

TESTING FOR SYPHILIS - RATIONAL USE AND INTERPRETATION

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ABSTRACT

Syphilis is a disease caused by a spiral organism, *Treponema pallidum*. Microscopy is useful in diagnosing syphilis in its primary stage while the serological tests are used for diagnosing primary secondary or latent stages of syphilis. The non specific serological tests are the non treponemal tests such as the Venereal Disease Laboratory Test (VDRL) and the Rapid Plasma Reagin Test (RPR). Both these tests are used as a screening test. The specific tests are the treponemal tests. The two common treponemal tests are the fluorescent treponemal antibody absorption test (FTA/ABS) and the Microhaemagglutination assay for *Treponema pallidum* (MHA-TP). These two tests can be used to confirm the diagnosis of syphilis.

The usefulness of laboratory tests in the diagnosis of syphilis depends on the selection of the appropriate standard tests.

Keywords: Syphilis, Microscopy, Non Treponemal test, Treponemal test, False positive.

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Syphilis is an infection with diverse clinical manifestations and the disease occurs in distinct stages. Establishing a diagnosis is difficult, thus all suspected cases should be referred for treatment and management by a venereologist.

The disease is caused by *Treponema pallidum*, which is a spiral organism. The diagnosis can be confirmed by the history of the patient, physical examination and one or all of the following laboratory tests: microscopy, serology and examination of cerebrospinal fluid. These tests can give a positive result depending on the clinical stage of the disease.

MICROSCOPY

Microscopic examination methods are the test of choice for the primary stage of syphilis. Darkfield direct microscopy examination of serous fluid is indicated when the patient has moist lesions. Darkfield microscopy was introduced as a diagnostic procedure in 1923, while direct fluorescent antibody test for *Treponema pallidum* was first described in 1964⁽¹⁾. Darkfield microscopy can not only be used to establish a diagnosis of primary lesions, it can occasionally be used to exclude the presence of treponemes from material obtained from puncture of

inguinal nodes. Darkground microscopy is unreliable for mouth lesions due to the presence of oral commensal treponemes.

The ideal specimen for darkfield examination is serous fluid and it should be free of red blood cells. The presence of red blood cells will mask the presence of treponemes.

Collection of a good specimen is critical if satisfactory results are to be obtained. The lesions should be cleaned with sterile normal saline and then by squeezing the lesion gently, the serum exudate that oozes out is transferred on to a microscope slide with the help of a sterile bacteriological loop. A cover slip is placed on the drop of the fluid and the specimen examined immediately under a darkground microscope. Internal vaginal and cervical lesions should be visualised using a speculum and the exudate collected for examination. A minimum of three samples from the lesions should be examined before an infection due to *Treponema pallidum* is excluded.

Specimens for darkfield microscopy should not be transported. This will result in the drying of the specimens. If there is a delay in examining the slides it should be stored in a moist chamber. Drying kills the organisms and the motility of the organism cannot be viewed. Considerable experience is required to recognise the characteristic morphology and movements of the treponema organism.

DIRECT FLUORESCENT ANTIBODY TEST

Specimens are collected by the same procedure as for darkground microscopy. In this instance the specimens and the slides are air dried fixed with acetone and stained with fluorescein labelled anti-T pallidum globulin. Paraffin

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embedded biopsy and autopsy material may be similarly stained and examined.

The serologic tests that are performed in the diagnosis of syphilis are either non specific or specific tests. The non specific tests are the non treponemal tests and the specific tests are the treponemal tests.

NON TREPONEMAL TEST

The most useful non specific test is the Venereal Research Laboratory test (VDRL). This test detects antibody (Reagin) in the serum. All non treponemal tests detect anti lipid IgG and IgM antibody formed by the host to the lipid on the treponemal cell surface as well as to lipoidal material released from the damaged host cells (2). It is a flocculation test in which the antigen antibody complexes remain suspended. This test is used as a screening as well as for monitoring the treatment of the patient.

The other common non treponemal test is the RPR card test (3). The sensitivity and the specificity of the VDRL and the RPR card tests are similar (4). The advantage of the RPR card test is that the reading of the results does not require a microscope, and it is read by the naked eye.

The VDRL tests are reported as either reactive or weakly reactive or non reactive, while the RPR test is reported as reactive or non reactive. Quantitative tests are reported as a reciprocal of the dilution, eg. 4 dil. Problems in non treponemal test performance can be avoided if the instructions for the test performance such as reagent control and quality control are carefully followed.

False positive non treponemal tests can occur in 1 to 2% of the population (5). The acute type of false positive reaction is transient and lasts a few weeks to 6 months. Such reactions occur after viral infections such as measles, mumps, Herpes simplex and after immunisation against typhoid fever and chicken pox while chronic false positive reactions occur in patients who have autoimmune diseases, haemolytic anaemia and Rheumatoid arthritis (6). The test may be positive even before the patient develops clinical features. In the above instances treponemal test for syphilis will be negative. As a rule 90% of the false positive titres are less than 8 dil.

TREPONEMAL TEST

The two specific tests that are used in our laboratory are the Fluorescent Treponemal Antibody Absorption test (FTA/ABS) and the Microhaemagglutination Assay for Treponema pallidum (MHA-TP). The treponemal tests use T pallidum as the antigen and hence detect antibodies to the sub species pallidum and therefore the tests are specific confirmatory tests for syphilis. The FTA-ABS test is the most specific and most sensitive treponemal test in primary syphilis. The FTA/ABS test will become positive before the MHA-TP test (4).

The FTA/ABS test is an indirect immunofluorescence test. The treponemal pallidum organisms are fixed to the slide, the serum is then placed on the antigen and finally the fluorescein labelled anti-human globulin is added. The presence of the antibody in the serum is indicated by fluorescence under a fluorescence microscope (4). The

test can detect both IgG as well as IgM. The laboratories that do not have fluorescent microscope may find the MHA-TP test simpler and less expensive to perform. This test is a qualitative haemagglutination test using tanned sheep red cells as a carrier of T pallidum antigen. In the presence of antibodies to T pallidum in patients sera, the cells haemagglutinate. Though the test is easier to perform than the FTA/ABS test, it is less sensitive in the diagnosis of primary syphilis (7). It is the last of the serological test to become positive. It is positive only in 60% of the patients presenting with primary syphilis while the FTA test is positive in 85-90% of cases of primary syphilis (8).

One of the newer treponemal tests is the enzyme-linked immunosorbent assay (ELISA) for syphilis. The metal beads are coated with the T pallidum antigen. The detection system consists of horseradish peroxidase conjugated antihuman IgG which is the second antibody. Studies have shown that the ELISA method is comparable to the FTA/ABS test and the MHA-TP test (8). Treponemal tests should not be used as a screening test. It should be noted that false positive results occur in 1% of the general population.

RATIONAL USE AND INTERPRETATION OF SEROLOGICAL TESTS

All blood samples from patients suspected to have syphilis should be sent for VDRL or RPR testing which are non treponemal test. A reactive result of the screening may indicate a past or a current infection due to treponemes. A reactive non treponemal test may also be a false positive. A negative non treponemal test may be either interpreted as the patient has been treated adequately or the patient has no infection. A fourfold increase in titre on paired sera taken 2 to 3 weeks apart indicates an infection, a reinfection or treatment failure. A decrease in the titre indicates adequate therapy.

Treponemal tests such as the FTA/ABS and the MHA-TP tests should be used only for confirmation of the non treponemal tests and also used to test patients suspected of having latent syphilis regardless of whatever status of the non treponemal test.

A reactive treponemal test usually indicates past or present infection with pathogenic treponemes. Once the treponemal test is reactive it remains so for life. However if treatment is begun early in primary syphilis about 10% of these patients will give a test result non reactive within 2 years after treatment (9). Generally a non reactive treponemal test indicates no past or present infection, however it may be non reactive if the patient is incubating the disease.

PRIMARY SYPHILIS

A direct microscopic examination should be performed from a specimen obtained from a lesion. The presence of treponemes indicates that the patient needs treatment. A reactive non treponemal test in patients with lesions indicates treatment. The non treponemal test could be confirmed with a FTA/ABS test or a MHA-TP test.

In primary syphilis the non treponemal test could be non reactive in 30% to 50% of the patients. In these instances the test should be repeated at intervals of 1 week, 1 month and 3 months. If the test is non reactive after three months this excludes the diagnosis of syphilis.

SECONDARY SYPHILIS

The non treponemal tests are always reactive in this stage and the titres are usually greater than 16. About 2% of these patients can give a prozone reaction i.e. the specimen gives a non reactive or a weakly reactive results on a undiluted specimen. However on dilution of the serum specimen the titre is usually greater than 16. All treponemal tests are also reactive in this stage.

LATENT SYPHILIS

Patients who have reactive non treponemal and treponemal tests in the absence of clinical symptoms are said to have latent syphilis. False positive non treponemal tests can occur in patients over 60 years but in these patient the treponemal will be negative.

Latent syphilis can be classified into early latent syphilis and late latent syphilis. A patient is categorised as having early latent syphilis if the serological tests are known to be non reactive in the past year, or if the patient had symptoms of syphilis during that time. Other patients are considered to have late latent syphilis and these patients should be investigated for neurosyphilis. Non treponemal tests may be non reactive in 20% of these cases ⁽¹⁰⁾.

NEUROSYPHILIS

The C.S.F. should be examined whenever the duration of syphilis is unknown or late syphilis is suspected. The tests to be performed are cell count, total protein and serological tests. A cell count of 5 white cells per cu mm of C.S.F. and at least 45 mg/dl of total protein are considered to be abnormal. A reactive C.S.F. VDRL test is also diagnostic. However VDRL test is unreliable in diagnosing neurosyphilis since it is negative in 50% of the patients with active neurosyphilis ⁽⁶⁾. A reactive FTA/ABS or a MHA-TP can result from a transudate of IgG antibody specific for *Treponema pallidum* in patients who have been adequately treated. Thus a reactive test result does not indicate an active disease of the nervous system ⁽⁶⁾. A negative test rules out neurosyphilis.

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PREGNANCY

Serological tests for syphilis should be performed at the beginning of prenatal care and at delivery. Intermediate testing should be performed at the beginning of the 3rd trimester (28 weeks) in high risk population. Expectant mothers should be treated if the non treponemal and treponemal tests are reactive and a false positive cannot be excluded ⁽¹¹⁾. In pregnancy non treponemal test titres may increase hence this should not be confused with a reinfection.

CONGENITAL SYPHILIS

Congenital syphilis is definite if *T pallidum* is demonstrated by direct examination of nasal discharge or skin lesions.

A four fold or greater rise in titre of non treponemal tests (VDRL or Rapid Plasma Reagent test) over a period of 6 months is diagnostic. This can be confirmed by one of the treponemal tests. A reactive treponemal or non treponemal test that does not revert to a non reactive result in 6 months is also diagnostic. By this time the maternal antibodies present in the baby should have disappeared.

The detection of IgM antibodies can contribute to the diagnosis of congenital syphilis. However in babies with congenital syphilis the IgM antibodies could be detected only after a few weeks to few months after the birth of the baby. If the mother is infected in late pregnancy the sera of the infected infant could be negative in all serological tests.

The usefulness of laboratory tests in the diagnosis of syphilis depends upon the selection of appropriate standard tests. It should be noted that quality of such test depends on the use of quality reagent and well trained technicians to perform the test. The laboratory should also participate regularly in quality assurance programme to ensure that the test results meet the standards.