RAPID PLASMA REAGIN 18-MM CIRCLE CARD TEST

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Rapid Plasma Reagin – NHANES 2001-2002

0. Public Release Data Set Information

This document details the Lab Protocol for NHANES 2001-2002 data.

A list of the released analytes follows:

Lab	Analyte	SAS Label	Description
l36_b	LBDSY3	Syphilis RPR Titer Level	Syphilis RPR Titer

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The rapid plasma reagin (RPR) 18-mm circle card test is a macroscopic, nontreponemal flocculation card test used to screen for syphilis (1 -4). The antigen is prepared from a modified Venereal Disease Research Laboratory (VDRL) antigen suspension containing choline chloride to eliminate the need to heat inactivate serum, ethylenediaminetetraacetic acid (EDTA) to enhance the stability of the suspension, and finely divided charcoal particles as a visualizing agent. In the test, the RPR antigen is mixed with unheated or heated serum or with unheated plasma on a plastic-coated card. The RPR test measures 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 (5,6). The anti lipoidal antibodies are antibodies that are produced not only as a consequence of syphilis and other treponemal diseases, but also in response to nontreponemal diseases of an acute and chronic nature in which tissue damage occurs (7). If antibodies are present, they combine with the lipid particles of the antigen, causing them to agglutinate. The charcoal particles coagglutinate with the antibodies and show up as black clumps against the white card. If antibodies are not present, the test mixture is uniformly gray. The test can be purchased in kit form or in component parts from many commercial sources. Without some other evidence for the diagnosis of syphilis, a reactive nontreponemal test does not confirm T. pallidum infection.(8).

2. SAFETY PRECAUTIONS

The risk of infection due to an occupational exposure to blood depends upon the prevalence of blood-borne pathogens in the population supplying the blood specimens, the probability of infection given a particular type of exposure to a blood-borne pathogen, and the frequency of exposures (9,10).

T. pallidum is present in circulating blood during primary and secondary syphilis. The minimum number (LD50) of T. pallidum organisms needed to infect by subcutaneous injection is 23 (11). The concentration of T. pallidum in patients' blood during early syphilis, however, has not been determined. The ability of blood inoculated with T. pallidum to infect animals is reduced by refrigerated storage (12,13). Although multiple instances of transmission of T. pallidum due to transfusion of an infected donor's blood were reported prior to the introduction of penicillin for treatment of syphilis and of refrigeration for blood storage (12). Subsequent reports have been rare (12,13). Infection of a health care or laboratory worker following exposure to T. pallidum infected blood has, apparently, not been reported.

Authoritative sources focus attention on infection with hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) as the principal concerns associated with exposure to blood (10,15-18). The prevalence of these infections varies greatly among patient populations tested for T. pallidum infection. HBV infection is most common. HBV viremia is indicated by tests for HBV surface antigen (HBsAg) in serum. Prevalence of anti-HBsAg, from published studies of patients in hospitals and emergency rooms cited in a recent review, ranged from 0.9% to 6% (9,19-22). Unlike initial HBV infection, in which only a minority of individuals continues to be viremic, initial HCV and HIV infections lead to persistent viremia in most individuals. Consequently, serum antibody to HCV and HIV are indicators of potential infectiousness. Seroprevalences of antibody to HCV in studies of patients in hospitals and emergency rooms cited in a recent review ranged from 2% to 18% (18,21-24). HIV prevalence ranged from 0.1% to 5.6% in patients enrolled in a national hospital surveillance system (9,25). All

three infections are more common among patients at increased risk for syphilis, especially patients with a history of illegal drug use. For example, seroprevalences of antibody to HCV were 10% among non-drug-using attendees at sexually transmitted diseases clinics and 60% among injection-drug users (26-28).

While infections with HBV (27,29) and HIV (17,30-32) can occur with skin and mucus membrane exposures to blood, needle stick and percutaneous injury with blood-coated sharp objects are the principal sources of laboratory associated acquisition of these agents. The risk of infection following exposure to blood from an infected patient is greatest for HBV, except for exposed individuals who are immune due to prior HBV infection or vaccination. The risk is highest if the source individual is HBSAG-positive (27,33-35) and is positive for envelope (E) antigen. A vaccine to prevent HBV infection has been available since 1982 and is strongly recommended for health care workers with potential exposures to blood or other body fluids (33,36,37). Individuals with anti-HBV antibody from vaccination or prior infection are considered to be immune to HBV infection.

The risk of HCV infection due to needle stick exposure to blood from an individual with antibody to HCV was 10% in one study (27,38,39) but HCV does not appear to survive long in serum held at room temperature (27,40). A vaccine is not yet available to immunize against HCV infection. Repeated infection with HCV appears to be possible in spite of detectable serum anti-HCV antibody, although the significance of reinfection is unknown (26,41,42).

The risk of infection with HIV following a single needle stick exposure to blood from a patient known to be infected with HIV is approximately 0.3% (9). The risks following mucous membrane or skin exposures to HIV-infected blood average approximately 0.1% and <0.1%, respectively (17,30,32,43). The lower rate of transmission for HIV than for HBV or HCV probably reflects a lower concentration of HIV in the blood of infected persons. A vaccine is not available to immunize against HIV infection. The frequency and significance of repeated exposures of individuals with prior anti-HIV antibody is unknown.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

   a. Each shipment of specimens received from the NHANES III mobile unit contains a corresponding transmittal sheet and an ASCII data file (KOUTPUT.TXT) on a 3 ½ " high density floppy diskette. The data file, containing the specimen ID, collection date, and type of sample (i.e., whole blood, serum, plasma) is checked against the information on the transmittal sheet and specimen label prior to the assay.

   b. After the data is calculated and the final values are approved by the reviewing supervisor for release, all results are entered onto the NHANES diskettes by using the program provided by National Center for Health Statistics (NCHS).

   c. After the results are entered on diskettes, back up copies are made and stored in locked areas.

   d. The original diskette containing analytical results are mailed to NCHS.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

   a. No special instruction such as special diet or fasting is necessary.

   b. Fresh serum samples are the specimens of choice for the RPR. Serum specimens may

be collected using regular red-top or serum separator Vacutainers. Specimens are allowed to clot at room temp and centrifuged. Transfer serum to 2-mL polypropylene screw-capped vials. Freeze at ≤-20°C. Each week, batches of frozen serum samples are placed in a Styrofoam-insulated shipping container with dry ice and sent to the laboratory by an overnight courier.

e. Serum specimens are stable up to 72 hours at 4° - 8°C. For longer periods, store the serum at ≤-20°C in glass or plastic vials, as long as the vials are tightly sealed to prevent desiccation of the sample.

f. Excessively hemolyzed, contaminated, or lipemic sera may give aberrant results and should not be used. A specimen is too hemolyzed for testing when printed material cannot be read through it. Heat-inactivated sera may be used (56°C for 30 minutes). Excessive inactivation time or temperature may increase nonspecific background activity which could result in equivocal results.

g. The optimal amount of serum is 0.5 mL to 1.0 mL. Specimen volumes of less than 0.4 mL are not acceptable.

h. Avoid repeated freeze-thawing cycles, which may compromise specimen integrity.

i. Specimens should generally arrive frozen.

j. Residual samples are frozen at ≤-20°C.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

a. Instrumentation

(1) Micropipettes to deliver 50 µL.

(2) Mechanical rotator, fixed-speed or adjustable to 100 ±2 rpm, circumscribing a circle 3/4-inch in diameter in a horizontal plane.

(3) High-intensity incandescent lamp.

b. Other Materials

(1) Disposable, calibrated 20-gauge needle without bevel, silicone treated, to deliver 17 µL per drop.
(2) Plastic antigen dispensing bottle, I dram.

(3) Plastic-coated RPR cards, with 10 circles, each approximately 18 mm in diameter. Store cards at room temperature.

(4) Dispensters, a disposable (plastic) dispensing-stirring device that delivers 50 µL.

(5) Humidifying cover.

(6) One-mL and 5-mL serologic pipettes

(7) Test-tube to hold 2-mL and 5-mL volumes

(8) Latex gloves, safety glasses, and protective clothing.

(9) Discard container and disinfectant.

c. Reagent Preparation

Each Serodia TP-PA kit contains enough reagents to test 92 samples and the controls. Reagents should be mixed gently to avoid possible deterioration of the antigen-carrier complex. Reagents are stable until the expiration date printed on the label. All reagents should be stored at 4° - 8°C.

(1) RPR antigen suspension.
Stabilized combination of 0.003% cardiolipin, 0.020-0.022% lecithin, 0.09% cholesterol, 10% choline chloride, 0.0125M EDTA, 0.01875% charcoal, 0.01M Na_2HP0_4, 0.01M KH_2P0_4, 0.1% thimerosal in distilled water (1). The antigen suspension is packaged in ampules. Store unopened ampules at 2° to 8°C; do not store the antigen in bright sunlight or in temperatures above 29°C; do not freeze. An unopened ampule of antigen is stable up to the expiration date.

(2) Control serum samples.
Liquid reactive (R), minimally reactive (Rm), and nonreactive (N) control serum specimens of graded reactivity. If quantitative tests are to be performed, a control serum that can be titered to at least a 1:4 dilution should be used. Store control cards or serum samples according to the manufacturer's directions

(3) 0.9% Saline.
Prepare a 2% solution of saline, by diluting 0.9 grams of sodium chloride in 100 mL of distilled water.

(4) Diluent (liquid).
Aqueous solution of 2% normal human serum made by diluting human serum 1:50 in 0.9% NaCl. Used to dilute serum specimens at dilutions of 1:32 and above.

d. Preparation of Control Serum Samples

(1) Positive Control Serum
Prepared from human serum samples containing antibodies to Treponema pallidum. Serum is ready to use. Bring to room temp before use.

(2) Reactive Minimal Control Serum
Prepared from human serum samples containing antibodies to T. pallidum. Serum should be nonreactive at 1:2 dilution, but reactive at 1:1 dilution. Serum is ready to use. Bring to room temp before use.

(2) Nonreactive Control Serum
Prepared from human serum samples free of T. pallidum antibodies. Serum is ready to use. Bring to room temp before use.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

a. Working Standards

The reactive control is used to determine level of reactivity of the test for lot to lot comparison and as an indication of whether reagents are deteriorating. Reactive control should have a titer of 1:8 ± 1 doubling dilution.

b. Pipettors and Tips

With the pipettors currently available, the measurement of small serum volumes is routine. Most manufacturers include in the specifications of the 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 pipette manufacturer are 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 of the substitute pipette tips in their system. Commercial kits to check the 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 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 nor do they substitute for an annual verification and repair by a company qualified to do this.

c. Needles

(1) Check the calibrated needle each time a new needle is used, when needle has been dropped or wiped, or when the control pattern is not met to ensure the delivery of the correct volume of antigen suspension (60 drops ± 2 drops per mL; 17 µL per drop).

(2) Place the needle on a 1-mL syringe or on a 2-mL pipette. Fill the syringe or pipette with RPR antigen suspension. Holding the syringe or pipette in a vertical position, count the number of drops delivered in 0.5 mL. The needle is correctly calibrated if 30 drops $\pm$ 1 drop is delivered in 0.5 mL.

(3) Replace the needle if it does not meet this specification. Be sure to test the calibration of the replacement needle.

d. 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 place 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 25. The rotator's speed should be calibrated each day it is 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.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. Preliminaries

(1) Bring all reagents and serum samples to room temp before beginning test.

(2) The reactive control should be titered every day that samples are tested. The reactive minimal and negative controls should also be run each day testing is done.

(3) To prepare antigen for testing, attach the hub of the dispensing needle to the fitting on the plastic dispensing bottle. Shake the antigen ampule to resuspend the particles. Open the ampule. Squeeze the dispensing bottle to collapse it. Insert the needle into the ampule and withdraw all the antigen suspension into the dispensing bottle.

b. Sample Preparation

All samples are initially tested undiluted.

c. Operation of Assay Procedure

Qualitative Test Procedure

(1) Place 50 µL of serum or plasma onto a 18-mm circle of the RPR test card, using a disposable Dispenstir or a safety pipetting device.

(2) Using the inverted Dispenstir (closed end) or flat toothpicks, spread the serum or plasma to fill the entire circle. Do not spread the specimen beyond the confines of the circle.

(3) Gently shake the antigen dispensing bottle to resuspend the particles.

(4) Holding the dispensing bottle and needle 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 or plasma. Do not mix (2, 3).

(5) Place the card on the mechanical rotator under a humidifying cover. Rotate the card for 8 minutes at 100 ± 2 rpm.

(6) Immediately remove the card from the rotator; briefly rotate and tilt the card by hand (three or four to-and-fro motions) to aid in differentiating nonreactive from minimally reactive results.

(7) Perform the quantitative test on serum specimens showing any degree of reactivity (clumping) or "roughness."

Quantitative Test (3)

(1) Dilute to an endpoint titer all serum specimens with rough nonreactive results in the qualitative test. Test each specimen undiluted (1:1), and in 1:2, 1:4, 1:8, and 1:16 dilutions (see Fig. 1).

(2) Place 50 µL of 0.9% saline in circles numbered 2 through 5. Do not spread the saline.

(3) Using a safety pipette device, place 50 µL of serum in circle 1 and 50 µL of serum in circle 2 (Fig. 1).

(4) Mix the saline and the serum in circle 2 by drawing the mixture up and down in a safety pipette eight times. Avoid forming bubbles.

(5) Transfer 50 µL from circle 2 (1:2) to circle 3, and mix.

(6) Transfer 50 µL from circle 3 (1:4) to circle 4, and mix.

(7) Transfer 50 µL from circle 4 (1:8) to circle 5 (1:16), mix, and then discard the last 50 µL.

(8) Using the broad end of a clean Dispenstir, spread the serum dilution to fill the entire surface of circle 5, the highest dilution (1:16). Using the same Dispenstir, repeat for circle 4(1:8), 3(1:4), 2(1:2), and 1 (undiluted).

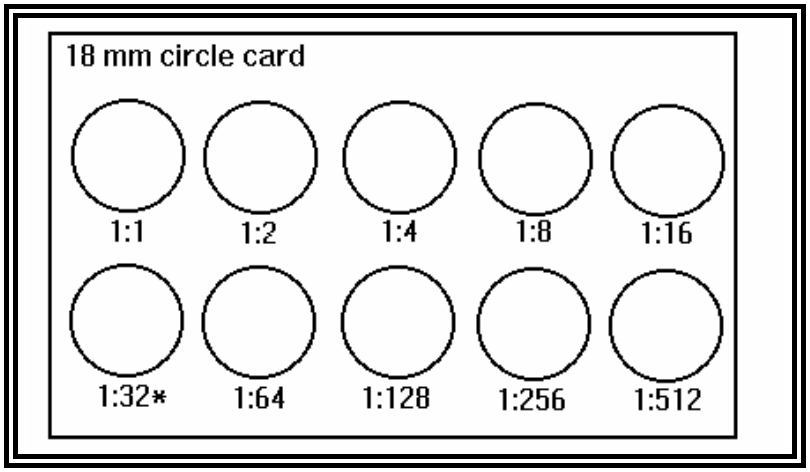
(9) Gently shake the dispensing bottle to resuspend the antigen particles.

(10) Holding the antigen dispensing bottle in a vertical position, dispense 1 or 2 drops to clear the needle of air. Then add exactly 1 free-falling drop (17 µL) of antigen suspension in each circle. DO NOT MIX.

(11) Place the card on the rotator under the humidifying cover and rotate the card for 8 minutes at 100 ± 2 rpm.

(12) Immediately remove the card from the rotator; briefly rotate and tilt the card by hand (three or four to-and-fro motions) to aid in differentiating nonreactive from minimally reactive results.

Figure 1. Diagram of card for quantitative test



* Begin dilutions in 2% normal human serum

(13) If the highest dilution tested (1:16) is reactive, continue as follows:

(a) Prepare a 1:50 dilution of nonreactive serum in 0.9% saline to be used for making 1:32 and higher dilutions of the specimen to be tested.

(b) Prepare a 1:16 dilution of the test specimen by adding 0.1ml of serum to 1.5ml of 0.9% saline. Mix thoroughly.

(c) Place 50 µL of the 1:50 nonreactive serum diluent in circles 2 through 5 of an RPR card.

(d) Using a safety pipetting device with disposable tip, place 50 µL of the 1:16 dilution of the test specimen in circle 1 and 50 µL in circle 2.

(e) Using the same pipette and tip, make serial twofold dilutions. Complete test as described in steps 4 through 13 (see "Quantitative Test"). Use a clean tip for each specimen tested. Prepare higher dilutions if necessary in 1:50 nonreactive serum diluent.

(14) After completing the day's tests, remove the needle from the antigen dispensing bottle. Rinse needle in distilled water, and air dry. Do not wipe needle (wiping removes the silicone coating). A satisfactory needle may be retained as a spare for replacement of an unsatisfactory needle.

(15) Recap the plastic dispensing bottle containing the antigen suspension and refrigerate at 2° to 8°C. Do not freeze the antigen. Antigen stored in the dispensing bottle will retain its reactivity for 3 months or until the expiration date, whichever is sooner.

Interpretation of results

Qualitative Test

1. Read the test reactions in the "wet" state under a high-intensity incandescent lamp. Read the test without magnification.

2. Report the results as given in Table 1.

Table 1. Reporting qualitative results

Reading	Report
Characteristic clumping ranging from marked and intense (reactive) to slight but definite (minimally to moderately) reactive	Reactive (R)
Slight roughness or no clumping	Nonreactive (N)

Note: Only two reports with the RPR card test are possible: reactive, no matter how much clumping, or nonreactive.

Quantitative Test

1. Read the test reaction in a "wet" state under a high-intensity incandescent lamp as for the qualitative test.

2. Report the results in terms of the highest dilution that has given a reactive result, including a minimally reactive result, as shown in Table 2.

Table2. Reporting quantitative results

	Serum Dilutions				Report
Undiluted (1:1)	1:2	1:4	1:8	1:16	
Rm	N	N	N	N	Reactive, undiluted 1:1, or R 1
R	R	N	N	N	Reactive, 1:2 dilution, or R 2
R	R	R	N	N	Reactive, 1:4 dilution, or R 4
R	R	R	Rm	N	Reactive, 1:8 dilution, or R 8

R=reactive, Rm=minimally reactive, N=nonreactive

e. Recording of Data

(1) Quality Control Data
Record lot number of kit, date of testing, and titer of reactive control serum

(a) The titer of the reactive control should be 1:8 ± 1 doubling dilution.

(b) The nonreactive control should have a uniform appearance with no clumping.

(2) Analytical results

Results should be recorded as reactive or nonreactive. If reactive, should be followed by a number indicating the titer or the serum.

f. Replacement and Periodic Maintenance of Key Components

All pipettors should be checked, repaired, and recalibrated at least yearly.

g. Calculations

Not applicable to this procedure.

h. Special Method Notes

(1) If the temperatures of the sera, reagents, or testing area are less than 23°C (73°F), test reactivity decreases; if temperatures are greater than 29°C (85°F), test reactivity increases.

(1) If the speed of the mechanical rotator is too fast or too slow, improper antigen-antibody interaction will cause unpredictable test results.

(3) If the time of rotation is too long test reactivity may be increased, or if too short test reactivity may be decreased.

(4) If the card is excessively rotated and tilted (to-and-fro motions) by hand after removal from the rotator, a false-reactive result may occur.

(5) If lighting produces a glare on the card, the reactions may be obscured.

(6) If the antigen is outdated or not adequately tested for standard reactivity, the results may be inaccurate.

(7) If the serum is unevenly spread in the circle, the antigen and antibody may not mix properly.

(8) If hemolyzed, contaminated, or improperly collected serum or plasma specimens are tested, the reaction may be masked.

(9) If the moistened humidifying cover is not used to cover tests as they are being rotated, proper humidity will not be maintained, and test components may dry on card giving rise to false reactive results.

9. REPORTABLE RANGE OF RESULTS

Results are reported as Reactive, Nonreactive, or Inconclusive.

10. QUALITY CONTROL (QC) PROCEDURES

a. Evaluation of RPR kits is the responsibility of the user. Reagents evaluated as described here must produce results comparable to those obtained with reference reagents. All glassware used must be free of contamination, and distilled water used as diluent must be pure.

b. Evaluation Procedure
Test 10 individual serum samples of predetermined reactivity on each of 2 days. The recommended distribution is three reactive serum samples, three minimally reactive serum samples, and four nonreactive serum samples. If necessary, prepare reactive serum samples of various levels of reactivity by diluting reactive samples with nonreactive serum samples. These pooled samples may be substituted for some of the individual serum samples.

c. Testing
The RPR reagents from the new and the reference lots are tested on 2 days by using reactive and nonreactive control serum samples from the new kit and the reference kit and 10 individual serum samples.

(1) Assemble the 10 individual serum samples described above in b.

(2) Perform the tests on reactive control, nonreactive control and individual serum specimens. Test all serum specimens in parallel, using new and reference (old) reagents.

(3) Read and record test results.

(4) Compare the results obtained with reference and new reagents. Determine whether new RPR reagents meet the criteria of acceptability.

(5) If results between reagent lots are discordant, additional testing may be necessary.

(6) If the new kit gives the established reactivity patterns for known controls other than the manufacturer supplied controls, further testing can continue.

Daily Control

1. Temperatures of refrigerators must be recorded daily.

2. At each routine test run, check expiration date on kit.

3. Test kit reactivity with control serum specimens of graded reactivity (reactive, minimal reactive, and nonreactive controls). Use only if results fall within ±1 doubling dilution of the titer of the reactive control.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

If the titer of the reactive control is more than ±1 doubling dilution pattern of agglutination for the unsensitized particles is other than, the test must be repeated.

If the controls are still out of compliance when repeated, a new kit should be used.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

Serum that is excessively lipemic, hemolyzed, or contaminated may interfere with the reaction.

Serum that has been repeatedly frozen and thawed may be falsely negative in the test.

Serum or reagents that have not reached room temperature before performing the test may cause false negative reactions.

Improperly diluting the serum samples will cause erroneous results. If the sample is diluted too much, it may be falsely negative. If not diluted enough, a false-positive result may occur.

A prozone reaction may be encountered occasionally. In a prozone reaction, complete or partial inhibition of reactivity occurs with undiluted serum (maximum reactivity is obtained only with diluted serum). The prozone phenomenon may be so pronounced that only a rough reading is produced in the qualitative test by a serum that will be strongly reactive when diluted. All test specimens producing any degree of roughness or reactivity with the RPR card test antigen in the qualitative test should be retested by using the quantitative procedure. In addition, a specimen should be tested for the prozone phenomenon when the clinician suspects syphilis, but the qualitative RPR is nonreactive.

13. REFERENCE RANGES (NORMAL VALUES)

Not applicable to this procedure.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable to this procedure.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens must be at room temp (18° - 25°C) during preparation and testing. Otherwise, store the serum at $\leq$-20°C. If the sample is going to be retested within 24 hours, store at 4° - 8°C to avoid a freeze-thaw cycle.

16. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)
Not applicable to this procedure.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

We recommend that records, including QA/QC dat, be retained for 2 years beyond the duration of the survey. Only numerical identifiers (e.g., NCHS ID numbers) should be used.

For the NHANES III study, residual samples are stored at $\leq$-20°C for 1 year after analysis, then

returned to the NCHS serum repository at Rockville MD.

19. SUMMARY STATISTICS AND QC GRAPHS

Qualitative assays are qualitative assays with a positive, negative or borderline/indeterminate result. The absorbance or reactivity values of specimens are compared with a cutoff value that is a ratio of the negative control mean and the positive control mean. Since the controls are read as cutoff values, plots of these values are not generated for quality control purposes.

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