Institute for Medical Research, Kuala Lumpur, an average of some 30,000 sera are examined annually. Most of these sera are from routine screening of blood donors, expectant mothers and routine medical examinations in addition to patients being investigated after known exposure.

Table I shows the number of serological tests for syphilis done in the Serology Division of the IMR[6]

Table 1

Tests	Year		
	1970	1971	1972
Kahn	24,614	26,023	10,993
Wasserman	23,603	25,649	30,480
VDRL	—	—	19,487
FTA	396	106	968

The most direct and early evidence of syphilitic infection is of course to demonstrate the presence of *Treponema pallidum* in the primary chancre in the mucous lesions of secondary syphilis. This is done by means of dark field microscopy[13] which requires the procedure to be performed at the patient's side and requires trained personnel who are able to recognize *T. pallidum* and to differentiate it from other commensal treponemas. Because of this and because the chances of finding treponemes are only during limited periods in the natural history of the disease one usually has to depend on the indirect evidence provided by serological examination.

Serological tests for syphilis

Serological tests for syphilis have evolved rapidly since they were first introduced and today the clinician is faced with a large gamut of tests to choose from. It is the purpose of this paper to review the current tests available, their interpretation and to make recommendations on a uniform testing system for this country.

All serological tests for syphilis can be divided broadly into either non-treponemal or treponemal.

Non-treponemal tests for syphilis are non specific tests that detect the presence of an antibody "reagin" that is found in the sera of patients suffering from syphilis and related treponematosis and from a multitude of other unrelated diseases like the collagen diseases, malaria, leprosy, infectious mononucleosis, certain viral infections, 10,5,3 some cases of pregnancy and even in blood donors after repeated donations.[4] Reagin is found in the gamma globulin fraction of serum but its exact nature is unknown. Reactive results in cases other than syphilis are referred to as biological false positive

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reactions and may be termed as either acute or chronic depending on whether the reaction persists for longer than six months.3

Treponemal tests on the other hands, detect the presence of treponemal antibodies and can be considered specific for syphilis and related treponematosis. It must be mentioned that there is no test at the moment that can differentiate syphilis from yaws, pinta and bejel which are the other treponeme caused diseases. One may wonder why, in the presence of treponemal tests, is there a need for non treponemal tests to be performed. The reasons are that treponemal tests are expensive, technically more difficult to perform, requiring specialised personnel and equipment and are usually only available in very specialised laboratories. Furthermore treponemal tests merely give information on the serological status of the patient indicating either present or past infection. The state of activity of the disease will not be known since treponemal tests remain reactive for long periods (sometimes for life) and usually do not show any change following treatment. Non-treponemal tests, on the other hand, are relatively cheap, easier to perform and allow quantitation. Titres will usually drop after successful treatment and hence they will be useful in following response to therapy.

It is the current practice therefore to use non treponemal tests as routine screening tests and to follow response to treatment. Treponemal tests are reserved for confirmation – to be performed at the special request of the clinician. They are of great value in distinguishing syphilitic reactions from biological false positive reactions in non treponemal tests and to aid in establishing a diagnosis of syphilis in a patient with clinical or epidemiological evidence of syphilis who shows non reactive non-treponemal tests.14

Non-treponemal tests

The following non-treponemal tests are available:

(1) The Kahn test
(2) VDRL test
(3) Complement fixation test (Wasserman)
(4) Rapid reagin tests
(5) Automated reagin tests

Present day non-treponemal tests do not use the crude lipoidal antigen (from beef heart) that is used in the Kahn Test. Tests like the VDRL utilise a purified preparation consisting of cardiolipin, lecithin and cholesterol, allowing for better control on sensitivity, specificity and reproducibility.

The Centre for Communicable Disease Control (C.D.C.) in Atlanta, Georgia, who in conjunction with WHO run an international proficiency testing programme for syphilis serology, recommend that only one cardiolopin test be done routinely. They recommend the VDRL as it is cheap, easily standardised, reproducible and easy to perform. There is no point in performing more than one cardiolipin test as no additional information can be gathered and in fact conflicting results may further confuse the clinician.

The VDRL flocculation test takes preference over the complement fixation tests because the latter, while requiring more reagents, more manipulation and more time is harder to standardise and is considered to be less sensitive.

The principle to remember is that it is better to perform a single test well than several tests poorly.14

The Rapid reagin tests are so called because they were originally designed to serve as a screening procedure for large groups of persons and could also be employed as a field test in mass surveys. The rapid plasma reagin and the RPR (teardrop) card test can be used for this purpose and while they may pick up many false positives, this is not really a disadvantage as all positive cases can then be subjected to the more standardised laboratory tests. A recent improvement on these tests, the RPR (circle) card test has the advantage that it has about the same sensitivity as the VDRL test and also allows for quantitation.

For laboratories performing a large volume of serological tests a suitable innovation is the automated reagin test which utilises the continuous flow antoanalyser system.8,9.

Treponemal tests

The treponemal tests utilise a specific antigen – either the pathogenic *Treponema pallidum* itself or a fraction of the non pathogenic Reiter treponeme. The tests now available are:–

(1) Treponema immobilisation test (TPI)
(2) Fluorescent treponemal antibody test (FTA)
(3) FTA – ABS
(4) Reiter protein complement fixation test
(5) *Treponema pallidum* haemagglutination test
(6) Microhaemagglutination test for *Treponema pallidum*
(7) Automated FTA – ABS

The Reiter protein complement is a modification of the cardiolipin complement fixation test except that a fraction of the non pathogenic Reiter treponeme is used as the antigen. It is more specific than the cardiolipin tests but less specific and less sensitive than the TPI or FTA tests which are described below.

The TPI (Treponema Pallidum Immobilisation Test), once considered the standard by which all serological test for syphilis are assessed employs living treponemes that have been grown in rabbit testicular tissue and extracted in survival medium. This test is expensive, time consuming, technically difficult and requires an elaborate system of controls. Standardisation of the test is difficult and this test is only performed in a few research laboratories.

The FTA – ABS test has now virtually taken over as the standard from the TPI as it has been found to be just as specific while being more sensitive (95% as compared to 89% in a study).2 The FTA – ABS test (introduced in 1964) is a modification of the earlier FTA where the antigen (cell components of *T. pallidum*-Nichol's strain) is absorbed with sorbent (an extract of Reiter treponeme cultures) to remove group specific antigens for *Treponema pallidum*.

The FTA, FTA-200 and FTA-ABS employ the indirect fluorescent antibody technique.

Recently the *T. pallidum* haemagglutination test was developed and has been assessed and some studies showed it to be just as specific and sensitive as the FTA-ABS.12 The TPHA test employs a haemagglutination reaction using *T.pallidum* (Nichol's strain) sensitised erythrocytes. The TPHA however appears to have a slightly lower positivity rate in early (primary) syphilis. The TPHA test is simpler and cheaper than the FTA-ABS and recently, a quantitative microhaemagglutination assay for *T. pallidum* has been introduced7 and shows great promise of being introduced as a test more practicable for routine use than the FTA-ABS. However, like the FTA-ABS, the haemagglutination tests have not proved useful in following the efficacy of treatment.

Automation has also been introduced in the treponemal tests and tests available are the Automated fluorescent treponemal test (AFTA)11 and the Quantitative Automated Micro-haemagglutination Assay for antibodies to *Treponema pallidum*.

The serological course of syphilis14

The primary chancre usually appears about three weeks after infection. The cardiolipin tests and the FTA-ABS become reactive about the forth to the sixth week. The TPI becomes reactive a bit later in the primary stage.

In secondary syphilis all serological tests are positive.

In late and latent syphilis it must be noted that in about a third of the cases the cardiolipin tests may be non-reactive, and the only means of laboratory detection may be the treponemal tests.

Of all tests assessed so far, the FTA-ABS appears to be the most sensitive test in primary syphilis, is fully reactive in secondary syphilis and maintains its high level of reactivity through latent and late syphilis.

Let us examine the effect of treatment on the serological course of syphilis.

If treatment is given before the primary chancre appears or before the cardiolipin tests become positive it is likely that the cardiolipin tests will remain non-reactive.

If treatment is given in the early stage after the cardiolipin test have become reactive it will take about six months before these tests become non-reactive. If treatment takes place in secondary syphilis reversion to non-reactivity may take between 12-18 months. If treatment is given very late the cardiolipin tests may persists for life.

As far as the treponemal tests are concerned reversion to non-reactivity will only occur if treatment is given very early in the disease. Otherwise it is very likely that these tests will remain reactive for life.

Recommendations

The serological tests for syphilis have been reviewed.

One flocculation test should be used for routine testing. At the moment, the VDRL test appears to be the most reliable and it should be adopted. Most laboratories doing serological tests can be easily equipped and staff trained so as to perform this test satisfactorily. All other non-treponemal tests should be discontinued.

All reactive sera should have a quantitative VDRL test done. A reactive result should be assessed in the light of clinical and epidemiological findings. If these do not support a diagnosis of syphilis, the test should be repeated at regular weekly intervals to establish the presence of a static or rising titre.

Verification may then be sought in doubtful and inconclusive cases by doing the FTA-ABS test. If this is positive it shows the presence of treponemal antibodies, indicating past or present infection.

Summary

The serological tests for detection of syphilis that are currently available have been reviewed. It is suggested that laboratories in this country utilise one non-treponemal test, the V.D.R.L. as a screening test and the FTA-ABS test as a confirmatory procedure.

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