

BHAT BIO-TECH INDIA (P) LTD.

RPR TEST

A Rapid Non-Treponemal test for the detection of reagin antibodies in serum or plasma for Syphilis

For Professional Use IVD

READ THIS PACK INSERT CAREFULLY BEFORE PERFORMING THE TEST

INTENDED USE :
RPR (Rapid Plasma Reagin) is a Rapid Non-Treponemal test for qualitative and semi quantitative in vitro determination of Reagin antibodies in serum or plasma for syphilis using modified cardiolipin antigen.

INTRODUCTION :
Detection of syphilis by serologic methods is related to both the stage of the disease and the test method.

Non-Treponemal (reagin Tests)	Primary	Secondary	Late
VDRL	70%	99%	1%
RPR	80%	99%	0%
Florescent Treponemal (Antibody Absorption Test (FTA-ABS)	85%	100%	95%
T-Pallidum Haemagglutination Assay (TPHA-TP)	65%	100%	95%
Treponemal Immobilization (TPI)	50%	97%	95%

In the primary stage, about 30% of cases become serologically active after one week and 90% of patients demonstrate reactivity after 3 weeks. Reagin titres increase rapidly during the first 4 weeks of infection and then remain stationary for approximately after 6 months. In late syphilis treponemal tests are generally reactive, non-treponemal tests are non-reactive. During latest syphilis there is a gradual return of non-reactive serologic manifestations with non-treponemal method. About one third of the patients in the late stage will remain seroactive and presumably infection.

PRINCIPLE :
The antigen used is a modified cardiolipin antigen in which micro particulate carbon particles are suspended; in addition, it contains a balanced quantity of cholesterol and lecithin.

In the presence of reagin antibodies, clumps or aggregates appear which can be visualized as black clumps against white background of the slide, macroscopically.

STORAGE AND STABILITY :
Storage :
Store the reagents at 2-8°C. Do not use the reagent beyond the expiry date mentioned on it. Before performing the test bring all the reagents to Room temperature. Replace the reagents to 2-8°C soon after performing the test. **DO NOT FREEZE THE REAGENTS.**

Stability :

- The unopened kit is stable for 24 months from the date of manufacturing as indicated on the package.
- The opened kit is stable for 6 months from the date of opening.
- Repeated freeze thaw of reagents from 2-8°C to Room temperature several times will reduce the stability of the kit.

PACK SIZE : Available in packs of 50, 100, 300 and 500 Tests.

CONTENTS OF THE KIT :

Materials	50 Tests	100 Tests	300 Tests	500 Tests
RPR Positive Control serum	0.3 ml	0.3 ml	0.5 ml	0.5 ml
RPR Negative Control serum	0.3 ml	0.3 ml	0.5 ml	0.5 ml
Carbon RPR Antigen suspension	1.2 ml	2.5 ml	7.5 ml	12 ml
Disposable white plastic slide with 8 cells	7 No.	14 No.	42 No.	70 No.
Disposable applicator sticks	50 Nos.	100 Nos.	300 Nos.	500 Nos.
Disposable plastic dropper with rubber teat	50 Nos.	100 Nos.	300 Nos.	500 Nos.
Antigen delivery dropper	1	1	1	1
Product pack insert	1	1	1	1

SPECIMEN :
Use fresh serum for testing. The specimen should be Non-hemolysed and free from contamination.

The specimen may be stored at 2-8°C for up to 8 days and at -20°C for up to 4 weeks.

SPECIMEN COLLECTION AND HANDLING :
Collect blood in a clean sterilized vial and allow it to clot. Separate the serum by centrifugation at 10000 rpm for 10 minutes at room temperature. It is recommended that fresh samples should be used. If serum is not used for testing immediately it should be stored at 2-8°C or Frozen at -20°C. Bring specimen to room temperature, and specimen should be mixed properly and centrifuged before use. Do not heat or repeatedly freeze thaw the specimens.

PRECAUTIONS :

- For invitro diagnostic use only.
- Bring all reagents and specimens to Room temperature, prior to testing.
- Avoid using lipemic, haemolysed or contaminated specimen.
- All human serum and plasma samples should be considered potentially infectious. It is recommended that all specimens of human origin should be handled as recommended for any potentially infectious human serum or blood specimen in the center for disease control/National Institute of Health Manual "Biosafety in Microbiological and Biomedical Laboratories", 1984.
- Never pipette by mouth.
- Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
- Wear disposable gloves while handling specimens and kit reagents. Afterwards wash hands carefully.
- Avoid splashing or forming aerosols.
- Discard all materials and specimens as if capable of transmitting infection. The preferred method of disposal is autoclaving for a minimum of one hour at 121°C. Liquid waste containing acid may be mixed with sodium hypochlorite in volumes such that the final mixture contains 50-500mg/l available chlorine. Allow 30 minutes for decontamination to be completed.

NOTE :

- Liquid waste containing acid must be neutralized with a proportional amount of base prior to the addition of sodium hypochlorite.
- Spills should be wiped thoroughly using either an iodophor disinfectant or sodium hypochlorite solution. Materials used to wipe up spills should be added to biohazardous waste matter for proper disposal.

NOTE :

- Liquid waste containing acid must be neutralized with a proportional amount of base prior to the addition of sodium hypochlorite.
- Spills should be wiped thoroughly using either an iodophor disinfectant or sodium hypochlorite solution. Materials used to wipe up spills should be added to biohazardous waste matter for proper disposal.
- Deterioration is indicated by a significant decrease by weak agglutination.
- Do not use reagents after the expiration date printed on the label.
- When removing reagents from the bottles, use aseptic technique to avoid contamination.
- Mix the reagent bottle gently before use.
- Do not use Contaminated Serum sample for testing.
- Ensure used glass slide is disinfected, washed thoroughly and rinsed free of detergents.

TEST PROCEDURE :
A. Qualitative Analysis :

- Bring the reagents and specimens to Room temperature. Mix the antigen suspension thoroughly prior to use. Micro-particulate Smooth sieved carbon particles suspended with cardiolipin and a balanced quantity of cholesterol and lecithin.
- Drop Specimen, Positive control serum, and Negative control serum onto separate cells of the slide.
- Add one drop of antigen suspension with antigen delivery dropper onto all cells of the slide.

- Mix with separate sticks and spread the fluid over the entire area of the particular cell.
- Tilt the test card back and forth slowly for 4 minutes or place the card on an automated rotator and rotate at 100 rpm for 4 minutes.

B. Semi quantitative Analysis :
Prepare dilutions of the specimen to be tested with physiological saline (0.9%) as indicated -1:2, 1:4, 1:8, 1:16, 1:32 and proceed as in qualitative analysis.

INTERPRETATION OF RESULTS :
A. Qualitative Analysis :
Read the result under strong source of light. Regardless of the degree of reactivity, clumping of carbon particles is reported as reactive or positive.
Complete absence of black aggregates and presence of a uniform greyish suspension indicates negative result.

B. Semi Qualitative Analysis :
The titre is reported as the reciprocal of the highest dilution which shows a positive result.

TROUBLE SHOOTING :

FALSE POSITIVE	
Cause	Remedy
1. Contaminated serum or reagents	Make sure that there is no contamination of serum or reagent. Use clean slide for testing. Do not read the result after 4 minutes.

FALSE POSITIVE	
Cause	Remedy
2. Drying in slide test	Do not read the result after 4 minutes
3. Improper mixing of Carbon RPR Antigen suspension	Mix the Carbon RPR Antigen suspension thoroughly before use.

FALSE NEGATIVE	
Cause	Remedy
Contamination of serum or reagents	Make sure that there is no contamination of Serum or reagent Use clean slide for testing. Do not read the result after 4 minutes.

WEAKLY / DELAYED REACTION	
Cause	Remedy
1. Prolonged storage of serum sample	Store the serum sample upto 8 days at 2-8°C and at 20°C for upto 4 weeks.
2. Expired reagent	Check the expiry date on the reagent
3. Improper storage conditions of the kit	The kit has to be stored at 2-8°C.
4. Improper mixing of Carbon RPR Antigen suspension	Mix the Carbon RPR Antigen suspension thoroughly before use.

PERFORMANCE CHARACTERISTICS :
Accuracy : Bhat Bio-Scan® RPR test meets the requirements when tested against DCI approved kits.

SPECIFICITY :

No. of Negative samples tested	No. of Negative by Bhat Bio-Scan® RPR Test	Specificity (%)
40	39	97.5

SENSITIVITY :

No. of Positive samples tested	No. of Positive by Bhat Bio-Scan® RPR Test	Sensitivity (%)
40	40	100

DIAGNOSTIC VALUE :
Syphilis is a sexually transmitted disease caused usually by direct but sometimes by indirect contact. The causative organism Treponema Pallidum can gain entrance to the body through minute lesions on the skin or mucous membrane. Infection is usually rendered within a month of infection, and is accompanied by enlargement of the local lymphatic nodes.
From 6 to 12 weeks after the appearance of the primary chancre, the secondary stage of the disease sets in, marked by constitutional symptoms, cutaneous lesions sometimes infection of the bones, joints, eyes and other organs. Thereafter, the disease becomes latent and can be detected by serological tests.
Certain non-syphilitic conditions, which cause tissue damage, can give false positive results. Common conditions like malaria, typhoid, leprosy, tuberculosis, certain viral diseases like viral pneumonia, infectious mononucleosis, pregnancy and some autoimmune disorders can cause positive reactions in low titer to appear. Positive sample should be confirmed with specific tests like TPHA, FTA, etc.

LIMITATIONS OF THE TEST :

- It is recommended that with every set of tests, positive and negative controls should be included. The result of positive and negative controls should be read before reading the test results. The satisfactory result of positive and negative control indicates that the reagents are working well.
- Factors other than reagents, which might affect the performance of the test, include cleanliness of glassware, meticulous follow up of the procedure and rouleaux formation.
- Do not use lipaemic, haemolysed, or contaminated specimens.
- Mix antigen suspension gently before use.
- While dispensing reagents/specimen hold pipette/dropper vertically straight.
- Improper mixing of specimen/control with antigen suspension may lead to erroneous results.
- Make sure that the cap of each reagent vial is properly and promptly applied to the same vial. Interchanging of the vial caps and or droppers will lead to contamination of reagents, which might lead to false results.
- The slide should be tilted back and forth gently to avoid disturbance to the reaction pattern.
- Contaminated blood specimen or reagents may interfere with test results. Peripheral drying in slide test should not be misinterpreted as agglutination.
- Interpret results exactly at 4 minutes.
- The reagents contain sodium azide as preservative. Do not swallow. Avoid contact with skin and mucous membrane.

REFERENCE :

- Portnou, J. Brewer, J. H. H. (1962). Pub. Health Rep., 77, 645-652.
- Portnoy, J. (1963). Amer. J. Clin Path., 40, 473-479.
- Falcone, V. H., Stout, G. V., Moore, M. B. (1964). Pub. Health Rep., 79, 491-495.
- Scotti, A. G., Mackey, D. M., Trautman, J. R. (1970). Arch. Derm., 101, 328-330.

EN 980:2008 (E) MEDICAL DEVICES SYMBOL

Temperature Limitation	Date of Manufacture 2001-06	In vitro Diagnostic Device	LOT	Batch Code
Company name & address	Refer Operating Instructions	Use by	Company	Company Name
Authorised Representative in European Community	Do Not Reuse	Sufficient for	KEEP AWAY FROM SUNLIGHT	
KEEP DRY	NON-STERILE	CONTROL -	NEGATIVE CONTROL	CONTROL +
		POSITIVE CONTROL		

Mfg. in India by
BHAT BIO-TECH INDIA (P) LTD.
11-A, 4th Cross, Weerasandra Industrial Area, Electronics City,
Bangalore - 560100, Karnataka Tel.: 080-4351 4000 (30 lines)
Fax: 080-4351 4001 Visit us at: www.bhatbiotech.com

M/S PDB Consulting
Bruno Rosen, Flehensburg
48, 42489 Walfrath, Germany
P : + 49 - 2058 - 8989881
Fax : + 49 - 2058 - 8989882