

Procedure	RPR - NCCLS
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Prepared by	Date Adopted	Supersedes Procedure #

Review Date	Revision Date	Signature

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PRINCIPLE:

RPR antigen is a cardiolipin suspension containing charcoal microparticles. This antigen detects an antilipid antibody, traditionally called a “reagin” present in the serum of syphilitic patients and also occasionally in the serum of patients with some other acute or chronic diseases. When a specimen contains reagins, a flocculation of the antigen is produced which coagglutinates with the charcoal. This results in black clumps which range from marked or intense clumping to slight but definite clumping, depending on the patient being tested. With non-reactive specimens, no reaction will take place and a gray homogeneous suspension will be maintained.

HANDLING AND PROCEDURAL NOTES:

- 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.
- **RPR** disposable cards are plastic coated and specifically designed to be used with the RPR antigen. In handling, take care not to finger mark the card areas, as this may result in an oily deposit and improper test result. When spreading specimen within the confines of the circle area, avoid scratching the card with the stirrer pipettes. If the specimen does not spread in the test area or spreads outside the test area, use another test circle.
- The needle assembly must be thoroughly washed in distilled or deionized water and air-dried after each shift; do not wipe the needle dry. Place the needle back into the plastic sieve. Do not remove bottle tip when washing needle assembly. Let the assembly air-dry. Before next use make

sure that no large water droplets remain in the dropping bottle by shaking the bottle and squeezing it.

- The 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 3 mL syringe. Fill the syringe with the antigen suspension and, holding 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. Replace the needle if it does not meet this specification.
- Do not use past the expiration date indicated on the kit.
- Do not interchange components of this kit with those of another product. Discard the needle and dropping bottle when the kit is exhausted.

STORAGE OF REAGENTS: Reagents and controls should be stored at 2-8°C.

DO NOT FREEZE

SPECIMEN COLLECTION AND STORAGE

- Use heated or unheated serum samples, and plasma specimens containing EDTA (3,4), CPD or CPDA-1 as anticoagulants. Plasma specimens should be from tubes or blood units which have been collected with adequate volume to provide the appropriate proportions of specimen to anticoagulant.
- Samples should be free from bacterial contamination, hemolysis, or lipemia.
- Serum samples should be tested within 72 hours and stored at 2-8°C. Samples that require longer storage periods must be removed from the red cells and may be stored at 2-8°C for 5 days or at -20°C or below until testing.
- Plasma samples stored longer than 48 hours should not be used in the assay because of the potential for false reactive results.
- If necessary before testing, centrifuge the specimens at a force sufficient to sediment cellular components.
- Samples to be sent out for testing should be placed on ice packs and packaged like any other bio-hazardous material that could potentially transmit infection.
- This test should not be used for testing spinal fluids.

MATERIALS REQUIRED:

- A mechanical rotator circumscribing a $\frac{3}{4}$ " diameter circle on a horizontal plane (100 ± 5 rpm).
- Humidifying cover to prevent evaporation during rotation
- Timer
- Saline solution (0.9% NaCl, only for quantitative technique)
- Automatic safety pipettes to deliver 50 μ L (only for quantitative technique)
- Non-reactive human serum for diluting specimens giving a positive result at the 1:16 dilution.
- High intensity incandescent lamp
- Disposable syringe, 1 or 3mL with accuracy of $\pm 5\%$ or pipette

MATERIALS PROVIDED:

- RPR antigen reagent
- Reactive control
- Minimally reactive control
- Non-reactive control
- Antigen dispensing vial
- Antigen dispensing needle
- Disposable dispensing/spreading pipettes
- Disposable cards

PROCEDURE

Antigen preparation:

- Allow the RPR antigen, controls and test specimens to reach room temperature (20-30C°).
- Thoroughly mix the reagents before use.
- **Vigorously agitate the CARBON antigen for 20-30 seconds** before each use to ensure homogeneity.

QUALITATIVE (STEP-WISE)

- Attach the needle to the dispensing vial, and transfer the required quantity of antigen from the glass vial to the plastic dispensing vial by collapsing it.
- Label the dispensing vial with the antigen lot number, expiration date and date antigen is placed in vial.
- Using a dispenser, place one drop of the specimen to be tested into a circle of the disposable card. When using an automatic pipette place 50 uL.
- Add one free-falling drop of reactive, weakly reactive or non-reactive control from the dropper vial supplied, to the appropriate test circle.
- Using the broad end of the dispenser, spread the specimen over the entire surface of the circle. Do not spread beyond confines of circle.
- Discard dispenser after use.
- Gently shake the antigen dispensing vial and holding the vial in a vertical position dispense several drops into the cap of the dispensing vial to assure that the needle passage is clear. Dispense one free-falling drop of antigen into each circle containing the specimen. Do not mix. Recover the antigen from the cap.
- Rotate the card for 8 minutes under the humidifying cover on mechanical rotator at 100 rpm.
- Immediately remove the card from the rotator; briefly rotate and tilt the card by hand (three or four to-and-fro motions) to differentiate minimally reactive from non-reactive results. Read macroscopically in the “wet” state under a high intensity incandescent lamp.
- Upon completion of daily test, rinse the needle with distilled water and air dry. Do not wipe the needle (wiping removes the silicone coating). Close the vials and store them at 2 - 8°C.

SEMI-QUANTITATIVE (STEPWISE)

- Allow the antigen, controls and test specimens to reach room temperature (20-30°C).
- Prepare the serum dilutions on the slide (see the descriptive diagram for the technique).
- Place 50 uL of saline into each one of the card circles 2 through 5.
- Using an automatic pipette, place 50 uL of specimen into circle 1 and 50 uL directly into the drop of saline in circle 2.
- Using the same pipette aspirate and expel eight times the mixture of specimen and saline in circle 2. Avoid forming bubbles.
- Transfer 50uL of the mixture made in circle 2 directly into the drop of saline in circle 3.
- Repeat the aforementioned operations, transferring 50 uL from circle 3 to 4 and so on in succession through circle 5, thereafter, discarding 50 uL.

Section	1	2	3	4	5
Saline uL	-	50	50	50	50
Serum uL	50	50	-	-	-
Mix and Transfer			50→	50→	50→
Dilution	1:1	1:2	1:4	1:8	1:16 Discard

- Using the broad end of a clean dispensing pipette, spread the specimen over the entire surface of the circle starting at the highest dilution (1:16). Using the same pipette end, proceed to the next lower dilution (1:8). Continue until the contents of all circles are spread.
- Discard dispenser after use.
- Gently shake the antigen dispensing vial and holding the vial in a vertical position, dispense several drops into the cap of the dispensing vial to assure the needle passage is clear. Dispense one free-falling drop of antigen into each circle containing the specimen. Do not mix. Recover the antigen from the cap.
- Rotate the card for 8 minutes under the humidifying cover on a mechanical rotator at 100 rpm.
- Immediately remove the card from the rotator; briefly rotate and tilt the card by hand (three or four to-and-fro motions) to determine end point of reactivity. Read macroscopically in the “wet” state under a high intensity incandescent lamp.
- Upon completion of daily test, clean the needle with distilled water and air dry. Do not wipe the needle (wiping removes the silicone coating). Close the vial and store at 2-8°C.

QUALITY CONTROL:

Controls with graded reactivity should be included in each test run to confirm optimal reactivity of the antigen. 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 kit and contact Biokit Technical Service.

INTERPRETATION OF RESULTS

- A **reactive** result is indicated by the presence of aggregates in the center or periphery of the test circle, ranging from slight but definite to marked and intense.
- A **non-reactive** result will give a smooth gray appearance within the test circle or a button of non-aggregated carbon particles in the center of the circle, showing none of the clumping characteristic of a reactive result.
- Results for the **RPR** should be reported only as Reactive or Non-reactive, regardless of the degree of reactivity. Minimal to moderate reactivity should always be reported as reactive.
- Slightly granular or “rough” reactions should be repeated using an alternative procedure. For donor screening, these should be reported as “indeterminate” pending further evaluation.
- If necessary, confirm reactive results by retesting the sample using the semi quantitative procedure.
- Reactive results may indicate past or present infection with a pathogenic-treponeme. Non-reactive results and no clinical evidence of syphilis may indicate no current infection or an effectively treated infection.

SPECIMENS WITH TITERS GREATER THAN 1:16

The approximate titer will correspond to the highest dilution that still presents a clearly visible positive reaction. If the 1:16 dilution is reactive, proceed as follows:

- In a test tube, prepare a 1:16 dilution of the specimen by adding 1.5 mL of saline to 0.1 mL of specimen.
- Prepare a 1:50 dilution of non-reactive human serum in saline.
- Follow quantitative procedure by using the 1:16 specimen dilution as a new sample and the 1:50 non-reactive human serum as a diluent.
- Proceed with the test under semi-quantitative procedure.
- Continue with additional dilutions as required until an endpoint titer is reached.

LIMITATIONS

- Prozone reactions occur in patients with secondary syphilis. False negative non-treponemal test results, arising from prozone, are also seen in incubating primary and in late syphilis. The reactive pattern is slightly granular or “rough” with specimens exhibiting prozone. When this pattern is exhibited, a dilution of the specimen should be prepared. Titer the diluted specimen until endpoint is reached or until non-reactivity is observed. All tests exhibiting a rough appearance should be further evaluated.
- Biological false positive reactions occur occasionally with the Carbon Antigen. 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, after smallpox vaccinations.
- Pinta, yaws, bejel and other treponemal diseases produce positive reactions in this test.
- Contaminated, lipemic, or grossly hemolyzed sera should not be used because of the possibility of nonspecific reactions.
- Reaction times longer than specified may cause false positive results due to a drying effect.
- Reactive RPR test samples should be substantiated using a confirmatory test as recommended in the Manual of Tests for Syphilis.
- Temperature of the reagents and samples is crucial to test outcome; it should be between 20 and 30°C.
- 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 RPR was compared to the CDC Reference RPR Card Antigen Suspension. The overall agreement between both products was 99.2%.

***Further information for this product can be found in the package insert.**