VDRL TEST

Venereal Disease Research Laboratory (VDRL) Test is a slide flocculation test employed in the diagnosis of syphilis. Since the antigen used in this test is cardiolipin, which is a lipoidal extracted from beef heart, it is not a specific test. This test is also classified as non-specific or non-treponemal or standard test. The antibodies reacting with cardiolipin antibodies have been traditionally (but incorrectly) termed “regain”.

**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.

**VDRL Antigen:** The cardiolipin antigen is an alcoholic solution composed of 0.03% cardiolipin, 0.21% lecithin and 0.9% cholesterol. The cardiolipin antigen must be freshly constituted each day of test. The working antigen is a buffered saline suspension of cardiolipin.

**VDRL Slide:** This is a glass slide measuring 2 x 3 inch with 12 concave depressions, each measuring 16 mm in diameter and 1.75 mm deep.

**Procedure:** Patients’ serum is inactivated by heating at 56°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.

**Qualitative test:** 0.05 ml of inactivated serum is taken into one well. 1/60th ml (or 1 drop from 18 gauge needle) of the cardiolipin antigen is then added with the help of a syringe (unbeveled) to the well and rotated at 180 rpm for 4 minutes. Every test must be accompanied with known reactive and non-reactive controls. The slide is then viewed under low power objective of a microscope for flocculation. The reactive and non-reactive controls are looked first to verify the quality of the antigen. Depending on the size the results are graded as weakly reactive (W) or reactive (R). Reactive samples are then subjected to quantitative test.

**Qualitative test:** this is performed to determine the antibody titres. The serum is doubly diluted in saline from 1 in 2 to 1:256 or more. 0.05 ml of each dilution is taken in the well and 1/60 ml of antigen is added to each dilution and rotated in a rotator. The results are then checked under the microscope. The highest dilution showing flocculation is considered as reactive titre. Sometimes, due to very high level of antibodies in the serum (prozone phenomenon) the qualitative test may be non-reactive. If the clinical findings are strongly suggestive of syphilis, a quantitative test may be directly performed on the serum specimen.
### 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.

### Significance of VDRL test:
VDRL test becomes positive 1-2 weeks after appearance of (primary lesion) chancre. The test becomes reactive (50-75%) in the late phase of primary syphilis, becomes highly reactive (100%) in the secondary syphilis and reactivity decreases (75%) thereafter. Treatment in the early stages of infection may completely suppress production of antibodies and result in non-reactive tests. Effective treatment in the primary or secondary stages results in rapid fall in titre and the test may turn non-reactive in few months. Treatment in latent or late syphilis has very little effect on the titre and the titres may persist at low levels for long periods. Since the titre falls with effective treatment, it can be used for assessment of prognosis. VDRL test is more suitable as a screening agent than a diagnostic tool.

VDRL test is also helpful in the diagnosis of congenital syphilis. Since passively transferred antibodies through placenta may give false reactive test in serum of the infant, a repeat test after a month showing no increase in titre may help rule out congenital syphilis.

Since the test employs a non-treponemal antigen, there are many chances of false positive results. False positivity (other than technical) may be due to physiological of pathological conditions. These are called biological false positives (BFP). If the remain positive for less than 6 months it is considered acute and they remain positive for longer than 6 months it is called chronic BFP. The physiological reasons for BFP include pregnancy, menstruation, repeated blood loss, vaccination, severe trauma etc while the reasons for pathological BFP include malaria, infectious mononucleosis, hepatitis, relapsing fever, tropical eosinophilia, lepromatous leprosy, SLE, rheumatoid arthritis etc.

A reactive VDRL test does not necessarily imply that the person is syphilitic. The diagnosis must be made in conjunction with clinical findings. Any reactive VDRL test must be confirmed with a specific or treponemal test such as TPHA, FTA-ABS test.

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