ARENA
BioScien

Block 5, Street 9 inside The Ismailia free zone. Ismailia- Egypt
Post Code-41511
Tel.-Fax: +202 21813500/ +202 21813600
Mob.: +2 01211550941/ +2 01119225860
E-mail: sales@arenabioscientific.com
Website: www.arenabioscien.com

RPR – carbon particles (slide agglutination)

Diagnostic reagent set for the in-vitro qualitative screening and semi-quantitative determination of Rapid Plasma Reagins in human serum or plasma manually.

REF: BS.1/RPR1.050.0050	50 tests	REF: BS.1/RPR1.100.0100	100 tests

CLINICAL SIGNIFICANCE

Reagins are a group of antibodies against some components of the damage tissues from patients infected by Treponema pallidum, the agent which causes the syphilis. This microorganism produces some damage to the liver and heart, releasing some tissue fragments. Immunological patient system reacts producing reagins, antibodies against these fragments.
The assay is useful to follow the antibiotic therapy answer.

METHOD PRINCIPLE

BioScien RPR-carbon antigen is a non-treponemal preparation specially developed for the rapid detection and semi-quantitation by coagglutination on a slide or microplate of plasma reagins, a group of antibodies directed against tissue components produced by almost every patient infected with T. pallidum. The assay also known as rapid plasma reagin (RPR) is performed by testing the antigen –an association of lipid complexes and particulate carbon- against unknown samples. The presence or absence of a visible agglutination indicates the presence or absence of circulating antibodies in the samples tested.

REAGENT COMPOSITION

Reagents:	Composition
RPR-carbon	Carbon particles coated with a lipid complex, cardiolipin, lecithin and cholesterol in phosphate buffer 20 mmol/L. Preservative. pH, 7,0.
Positive Control	Human serum with reagin titer ≥ 1/4.
Negative Control	Animal serum.

All reagents contain sodium azide (0.1%) as a preservative.

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent (R) contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the RPR reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

BioScien RPR reagent is ready-to-use and is stable until expiration date stated on label when properly stored in an upright position and refrigerated at 2-8°C (do not freeze).

Deterioration

Particles and turbidity indicate deterioration and should not be used.

SPECIMEN COLLECTION AND PRESERVATION

Fresh serum or plasma.
Stable 7 days at 2-8ºC or 3 months at –20ºC. The samples with presence of fibrin should be centrifuged before testing. Do not use highly hemolyzed or lipemic samples.

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes
- Centrifuge
- Stop watch
- Variable Micropipettes

ASSAY PROCEDURE

Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperatures. Shake and mix well before dispensing

Qualitative procedure

1. Identify each reaction circle of the slide test to make one positive control, one negative control and the desired number of samples respectively.
2. Place one drop (50 µl) of positive control reagents, one drop (50 µl) of negative control reagents and one drop (50 µl) of patient's serum to be tested onto each reaction circles.
3. Add one drop of RPR (20 µl) to the reaction circles containing positive controls, negative control and patient's serum.
4. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
5. Rock the slide gently back and forth, and observe for agglutination macroscopically at 8 minute.
   False positive results could appear if the test is read later than 8 minutes.

* Sample that are positive in the screening test should be retested in the titration test (semi-quantitative test) to provide verification for borderline interpretations.

Qualitative Test in microplate (flat bottom)

1. place 50 µl of each sample into a separate well on the microplate. Use a separate tip for each sample and discard after use. Dispense 1 drop of each of the two serum controls into two additional wells.
2. Dispense 1 drop of antigen (20 µl) in each well of the microplate that contain the samples to be tested.
3. Place the microplate on a mechanical rotator and rotate at 200±50 r.p.m. for 20 minutes.
4. Observe macroscopically for agglutination under a high intensity

lamp over a white surface, within a minute after removing the microplate from the rotator.

Semi-quantitative method

1. Serum to be titrated is serially diluted (1:2, 1:4, 1:8, 1:16, 1:32) in physiological saline.
2. Test each dilution as described in steps 2-5 for the Qualitative Test.

READING AND INTERPRETATION

Qualitative procedure	
Positive	Marked and intense visible aggregates are seen.
Negative	Smooth suspension with no visible aggregates.
Semi-Quantitative procedure	
Titer	The titer of the patient serum corresponds to the visible agglutination in the test circle with the smallest amount of serum sample.

QUALITY CONTROL

Positive and negative controls should be run daily following the steps outlined in the Qualitative Test, in order to check the optimal reactivity of the latex reagent.
The positive control should produce clear agglutination. If the expected result is not obtained, do not use the kit. For more information please contact ***BioScien*** technical support.

PERFORMANCE CHARACTERISTICS

1. **Analytical sensitivity**: Accurate titer determination of the Reference Material, under the described assay conditions.
2. **Prozone effect**: No prozone effect was detected up to titers ≥1/128.
3. **Diagnostic sensitivity**: 100 %.
4. **Diagnostic specificity**: 100 %.

LIMITATIONS OF PROCEDURE

1. RPR carbon test is non-specific for syphilis. All Reactive samples should be retested with treponemic methods such as TPHA and FTA-Abs to confirm the results.
2. False negatives may be seen in primary early syphilis and in late syphilis, and also as a result of the prozone reaction (≥1/128). A negative result for a patient strongly suspected of having syphilis, should be tested by semi-quantitative method in order to eliminate the possibility of this effect.
3. False positive results have been reported in diseases such as infectious mononucleosis, viral pneumonia, toxoplasmosis, pregnancy and autoimmune diseases.
4. A non Reactive result by itself does not exclude a diagnosis of syphilis. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.
5. With cardiolipin type antigens, biological false positive reactions have been reported in diseases such as infectious mononucleosis, hepatitis, brucellosis, leprosy, malaria, measles, lupus erythematosus, virus pneumonia and other virus infections. Pregnancy, malignancy, narcotic addiction and autoimmune diseases also may give false positive reactions.
6. Do not use on spinal fluid.

INTERFERENCES

Bilirubin (20 mg/dL), hemoglobin (10 g/L) and lipids (10 g/L), do not interfere. Rheumatoid factors (300 IU/mL), interfere. Other substances may interfere.

REFERENCES

1. George P. Schimid. Current Opinion in Infectious Diseases 1994; 7: 34-40.
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3. Sandra Larsen et al. A manual of Test for SisypAhmilerican Public Health Association 1990: 1-192.
4. Guide to Clinical Preventive Services. 2nd Ed. U.S. Dept. of Health and Human Services, Washington, DC (1996).
5. Young, D.S. Effects of Drugs on Clinical Laboratory Tests. 4th Edition. AACC Press (1995).

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number	⚠	Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		

EC REP

Medical Device Safety Service
MDSS GmbH
Schiffgr aben 41
30175 Hannover, Germany

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