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VDRL Antigen

REF

Catalog No.S15-711

IVD

INTENDED USE

The Immunospec's VDRL Antigen is for both qualitative and quantitative slide flocculation tests for the detection of Treponema pallidum antibodies.

SUTIMARY AND EXPLANATION

Several serological tests are presently used for detecting antibody in serum and spinal fluid to the etiological agent for syphilis, Treponema pallidum. Most of these tests fall into two categories, nontreponemal and treponemal'. Nontreponemal tests include precipitation, flocculation and complement fixation methods which utilize antigens derived from animal tissue extracts (e.9.cardiolipin, lecithin, cholesterol) in alcoholic solution.Treponemal tests include agglutination, fluorescent antibody, treponema immobilization, methods and complement fixation methods which utilize antigens extracted from virulent strains of treponema pallidum.

PRINCIPLES OF THE PROCEDURE

The principle of the test is an immunologic reaction between Treponema pallidum antibodies and and a cardiolipin, lecithin, cholesterol antigen (VDRL Antigen) in an alcoholic solution. The antibody detected is called"Reagin".

REAGENTS

Immunospec's VDRL Antigen is a colorless, alcoholic solution containing 0.03% cardiolipin, 0.9%cholesterol, and sufficient purified lecithin to produce standard reactivity.

Immunospec's VDRL Buffered Saline Diluent, pH 6.0± 0.1, has the following composition:

Formaldehyde, neutral.....	0.Sml
Secondary Sodium Phosphate anhydrous, (Na2HPO4.).....	0.037 grams
Primary Potassium Phosphate, (KH2PO4)	0.170 grams
Sodium Chloride.....	10.0grams
Distilled Water	1000.0ml

Both antigen and buffer have a shelf life of 5 years.

WARNING

For in vitro diagnostic use.

STORAGE CONDITIONS

Store at room temperature.

STABILITY

When an unexplained change in test reactivity occurs, check the pH of the VDRL Buffered Saline Diluent to determine if this is a contributing factor. Saline outside the pH 6.0 ± 0.1 should be discarded.

PROCEDURE

Materials provided:

1. VDRL Antigen
2. VDRL Buffered Saline Diluent
3. VDRL Control Sera

Materials required but not provided:

1. Rotating machine (adjustable to 180 rpm, circumscribing in a circle $1/4$ " in diameter on a horizontal plane).
2. Hypodermic needles with bevels
 - a. 18, 19 and 23 gage (for serum test)
 - b. 21 and 22 gage (for spinal fluid test)
3. Syringe, Luer-type, 1 or 2 ml
4. Bottles, 30 ml, round, glass-stoppered, narrow mouth, approximately 35 mm in diameter with flat innerbottom surfaces.
5. Serological microscope glass slide.

Note: Some of the bottles now available are unsatisfactory for preparing antigen suspension because the convex innerbottom surface causes the saline to be distributed only at the periphery.

METHOD

VDRL Slide Test On Serum

Preparation of antigen suspension:

Temperature of buffered saline and antigen should be in the range of 73° - 85° F (23° - 29° C) at time antigen suspension is prepared.

1. Pipet 0.4 ml of buffered saline to the bottom of a 30 ml round, glass-stoppered bottle.
2. Add 0.5 ml of antigen (from the lower half of a 1.0 ml pipet graduated to the tip) directly onto the saline while continuously but gently rotating the bottle on a flat surface.

Note: Antigen is added drop by drop, but rapidly, so that approximately 6 seconds are allowed for each 0.5ml of antigen. Pipet tip should remain in upper third of bottle and rotation should not be vigorous enough to splash saline onto pipet. Proper speed on

rotation is obtained when the center of the bottle circumscribes a 2" diameter circle approximately 3 times per second.

3. Blow last drop of antigen f from pipet without touching pipet to saline.
4. Continue rotation of bottle for 10 seconds.
5. Add 4.1ml of buffered saline from 5 ml pipet.
6. Place top on bottle and shake from bottom to top and back approximately 30 times in 10 seconds.
7. Antigen suspension is ready for use and may be used during one day.
8. A double volume of antigen suspension may be prepared at one time by using double quantities of antigen and saline. A 10 ml pipet should be used for delivering the 8.2ml volume of saline. If larger quantities are required, more than one antigen suspension should be prepared. Test the suspensions with control sera and pool the ones with satisfactory reactivity.
9. Mix antigen suspension gently each time it is used. Do not mix the suspension by forcing back and forth through the syringe and needle since this may cause breakdown of particles and loss of reactivity.

TESTING ACCURACY OF DELIVERY NEEDLES

1. It is of primary importance that the proper amounts of reagents be used, and for this reason, the needles used each day should be checked. Practice will allow rapid delivery of antigen suspension and saline, but care should be exercised to obtain drops of uniform size.
2. For the slide qualitative test on serum, dispense antigen suspension from a syringe fitted with an 18-gage needle without bevel which will deliver 60 drops ± 2 drops of antigen suspension per millimeter when the syringe and needle are held vertically.
3. For the slide quantitative test on serum, dispense antigen suspension from a syringe fitted with a 19-gage needle without bevel which will deliver 75 drops ± 2 drops of antigen suspension per millimeter when the syringe and needle are held vertically.
4. For the slide quantitative test on serum, dispense 0.9 percent saline from a syringe fitted with a 23-gage needle (with or without bevel) which will deliver 100 drops ± 2 drops of saline per millimeter when the syringe and needle are held vertically.

PRELIMINARY TESTING OF ANTIGEN SUSPENSION

1. Test control sera of graded reactivity.
 2. Reaction of control sera should reproduce the established reactivity pattern. Non reactive serum should show complete dispersion of antigen particles.
 3. Do not use an unsatisfactory antigen suspension or pool of antigen suspension.
- Note: Control sera of graded reactivity (Reactive, Weakly Reactive and Nonreactive) should always be included during a testing period to insure proper reactivity of antigen suspension at time tests are performed.

PREPARATION OF SERUM

1. Heat clear serum, obtained from centrifuged, clotted blood, in 56° C water bath for 30 minutes before testing.
2. Examine all sera when removed from the water bath and recentrifuge those found to contain particulate debris.

3. Reheat at 56° C for 10 minutes those sera to be tested more than four hours after the original heating period.
4. When tested, the sera must be at room temperature.

INTERFERING SUBSTANCES

Sera that contain particulate matter should not be used.

VDRL SLIDE QUALITATIVE TEST ON SERUM

Note: Slide flocculation test for syphilis are affected by room temperature. For reliable and reproducible results, test should be performed within the temperature range 73°-85° F (23°-29° C). At lower temperatures, test reactivity is decreased; at higher temperatures, test reactivity is increased.

1. Pipet 0.05 ml of heated serum into one ring of a paraffin-ringed or a ceramic-ringed slide. Glass slides with concavities, wells, or glass rings are not recommended for this test.
2. Add one drop (1/60ml) of antigen suspension onto each serum with an 18-gage needle and a syringe.
3. Rotate slide for 4 minutes (mechanical a rotator that circumscribes a 3/4" diameter circle should be set at 180 rpm).

Note: When tests are performed in a dry climate, slides may be covered with a box lid containing a moistened blotter during rotation to prevent excessive evaporation.

4. Read tests microscopically with a 10X ocular and a 10X objective immediately after rotation.
5. Report the results as follows:

Reading

Medium and large clumpsReactive(R)
 Small clumps..... Weakly Reactive (W)
 No clumping or very slight roughness Nonreactive (N)

VDRL SLIDE QUANTITATIVE TEST ON SERUM

Retest quantitatively, to an endpoint titer, all sera that produce Reactive, Weakly Reactive, or "rough" Nonreactive results in the qualitative VDRL Slide Test. The dilutions of the serum to be tested are: undiluted (1:1), 1:2, 1:4, 1:8, 1:16, and 1:32. Two quantitative tests may be performed on one slide.

1. Place tubes of serum for quantitation in the front row of rack with a tube containing 0.7 ml of 0.9 percent saline directly behind each serum.
2. Prepare a 1:8 dilution of each serum by adding 0.1 ml of the serum to 0.7 ml of the 0.9 percent saline by using a 0.2ml pipet graduated in 0.01ml subdivisions.
3. Mix thoroughly and allow the pipet to stand in the dilution tube until all dilutions are prepared.
4. Using this pipet, transfer 0.04 ml, 0.02 ml, and 0.01 ml of the 1:8 serum dilution into the fourth, fifth and sixth paraffin rings, respectively.
5. Blow out remaining serum dilution into the dilution tube.

6. With the same pipet, transfer 0.04 ml, 0.02ml, and 0.01 ml of the undiluted serum into the first, second, and third paraffin rings, respectively.
7. Add two drops (0.01ml/drop)of 0.9 percent saline to the second and fifth rings of each serum with a 23-gage needle and a syringe.
8. Add three drops (0.01mldrop) of 0.9 percent saline to the third and sixth rings of each serum with a 23-gage needle and a syringe.
9. Rotate slides gently by hand for about 15 seconds to mix the serum and saline.
10. Add one drop (1/75 ml) of antigen suspension to each ring with a 19-gage needle and syringe.

Note: The amount of antigen suspension used in this method has been reduced to 1/75ml to correspond with the reduced serum volume of 0.04ml.

11. Complete tests in the manner described for the "VDRL SLIDE QUALITATIVE TEST ON SERUM" and read results microscopically immediately after rotation.

12. Report results in terms of the greatest serum dilution that produces a Reactive (NOT Weakly Reactive) result.

13. If all serum dilutions tested produce Reactive results, prepare a 1:64 dilution of the serum in saline by adding 0.1 ml of the 1:8 serum dilution of 0.7 ml of saline. Mix, and test this 1:64 dilution in three amounts as was done for the 1:8 serum dilution. This will be equivalent to 1:64, 1:128 and 1:256 dilutions.

VDRL SLIDE TEST ON SPINAL FLUID

Preparation of the "Sensitized Antigen Suspension"

1. Prepare antigen suspension as described for the VDRL Slide Test.
2. Add one part of 10 percent saline(sodium chloride solution) to one part of VDRL Slide Test suspension.
3. Mix by gently rotating the bottle or inverting the tube and allow to stand at least 5 minutes, but no more than 2 hours before use.

TESTING ACCURACY OF DELIVERY NEEDLES

1. It is of primary importance that the proper amount of reagent be used, and for this reason, needles used each day should be checked. Practice will allow rapid delivery of antigen suspension, but care should be exercised to obtain drops of uniform size.

2. For the Slide Qualitative and Quantitative tests on spinal fluid, dispense sensitized antigen suspension from a syringe fitted with a 20 or 22-gage needle which will deliver 100 drops \pm 2 drops of sensitized antigen suspension per milliliter when the syringe and needle are held vertically.

3. Adjust needles meeting these specifications to deliver the correct volume before being used.

PRELIMINARY TESTING OF SENSITIZED ANTIGEN SUSPENSION

1. For daily use, control sera of graded reactivity (R,WR, N) are tested without preliminary heating in the slide test.

2. Test the control sera as described under "VDRL SLIDE QUALITATIVE TEST ON SPINAL FLUID"

3. Reactions on the Reactive and Weakly Reactive control sera should reproduce the established reactivity pattern and the Nonreactive should show complete dispersion of antigen particles,
4. Do not use an unsatisfactory sensitized antigen suspension.

PREPARATION OF SPINAL FLUID

1. Centrifuge and decant spinal fluid.
2. The spinal fluid is tested without preliminary heating.

INTERFERING SUBSTANCES

Spinal fluids which are visibly contaminated or contain gross blood are unsatisfactory for testing.

VDRL SLIDE QUALITATIVE TEST ON SPINAL FLUID

Note: Slide flocculation tests for syphilis are affected by room temperature. For reliable and reproducible results, tests should be performed within the temperature range 73°-85° F (23°-29° C). At lower temperatures, test reactivity is increased.

1. Pipet 0.05 ml of spinal fluid into one ring of an agglutination slide.
2. Add one drop (0.01ml) of sensitized antigen suspension to each spinal fluid with a 21 or 22-gage needle.
3. Rotate slide for 8 minutes on a mechanical rotator at 180 rpm.

Note: When tests are performed in a dry climate, the slides may be covered with a box lid containing a moistened blotter during rotation to prevent evaporation.

4. Read tests microscopically, with a 10X objective, immediately after rotation.
5. Report the test results as follows:

Reading

Definite clumpingReactive(R)
 No clumping or very slight roughness Nonreactive (N)

VDRL SLIDE QUALITATIVE TEST ON SPINAL FLUID

Quantitative tests are performed on all spinal fluid found to be Reactive in the Qualitative test.

1. Prepare spinal fluid dilutions as follows:
 - a. Pipet 0.2 ml of 0.9 percent saline into each of 5 or more tubes.
 - b. Add 0.2 ml of unheated spinal fluid to tube 1, mix well and transfer 0.2 ml to tube 2.
 - c. Continue mixing and transferring 0.2ml from one tube to the next until the last tube is reached. The respective dilutions are 1:2, 1:4, 1:8, 1:16, 1:32, etc.
2. Test each spinal fluid dilution and undiluted spinal fluid as described under "VDRL SLIDE QUALITATIVE TEST ON SPINAL FLUID".
3. Report results in terms of the greatest spinal fluid dilution (dils) that produces a Reactive result.

QUALITY CONTROL PROCEDURE

Control sera with established reactivity patterns such as the control sera, are intended to help laboratories achieve reliable and reproducible test results in syphilis serology and maintain a stable day-to-day consistency. The reactivity patterns were established using

VDRL reagents and standard reference reagents available from the Center for Disease Control. Each lot of reagent has been tested by the Reagents Evaluation Unit of the U.S. Public Health Service, Center for Disease Control and found to reproduce the reactivity of its standard reference reagent.

LIMITATIONS OF PROCEDURE

A prozone reaction is encountered occasionally. This type of reaction is demonstrated when complete or partial inhibition of reactivity occurs with undiluted serum and maximum reactivity is obtained only with diluted serum. The prozone phenomenon may be so pronounced that only a weakly reactive or "rough" non-reactive result is produced in the qualitative test by a serum which will be strongly reactive when diluted.

It is, therefore, recommended that all sera producing weakly reactive or "rough" nonreactive results in the qualitative test be retested using the quantitative procedure before a report of the VDRL Slide Test is submitted. When a reactive result is obtained on some dilution of a serum that produced only a weakly reactive or a "rough" nonreactive result before dilution, report the test as reactive and include the qualitative titer.

Although serological tests for syphilis are not absolutely specific and some sera are reactive in one test and non-reactive in another, analysis of conflicting serologic results in terms of diagnosis or prognosis is only within the province of the physician.

While it is agreed that most of the positive serologic reactions obtained with non-specific lipid antigen sera due to syphilis, tests employing such antigens have been criticized on the grounds that they also yield positive serologic reactions when applied to serum or spinal fluid samples from nonsyphilitic individuals known as biologically false positive (BFP) reactors.

Biologically false positive reactors may be due to: (a) the presence of antibody-like substances similar to the antibodies produced in syphilis diseases; (b) an increase or alteration of the seroglobulin fraction; or (c) an increase or alteration of some other chemical substance or substances in the blood. Disorders which induce pseudo-syphilitic positivity include upper respiratory infections, hyperproteinemia, and varicella, infections of foreign protein such as tetanus toxoid, infectious hepatitis, malaria, leprosy, tuberculosis, lymphopathia venerea, leishmaniasis and scarlet fever. A positive reaction can occur in yaws, pinta, rat bite spirochetosis, relapsing fever, and other spirochetal infections. Moreover, biologically false positivity, lifelong when it occurs in normal human beings, has been difficult to identify by serologic methods.

EXPECTED VALUES

Those patients that contain the antibody to the etiological agent for syphilis, *Treponema pallidum*, will react to some degree with the VDRL Antigen.

SPECIFIC PERFORMANCE CHARACTERISTICS

There are many factors such as equipment, reagents, measurements, time periods, temperature and orders of procedure which influence test performance. Technologists who make arbitrary changes in recommended technique must assume full responsibility for the test results.

In addition, interlaboratory and intralaboratory checks are strongly recommended. These include the daily use of controls of graded reactivity, periodic check readings to maintain uniform reading levels among the laboratory personnel and comparison of results obtained on evaluation specimens with those of a reference laboratory.

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