Lecture 8 **Dr. Maytham Ahmed**

Serological tests: VDRL, ASO-Titer

Serology is the branch of laboratory medicine that studies blood serum for evidence of infection and other parameters by evaluating antigen-antibody reactions in vitro.

Serological tests are blood tests that look for antibodies in the blood (antigen–antibody reaction).They can involve a number of laboratory techniques. Different types of serologic tests are used to diagnose various disease conditions.

The Venereal Disease Research Laboratory test

Syphilis is a systemic, infectious disease caused by spirochete Treponema pallidum (T. pallidum). The organism is transmitted primarily through direct sexual contact. It can also be transmitted via the placenta from mother to fetus. If untreated, infected individuals can develop irreversible complications such as chronic inflammation of the joints, cardiovascular problems such as valvular involvement, and central nervous system problems such as mental illness and paralysis.

Laboratory diagnosis of syphilis can be made through direct and indirect tests. Direct tests, such as scraping of syphilis lesions, identify the causative organism. Indirect tests, such as the syphilis serologic tests, identify antibodies of the causative agent. These antibodies do not appear in the serum until 3 to 4 weeks after the appearance of syphilis chancre, an ulcer located at the site where the organism initially enters the body.

The syphilis serology includes the venereal disease research laboratory test (VDRL), rapid plasma regain (RPR), and fluorescent treponemal antibody absorption (FTA-ABS) tests. The VDRL and the RPR test are screening tests. In both of these tests, agglutination occurs in the presence of the syphilis antigen. Both these tests have a high false-positive rate. Conditions such as infectious mononucleosis, rheumatoid arthritis, and malaria can cause false-positive reactions. Because of the high possibility of a false- positive result, any positive, or reactive VDRL or RPR test must be followed with a confirmatory test, such as the FTA-ABS. This test identifies the antibodies that are specific against T. pallidum. The FTA-ABS test is the most sensitive test used to diagnose syphilis

following a positive VDRL or RPR. The FTA-ABS test will remain positive for life even if an individual has received appropriate treatment.

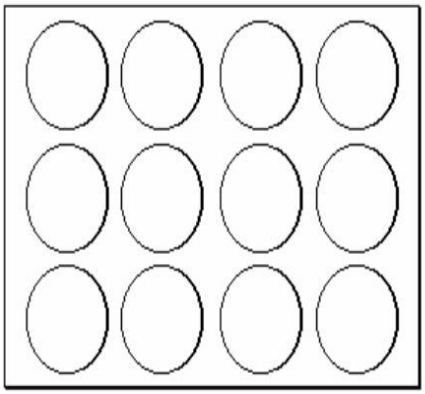
The basis of the VDRL test is that an antibody produced by a patient with syphilis reacts with an extract of ox heart (diphosphatidyl glycerol). It therefore detects anti-cardiolipin antibodies (IgG, IgM or IgA), visualized through foaming of the test tube fluid, or "flocculation". This test is very useful as the trend of titres are correlated to disease activity (i.e. falling titres indicate successful treatment). It has a very good sensitivity for syphilis, except in late tertiary form.

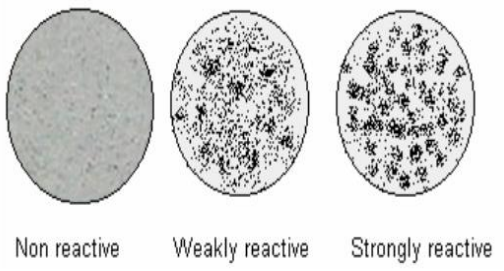
The rapid plasma reagin (RPR) test uses the same antigen as the VDRL, but in that test, it has been bound to several other molecules, including a carbon particle to allow visualization of the flocculation reaction without the need of a microscope.

Principle: Patients suffering from syphilis produce antibodies that react with cardiolipin antigen in a slide flocculation test, which are read using a microscope. It is not known if the antibodies that react with cardiolipin are produced against some lipid component of Treponema pallidum or as a result of tissue injury following infection.

Requirements: Patient's serum, water bath, freshly prepared cardiolipin antigen, VDRL slide, mechanical rotator, pipettes, hypodermic syringe with unbeveled needle and microscope. Known reactive and non-reactive serum controls are also required.

Procedure: Patients' serum is inactivated by heating at 56 ^{o}C for 30 minutes in a water bath to remove non-specific inhibitors (such as complement). The test can be performed both qualitatively and quantitatively. Those tests that are reactive by qualitative test are subjected to quantitative test to determine the antibody titres.





Normal values

Negative (Nonreactive). Negative test is normal. It means that no antibodies to syphilis have been seen in your blood sample. The screening test is most likely to be positive in the secondary and latent stages of syphilis. This test may give a false-negative result during early- and late-stage syphilis.

Possible meaning of abnormal values

Positive **(**Syphilis). The VDRL blood test is not always accurate. Infections, such as HIV or pneumonia, as well as other autoimmune disorders, can trigger a false-positive result. If the result is positive, a doctor will perform another test, such as the fluorescent treponemal absorption assay.

Contributing factors to abnormal values

1- Hemolysis of the blood sample, presence of lipemia, and the intake of alcohol may alter test results.

2- Conditions that can cause false - positive VDRL or RPR results: atypical pneumonia, brucellosis, HIV, infectious hepatitis, leprosy, Lyme disease, malaria, mononucleosis, pinta, pregnancy, systemic lupus erythematosus, typhus, yaws.

3- The presence of antinuclear antibodies (ANA) can cause false - positive RPR results.

4- Conditions that can cause false - positive FTA-ABS results: diseases with increased or abnormal globins, patients with systemic lupus erythematosus, positive ANA, yaws, pinta, and pregnancy.

Interventions / implications

Pretest

- Explain to the patient the purpose of the test and the need for a blood sample to be drawn. Only serum (plasma cannot be used) or cerebrospinal fluid (CSF).

- No fasting is required before the test.

- No alcohol is allowed for 24 hours before the test.

Clinical alerts

1- Monitoring of RPR is helpful in assessing effectiveness of therapy.

2- The VDRL test is most sensitive to detect syphilis during the middle stages; it is less sensitive during the earlier and later stages. Sensitivity of the VDRL and RPR tests are estimated to be 78-86% for detecting primary syphilis, 100% for detecting secondary syphilis, and 95-98% for detecting tertiary syphilis.

3- The VDRL turn negative after treatment. The VDRL test titers should decline at least 4-fold within 3–6 months after therapy for primary or secondary syphilis, and within 12–24 months for latent syphilis.

4- Positive screening test results require follow-up confirmatory testing. A treponemal test is often used to confirm the positive test. Treponemal tests check whether your immune system has produced specific antibodies in direct response to the syphilis-causing Treponema pallidum.

5- Positive confirmatory test results should be followed with appropriate antibiotic therapy and education of the patient.

6- A positive FTA-ABS will remain positive for life even if an individual has received appropriate treatment.

Note: After successful treatment, a positive nontreponemal test usually becomes negative, whereas the treponemal test usually remains positive for life.

Anti-streptolysin O

- Anti-streptolysin O (ASO or ASLO) is the antibody made against streptolysin O, an immunogenic, oxygen labile streptococcal hemolytic exotoxin produced by most strains of group A and many strains of groups C and G streptococcus bacteria.

- The "O" in the name stands for oxygen-labile; the other related toxin being oxygen-stable streptolysin-S. The main function of streptolysin O is to cause hemolysis (the breaking open of red blood cells) — in particular, beta-hemolysis.

- Increased levels of ASO titer in the blood could cause damage to the heart and joints. In most cases, penicillin is used to treat patients with increased levels of ASO titer.

- The ASO titer helps direct antimicrobial treatment and is used to assist in the diagnosis of scarlet fever, rheumatic fever, and post infectious glomerulonephritis.

- A positive test usually is >200 units/mL, but normal ranges vary from laboratory to laboratory and by age.

- The false negatives rate is 20-30%. If a false negative is suspected, then an anti-DNase B titer should be sought.

- False positives can result from liver disease and tuberculosis.

Clinical significance

- When the body is infected with streptococci, it produces antibodies against the various antigens that the streptococci produce, ASO is one such antibody. A raised or rising levels can indicate past or present infection. Historically it was one of the first bacterial markers used for diagnosis and follow up of rheumatic fever or scarlet fever . Its importance in this regard has not diminished.

- Since these antibodies are produced as a delayed antibody reaction to the above-mentioned bacteria, there is no normal value. The presence of these antibodies indicates an exposure to these bacteria. However, as many people are exposed to these bacteria and remain asymptomatic, the mere presence of ASO does not indicate disease.

- Acceptable values, where there is no clinical suspicion of rheumatism are as follows:

-Adults: less than 200 units

-Children: less than 400 units

- This titer has a significance only if it is greatly elevated (>200), or if a rise in titer can be demonstrated in paired blood samples taken days apart. The antibody levels begin to rise after 1 to 3 weeks of streptococcal infection, peaks in 3 to 5 weeks and falls back to insignificant levels in 6 months. Values need to be correlated with a clinical diagnosis.

Sample Collection and Handling

Only fresh serum specimens should be used. Plasma must not be used since fibrinogen may cause non-specific agglutination of the latex. It is preferable to test samples on the same day as collected. Serum samples may be stored at 2-8 ºC for up to 48 hours prior to testing. If longer storage is necessary, sera should be stored frozen at -20ºC.

Estimation

- It is done by serological methods like latex agglutination or slide agglutination. ELISA may be performed to detect the exact titer value.

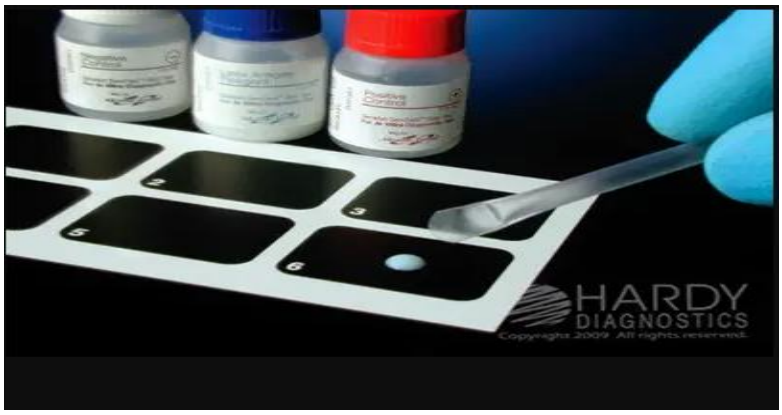
- To detect the titer value, by a non-ELISA method, one has to perform the above agglutination using a serial dilution technique.

Mechanism of action

The antibodies produced against the bacteria crossreact with human antigens (mainly collagen) and hence attack the cellular matrix of various organs, mainly the heart, joints, skin, brain, etc.

ASO Latex test

- When the latex reagent is mixed with a serum containing ASO, agglutination occurs. The sensitivity of the latex reagent has been adjusted to yield agglutination when the level of ASO is greater than 200 IU/ml, a level determined to be indicative of disease by epidemiological and clinical studies.



Materials used in the ASO Test

1-ASO Antigen: A stabilized buffered suspension of polystyrene latex particles coated with Streptolysin O and 0.1% sodium azide as preservative. Shake well prior to use.
2-ASO Positive Control: Human serum containing more than 200 IU/ml ASO and 0.1% sodium azide as preservative.
3-ASO Negative Control: Human serum containing 0.1% sodium azide as preservative.
4-Sufficient disposable pipettes and glass test slide.

Procedure for Antistreptolysin O Test

1-Bring all test reagents and samples to room temperature.

2-Use a disposable pipette to draw up and place one free-falling drop of each undiluted sample into its identified circle of the slide. Retain each pipette for mixing in step 5.

3-Deliver one free-falling drop of positive and negative control into its identified circle.

4-Mix the ASO latex reagent by gently shaking. Add one free-falling drop of reagent to each control and sample.

5-Using the flattened end of the appropriate plastic pipette as a stirrer (step 2), thoroughly mix each sample with reagent within the full area of the circle.

6-Discard the disposable pipette.

7-Slowly rock the slide for exactly two (2) minutes and observe for agglutination under a high intensity light.

8-Record results.

9-Re-wash glass slide for future use

Test Result: A test sample is considered to contain ASO antibodies in excess of 200 IU/ml when agglutination (clumping) is observed when compared to the result of the negative control.