Serological Diagnosis of Syphilis

ROBERT GIBB, B. APP. SC. M. APP. SC, MASM
SUPERVISING SCIENTIST
SEROLOGY
PRINCESS ALEXANDRA HOSPITAL

Syphilis is a serious systemic disease caused by the spirochaete, Treponema pallidum. Because the organism is unable to be cultured, diagnosis of syphilis is based on clinical findings, examination of lesion exudates and serologic tests for syphilis. Direct examination of exudates from primary lesions by dark field microscopy is not often possible and is rarely performed. Hence the primary method for laboratory diagnosis of syphilis is by serology.

Serologic tests have been divided into two groups, the non-specific non-treponemal tests and the specific treponemal tests.

The non-treponemal tests include the Venereal Diseases Research Laboratory Test (VDRL) and the Rapid Plasma Reagin card circle test (RPR). These use a cardiolipin-lecithin-cholesterol antigen to detect reagin antibodies in serum or CSF. These antibodies are directed against lipids in the cell wall of the treponeme and the products of inflammation following infection of tissues with the organism.

The VDRL and RPR tests are used as a screening test for syphilis as well as quantitative test for monitoring the course of infection and response to therapy. The VDRL requires microscopic examination to detect the agglutination and is rarely performed. The RPR uses carbon particles to enhance the agglutination pattern and allow visual examination of the assay. The RPR titre is usually greater than 1:8 in primary or secondary syphilis, and somewhat lower in latent syphilis. The titre will fall to negative or a very low titre within 12 months after successful therapy, provided treatment is commenced soon after infection. Delayed commencement of treatment does not produce the same significant fall in RPR titre. The rate at which the titre falls is dependant on the stage of the infection, the initial titre and the patient's history of previous syphilis. When interpreting results, it is important to recognise that a titre variation of only one doubling dilution is inherent in the test and is not significant.

These tests are not specific and biological false positive (BFP) reactions do occur in 1-2% of the population. BFP's occur in a variety of conditions including autoimmune diseases, intravenous drug users and during pregnancy. All reactive non-treponemal tests must be confirmed by a specific treponemal test.

The specific treponemal tests include the Treponema pallidum haemagglutination test (TPHA), the fluorescent treponemal antibody absorption test (FTA-ABS) and also several ELISA tests. These tests use the specific antigens of the treponeme to detect only antibodies against T. pallidum. These specific treponemal tests cannot be used for monitoring therapy or reinfection because they generally remain reactive for life, even after successful treatment. In some cases of early primary syphilis, successful treatment will produce seroreversion of the treponemal tests to non-reactive within 3 years. Also some HIV positive patients will have non-reactive treponemal tests following successful therapy.

Most laboratories perform both the RPR and the TPHA tests for routine screening. The use of the RPR alone has been used in the past with specific serology only used to confirm reactive results. However, the presence of the prozone phenomenon where highly reactive sera may give no agglutination due to excess antibody may give a false non-reactive result. For this reason, the TPHA is the preferred single screening test where the RPR can be performed on reactive samples to aid in the diagnosis.

The first test to become reactive is the FTA-ABS, usually 3-5 weeks after initial contact. The TPHA and VDRL/RPR follow one or two weeks later. Although the FTA-ABS is rarely performed as a screening test, it is recommended in cases of suspected early primary syphilis. Alternatively, the screening tests should be repeated 7 days later if initially non-reactive.

The diagnosis of syphilis is complex and the picture varies markedly according to the stage of the disease. In late primary and secondary syphilis, both non-treponemal and treponemal tests are almost invariably reactive. The VDRL/RPR titre is usually greater than 8 and declines naturally as the disease progresses to the latent stage and may even become non-reactive. However, in early primary syphilis, the VDRL/RPR may be non-reactive whilst the specific treponemal tests, particularly the FTA-ABS may already be reactive. This same profile is also seen in successfully treated syphilis and in latent disease. This demonstrates the importance of considering the clinical findings when making a diagnosis.

Similarly, a low titre of the non-treponemal tests following treatment may reflect either inadequate treatment persistent antibody levels following successful therapy which was not commenced in the early stages of disease. Treatment commenced in the latent stage, or in patients with a previous history of syphilis may never produce a non-reactive RPR/VDRL.

For diagnosis of congenital syphilis in new born babies is complicated by maternal antibodies which cross the placenta giving reactive serology. Diagnosis can be made by demonstration of FTA-ABS IgM antibodies which do not cross the placenta, although a negative result does not exclude syphilis. Alternatively, a decrease in the baby's VDRL/RPR titre over several months is required to rule out congenital syphilis.

Neurosyphilis is a result of invasion of the central nervous system by T. pallidum. The VDRL is the only test recommended for

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examination of cerebrospinal fluid. Since these antibodies do not cross from the blood into the CSF, a reactive CSF VDRL is diagnostic of neurosyphilis. However, the VDRL is non-reactive in up to 50% of patients with neurosyphilis and other laboratory findings such as raised cell count and protein levels need to be used.

Using the current serologic tests, syphilis cannot be diagnosed on laboratory findings alone and careful examination of the patient's clinical condition is required. More sensitive and specific methods are required to confirm the diagnosis of syphilis and monitor therapy. Future developments such as the polymerase chain reaction (PCR) and the Western Blot assay may be able to provide the confidence to accurately diagnose syphilis and assess response to therapy.

Varicella Zoster Virus

The Virus

Varicella-Zoster Virus (VZV), the causative agent of both chicken-pox (varicella) and shingles (zoster, or herpes zoster), is a member of the family Herpesviridae, which also includes the herpes simplex viruses, cytomegalovirus, and the Epstein-Barr (infectious mononucleosis) virus.

These viruses are enveloped viruses, consisting of an icosahedral nucleocapsid surrounding double-stranded DNA. The envelope consists of two layers, and also has glycoprotein spikes. The DNA codes for approximately 75 proteins.

VZV is similar to most other enveloped viruses, in being particularly susceptible to disinfection and drying.

Herpesviruses have a diameter of approximately 180 to 200nm.

Laboratory Diagnosis

VZV was first cultured in the laboratory in 1952, and it was subsequently proven that Varicella and Zoster were identical immunologically, morphologically, and genetically. Cells such as Human diploid fibroblasts will support the growth of VZV, and the virus produces characteristic cytopathic effects, such as formation of 'giant cells', intranuclear inclusion bodies and micronuclei.

Fortunately we now have quicker and easier methods for diagnosing VZV infection. Stained direct smears (Tzanck smear) from the base of vesicles may show the characteristic multinucleated giant cells. More often, however, viral antigen can be demonstrated in smears by using direct fluorescent antibody (DFA) staining. In addition, anti-VZV antibody can be detected in serum, and a rise in antibody titer between acute and convalescent serum samples can confirm the diagnosis.

Carolyn Wills
Infection Control Scientist
Princess Alexandra Hospital
Brisbane

Chickenpox (Varicella)

Chickenpox is a herpes virus.

At risk:	Immunosuppressed patients, e.g. patients with leukaemia or lymphoma etc and non-immune pregnant health care workers.
Mode of transmission:	Predominantly respiratory e.g. droplet. Contact with vesicle fluid.
Infectious period:	From day 10 to 21 post exposure. Day 10 is determined by a calculation of 3 days before contact with the infected person and seven days after contact.
Diagnosis:	Using a Herpes DFA kit swab fluid from the vesicle according to instructions.
Management:	Contact tracing will be performed to ensure staff that have had any contact with the infected person will be investigated regarding immune status. Immune status will be obtained by questioning, for definite past medical history of chickenpox, or obtaining a blood sample for VZV IgG if immune status uncertain. If staff are not immune they will be relieved from patient care duties from day 10 through to day 21. If staff contract chickenpox they must be placed on sick leave until 5 to 7 days after the last vesicle has appeared or until all the vesicles have dried and crusted.
Treatment:	Conservative management in most cases. Acyclovir (Zovirax) or VZV immunoglobulin may be prescribed in specific circumstances.

Infection Control Team
Princess Alexandra Hospital
Brisbane