

ASI VDRL Antigen Test

For *in vitro* diagnostic use

Catalog Number

950010

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INTENDED USE

The *ASI VDRL Antigen Test* is a qualitative and semiquantitative nontreponemal flocculation test for the detection of reagin antibodies in human serum and cerebrospinal fluid (CSF). 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 TPPA, TPHA (as examples), and anti-nontreponemal antibodies (reagin) that can be detected by the VDRL test¹.

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 or CSF.

REAGENTS

ASI VDRL ANTIGEN - 0.03% Cardiolin 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.

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 to avoid vapors. 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 Practice².
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.

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 manufacturer's kit.
4. Keep the VDRL ANTIGEN and VDRL BUFFERED SALINE tightly closed at all times to prevent evaporation.
5. All glassware, needles and glass syringes must be clean and dry before use.

Clean syringes, needles and glassware by:

1. Rinse with tap water.
 2. Soak and wash thoroughly in a glassware detergent solution.
 3. Rinse with tap water 6-8 times.
 4. Rinse with distilled or deionized water.
 5. Rinse with absolute alcohol.
 6. Rinse with acetone.
 7. Air dry until the acetone odor is gone.
 8. Remove needles from syringes for storage.
6. Needle Calibration: The 18-gauge needle should deliver 60 ± 2 drops of antigen suspension per milliliter when held in a vertical position. To perform accuracy check on the needle, attach the needle to a 1- or 2-ml glass syringe. Fill the syringe with the antigen suspension and hold the syringe in a vertical position, count the number of drops delivered in 0.5 ml. The needle is considered satisfactory if 30 ± 1 drops are obtained in 0.5 ml.

The 21 or 22-gauge needle should deliver 100 ± 2 drops of antigen suspension per milliliter when held in a vertical position. To perform accuracy check on the needle, attach the needle to a 1- or 2-ml syringe. Fill the syringe with the antigen suspension and hold the syringe in a vertical position, count the number of drops delivered in 0.5 ml. The needle is considered satisfactory if 50 ± 1 drops are obtained in 0.5 ml.

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 the antigen or buffer 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. Serum and CSF are suitable for use in this test. Plasma is not acceptable.
2. Serum 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. If testing is to be delayed more than 4 hours, refrigerate the CSF specimen (2-8° C). If testing is to be delayed for more than 5 days, freeze the specimen at temperatures at or below -20° C. Avoid repeated freezing/thawing of specimens. Specimens must be at room temperature, 20-30° C, when tested.¹
5. 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 it¹.
6. If necessary, before testing, centrifuge the specimens at a force sufficient to sediment the cellular components.
7. 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.
8. The test procedure can be modified for testing cerebrospinal fluid (CSF)¹.

PERFORMANCE OF THE TEST

Materials Provided:

- | | |
|-------------------------|-----------|
| 1. VDRL ANTIGEN | 2 x 5 ml |
| 2. VDRL BUFFERED SALINE | 2 x 60 ml |

Additional Material Required:

1. Mechanical rotator adjustable to 180 ± 5 rpm and circumscribing 3/4-inch diameter (19.05mm), 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 for serum testing and calibrated 21 or 22 gauge needle for CSF testing.
5. Non-disposable glass syringe, 1 ml or 2 ml
6. Flasks, 25 ml, round, approximately 40 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. (Used for serum and CSF)
 - 4.3 x 2.2" with approximately 14 mm diameter well Boerner Micro Test Slide. (10 well) (Used for serum only)
 - 2 x 3" with approximately 16 mm diameter well, (12 well) Kline concavity slide. (Used for CSF only)¹
8. Micropipettor, calibrated to deliver 50 μ l
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
12. Acetone
13. Absolute alcohol
14. Distilled or deionized water

TEST PROCEDURE

PREPARATION OF THE VDRL ANTIGEN SUSPENSION

1. The VDRL ANTIGEN SUSPENSION must be prepared fresh each day.
2. The temperature of the VDRL BUFFERED SALINE, VDRL ANTIGEN and equipment should be between 23 and 30°C before preparing the suspension. All glassware, pipets and equipment must be dry.
3. With a 1 ml serological pipet, deliver 0.4 ml of VDRL BUFFERED SALINE to the bottom of a 25 ml round, glass stoppered flask with flat inner bottom.
4. Pipet 0.5 ml of VDRL ANTIGEN gradually into the VDRL BUFFERED SALINE while continuously but gently rotating the flask on a flat surface. Add the VDRL ANTIGEN drop by drop at a rate allowing approximately 6 seconds for 0.5 ml of VDRL ANTIGEN. Keep the pipet tip in the upper third of the flask. Do not splash the BUFFERED SALINE onto the pipet. The proper rotation speed is obtained when the center of the flask circumscribes a 2-inch (5 cm) diameter circle approximately three (3) times per second. Expel the last drop of VDRL ANTIGEN from the pipet without touching the pipet to the BUFFERED SALINE and continue rotation of the flask for 10 seconds.

5. Add 4.1 ml of VDRL BUFFERED SALINE from a 5 ml pipet.
6. Cap the flask and shake it from bottom to top and back approximately 30 times in 10 seconds. The VDRL ANTIGEN SUSPENSION is ready for use and is usable for 8 hours.
7. Mix the VDRL ANTIGEN SUSPENSION by gently swirling it each time it is used. Do not mix the SUSPENSION by forcing it back and forth through the syringe and needle since this may cause breakdown of particles and loss of reactivity.
8. To achieve reliable and reproducible test results, the VDRL ANTIGEN SUSPENSION, controls and test specimens must be at 20-30°C when the tests are performed.

PREPARATION OF THE SENSITIZED VDRL - (Cerebrospinal Fluid) CSF Antigen Suspension

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

PREPARATION OF THE SAMPLES

1. Each serum sample (including control sera) must be heat-inactivated for 30 minutes at 56°C prior to testing. Control sera should be heat-inactivated for 30 minutes each day of use.
2. If heat-inactivation occurs more than four (4) hours prior to testing, reheat the serum or controls for an additional 10 minutes at 56°C before use.
3. DO NOT heat-inactivate CSF.

ASSAY PROTOCOL - QUALITATIVE

1. Using a micropipettor, pipet 50 µl of serum or CSF into one test circle.
2. Gently resuspend the VDRL ANTIGEN SUSPENSION or SENSITIZED VDRL-CSF ANTIGEN SUSPENSION.
3. 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 25 ml SUSPENSION flask to make sure the passage is clear.
4. 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.
5. 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.
6. 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. To quantitate serum or CSF samples for determination of endpoint, dilutions can be prepared directly on the glass slide.
2. Using a pipettor, 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:

CIRCLE	1	2	3	4
DILUTION	1:1	1:2	1:4	1:8

6. Gently resuspend the VDRL ANTIGEN SUSPENSION or SENSITIZED VDRL-CSF ANTIGEN SUSPENSION in the 25-ml flask 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 25-ml flask to clear the needle of air. Then add exactly one (1) free-falling drop of ANTIGEN SUSPENSION to each circle.
8. 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.
9. Immediately after rotation, read the test microscopically using 100x magnification.
10. If the highest dilution tested (1:8) is reactive, further serial dilutions must be performed until endpoint is observed.

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.

INTERPRETATION OF RESULTS

INTERPRETATION OF RESULTS - QUALITATIVE

1. Read and record the results as follows:

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

INTERPRETATION OF RESULTS - SEMIQUANTITATIVE

1. 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.

TITER	1:1	1:2	1:4	1:8	1:16
RESULTS	R	R	W	N	N

INTERPRETATION OF CSF RESULTS - SEMIQUANTITATIVE

1. Read the test microscopically, using a 10x ocular and a 10x objective.
2. Report the results in terms of the highest spinal fluid dilution that produces a reactive result in the table below.

CSF Dilutions						Report
Undiluted (1:1)	1:2	1:4	1:8	1:16	1:32	
R	N	N	N	N	N	Reactive, undiluted, 1 dil.
R	R	N	N	N	N	Reactive, 1:2 dilution, 2 dils.
R	R	R	N	N	N	Reactive, 1:4 dilution, 4 dils.
R	R	R	R	N	N	Reactive, 1:8 dilution, 8 dils.
N (ROUGH)	R	R	R	R	N	Reactive, 1:16 dilution, 16 dils.

LIMITATIONS OF THE PROCEDURE

1. Biological false positive reactions occur occasionally with the VDRL test. Such reactions sometimes occur in samples from individuals with a history of drug abuse, or with diseases such as lupus erythematosus, malaria, vaccinia, mononucleosis, leprosy, viral pneumonia and after smallpox vaccinations.
2. Prozone reactions may occur in which reactivity with undiluted serum is inhibited. The prozone phenomenon often gives weakly reactive or "rough" nonreactive results in the qualitative test. Therefore, all specimens with these results must be quantitatively tested until endpoint is reached or until no reactivity is observed.
3. Pinta, yaws, bejel and other treponemal diseases produce positive reactions in this test¹.
4. The results of the *ASI VDRL Antigen Test* must be confirmed by a treponemal test.
5. In accord with all diagnostic methods, a final diagnosis should not be made on the result of a single test but should be based on a correlation of test results with other clinical findings.

EXPECTED VALUES

The *ASI VDRL Antigen Test* is evaluated for the equivalence, in its pattern of reactivity, against a reference VDRL antigen suspension.

REFERENCES

1. Larsen SA, Pope V, Johnson R, Kennedy E, (ed.). 1998. *Manual of Tests for Syphilis, Public Health Service*, Washington, D.C.
2. *Biosafety in Microbiological and Biomedical Laboratories*, 3rd ed. 1993. HHS Publication No. (CDC) 93-8395, Public Health Service, Washington, D.C.

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