YALE-NEW HAVEN HOSPITAL	TITLE: **VDRL Slide Test** **SOFT codes: Serum -VDRLG, VDRLN** **CSF- CSFVD, CVDSN**		**DEPT OF LAB MEDICINE** **Policy and Procedure Manual** **DOCUMENT # IMM 176** **Page 1 of 10**
WRITTEN BY: Voula Golfis MT (ASCP) Penny Smith, MT(ASCP)	**EFFECTIVE DATE:** November 2, 2011	**REVISION:** December 13, 2012 Penny Smith	**SUPERCEDES:** VDRL Slide Test (IMM 103) Reporting of Reactive VDRL/FTA (IMM 104)

I. Principle of the Assay:

VDRL antigen is a cardiolipin-lecithin antigen. Spinal fluid or inactivated serum and VDRL antigen emulsion, prepared from VDRL antigen and VDRL buffered saline, are mixed with an aid of a rotating machine for a prescribed period of time. During the mixing time, the cardiolipin-lecithin coated cholesterol particles flocculate in the presence of an antibody like substance (reagin) that is present in the sera from syphilitic persons and, occasionally, in the sera of persons with other acute and chronic conditions.

Qualitative testing is performed first on all specimens. Each serum that produces a Reactive, Weakly Reactive or "Rough" Non-Reactive result is retested quantitatively to an end point titer.

A VDRL can be requested as a VDRL with reflex to titer (Soft code VDRLN) or a VDRL with reflex to titer and FTA (Soft code VDRLG).Any orders received that do not specify which VDRL reflex algorithm to perform will VDRL with reflex to titer and FTA.

RPR (Rapid Plasma Reagin) screening test for Syphilis is not offered by this laboratory. Request for RPR testing will be given the equivalent VDRL assay. VDRL with reflex to titer and FTA will be ordered (VDRLG).

II. Specimen Collection:

A. Blood

Clotted blood; Serum; Red top tube, spin at 3000 rpm for 15 minutes; specimen should be absolutely free of cells. Serum samples may be stored refrigerated (2-8°C) up to 7 days. If the sample cannot be tested in this period, store frozen at -20°C for up to 6 months. Repeat freezing and thawing should be avoided. Grossly hemoylzed samples should be rejected.

Standard Aliquot volume = 250 uL
Minimum Aliquot volume = 100 uL
Grossly Hemolyzed: Reject

Grossly Lipemic: Spin at 10,000 rpm for 20 min.

B. CSF

Spin at 3000 rpm for 15 minutes to spin out any cells or debris. Store at 2°–8°C. Freeze an aliquot at -20°C for 6 weeks. Spinal fluids may that are visibly contaminated or contain gross blood are unsatisfactory for testing. Perform the test and enter one of the following suggested result comments:

@1CTP (Blood Present in CSF)
free text comment: Sample Xanthochromic
free text comment: Sample contaminated

Standard Aliquot volume = 250 uL
Minimum Aliquot volume = 100 uL
Grossly Hemolyzed: Comment @1CTP

III. Reagents

A. BD VDRL Antigen Kit: Cardinal Healthcare (Cat. No. 4340765)

1. Antigen for this test is a colorless, alcoholic solution containing 0.03% cardiolipin, 0.9% cholesterol, and sufficient purified lecithin to produce standard reactivity. Antigen is dispensed in hermetically sealed glass ampules and should be stored in the dark at room temperature.

2. VDRL Buffered Saline: This contains 1% sodium chloride, pH 6.0 ±0.1. Once opened, this is stored at 2°–8°C.

B. 0.9% Saline: (YNHH Pharmacy).

C. 10% Saline: Add 10 g of dry sodium chloride (A.C.S.) to 100 mL of Clinical Laboratory Reagent Water (CLRW). Store at 4°C. Stable for 6 months.

IV. Supplies and Equipment

A. Rotating Machine: Adjustable to 180 rpm, circumscribing a circle 3/4 inch in diameter on a horizontal plane. (Fisher).

B. Ringmaker: To make paraffin rings approximately 14 mM in diameter (Eberbach).

C. Slide Holder: For 2 × 3 inch microscope slides (YNHH).

D. Hypodermic Needles: Without bevels. (Becton-Dickinson).

1. For Serum Test: 18 gauge.

E. For Spinal Fluid test: 10 ul MLA Pipet.
50 ul MLA Pipet.
Pipet Tips (MLA)

F. BD Dispenstirs (Cardinal Health cat#B6940-21)

G. Glassware

1. Slides, 2 × 3 inch, with 12 rings approximately 14 mM in diameter (Baxter).

2. Syringe, Luer-type, 1.0 or 2 mL (Micro-Mate, Popper and Sons).

3. Bottles, 25 mL round glass - stoppered, Ehrlenmeyer flask.

4. Paraffin block (YNHH).

5. Glass pipets: 5 ml (Fisher)
1 ml (Fisher)

V. Controls

A. BD Difco VDRL Controls, Cardinal Healthcare (Cat. No. 3520-32): Reactive, Weakly Reactive, Nonreactive. Lyophilized. Stored at 2°–8°C. Stable until expiration date on bottle.

B. Reconstituted controls: once reconstituted, aliquots are made and stored at -70°C. Use within one month of rehydration.

C. Routine Use of Control Serum — Daily

1. Run Reactive, Weakly Reactive, and Nonreactive control each day the VDRL test is performed. Remove a set of controls from the freezer, thaw, and mix thoroughly. Inactivate at 56°C for 30 minutes along with the patient samples. If testing is not performed within 4 hours, inactivate for an additional 10 minutes at 56°C. The control sera must be at room temperature when tested.

2. Check the reactivity of the test Antigen Suspension with the control sera as described in this technique (Section VI).

3. Do not use an Antigen Suspension that does not reproduce the established reactivity pattern of the control sera. If necessary, prepare a new Antigen Suspension.

VI. Methodology

A. VDRL Slide Qualitative Test on Serum

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

1. Heat Inactivate: Inactivate QC and Serum samples at 56°C for 30 minutes. If testing is not performed within 4 hours, inactivate for an additional 10 minutes at 56°C. The control sera and patient sera must be at room temperature when tested.

2. Preparation of Antigen Suspension
   a. Pipette 0.4 mL of buffered saline to the bottom of a 30 mL, round, glass-stoppered bottle or 25 ml round glass - stoppered, Ehrlenmeyer flask.

   b. Add 0.5 mL of antigen (from the lower half of a 1.0 mL pipette 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 0.5 mL of antigen. Pipette tip should remain in upper third of bottle and rotation should not be vigorous enough to splash saline onto pipette. Proper speed of rotation is obtained when the center of the bottle circumscribes a 2-inch diameter circle approximately three times per second.**

   c. Expel the last drop of antigen from the pipette, using a pipette bulb, without touching the pipette to the saline.

   d. Continue rotation of bottle for 10 seconds.

   e. Add 4.1 mL of buffered saline using a 5 mL pipette.

   f. Place the top back onto the bottle and shake from bottom to top and back approximately 30 times in 10 seconds.

   g. The antigen suspension is ready for use and may be used during one day.

h. Mix the antigen suspension gently each time it is used. Do not mix suspension by forcing back and forth through the syringe and needle since this may cause breakdown of particles and a loss of reactivity.

3. Testing Accuracy of Delivery Needles

a. Dispense the antigen suspension from a syringe fitted with an 18-gauge needle without a bevel which will deliver 60 drops ±2 drops (30 drops $\pm$ 1 drop in 0.5 mL) of antigen suspension per milliliter when the syringe and needle are held vertically.

b. Adjust needles not meeting these specifications to deliver the correct volumes before being used. (See "Preparation and Calibration of Needles for Slide Flocculation Tests," *Manual of Tests for Syphilis,* kept on the VDRL testing bench.)

4. Test the Revolutions per Minute (RPM) of the rotator

a. Verify the RPM by counting the revolutions for 15 seconds
b. Multiply the number by 4 to determine the revolutions per minutes
c. RPM should be 180 +/- 2 rpm
d. Record result in the VDRL logbook.

5. Preliminary Testing of Antigen Suspension

a. Reactions with control sera should reproduce the established reactivity pattern. The Nonreactive serum should show complete dispersion of antigen particles.
b. Do not use an unsatisfactory antigen suspension or pool of antigen suspensions.

NOTE: **Control sera of graded reactivity (Reactive, Weaklyly Reactive, and Nonreactive) are always included during a testing period to insure proper reactivity of antigen suspension at time tests are performed.**

c. Check controls for established reactivity pattern. Any controls not showing proper reactivity should be reported to a supervisor.

d. Using a dispenstir, place 1 drop (50 µL) of heated serum in one paraffin-ring.

e. Add one drop (1/60 mL) of antigen suspension onto each serum with an 18-gauge needle and a syringe.

f. Rotate slides for 4 minutes. (Mechanical rotators that circumscribe a 3/4 inch diameter circle should be set at 180 rpm.)g.Read tests microscopically with a 10× ocular and a 10× objective immediately after rotation.

h. Report the results as follows:

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

i. 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. This prozone phenomenon may be so pronounced that only a Weakly Reactive or "rough" Nonreactive result is produced in the qualitative test by a serum that 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 by 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 "rough" Nonreactive result before dilution, report the test as Reactive and include the quantitative titer.

j. Retest, quantitatively, to an endpoint titer, all sera that produce Reactive, Weakly Reactive, or "rough" Nonreactive results in the qualitative VDRL Slide Test (See Section D). The initial dilutions of the serum to be tested are: undiluted (1:1), 1:2, 1:4, 1:8, 1:16, and 1:32. Two serum quantitative tests may be performed on one slide. For titers greater than 1:32, retest <u>all dilutions</u> up to 12 wells.

k. Record all the following pertinent information each day in the VDRL logbook kept on the VDRL testing bench:

Antigen lot # and expiration date

Buffered Saline lot # and expiration date
Control lots # and expiration date
Room temperature
Rotator speed
Needle calibration
Control results
pH of Buffered Saline
pH of 6.0 pH standard

C. VDRL Slide Tests on Spinal Fluid

1. Preparation of the Spinal Fluid
   Centrifuge and decant each spinal fluid. The spinal fluid is tested without preliminary heating.

2. Preparation of the "Sensitized Antigen Suspension"
   a. Prepare antigen suspension as described for the VDRL Slide Tests.

   b. Add one part (1 mL) of 10% saline to one part (1 mL) of VDRL Slide Test suspension.

   c. Mix by gently rotating the bottle or inverting the tube; allow to stand at least 5 minutes but not more than 2 hours before use.

3. VDRL Controls for CSF Testing
   a. Reactive control serum used for VDRL-CSF are run against current controls to establish working dilutions of reactivity: Reactive, Reactive Minimal, and Nonreactive.

   b. Refer to VDS workbook for lot number in current use and working dilutions.

   c. Dilutions are made with 0.9% saline solution.

   d. Remove vial from freezer; thaw; mix well by inversion.

   e. Serum is delivered using a 50 µL MLA pipette.

   f. Rinse tip by emptying and filling pipette at least six times. Avoid foaming.

   g. Vortex dilutions 5 seconds to mix.
   h. The controls are tested without preliminary heating.

i. Any controls not falling within the established range of reactivity require a supervisor's notification.

4. Testing

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

a. Pipette 50 µL of each control into one concavity of an agglutination slide.

b. Pipette 50 µL of spinal fluid into one concavity of an agglutination slide.

c. Add 10 µL of sensitized antigen suspension to each spinal fluid with a 10 µL MLA pipette.

d. Rotate slides for 8 minutes on a mechanical rotator at 180 rpm.

e. Read tests microscopically, with a 10× ocular and a 10× objective, immediately after rotation.

f. Report the patient results as follows:

Reading	Report
Definite clumping of any degree	Reactive (R)
No clumping or very slight roughness	Nonreactive (N)

g. Record VDS Control Readings in VDRL logbook.

D. Quantification of VDRL (CSF and Serum)

1. In testing each serum or CSF, add 50 µL 0.9% saline to all wells used for that sample except the first well. (Number of wells is dependent on the degree of reactivity: 6 wells for strong reactors, 3 wells for less intense reactors).

2. Add 50 µL of patient serum or CSF to the first and second wells.

3. Mix the contents of the second well by aspiration into and expulsion from the pipette tip six times. Use the same pipette tip to transfer 50 µL of the contents into the next well. Repeat this step as often as required by the number of wells.

4. Discard 50 µL from the last well.

5. **For Serum** - add one drop of VDRL Antigen to each well for serum using a 10 µL MLA pipette.

   For CSF - add 10 µL of VDS antigen to each well for CSF. Using a 10 µL MLA pipette

6. **For Serum** - Rotate the slide at 180 rpm for 4 minutes.

   For CSF - Rotate the slide at 180 rpm for 8 minutes.

7. Read under microscope.

   For serum: The titer is equal to the last well that is reactive. If the VDRL serum is weakly-reactive at 1:1, titer is reported out as weakly-reactive.

   For CSF: Report out last dilution with any reactivity.

VII. Interpretation Reporting of results:

1. Serums are reported as Reactive, Weak Reactive or Non-Reactive. CSF's are report as Reactive, Minimal Reactive or Non-Reactive.

2. Do not report Screen Results until after the titer is performed.

3. Titer end points are determined by the last Reactive (not Weak or Minimal reactive) dilution.

4. VDRL titers that are Reactive only in the undiluted well are reported as See Comment. Add the following comment: Reactive, Undiluted
5. Report Weak Reactive serum screen results as follows:

   - Results Weak Reactive at only the 1:1 dilution are reported as Weak Reactive for both screen and titer.

   - Results Reactive 1:2 or greater- report Screen as Reactive and report titer value.

Use the following chart as a guideline:

Undiluted 1:1	1:2	1:4	1:8	1:16	1:32	Screen Result	Titer Result
R	W	N	N	N	N	Reactive	Reactive, Undiluted
R	R	W	N	N	N	Reactive	1:2
R	R	R	W	N	N	Reactive	1:4
W	W	R	R	W	N	Reactive	1:8
N (rough)	W	R	R	R	N	Reactive	1:16
W	N	N	N	N	N	Weak Reactive	Weak Reactive

For Serum: Batch Result by Tasklist. Result **VDRLO and VDRL.** Result Reactive, Weakly Reactive and titers first, then Nonreactive. Refer to the Immunology Soft Manual (Doc#120).

For CSF: Enter result of reactive or non-reactive in Result Entry using the keypad.

VIII. Reporting of Significant Findings:

Serum : All Reactive and Weakly Reactive VDRL samples are written in the Reactive VDRL book. If the sample does not require FTA confirmation, the book is given to an LA by the VDRL tech for reporting of Significant Findings. If an FTA confirmation is to be performed, the Significant Findings report will be held until after the FTA is completed. The FTA tech will then give the book to an LA.

CSF : All Reactive and Minimal reactive samples are written in the in the Reactive VDRL book. The book is given to an LA by the VDRL tech for reporting Significant Findings.

A Laboratory Form of Significant Findings (Form OL-15C) is completed by the Laboratory Associate on all reactive VDRLs. This report is sent to the Connecticut Department of Public Health, Infectious Diseases Division, a copy to the Local Health Department where the patient resides and a copy filed in the Immunology laboratory.

IX. References:

Manual of Tests for Syphilis, American Public Health Association, 1990.

X. Appendix

IMM 176-A VDRL Checklist log
IMM 176-B Reactive VDRL/FTA log

Document Author
Voula Golfis MT (ASCP)
Penny Smith, MT(ASCP)
November 2, 2011

Signature Approval for Annual Review
Name: VDRL Slide Test – Serum and CSF
Document #: IMM 176

Name (Print)	Title	Signature	Date of Review	Revision Page and Section # (Use Procedure Review Log to document staff review)	Issue Date for Training if Applicable	Effective Date for Use
TEODORICO LEE	LAB MANAGER	Teodorico Lee	11/2/12	NEW		11/2/11
BRIAN SMITH	LAB DIRECTOR	Brian R. Smith MD	11/2/12	NEW		11/2/11
TEODORICO LEE	LAB MANAGER	Teodorico Lee	12/13/12	**Section I Principle:** Changed procedure for RPR requests from calling the physician to using the default order of VDRLG. **Section 3A Reagents:** Removed the requirement of having to measure the pH of the buffer. **Section VII Interpretation and Reporting of Results:** Added more detail to, including a chart, to the reporting section to clarify reporting of Weak reactive and reactive undiluted specimens.		12/13/12
BRIAN SMITH	LAB DIRECTOR		12/13/12			