CE

Stanbio RPR Quicktest (Syphilis) Procedure No. 1170

Carbon flocculation slide test for the qualitative and quantitative determination of reagin antibodies in serum or plasma.

Summary and Principle

The Stanbio RPR test is a macroscopic non-treponemal flocculation test that is used to detect and quantify reagin, an anti-lipoidal antibody found in serum or plasma from persons with syphilis and occasionally in persons with acute or chronic infections or conditions other than syphilis.

The antigen used in the kit is a modification of VDRL antigen which contains microparticulate charcoal to enhance the visual difference between a reactive and non-reactive result. If a specimen contains reagin, flocculation occurs with the carbon particles contained in the antigen suspension, which appear as black clumps. Non-reactive specimens appear as a light gray color.

Reagents

RPR Antigen Suspension (White Cap), Cat. No. 1171
A Cardiolipin suspension,[1] containing micro-particulate charcoal.

RPR Reactive Control (Red Cap), Cat. No. 1172
Stabilized liquid control, reactive with RPR antigen.

RPR Weakly-Reactive Control (Yellow Cap), Cat. No. 1174
Stabilized liquid control, weakly-reactive with RPR antigen.

RPR Non-Reactive Control (Green Cap), Cat No. 1173
Stabilized liquid control, non-reactive with RPR antigen.

Precautions: For In Vitro Diagnostic Use Only.
The controls used in this kit have been tested by an FDA-approved method and found non-reactive for the presence of HBsAg and antibody to HIV. While these methods are highly accurate, no test can offer complete assurance that infectious agents are absent. This material, as well as all patient samples, should be handled as though capable of transmitting infectious disease. The United States Food and Drug Administration recommends such samples be handled at Center for Disease Control's Biosafety Level 2.

Reagent Preparation: Antigen suspension should be shaken for 5 to 10 seconds prior to opening, to resuspend the charcoal particles. Controls are ready for use.

Reagent Storage and Stability: Upon receiving the test kit, remove the test cards from the kit and store at room temperature (23°-29°C). The antigen suspension should always be refrigerated when not in use. Do not store in bright sunlight **Do not freeze!** The antigen is stable if unopened and stored at 2-8°C, until expiration date on label. Once the antigen has been placed in the dispensing bottle, the reactivity remains satisfactory for approximately three months, or until expiration date on label whichever occurs first. Controls are stable until expiration date on label when stored at 2-8°C.

Bring antigen suspension and controls to room temperature (23°-29°C) before use.

Material Provided

20 G needle	Use to deliver approximately 17 µL of antigen suspension.
Dispensing bottle	Use to store and deliver antigen suspension.
Pipette/Stirrers	Use to dispense 50 µL (0.05 mL) specimens.
Test Cards	(18 mm circles) Use as testing surface.
Needle:	Accuracy of needle may be determined by placing needle on a 1 mL pipette; filling the pipette with 0.5 mL Antigen Suspension, and holding the pipette in a vertical position. There should be 30 drops ± 1 drop delivered from the needle.
Dispensing bottle	Attach the needle to the plastic dispensing bottle. After Antigen Suspension has been well mixed (see Reagent Preparation) withdraw it from vial using the dispensing bottle as a suction device. The dispensing bottle should be recapped for storage. The needle should be removed after each day's testing, rinsed with distilled water and allowed to air dry. Label dispensing bottle with Antigen lot number, expiration date, and date Antigen was placed in bottle.
Test Cards:	Care should be taken when handling test cards so as not to introduce contaminants to test circle. Spread each specimen over entire area of test circle. Store test cards at room temperature. DO NOT REFRIGERATE.
Note:	Discard needle and dispensing bottle when the kit is completely used.

Materials Required But Not Provided

Rotator (100±2 rpm) with humidifying cover, that circumscribes a circle 3/4 inch in diameter on a horizontal plane
Saline (0.9%)
Interval timer
High intensity lamp

Specimen Collection and Preparation

Specimens may be heated or unheated serum, or plasma collected with EDTA as anticoagulant. Avoid hemolysis. Lipemia will not interfere with Antigen Suspension, however, if specimen is so severely lipemic that it obscures the state of the antigen particles, the specimen should not be used.

Sample Stability: Serum samples are reportedly stable for 5 days, stored at 2-8°C. Plasma collected with EDTA and stored at 2-8°C is reportedly stable up to 24 hours.[2]

Interfering Substances: As with all cardiolipin type antigens, biological false positives may result. These results can be caused by diseases such as leprosy, lupus erythematosus, infectious mononucleosis, malaria vaccina and viral pneumonia. Several reports indicate the occurrence of false positives in pregnancy.[3, 4] Autoimmune diseases and narcotic addiction also may give false positive reactions.[5]

Procedure

Qualitative Test

1. Bring the RPR Antigen Suspension, controls and samples to room temperature (23°-29°C).
2. Hold pipette/stirrer near sealed end. While squeezing and maintaining pressure, insert pipette end into specimen. Release pressure to draw up specimen.
3. Hold pipette/stirrer in vertical position directly over circle on test card and squeeze pipette/stirrer near sealed end to allow one (1) drop of specimen to "free fall" onto test circle.
4. Using flat end of pipette/stirrer, spread the specimen to fill the entire surface of circle. Dispose of pipette/stirrer. Repeat procedure for number of specimens tested.
5. Shake the Antigen Suspension dispensing bottle prior to use. Hold in vertical position and dispense several drops into dispensing bottle cap to insure needle passage is clear. Do not wipe the needle. Place one (1) drop of "free falling" Antigen Suspension onto each test specimen. **DO NOT RESTIR!**

6. Rotate for 8 minutes at 100 rpm on mechanical rotator with humidifying cover.
7. Immediately remove the card from the rotator; briefly rotate and tilt the card by hand three or four to-and-fro motions.
8. Read macroscopically under a high intensity incandescent lamp.

Qualitative Results

Reactive – Indicated by large or small floccules in the center or the periphery of the test circle.

Minimally Reactive – Slight but definite floccules present.

Non-Reactive – Indicated by a smooth, even appearance with no floccules visible.

Reactive results should be confirmed by retesting the specimens using the quantitative procedure. Confirming serological test such as the microhemagglutination assay for Treponemal antibodies (MHA-TP) is also recommended. The final diagnosis should be based on a correlation of test results with other clinical findings. (See Limitations).

Quantitative Procedure

1. Bring the RPR Antigen Suspension, controls and samples to room temperature (23°-29°C).
2. For each specimen to be tested, place 50 µL (0.05 mL) of 0.9% saline into test circles, numbered 2 to 5. A calibrated capillary or serological pipette, 1 mL or less, may be used. DO NOT SPREAD SALINE!
3. Place 50 µL (0.05 mL) of specimen onto test circle 1.
4. Place 50 µL (0.05 mL) of specimen onto test circle 2, mix 6-8 times then transfer 50 µL to circle 3, mix and continue preparing twofold serial dilutions to circle 5. Discard the last 50 µL.
5. Using a new pipette/stirrer (flat end) for each specimen, start at highest dilution of serum (circle 5) and spread serum over entire area of test circle. Proceed to circles 4, 3, 2 and 1.
6. Shake the Antigen Suspension dispensing bottle prior to use. Hold in vertical position and dispense several drops into dispensing bottle cap to insure needle passage is clear. Place one (1) drop of "free falling" Antigen Suspension onto each test specimen.
7. Rotate for 8 minutes at 100 rpm on mechanical rotator with humidifying cover.
8. Immediately remove the card from the rotator; briefly rotate and tilt the card by hand three or four to-and-fro motions.
9. Read macroscopically under a high intensity incandescent lamp.

Quantitative Results

The last dilution step that contains macroscopic aggregates indicates the titer of the sample.

Example:

	Circle 1	Circle 2	Circle 3	Circle 4	Circle 5
Report	1:1 (Und)	1:2	1:4	1:8	1:16
Reactive 1:2	R	R	N	N	N
Reactive 1:8	R	R	R	R	N
Reactive 1:16	R	R	R	R	R

If the last dilution (circle 5) is reactive, the dilution series should be extended as follows:

Do not use plasma in this series.

1. Prepare a 1:50 dilution of non-reactive serum in 0.9% saline. (This will be used for making the 1:32 and higher dilutions)
2. Prepare the 1:16 dilution of specimen by adding 100 µL (0.1 mL) of undiluted serum to 1.5 mL of 0.9% saline. Mix thoroughly.
3. Place 50 µL (0.05 mL) of the 1:50 non-reactive serum (prepared in step 1) in circle 2, 3, 4 and 5.
4. Place 50 µL (0.05 mL) of the 1:16 diluted serum in circle 1.
5. Continue test as described under steps 4 to 8.
   If the serum titer exceeds a 1:512 dilution, higher dilutions may be prepared, again using the 1:50 nonreactive control serum.

Limitations

The diagnosis of syphilis should not be made on a single reactive result in a nontreponemal test, without the support of a positive history or clinical evidence. Serum specimens which are reactive in qualitative testing should be quantitated to establish a baseline from which changes in titer can be determined, as an indicator of response to treatment. Plasma samples should not be used in the quantitative test. Specimens which are non-reactive, but appear rough should be repeated and quantitated so that an infrequent prozone reaction may be detected.

Performance Characteristics[6]

Six hundred twenty-five specimens were obtained from a public health laboratory and subjected to a comparison between Stanbio Syphilis RPR Test and Macro-Vue™ RPR Card Test (BBL Division of Becton Dickinson and Company) with the following results:

	Stanbio Syphilis RPR	Macro-Vue™
Non-Reactive	431	433
Reactive	194	192

There was agreement on 431 of the non-reactive sera, and on 192 of the reactive sera. This represents a 99.4% agreement.

Twenty-five of the sera testing reactive to both procedures were selected at random and compared in the semi-quantitative procedure as described above. The titers ranged from 1:4 to 1:512. There was agreement to the final dilution in 22 cases. The other three results were as follows:

	Stanbio Syphillis RPR	MacroVue™
A.	1:4	1:8
B.	1:128	1:64
C.	1:32	1:16

This represents agreement within one dilution in all cases.

References

1. MANUAL OF TESTS FOR SYPHILIS, 1990, APHA publication.
2. Larsen, SA, Pettit DE, Perryman MW, Hambie EA, Mullally R, Whittington W. EDTA-treated plasma in the rapid plasma reagin card test and the toluidine red unheated serum test for serodiagnosis of syphilis. J Clin Microbiol 1983; 17:341-345.
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4. Garner, M.F. & Backhouse, J.L.: Med. Jr. Australia, 1:737-739, April 1973.
5. Kaufman, R.E.: Weiss, S, Moore, J.D.; Falcone, V. and Weisner, P.J.: Brit. Jr. Ven. Dis., 50:350-353, 1974.
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DN: RBR.1170.CE.00-M
Last Revision: 09/05
Procedure No. 1170

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