INTERPRETATION OF CSF RESULTS – QUANTITATIVE

The highest dilution that produces a reactive, not weakly reactive, result is the endpoint titer. In the following example, the titer reported would be 1:2.

<table>
<thead>
<tr>
<th>TITER</th>
<th>1:1</th>
<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
<th>1:16</th>
</tr>
</thead>
<tbody>
<tr>
<td>RESULT</td>
<td>R</td>
<td>R</td>
<td>W</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

INTENDED USE:
The ASI VDRL Antigen Test is a qualitative and semiquantitative nontreponemal flocculation test for the detection of reagin antibodies in human serum. These materials are intended to be acquired, possessed and used only by health professionals.

SUMMARY AND EXPLANATION:
Treponema pallidum, the etiological agent of syphilis, induces the production of at least two types of antibodies in human infection: anti-treponemal antibodies that can be detected by FTA-ABS antigen, and anti-nontreponemal antibodies (reagin) that can be detected by the VDRL test.

PRINCIPLE OF THE PROCEDURE:
The ASI VDRL Antigen Test is a microscopic nontreponemal flocculation test to be used for the detection of reagin. The procedure is based on the VDRL antigen being combined at a correct ratio with buffered saline and then mixed with heat-inactivated serum.

REAGENTS
ASI VDRL ANTIGEN - 0.03% Cardiolipin and 0.9% Cholesterol in absolute alcohol. Sufficient Lecithin (approximately 0.20 to 0.22%) is added to produce standard reactivity.

ASI VDRL BUFFERED SALINE - Phosphate-buffered saline, pH 5.9-6.1, containing 0.05% formaldehyde as preservative.

WARNINGS AND PRECAUTIONS
For In Vitro Diagnostic Use
1. ASI VDRL ANTIGEN is highly flammable and is irritating to eyes, respiratory system and skin. There is possible risk of irreversible effects and there is risk to an unborn child. Avoid contact with skin and eyes. Do not breathe aerosols. Wear suitable protective clothing. Keep container tightly closed. Keep away from sources of ignition. No smoking. Target organs are blood, intestines, liver, muscle and nervous tissue.
2. Observe universal precautions in handling and disposing of the specimens utilized in this test. The CDC/NIH Health Manual "Biosafety in Microbiological and Biomedical Laboratories" describes how these materials should be handled in accordance with Good Laboratory Practice.
3. Do not pipet by mouth.
4. Do not smoke, eat, drink or apply cosmetics in areas where serum samples are handled.
5. Any cuts, abrasions or other skin lesions should be suitably protected.

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Handling and Procedural Notes
1. In order to obtain reliable and consistent results, the instructions in the package insert must be strictly followed. Do not modify the handling and storage conditions for reagents or samples.
2. Do not use past the expiration date indicated on the kit.
3. Do not interchange components of one kit with those of another kit.
4. Keep the VDRL ANTIGEN and VDRL BUFFERED SALINE tightly closed at all times to prevent evaporation.
5. All glassware, needles and syringes must be clean and dry before use. Rinse all equipment with water, alcohol and acetone in this specific order.
6. Do not use glass slides with concavities, wells or glass rings.

Storage Instructions
Store VDRL ANTIGEN and BUFFERED SALINE at room temperature (15-30°C). The VDRL ANTIGEN should be protected from light.
Indications of Deterioration
1. Turbidity or precipitation in controls is indicative of deterioration and the control should not be used.
2. Bacterial contamination of reagents or specimens may cause false positive results.
3. Any visible discoloration of VDRL ANTIGEN or VDRL BUFFERED SALINE may be indicative of deterioration and the reagent should not be used.

SPECIMEN COLLECTION AND STORAGE
1. Only serum is suitable for use in this test. Plasma is not acceptable.
2. Samples may be maintained in their original tubes at 2-8°C for up to four (4) hours. If longer storage is required, the serum must be separated from the red cells and stored at 2-8°C for 5 days or at -20°C indefinitely.
3. Frozen samples must be thawed at room temperature before use. Avoid repeated freezing and thawing.
4. Samples should be free from bacterial contamination, gross hemolysis or lipemia. A specimen is too hemolyzed for testing when printed matter cannot be read through it.
5. If necessary before testing, centrifuge the specimens at a force sufficient to sediment the cellular components.
6. Samples to be sent out for testing should be placed on ice packs and packaged like any other biohazardous material that could potentially transmit infection.
7. The test procedure can be modified for testing cerebrospinal fluid (CSF)...

PERFORMANCE OF THE TEST
Materials Provided:

<table>
<thead>
<tr>
<th>Material</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>VDRL ANTIGEN</td>
<td>2 x 5 ml</td>
</tr>
<tr>
<td>VDRL BUFFERED SALINE</td>
<td>2 x 60 ml</td>
</tr>
</tbody>
</table>

Additional Materials Required
1. Mechanical rotator adjustable to 180 ± 5 rpm and circumscribing 3/4 inch diameter, with humidity cover
2. VDRL control sera: reactive, weak reactive, nonreactive
3. Saline (0.9% NaCl solution)
4. Non-disposable calibrated 18-gauge needle without bevel
5. Non-disposable glass syringe, 1 ml or 2 ml
6. Bottles, 30 ml, round, narrow-mouth, approximately 35 mm in diameter with ground glass stoppers and flat inner bottom surfaces
7. Slides, 2 x 3” with rings approximately 14 mm in diameter. The rings can be paraffin or ceramic, but must be sufficiently high to prevent spillage during rotation.
8. Micropipettor, calibrated to deliver 50 µl
9. Pipets, glass serological: 1 ml in 1/10 increments, 5 ml in 1/10 increments, 10 ml in 1/10 increments
10. Timing device, minute and second capability
11. Microscope capable of 100x magnification

ASSAY PROTOCOL - QUALITATIVE
1. To quantitate serum samples for determination of endpoint, dilutions can be prepared directly on the glass slide.
2. Using a micropipettor, dispense 50 µl of saline into the circles numbered 2 through 4. Do not spread.
3. Dispense 50 µl of serum or CSF onto circles 1 and 2 of the glass slide.
4. Mix the saline and the serum or CSF in circle 2 by drawing the mixture up and down in the pipet 5 or 6 times. Avoid any bubble formation.
5. Transfer 50 µl from circle 2 to circle 3 and mix as in step (4) above. Repeat this serial dilution procedure to circle 4 and discard 50 µl from the last circle. Circles 1 through 4 represent a dilution series as follows:

<table>
<thead>
<tr>
<th>Circle</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution</td>
<td>1:1</td>
<td>1:2</td>
<td>1:4</td>
<td>1:8</td>
</tr>
</tbody>
</table>

6. Gently resuspend the VDRL ANTIGEN SUSPENSION or SENSITIZED VDRL-CSF ANTIGEN SUSPENSION in the 30-ml bottle, and draw sufficient volume into the syringe and needle assembly.
7. Holding the VDRL ANTIGEN SUSPENSION or SENSITIZED VDRL-CSF ANTIGEN SUSPENSION dispensing needle and syringe in vertical position, dispense several drops into the 30 ml SUSPENSION bottle to make sure the passage is clear.
8. Hold the VDRL ANTIGEN SUSPENSION or SENSITIZED VDRL-CSF ANTIGEN SUSPENSION into the needle and glass syringe assembly. Dispense several drops into the 30 ml SUSPENSION bottle to make sure the passage is clear.
9. Place the slide onto the automatic rotator and cover to maintain humidity. Rotate the slide at 180 ± 5 rpm for four (4) minutes for serum or eight (8) minutes for CSF.
10. Immediately after rotating the slide, remove it from the rotator and read the test microscopically, using 100x magnification. Record the results.

ASSAY PROTOCOL - SEMIQUANTITATIVE
1. Preparation of the SENSITIZED VDRL – (Cerebrospinal Fluid) CSF ANTIGEN SUSPENSION

   a. Prepare the VDRL ANTIGEN SUSPENSION as described for the VDRL Slide tests on serum.
   b. Add one part of 10% saline to one part of VDRLANTIGEN SUSPENSION.
   c. Mix by gently rotating the bottle or inverting the tube. Allow the mixture to stand at least 5 minutes.
   d. The SENSITIZED VDRL-CSF ANTIGEN SUSPENSION is good for only 2 hours after preparation.

   e. Each serum sample (including control sera) must be heat-inactivated for 30 minutes at 56°C prior to testing.
   f. If heat-inactivation occurs more than four (4) hours prior to testing, reheat the serum for an additional 10 minutes at 66°C before use.

   g. Preparation of the Samples

   i. Using a micropipettor, pipet 50 µl of serum or CSF into one 14 mm test circle.
   j. Gently resuspend the VDRL ANTIGEN SUSPENSION or SENSITIZED VDRL-CSF ANTIGEN SUSPENSION.
   k. Draw a sufficient volume of VDRL ANTIGEN SUSPENSION or SENSITIZED VDRL-CSF ANTIGEN SUSPENSION into the needle and glass syringe assembly. Dispense several drops into the 30 ml SUSPENSION bottle to make sure the passage is clear.
   l. Holding the VDRL ANTIGEN SUSPENSION or SENSITIZED VDRL-CSF ANTIGEN SUSPENSION dispensing needle and syringe in a vertical position, dispense exactly one (1) free-falling drop of ANTIGEN SUSPENSION into each circle containing serum or CSF.
   m. Place the slide onto the mechanical rotator and cover to maintain humidity. Rotate the slide at 180 ± 5 rpm for four (4) minutes for serum or eight (8) minutes for CSF.
   n. Immediately after rotating the slide, remove it from the rotator and read the test microscopically, using 100x magnification. Record the results.

   o. Preparation of the SENSITIZED VDRL – (Cerebrospinal Fluid) CSF ANTIGEN SUSPENSION...

Quality Control
Controls with graded reactivity should be included in each test run to confirm optimal reactivity of the ANTIGEN SUSPENSION. If control samples do not yield the expected response, the assay should be considered invalid and the assay repeated. If the repeat assay does not elicit the expected results for the control samples, discontinue use of the ANTIGEN SUSPENSION and contact ASI Technical Support at (800) 654-0146.