

Chapter 8

VENEREAL DISEASE RESEARCH LABORATORY (VDRL) SLIDE TEST

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CONTENTS

Test Principles

Specimen Collection and Handling

- Specimen
- Collection
- Handling

Materials

- Reagents
- Equipment

Calibration

- Pipettors and Tips
- Needles
- Rotator

Quality Control

- VDRL antigen with buffered saline
- Daily Controls for Serum Test
- Daily Controls for Cerebrospinal Fluid Test

Test Procedures for Serum

- Preparing the Antigen Suspension
- Qualitative
- Quantitative

Test Procedures for Cerebrospinal Fluid (VDRL-CSF)

- Preparing the Sensitized Antigen Suspension
- Qualitative
- Quantitative

Calculations and Ranges

Interpretation of Results

- Serum

Cerebrospinal Fluid
Acceptable Variations

Sources of Error

Test Limitations

References

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VENEREAL DISEASE RESEARCH LABORATORY (VDRL) SLIDE TESTS

TEST PRINCIPLES

The Venereal Disease Research Laboratory (VDRL) tests¹⁻³ are slide microflocculation tests for syphilis that use an antigen containing cardiolipin, lecithin, and cholesterol. The antigen, suspended in a buffered saline solution, forms flocculates when combined with lipoidal antibodies in serum or cerebrospinal fluid from syphilis patients. The VDRL tests are fast, easy to perform, and excellent for screening of samples. The VDRL tests measure IgM and IgG antibodies to lipoidal material released from damaged host cells as well as to lipoprotein-like material, and possibly cardiolipin released from the treponemes.^{4,5} The antilipoidal antibodies are antibodies that are not only produced as a consequence of syphilis and other treponemal diseases, but also may be produced in response to nontreponemal diseases of an acute and chronic nature in which tissue damage occurs.⁶ Without some other evidence for the diagnosis of syphilis, a reactive nontreponemal test does not confirm *T. pallidum* infection.

SPECIMEN COLLECTION AND HANDLING

Specimen

Avoid accidental infection from needle sticks or cuts when collecting and processing specimens by observing universal precautions (Chapter 2).

1. Only serum and cerebrospinal fluid (CSF) are appropriate specimens for the VDRL tests.
2. Acceptable specimens
 - a) Serum - An acceptable serum specimen should not contain particulate matter that would interfere with reading test results. Serum specimens that are excessively hemolyzed, grossly contaminated with bacteria, chylous or otherwise extremely turbid are unsatisfactory for testing. A specimen is too hemolyzed for testing when printed matter cannot be read through it.

Note: Hemolysis may be caused by transporting blood in freezing or extremely hot weather without proper insulation.

- b) Spinal fluid- An acceptable CSF specimen should not contain particulate matter that would interfere with reading test results. CSF specimens with even traces of blood are unsuitable for testing.

4. Unacceptable specimens

Not all unsuitable samples should be discarded or not analyzed. When an unsatisfactory sample is received in the laboratory, notify the requesting physician and discuss whether testing is appropriate for that specimen. If a test result is still desired by the ordering physician, then the condition of the sample must be stated on the report, and a notation made of any limitation on interpretation of the test result.⁷

Collection

The procedures for the collection and processing of venous blood and spinal fluid are given in detail in Chapter 3.

1. Collect whole blood or spinal fluid into a clean, dry tube without an anticoagulant.
2. Label each specimen with patient identifier, and date.

Handling

Serum

1. Allow sufficient time (approximately 20 minutes) at room temperature for the specimen to clot.
2. Centrifuge the specimen at room temperature at 1000 to 1200 x g for at least 5 minutes to sediment cellular elements (see chapter 3).
3. Transfer the serum into a clean, dry, labeled test tube.
4. If serum samples are to be shipped to a testing site, specimen containers must be leakproof and placed within a leakproof plastic bag. Paper work should be submitted in a separate plastic bag, if included with the specimen.⁸
5. On the day of testing, heat the serum in a 56°C water bath for 30 minutes.
6. Remove the serum from the water bath and examine for debris. Recentrifuge all serum specimens containing particulate debris.
7. Reheat serum at 56°C for 10 minutes if testing is delayed more than 4 hours.
8. Specimens must be at room temperature, 23° - 29°C (73° - 85°F) when tested.
9. If testing is to be delayed more than 4 hours, stopper the specimen tube and store serum at refrigerator temperature (2° - 8°C). If testing is to be delayed more

than 5 days, freeze the specimen at temperatures at or below -20°C. Avoid repeated freezing - thawing of specimens.

10. If specimens have been stored before heating, heat to 56 °C for 30 minutes. Specimens heated before storage should be reheated to 56°C for 10 minutes. Specimens must be at room temperature, 23° - 29°C (73° - 85°F) when tested.

Spinal Fluid

1. Perform total protein determinations according to standard procedures.
2. Cell counts must be done within 2 hours after lumbar puncture.
3. Centrifuge at 1000-1200 x g for 10 ± 5 minutes and transfer to a clean labeled tube.
4. If serum samples are to be shipped to a testing site, specimen containers must be leakproof and placed within a leakproof plastic bag. Paper work should be submitted in a separate plastic bag, if included with the specimen.⁸
5. Do not heat spinal fluid before testing.
6. Do not test spinal fluid specimens that are visibly contaminated or that contain gross blood.
7. If testing is to be delayed more than 4 hours, refrigerate the CSF specimen (2° - 8°C). If testing is to be delayed more than 5 days, freeze the specimen at temperatures at or below -20°C. Avoid repeated freezing - thawing of specimens.
8. Specimens must be at room temperature, 23° - 29°C (73° - 85°F) when tested.

MATERIALS

Reagents

Purchased

1. **VDRL antigen.** A colorless alcohol solution containing 0.03% cardiolipin, 0.9% cholesterol, and sufficient purified lecithin to produce standard reactivity. During recent years, the VDRL antigen has contained 0.21% ± 0.01% lecithin. The antigen is packaged in screw-capped (vinylite liners) bottles or hermetically sealed glass ampules and are stored either in the dark at room temperature (23°-29°C) or refrigerated at 2° - 8°C. At these temperatures, the antigen components remain in solution. Discard bottles that contain a precipitate.

2. **VDRL-Buffered Saline, pH 6.0 ±0.1 (1.0% NaCl).** VDRL-buffered saline may be purchased or prepared in the laboratory.

Prepare a solution consisting of:

Formaldehyde, neutral (ACS)	0.5 ml
Na ₂ HPO ₄ , anhydrous	0.037 g
KH ₂ PO ₄	0.170 g
NaCl	10.00 g
Distilled water	1,000.0 ml

If the buffer is prepared in the laboratory, use reagent-grade chemicals. Check the pH of the solution and store in screw-capped or glass-stoppered bottles.

Note: When an unexplained change in reactivity of the controls occurs, check the pH of the buffered saline to determine whether it is a factor. Discard buffered saline that is outside the range of pH 6.0 ±0.1.

3. **Control serum samples.** Reactive (R), weakly reactive (W) and nonreactive (N) sera in dehydrated or liquid form are used as controls in the test. If quantitative tests are to be performed, a control serum that can be titered to at least a 1:4 dilution should be used.

(Reagents may be purchased from **Lee Laboratories, Grayson, GA; Difco, Detroit, MI**)

4. Acetone
5. Alcohol, 95% ethanol
6. Paraffin

Prepared

1. **0.9% Saline.** Add 0.9 g of dry sodium chloride (ACS) to each 100 ml of distilled water.
2. **10.0% Saline.** Add 10 g of dry sodium chloride (ACS) to each 100 ml of distilled water.

Equipment

1. Nondisposable calibrated needles without bevel
 - a. Serum test: 18-gauge

- b. Spinal fluid test: 21- or 22-gauge
2. Bottles, 30 ml, round, narrow-mouthed, 35 mm in diameter, with glass stoppers and **flat inner-bottom surface**.

Note: These bottles are no longer commercially available. Studies indicate that properly washed 25 ml glass stoppered erlenmeyer flasks can be used without affecting the sensitivity of the antigen (Pope, personal communication).
3. Safety pipetting device with disposable tip delivering 50 μ l
4. Pipettes, serologic, graduated to tip:
 - 1.0 ml, graduated in 1/100 ml
 - 5.0 ml, graduated in 1/10 ml
 - 10.0 ml, graduated in 1/10 ml
5. Slides
 - a. Serum test: 2 x 3 inches, with 12 paraffin or ceramic rings approximately 14 mm in diameter (Glass slides with ceramic rings may be purchased from **Technical Glass, 10903 Chandler Blvd., N. Hollywood, CA.**).

Note: Ceramic rings must be high enough to prevent spillage during rotation.
 - b. Spinal fluid test:
 - 1) Serum test slides as above, or
 - 2) Kline concavity slides, 3 x 2 1/4 inch x 3 mm thick, 12 concavities measuring 16 mm in diameter and 1.75 mm in depth
6. Slide holder, for 2 x 3 inch slides
7. Ringmaker, to make paraffin rings approximately 14 mm in diameter (**Cat.# 2600, Eberbach Corp., Ann Arbor, MI**)
8. Mechanical rotator adjustable to 180 \pm 2 rpm, circumscribing a circle 19 mm in diameter on a horizontal plane
9. Binocular or monocular microscope with 10X ocular(s), 10X objective, and built-in adjustable light source
10. Discard containers; disinfectants

11. Disposable latex gloves, safety glasses, and protective clothing
12. Humidifying cover (needed for dry climates)
13. Glass syringe, 2 ml or 5 ml

CALIBRATION

Pipettors and Tips

With the pipettors currently available, the measurement of small serum volumes is routine. Most manufacturers include in the specifications of their pipettors the accuracy for frequently used microliter volumes. Daily use may affect pipettors, making them lose their initial accuracy. The differences in disposable tips from sources other than the manufacturer of the pipettor, is probably the most common error. For budgetary reasons, a less expensive brand of pipette tips may be substituted for those of the manufacturer. Although the less expensive brand may be satisfactory, the laboratory should verify the accuracy and precision of the substitute pipet tips in their test system. Commercial kits to check pipettor accuracy are available. Also, manufacturers provide procedures for checking the accuracy of their equipment. Historically, the gravimetric or spectrophotometric procedures, which use the weight of water or the absorbance of a substance at a given wavelength, have been the most accepted methods used to calibrate pipettors. These procedures should not be used instead of those specified by the manufacturer's.

Needles

Preparation and calibration of stainless steel needles for slide flocculation tests:

1. File a deep notch in nondisposable stainless steel needles just above the bevel. Use pliers to break the point off the needle.
2. Stainless steel cannula with square-cut tip and Luer may be used if they deliver equivalent volume to meet the drop size specified for the test.
3. A calibrated stainless steel needle without bevel is the most satisfactory tool for delivering a given volume of reagent. However, any device that consistently and accurately delivers a given volume of reagent can be used.
4. Adjust needles NOT meeting these specifications to deliver the correct volumes before use.
 - a. If too many drops per milliliter are delivered by the needle, the opening of the tip is too small. Adjust by reaming out the tip with a sharp pointed instrument, such as the sharpened end of a triangular file.
 - b. If too few drops per milliliter are delivered by the needle, the opening of the tip is too large. Adjust by pressing together slightly or by filing the edges of the needle inward.

5. Once calibrated, protect the tips of needles against dropping on the floor, sink, or to the bottom of bottles.
6. Check the needles once a month for delivery of correct volume, or when the needle has been dropped, wiped, or the pattern of the control sera is not met.
7. Clean stainless steel needles and glass syringes by rinsing with water, alcohol, and acetone. Remove needle from syringe after cleaning.

Testing Accuracy of Antigen Suspension Needle for Serum Test

1. The accuracy of the test depends on the volume of antigen suspension used. Check the calibrated needle once a month to ensure the delivery of the correct volume of VDRL antigen suspension.
2. For the qualitative and quantitative tests on serum, dispense antigen suspension from a syringe fitted with a 18-gauge needle without bevel, which will deliver 17 μl /drop of antigen suspension per ml (60 ± 2 drops) when held vertically.
3. Place the needle on a 2-ml syringe or a 1-ml pipette. Fill the syringe or pipette with VDRL antigen suspension. Holding the syringe or pipette in a vertical position, count the number of drops delivered in 0.5ml. The needle is correctly calibrated if 30 drops ± 1 drop are delivered in 0.5 ml.

Testing Accuracy of Antigen Needle for VDRL-CSF Test

1. The accuracy of the test depends on the amount of antigen used. Check the 21- or 22-gauge calibrated needle monthly to ensure the delivery of the correct volume of sensitized antigen suspension (100 drops ± 2 drops per ml, 10 μl per drop).
2. Place the needle on a 2-ml syringe or a 1-ml pipette. Fill the syringe or pipette with VDRL-CSF sensitized antigen suspension. Holding the syringe or pipette with the needle in a vertical position, count the number of drops delivered in 0.5 ml. The needle is considered correctly calibrated if 50 drops ± 1 drop are delivered in 0.5 ml.
3. Adjust or replace the needle if it does not meet this specification. Be sure to test the calibration of the adjusted or replaced needle (see chapter 4).

Rotator

1. Speed - For rotators without a digital readout, the speed can be estimated by counting the number of rotations made per minute. To count the rotations hold

your finger next to the rotator and count the number of times the rotator touches your finger in 15 seconds. If the rotator is properly adjusted, the count should be 45. The rotator should be calibrated each day it is to be used.

2. Time - The rotator's timer should be checked against another laboratory timer or stop watch. The rotator's timer should be within ± 15 seconds of the set time.

QUALITY CONTROL

It is the responsibility of the laboratorian to ensure that reagents are of good quality and standard reactivity. Chemicals and distilled water should be of high quality, and solutions should be prepared according to the directions specified for each technique. Each new lot of cardiolipin antigen for the Venereal Disease Research Laboratory (VDRL) should be tested in parallel with reference reagents to verify that it is of standard reactivity. Parallel testing should be performed on more than one testing day, by using different specimens of graded reactivity for each test period. Tests should be performed in accordance with the techniques described below. Record the results of all check testing.

Individual specimens of graded reactivity for check testing may be obtained by selecting specimens from the daily tests runs and storing them in the freezer. Reactive serum diluted with nonreactive serum to produce various degrees of reactivity may also be used. If possible, fresh serum specimens from routine test runs should be used for the nonreactive specimens.

VDRL Antigen with VDRL-Buffered Saline

Criteria of Acceptability

1. The reportable test results (reactive, weakly reactive, and nonreactive) with reference controls of graded reactivity and individual serum specimens in the qualitative and the quantitative tests should be comparable to those obtained with the reference antigen.
2. In tests with nonreactive specimens, the antigen suspension should show complete dispersion of particles with no more "roughness" than is seen with the reference antigen suspension.

Procedure for Testing

1. Before the antigen suspension is prepared, determine the pH of the VDRL-buffered saline. If the pH of the VDRL buffered saline is outside the specified range of 6.0 ± 0.1 , the product is unsatisfactory and may not be used for testing.
2. If the pH of the VDRL-buffered saline is satisfactory, prepare VDRL antigen suspension from the new reagents and the reference reagents that meet the established reactivity of the control sera.

3. Compare the antigen suspensions by qualitatively testing individual serum specimens of graded reactivity. Test at least 3 reactive serum samples and 10 serum samples of intermediate reactivity (ranging from weakly reactive to reactive 1:2). In addition, test 7 nonreactive samples. Test serum samples side by side with the new and the reference reagents.
4. Select three strongly reactive serum samples for quantitative testing. In test tubes, prepare serial dilutions of each serum in 0.9% saline (1:2, 1:4, 1:8, 1:16). Test each serum dilution side by side with the new and reference antigen suspensions.

Note: It is important to make these master serial dilutions so that antigens are being compared with the same serum dilutions.

5. Prepare sensitized VDRL antigen suspensions for spinal fluid testing from the new and the reference standard suspensions that produce the established reactivity pattern on spinal fluid controls of graded reactivity.
6. Compare new versus old sensitized antigen suspensions by the qualitative testing of at least six simulated spinal fluid specimens of graded reactivity (two nonreactive and four reactive). Prepare simulated spinal fluid specimens by diluting a serum found to be reactive in the serum VDRL test at a dilution of 1:80 or higher when diluted in 0.9% saline.
7. Record results of all testing.
8. Review test results to determine whether the new antigen meets the criteria of acceptability.

Daily Controls

Serum Test

1. Each testing day, prepare a fresh antigen suspension.
2. Store the antigen suspension at room temperature, 23°C - 29°C (73°F - 85°F); it is good for only that testing day (not to exceed 8 hours). The antigen loses reactivity and false nonreactives may occur if the working antigen suspension is more than 8 hours old.
3. Test the antigen suspension reactivity with control sera specimens of graded reactivity (reactive, weakly reactive and nonreactive). If quantitative tests are to be performed, a control serum that can be titered to at least a 1:4 dilution should be used.

4. For routine testing, use only the antigen suspensions that reproduce the established reactivity pattern of the controls (Reactive, Weakly Reactive, and Nonreactive).
5. After completing the day's tests, discard the antigen suspension and clean dispensing needle and syringe by rinsing with water, alcohol, and acetone, in that order. Remove needle from syringe after cleaning.

CSF Test

1. Prepare a fresh sensitized antigen suspension. The sensitized antigen suspension is good for only 2 hours after preparation.
2. Store the sensitized antigen suspension at room temperature, 23°C - 29°C (73°F - 85°F).
3. Satisfactory control samples for testing the reactivity of the sensitized antigen suspension are conveniently prepared by diluting reactive serum in 0.9% saline (see instructions in step f of quality control for VDRL antigen).
4. For daily use, remove one tube of the reactive control serum from the freezer, thaw, and mix thoroughly. Prepare the designated serum dilutions in 0.9% saline. Test the controls without preliminary heating.
5. Test the control serum dilutions as described in Performing the Qualitative Test on Cerebrospinal Fluid.
6. Reactions of the VDRL-CSF sensitized antigen suspension with the control serum dilutions should reproduce the established reactivity pattern. The nonreactive dilution should show complete dispersion of antigen particles. Always include control serum dilutions of graded reactivity (reactive, minimally reactive, and nonreactive) during a testing period to ensure proper reactivity of the sensitized antigen suspension at the time tests are performed.
7. Do not use an unsatisfactory sensitized antigen suspension.

TEST PROCEDURES FOR SERUM

Preparing the Antigen Suspension

1. Prepare a fresh VDRL antigen suspension each testing day. The temperature of the buffered saline, antigen and equipment should be between 23°C and 29°C (73°F to 85°F) at the time the antigen suspension is prepared.

2. Pipette 0.4ml of VDRL-buffered saline to the bottom of a round, 30-ml, glass-stoppered bottle with a flat inner-bottom surface or a 25 ml glass-stoppered erlenmeyer flask.
3. Add 0.5 ml of VDRL antigen suspension (from the lower half of a 1.0-ml pipette graduated to the tip) directly onto the saline, while rotating the bottle continuously but gently on a flat surface. Add antigen drop by drop at a rate allowing approximately 6 seconds for each 0.5ml of antigen. Keep the pipette tip in the upper third of the bottle. Do not splash saline onto the pipette. The proper speed of rotation is obtained when the center for the bottle circumscribes a 2-inch-diameter circle approximately three times per second.
4. Expel the last drop of antigen from the pipette without touching the pipette to the saline and continue rotation of the bottle for 10 seconds.
5. Add 4.1 ml of buffered saline from a 5-ml pipette.
6. Cap the bottle and shake it from bottom to top and back approximately 30 times in 10 seconds. The antigen suspension is ready for use and may be used during that day (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. Do not use a suspension that does not meet the control pattern.

Qualitative Test

Serum

1. Slide flocculation tests for syphilis are affected by room temperature. For reliable and reproducible test results, the VDRL antigen suspension, controls, and test specimens must be at room temperature, 23°C - 29°C (73°F - 85°F), when tests are performed.
2. Place 50 µl of serum into one ring of a paraffin or ceramic-ringed slide using a safety pipetting device. Do not use glass slides with concavities, wells, or glass rings.
3. Gently resuspend the VDRL antigen suspension.
4. Holding the VDRL antigen suspension dispensing needle and syringe in a vertical position, dispense several drops to clear the needle of air. Then add exactly 1 free-falling drop (17 µl) of antigen suspension to each circle containing serum.

- Place the slide on the mechanical rotator. Rotate the slide for 4 minutes at 180 ± 2 rpm. When testing in a dry climate, cover the slides with a moist humidifying cover during rotation to prevent excessive evaporation.
- Immediately after rotating the slide, remove it from the rotator and read the test results.
- Test quantitatively, to an endpoint, all serum specimens that produce reactive, weakly reactive, or “rough” nonreactive results in the qualitative VDRL slide test.

Reading and Reporting Results

- Read slide microscopically, using 10X oculars and a 10X objective.
- Report the results as follows:

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

Quantitative Test

- Dilute serum samples to an endpoint titer. Quantitative tests for 3 serum specimens through the 1:8 dilution may be performed on one slide (Fig. 8:1).

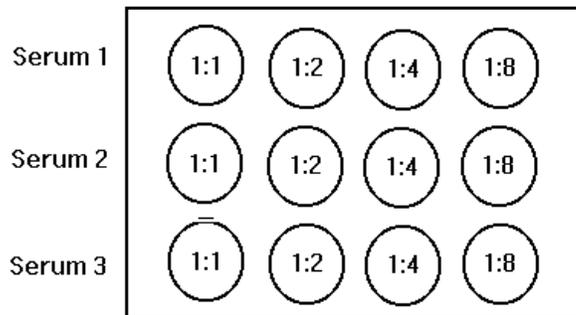


Figure 8:1. Example of titration of serum.

- Place 50 μ l of 0.9% saline in circles numbered 2 through 4. Do not spread saline.
- Using a safety pipette device, place 50 μ l of serum in circle 1 and 50 μ l of serum in circle 2.

4. Mix the saline and the serum in circle 2 by drawing the mixture up and down in the safety pipette eight times. Avoid forming bubbles.
5. Transfer 50 μ l from circle 2 (1:2) to circle 3, and mix.
6. Transfer 50 μ l from circle 3 to circle 4, mix, and then discard the last 50 μ l.
7. Gently resuspend the antigen suspension.
8. Holding the VDRL antigen suspension dispensing needle and syringe in a vertical position, dispense several drops to clear the needle of air. Add exactly 1 free-falling drop (17 μ l) of antigen suspension to each circle.
9. Place the slide on the mechanical rotator. Rotate the slide for 4 minutes at 180 ± 2 rpm. When performing the test in a dry climate where drying may be a problem, place the slides under a moist humidifying cover during rotation to prevent excessive evaporation.
10. Immediately after rotation, read the test.
11. If the highest dilution tested (1:8) is reactive, continue as follows:
 - a. Prepare a 1:8 dilution of the test specimen in a test tube. Add 0.1 ml of serum to 0.7 ml of 0.9% saline. Mix thoroughly.
 - b. Place 50 μ l of 0.9% saline into paraffin rings 2, 3, and 4. Prepare additional serial dilutions for strongly reactive specimens.
 - c. Add 50 μ l of the 1:8 dilution of the test specimen to paraffin rings 1 and 2.
 - d. Prepare serial twofold dilutions beginning with ring 2. Complete the test as described in steps 5-11.
12. After completing the day's tests, discard the antigen suspension and clean dispensing needle and syringe by rinsing with water, alcohol, and acetone, in that order. Remove needle from syringe after cleaning.

Reading and Reporting of Results

1. Read the results microscopically using 10X oculars and a 10X objective as for the qualitative test.
2. Report titers as the highest dilution that gives a reactive (not weakly reactive) result according to Table 8:1.

Table 8.1. Reporting Quantitative Serum Results

	Serum Dilutions					Report
	1:2	1:4	1:8	1:16	1:32	
Undiluted (1:1)	1:2	1:4	1:8	1:16	1:32	
R	W	N	N	N	N	Reactive, undiluted, 1 dil.
R	R	W	N	N	N	Reactive, 1:2 dilution, 2 dils.
R	R	R	W	N	N	Reactive, 1:4 dilution, 4 dils.
W	W	R	R	W	N	Reactive, 1:8 dilution, 8 dils.
N (rough)	W	R	R	R	N	Reactive, 1:16 dilution, 16 dils.
W	N	N	N	N	N	Weakly reactive, undiluted, 1 dil

TEST PROCEDURES FOR CEREBROSPINAL FLUID (VDRL-CSF)

Preparing the Sensitized 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.

Qualitative Test

Performing the Test

1. Slide flocculation tests for syphilis are affected by temperature. For reliable and reproducible test results, the VDRL-CSF sensitized antigen suspension, controls and spinal fluid specimens must be at room temperature (23°C - 29°C; 73°F - 85°F), when tested.
2. Place 50 µl of spinal fluid into one cavity of a Kline concavity slide or 14 mm circle of a flat slide, using a safety pipetting device.
3. Gently resuspend the sensitized antigen suspension.
4. Holding the VDRL-CSF sensitized antigen suspension dispensing needle (21- or 22-gauge) and syringe in a vertical position, dispense several drops to clear the needle of air. Then add exactly 1 free-falling drop (10 µl) of sensitized antigen suspension to each slide concavity that contains spinal fluid.

5. Place the slide on the mechanical rotator. Rotate the slide for 8 minutes at 180 ± 2 rpm. When performing the test in a dry climate, cover the slides with a moist humidifying cover during rotation to prevent excessive evaporation.
6. Immediately after rotating, remove the slide from the rotator and read the test.
7. Test quantitatively, to an endpoint, all spinal fluid specimens that are reactive in the qualitative VDRL-CSF test.

Reading and Reporting Results

1. Read test results microscopically, using 10X oculars and a 10X objective.
2. Report the results as follows.

Reading

Definite clumping of any degree
 No clumping or very slight roughness

Report

Reactive (R)
 Nonreactive (N)

Quantitative Test

1. Test each specimen undiluted and in 1:2, 1:4, and 1:8 dilutions (see Table 8:2).
2. Place 50 μ l of 0.9% saline into concavities numbered 2 through 4 of a Kline concavity slide or circles numbered 2 through 4 if using a flat 14 mm circle slide.
3. Using a safety pipetting device, place 50 μ l of spinal fluid in concavity or circle 1 and 50 μ l of spinal fluid in concavity or circle 2.
4. Mix the saline and spinal fluid in circle 2 by drawing the mixture up and down in the safety pipette eight times. Avoid forming bubbles.
5. Transfer 50 μ l from circle 2 (1:2) to circle 3, and mix.
6. Transfer 50 μ l from circle 3 to circle 4, mix, and then discard the last 50 μ l.

NOTE: Additional serial dilutions may be needed for strongly reactive spinal fluid specimens.

7. Holding the VDRL-CSF sensitized antigen suspension dispensing needle (21- or 22-gauge) and syringe in a vertical position, dispense several drops to clear the needle of air. Then add exactly 1 free-falling drop (10 μ l) of sensitized antigen suspension to each concavity or circle.

8. Place the slide on the mechanical rotator. Rotate the slide for 8 minutes at 180 ±2 rpm. When performing the test in a dry climate, cover the slides with a moist humidifying cover during rotation to prevent excessive evaporation.
9. Immediately after rotating the slide, remove it from the rotator and read the test.
10. After completing the day's testing, discard the remaining suspension and clean the needle. Remove needle from syringe after cleaning.

Reading and Reporting Results

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 as in Table 8:2.

Table 8:2. Reporting Quantitative CSF Results

	Serum 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.

CALCULATIONS⁹ AND RANGES

Sensitivity

$$\frac{TP}{TP + FN}$$

Specificity

$$\frac{TN}{TN + FP}$$

TP = True Positive, the number of individuals who test reactive that actually have syphilis

FN = False Negative, the number of persons who test nonreactive that have syphilis

TN = True Negative, the number of persons who test nonreactive that do not have syphilis

FP = False Positive, the number of persons who test reactive that do not have syphilis

Table 8:3. Performance of Serum VDRL by Stage of Untreated Syphilis*

Stage	Sensitivity		Specificity
	Percent	Range	PercentRange
Primary	78	74-87	
Secondary	100		

Latent	95	88-100		
Late	71	37-94		
Nonsyphilis			98	96-99

*Results of CDC studies

Table 8:4 Performance of the VDRL CSF Test¹⁰

	Sensitivity	Specificity	Predictive Value Positive	
Negative				
VDRL CSF	50%	99.8%	83.3%	99.1%

INTERPRETATION OF RESULTS

Serum

1. The VDRL test is an aid in the diagnosis of syphilis. To diagnose syphilis, clinicians combine VDRL test results with the results of other serologic tests, darkfield examinations, clinical signs and symptoms, and risk factors. Without other supporting evidence for a diagnosis of syphilis, a reactive VDRL may be unrelated to *T. pallidum* infection. The predictive value of a reactive VDRL in the serologic diagnosis of syphilis is increased when combined with a reactive treponemal test, such as the fluorescent treponemal antibody absorption (FTA-ABS) test or the microhemagglutination assay for antibodies to *T. pallidum* (MHA-TP).
2. A reactive VDRL test may indicate past or present infection with a pathogenic treponeme; however, the result may also be a false-positive reaction. False-positive reactions can result from laboratory error and also from serum antibodies that are unrelated to syphilis infection. Laboratory errors are detected by a nonreactive VDRL with a second serum specimen. False-positive VDRL tests that result from infection with nontreponemal diseases or other disease conditions are identified by an accompanying nonreactive treponemal test.
3. A nonreactive VDRL test without clinical evidence of syphilis may indicate no current or past infection, an effectively treated infection, or occasionally, long standing infection. A nonreactive VDRL test with clinical evidence of syphilis can be seen in early primary syphilis, in secondary syphilis as a result of the prozone reaction, and in some cases of late syphilis. If a prozone reaction is suspected, the test should be repeated with the patient's serum diluted 1:16 before

determining that the serum sample is nonreactive. A nonreactive VDRL result does not rule out an incubating syphilis infection.

4. When the quantitative VDRL test is performed on patients with syphilis, a fourfold rise in titer, e.g. 1:4 to 1:16, on a repeat specimen may indicate infection, a reinfection, or a treatment failure. If the increase in titer occurs over a short period of time, for example two weeks, the immunologic response is most likely due to treponemal response rather than a biologic false-positive lipoprotein response. A fourfold decrease in titer, e.g. 1:8 to 1:2, 6 to 12 months following treatment for early syphilis usually suggests that therapy was adequate.¹¹
5. All reactive qualitative VDRL tests should be quantitated to an endpoint, and the endpoint titer should be reported. Unusually high VDRL titers may be seen with concurrent HIV-1 infection. Unusually high false-positive titers may be seen in serum from some patients with lymphomas.

Cerebrospinal Fluid

1. A reactive VDRL test on CSF, free of blood or other contaminants, usually suggests past or present syphilis infection of the central nervous system. A biologic false-positive VDRL test result for syphilis is rare in spinal fluid.
2. A nonreactive VDRL test on CSF may indicate that the patient does not have neurosyphilis. However, a negative result may occur in some serum from neurosyphilis patients.¹⁰ Nonspecific changes in the CSF of neurosyphilis patients include an increase in total protein and an increased cell count. However, pleocytosis is also characteristic of HIV infection.

ACCEPTABLE VARIATIONS

1. If larger quantities of antigen suspension are needed, prepare a double volume of VDRL antigen suspension by doubling quantities of antigen and saline. Use a 10-ml pipette to deliver the 8.2-ml volume of saline. Test these suspensions with control serum and pool those with satisfactory reactivity.
2. A calibrated 18-gauge needle without bevel is the most satisfactory tool for delivering 17 μ l (1/60ml) of antigen suspension; however, any device that consistently and accurately delivers 17 μ l can be used.
3. The flat slide used for the VDRL serum test may be used for the VDRL-CSF test.

SOURCES OF ERROR

1. Reactivity is decreased if the temperature of the testing area, specimens, or reagents is less than 23°C (73°F) test; test reactivity is increased if the temperature is greater than 29°C (85°F).

2. If hemolyzed, contaminated, or extremely turbid serum specimens are tested, the reliability of test results will be questionable.
3. If the procedure for preparing the antigen suspension is not strictly followed, the suspension may not be of standard reactivity.
4. If outdated or inadequately tested antigen is used, test results may be erroneous.
5. If the specimens and antigen are not rotated for the correct speed and length of time, test results may be incorrect.
6. If the antigen suspension is frequently forced through the syringe and needle, the suspension may lose reactivity.

TEST LIMITATIONS

1. A prozone reaction may occur. In a prozone reaction, reactivity with undiluted serum is inhibited. The prozone phenomenon may be suspected when a specimen produces only a weakly reactive or a rough nonreactive result in a qualitative test. Therefore, all specimens producing weakly reactive or rough, nonreactive results in the qualitative test should be retested by using the quantitative procedure. When a reactive result is obtained on a dilution of a serum that produced only a weakly reactive or rough nonreactive result before dilution, report the test as reactive and include the quantitative titer (see Table 8:1). In addition, a specimen should be tested for the prozone phenomenon when the clinician suspects syphilis, but the qualitative VDRL is nonreactive.
2. VDRL antigen suspension for the VDRL test on serum must be prepared fresh each day of testing. The VDRL-sensitized antigen suspension for the VDRL-CSF test is good for only 2 hours after preparation.
3. The VDRL-CSF test should be performed only when patient's serum treponemal test result is reactive.
4. Plasma cannot be used for the VDRL test.
5. Biological false-positive reactions can occur with cardiolipin antigens, mainly in specimens from persons who abuse drugs; who have diseases such as lupus erythematosus, mononucleosis, malaria, leprosy or viral pneumonia; or who have recently been immunized.
6. The VDRL may be reactive in persons from areas where yaws is endemic. As a rule, residual titers from these infections will be $<1:8$.^{12,13}
7. Nontreponemal test titers of persons treated in latent or late stages of syphilis or who have become reinfected 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.⁹

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