INSTRUCTIONS FOR USE

RPR Carbon Antigen Test Kit

Catalog No. 52027-100, 52027-500

Intended use

The Rapid Plasma Reagin (RPR) Carbon Antigen Test Kit is a non-Treponemal Flocculation Test that is used to detect and quantify reagin, an antibody present in serum or plasma from persons with syphilis, or with other treponemal diseases. Occasionally individuals with other diseases or conditions may also be reactive to the non-Treponemal Tests.

Summary and Explanation of the Test

Treponema pallidum, the etiologic agent responsible for syphilis produces at least two kinds of antibodies in human infections. Treponemal antibodies can be detected by tests such as the Fluorescent Treponemal Antibody-Absorption (FTA-ABS) Test[1] or MHA-TP whereas the reagin antibody is detected by non-treponemal tests such as the RPR Antigen Card Test[2]. In the presence of the reagin antibody in the reactive sample, the RPR Antigen preparation will produce flocculation consisting of black clumps against the white background of the test card. By contrast, non-reactive samples will yield an even light-grey homogenous suspension.

Test principle

The test kit employs purified cardiolipin, lecithin, and cholesterol immobilized onto micronized carbon particulate. The bound carbon particles are combined with patient sera samples to detect the present of anti-lipoidal antibodies. The antibodies are generated by the patient in response to Treponemal infections such as syphilis.

The test kit contains 100 qualitative tests worth of micronized carbon particles bound to cardiolipin, lecithin, and cholesterol. The test procedure has a single rection step performed by adding the RPR Carbon Antigen suspension directly to the specimen on the RPR Carbon test card. This mixture is incubated for eight minutes on an orbital rocking platform to facilitate flocculation of carbon particles in the presence of anti-lipoidal antibodies. Reactions are read visually and reported differently for qualitative and semi-quantitative assays. Qualitative assays are reported as a binary positive or negative result for the flocculation of the RPR Carbon particulate. Semi-quantitative results are reported as an approximate titer value being the inverse of the lowest dilution providing a positive flocculate result.

Contents of the Kit 52027-100

Component	Spec.	Component Description	Qty
RPR Carbon Antigen	2mL	Micronized Carbon bound with VDRL Antigen	1
Reactive Control[1]	1mL	Stabilized Human Sera	1
Minimal Reactive Control[1]	1mL	Stabilized Human Sera	1
Non-Reactive Control[1]	1mL	Stabilized Human Sera	1
19-Gauge Needle	Each	19- Gauge blunt end needle with cap	1
RPR Carbon Test Card	Each	Low absorption coated test cards	10
3mL RPR Dispensing Bottle	Each	Squeeze bottle for RPR Carbon dispensing	1
Dispensing pipet	Each	0.05mL volumetric dispensing pipet	100

Note: Exchange of components from different lots is not allowed. Store all reagents at 2–8°C; do not freeze.

Contents of the Kit 52029-100

Component	Spec.	Component Description	Qty
RPR Carbon Antigen	10mL	Micronized Carbon bound with VDRL Antigen	1
Reactive Control[1]	2.5mL	Stabilized Human Sera	1
Minimal Reactive Control[1]	2.5mL	Stabilized Human Sera	1
Non-Reactive Control[1]	2.5mL	Stabilized Human Sera	1
19-Gauge Needle	Each	19- Gauge blunt end needle with cap	1
RPR Carbon Test Card	Each	Low absorption coated test cards	50
3mL RPR Dispensing Bottle	Each	Squeeze bottle for RPR Carbon dispensing	1
Dispensing pipet	Each	0.05mL volumetric dispensing pipet	500

Note: Exchange of components from different lots is not allowed. Store all reagents at 2–8°C; do not freeze.

WARNING – Human Sera

Each donor unit used in the preparation of this product has been tested by an FDA approved method and found non-reactive for the presence of HbsAg and antibody to HIV Virus. However, as no known test method can offer complete assurance that hepatitis B virus, HIV Virus, or other infectious agents are absent, all human serum products and patient specimens should be handled in accordance with good laboratory practices.

Additional Materials and Equipment (not supplied with the test kit)

- Orbital platform shaker
- 0.85% Saline solution
- Micropipettes and associated tips
- Timer

Precautions

Strict adherence to assay instructions is required. Any variation in pipetting technique, incubation time or temperature, and use of reagents beyond the expiration dates kit age can cause result difference can affect the results.

- The kit/reagents should not be used beyond the expiration date on the kit label.
- To avoid cross contamination, disposable pipette tips should be used.
- All reagents and samples should be prepared before starting the procedures.
- If the kit will be used multiple times, ensure the reagents are sealed fully and store components in original kit box at recommended storage temperature.
- Positive and negative controls should be included in each run of the assay.
- Modifications to the kit components or procedures may provide false results.
- Serum or plasma samples are recommended. Store specimens at 2–8°C for no longer five days. For longer storage, sera specimens should be frozen. It is recommended that frozen specimens be tested within one year. Avoid repeated freezing and thawing of samples

Test Procedure

Reagent Preparation

- All reagents must be brought to room temperature (20–30°C) and mixed thoroughly prior to use.
- All specimens must be brought to room temperature (20–30°C) and mixed thoroughly prior to use.
- Transfer RPR Carbon Antigen suspension from the source container to the RPR Carbon dispensing bottle.
  - Attach a clean blunt 19-gauge needle to the 3mL bottle.
  - Vigorously mix the RPR Carbon Antigen suspension by inversion multiple times to fully homogenize the micronized carbon material.
  - Squeeze the bottle to remove some of the air from the bottle.
  - Place the needle into the source RPR Carbon Antigen suspension.
  - Gently release tension on the bottle, allowing the expansion of the bottle to slowly aspirate the RPR Carbon antigen suspension into the dispensing bottle.
- Tightly close unused kit reagents and store at 2–8°C in original kit packaging.
- Do not interchange components of different lots. Do not use reagents beyond the expiration date indicated on labels.

Sample Preparation

- Only serum and plasma specimens should be used in this assay. Specimens with gross hemolysis, elevated lipids or microbial contamination may interfere with the performance of the test and therefore must not be used. Store specimens at 2–8°C for no longer than 5 days. For longer storage, sera specimens should be frozen. Avoid repeated freezing and thawing of samples.
- Sera specimens are tested without dilution for qualitative results and diluted at 1:2 serial dilution steps with 0.85% saline for semi-quantitative results.
  - For example: Add 500µl of sample into 500µl 0.85% saline and mix well by vortex. Diluted samples must be tested within one working day.
- Reactive controls are processed as specimens.

Assay Procedure

1. Prepare reagents prior to starting the procedure as described above.
2. Prepare diluted patient samples prior to starting the procedure as described above.
3. Label test card(s) with sample ID as needed for the number of tests, dilutions, or samples being assayed.
4. Using the dispensing pipet or micropipette and tip add 50µl of sample to the corresponding well on the RPR Test Card.
5. Using the flat end of the dispensing pipet carefully distribute each sample evenly throughout the well on the test card.

5.1. Each sample should be processed with its own individual dispensing pipet to prevent cross contamination of samples and controls.

6. Mix the RPR Carbon Antigen dispensing bottle by vigorous inversion to ensure a homogeneous suspension of the micronized carbon.
7. Add one drop of RPR Carbon Antigen suspension to each sample will well on the RPR Carbon Antigen test card.

7.1. Dispense a few drops of RPR Carbon antigen suspension into the cap of the dispensing bottle or other clean surface to ensure the needle is dispensing the complete 17µL volume, with no air bubbles.

7.2. Ensure the drop falls freely from the end of the needle to dispense the full 17µL volume.

7.3. Do not touch the end of the need to the specimen in the well to prevent contamination across samples.

7.4. Do not premix the RPR Carbon with the specimen, all required mixing takes place on the orbital rotating platform.

8. place the test card(s) on the orbital rotating platform.

8.1. Add damp sponges for added humidity to prevent sample drying.

8.2. Cover cards and sponges with a humidity cover to prevent sample drying.

8.3. Care should be taken to ensure the final set-up of cards, sponges, and humidity cover will not touch or disrupt the sample mixing on the test cards. Contact of materials to the specimen on the test card can induce incorrect results.

9. Incubate the test cards on the rotating platform for eight minutes while rotating at 100rpm.

9.1. Orbital distance should be 10 millimeters

9.2. Increased speed or orbital distance can impact the specimen's ability to remain in the test card well and impact the level of flocculation observed in the test.

10. Following the incubation, observe the level of flocculation observed in each well within 1-2 minutes of completing the incubation time.

10.1. Drying of the specimen negatively impacts the ability to accurately determine flocculation level.

10.2. Refer to the reference image in next column to grade the level of flocculation for each specimen.

Strongly Reactive 4+ | Reactive 3+ | Weakly Reactive 2+ | Trace Reactive 1+ | Non-Reactive

Quality Control

Use one positive control and one negative control every time the test is performed.

Interpretation of the Results

Visual Evaluation: Using the reference image above as a guide, rotate the test card while in the hand and inspect each well for flocculation. Flocculation can be seen as large aggregates of carbon particles indicating high levels of antibodies in the sample, very fine aggregates of carbon particles indicating low levels of antibodies in the sample, or no aggregation of carbon particles indicating an absence of antibodies in the sample. If the positive control sample does not show flocculation, and the negative sample show an absence of flocculation, the test should be considered inconclusive and all samples in the assay run should be retested

Limitation of the Test

- This assay should only be used for testing human serum/plasma samples.
- The results obtained from this assay are aid to diagnosis and should not be considered as the sole basis for disease diagnosis. The results should be correlated with clinical findings and other disease specific laboratory tests and work up.
- Avoid cross contamination of samples or reagents by changing dispensing pipets or tips between samples and controls.
- Accurate volumetric dispensing is essential for obtaining test result.

Performance Characteristics

The RPR Carbon Antigen Test Kit is evaluated for equivalence in reactivity against a reference RPR carbon antigen suspension. A total of 100 samples were evaluated across the full range of reactivity for each sample resulting in over 400 comparative assays. The data located in the table below summarizes the results of the testing. Of the samples tested, there was 100% agreement across all non-reactive samples, and 100% agreement for final titer endpoint for both the RPR Carbon Suspension kit and reference RPR carbon suspension product tested.

		KSL RPR Carbon Antigen Test Kit	
		Reactive	Non-Reactive
Competitor RPR Carbon Antigen Suspension	Reactive	50	0
	Non-Reactive	0	50

References

1. Hunter, E.F. Deacon, W.E. and Meyer, P.E., An improved FTA Test for Syphilis, The Absorption Procedure (FTA-ABS). Public Health Reports, 79, 410-412, 1964.
2. Manual of Tests for Syphilis, Public Health Service Publication, No. 411, 1969.

Symbol Key

- Consult Instructions for Use
- Manufacturer
- Temperature Limit
- Date of Manufacture
- Use by Date
- LOT — Batch Code / Lot Number
- REF — Catalogue / Part Number
- IVD — In Vitro Diagnostic Use
- Σ — Number of Tests

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