



The VDRL Test

Principle, Procedure and Interpretation.

Dr. Mohanad Khalid Ahmed

VDRL Test

Introduction of the (VDRL)

Syphilis is sexually transmitted (venereal) disease caused by the bacterium *Treponema pallidum*. As the organism cannot be cultured in artificial media, the diagnosis of syphilis depends upon the correlation of clinical data either with the demonstration of microorganism in the lesion or serological testing. Serological procedures for syphilis include the following:

- Treponemal tests: detect the antibodies to *Treponema pallidum*. eg. Fluorescent *Treponema pallidum* antibody absorption (FTA-ABS) and microhemagglutination *Treponema pallidum* (MHA-TP).
- Non-treponemal tests: detect the antibodies produced in response to lipoidal material released from the damaged host cell. These antibodies are traditionally referred to as 'REAGINS'. eg; Venereal Disease research laboratory (VDRL)

The Venereal disease research laboratory (VDRL) test is a non-treponemal microflocculation test, which is used for screening of syphilis. It detects the IgM and IgG antibodies to lipoidal material released from the damaged host cells, as well as to lipoprotein-like material and possibly cardiolipin released from the treponemes.

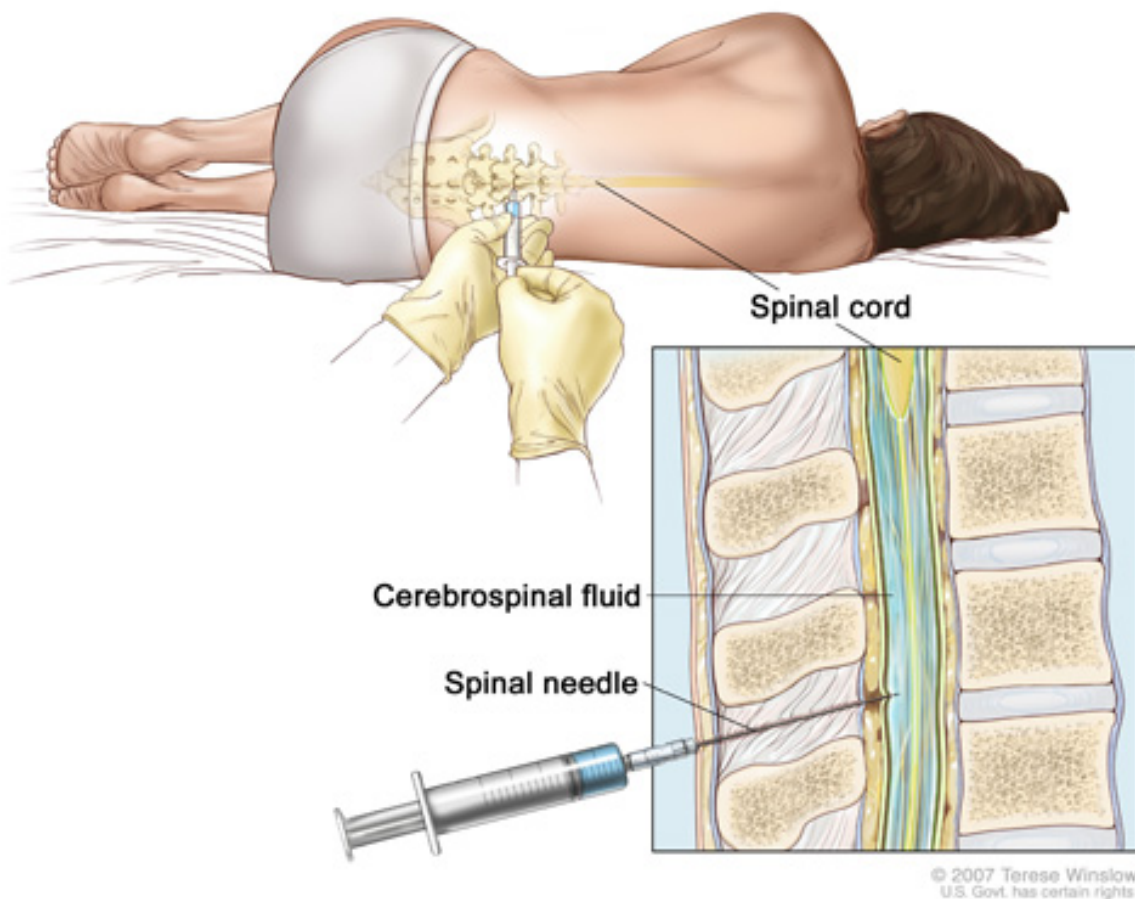
Principle:

The presence of lipoidal antibodies in patient's serum or cerebrospinal fluid (CSF) is detected by using non-specific antigen, suspended in buffered saline solution. The cardiolipin antigen is an alcoholic solution composed of 0.03% cardiolipin, 0.21% lecithin and 0.9% cholesterol.

The heat inactivated (to destroy complement) serum or CSF is mixed with VDRL antigen. If the specimen contains Reagan, flocculation occurs which can be observed using microscope. Non-reactive specimens appear as homogeneous suspension.

Sample:

Only Serum (plasma can not be used) or Cerebrospinal fluid (CSF). Acceptable Serum should be heated to 56°C for 30 minutes to destroy or remove non-specific inhibitors, while no heat treatment is required for CSF. 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.



Procedure

1. Bring the VDRL antigen suspension, controls and samples to room temperature.
2. Pipette one drop (50 μ l) of the test specimen, positive and negative controls onto separate reaction circles of the disposable slide.
3. Add one drop of well-mixed VDRL antigen next to the test specimen, positive control and negative control.
4. Using a mixing stick mix the test specimen and the VDRL reagent thoroughly spreading uniformly over the entire reaction circle.
5. Rotate the slide gently and continuously either manually or on a mechanical rotor at 180 r.p.m.
6. Observe for flocculation microscopically at 8 minutes.



Reactive



Weak Reactive



Weak Reactive
(Minimally)



Nonreactive

Result;

- Large aggregates in the centre or periphery of the circle indicate strongly or moderately reactive sera.
- Negatives display a macroscopically smooth and even appearance.

SEMI-QUANTITATIVE METHOD

1. Using isotonic saline prepare serial dilutions of the test sample positive in the qualitative method 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and so on as follows:
 1. For each specimen to be tested, add 100 μ L of 0.9% saline into test tubes numbered 1 to 5.
 2. Add 100 μ L of specimen onto test tube 1.
 3. Mix the mixture. Avoid formation of bubbles.
 4. Transfer 100 μ L of mixed sample from tube 1 to 2.
 5. Repeat this serial dilution procedure in tube 3 to 4, and then 5. Dispose 100 μ L from test tube 5 after mixing.
 6. Tubes 1 to 5 now represent a dilution series as follows:

Tube Number	1	2	3	4	5
Dilution	1:2	1:4	1:8	1:16	1:32

2. Perform the qualitative test procedure using each dilution as test specimen.

Interpretation

The titer is reported as the alternate of the highest dilution, which shows a positive test result.

Limiation of the Test

A. False positive VDRL test result

1. Reagin antibodies may be produced in response to nontreponemal diseases of an acute and chronic nature in which tissue damage occurs such as:
 - Leprosy

- Hepatitis B
 - Infectious Mononucleosis
 - Various autoimmune Diseases
2. VDRL may be reactive in persons from areas where yaws is endemic. As a rule, residual titers from these infections will be $<1:8$.
 3. Nontreponemal test titers of persons treated in late stages of syphilis or who have become re-infected do not decrease as rapidly as do those from persons in the early stages of their first infection. In fact, these persons may remain “serofast,” retaining a low -level reactive titer for life.

B. False negative VDRL test

It can be seen because of prozone phenomenon (no flocculation due to antibody excess). In that case test serum has to be diluted further to obtain zone of equivalence (where maximum flocculation of Ag-Ab occurs).

CSF VDRL:

VDRL test may also be performed on CSF samples in the diagnosis of Neurosyphilis. Quantitative VDRL is the test of choice on CSF specimens. However, there are some variations in this test. The antigen is diluted in equal volumes with 10% saline, CSF must not be heated (or inactivated), the volume of antigen solution taken is 0.01 ml (or 1 drop from 21 gauge needle) and rotation time is 8 minutes. Rest of the procedure remains same.

Watch the test in youtube;

<https://www.youtube.com/watch?v=cFRk6CoupDs>