RPR TEST KIT

INTENDED USE
RPR Carbon antigen is used in a Non-Treponemal test for the qualitative and semi-quantitative detection of syphilis using serum (Heated or unheated) and Plasma.

CLINICAL PRINCIPLE:
Syphilis is a venereal disease caused by the spirochaete micro-organism *Treponema pallidum*. As the organism cannot be cultured on artificial media the diagnosis of syphilis depends on the correlation of clinical data with the detection of specific antibody by serological tests.
The VDRL antigen test is "Non Treponemal" which means antibodies detected are not specific to *T. Pallidum*, although the presence strongly indicates infection by the organism. The test measures antibody (IgG and IgM) produced in response to lipoidal material released from damaged host cells as well as lipoprotein like material released by the spirochaetes. After successful treatment the antibody titre will fall quickly.

Serological screening tests for syphilis using cardiolipin and lecithin as antigens are simple to perform but may give rise to a small proportion of false positive results because as stated above, the tests use non-treponemal antigens.
The VDRL Carbon Particle Antigen is a modified form of VDRL Antigen containing micro-particulate carbon[1]. It is designed for use in flocculation tests for the sero-diagnosis of syphilis. The carbon particles aid the macroscopic reading of the results. Weak reactive results can be easily and clearly distinguished from non-reactive patterns which display a macroscopically smooth and even appearance. This antigen is suitable for use in both manual slide tests and Automated Reagin tests[2].

WARNINGS AND PRECAUTIONS
For *in vitro* diagnostic use only. For professional use only
Health and Safety warnings:
All patient samples and reagents should be treated as potentially infectious and the user must wear eye protection, gloves and laboratory coats when performing the test.
Non disposable apparatus must be sterilised after use by an appropriate method.
Disposable apparatus must be treated as bio-hazardous waste and autoclaved or incinerated.
Spillages of potentially infectious material should be absorbed and disposed of as above. The site of spillage must be sterilised with disinfectant or 70% alcohol.
Do not pipette by mouth.
The test reagent is a modified form of VDRL antigen containing micro-particles.
Control reagents contain human serum. The human serum used has been tested and found to be negative for HIV and HbsAg and has also been heat treated. Nonetheless the reagent must be treated as potentially infectious and appropriate precautions should be taken when handling and on disposal. The product also contains aqueous buffer salts including sodium azide as preservative- see material safety data sheet available on request.
Analytical precautions:
Do not modify the test procedure.
Do not dilute or modify the reagents in any way.
Allow all reagents and samples to reach room temperature (18 to 30ºC) before use.
Do not interchange reagents from different kit batches.

KIT COMPOSITION
Standard Kit Contents. May vary depending on the format supplied.
Antigen (2ml for the 100 test kit; 10ml for the 500 test kit)
This reagent is ready for use and is supplied in 2ml/10ml capped vials. Time must be allowed for the antigen to reach room temperature prior to testing and should be *WELL SHAKEN* to ensure homogeneity.

Positive/negative controls sera
Controls are supplied so the validity of the test can be checked periodically.
The controls are supplied ready for use.
Dispensing bottle (3ml) and needle
These components are designed for dispensing the RPR test antigen. For use, attach the needle to the end of the bottle and draw the *WELL SHAKEN* antigen into the bottle. Expel a drop or two of antigen to eliminate the possibility of an inadequate amount of antigen being added to the sample due to the presence of air in the needle. It is extremely important to maintain bottle and needle in a **vertical position** when dispensing the antigen. At the end of each day's testing the needle should be removed, rinsed with distilled water and air dried. The dispensing needle should not be wiped. Doing so may remove the silicon coating thereby allowing some antigen to adhere to the needle, which may result in insufficient antigen being delivered.
Test Cards
These cards are for use with the RPR antigen suspension and are specially prepared, plastic coated cards. The circles of the test cards should never be touched with the fingers, as this may invalidate test results. Each test area should only be used once and then the card should be discarded or filed for future reference.
Pipette stirrers (100 or 500)
The droppers are used to transfer serum or plasma to the test card surface and one drop is equivalent to approximately 50µl. In qualitative tests, a new dropper must be used for each test specimen.
Pack Insert
ALL REAGENTS ARE SUPPLIED READY TO USE.

STORAGE AND SHELF LIFE
Store reagents, upright at 2-8ºC.
DO NOT FREEZE THE ANTIGEN REAGENT
Do not use reagents after the stated expiry date.
Discard reagents if they become contaminated or do not demonstrate the correct activity with controls.

ADDITIONAL EQUIPMENT REQUIRED
Micropipettes for delivering 50 µl, Automatic rotating table.

SPECIMEN PREPARATION
Plasma, unheated or heated serum may be used. Test material should be free from bacterial contamination and non-haemolysed. Specimens may be stored at 2-8 C for up to 7 days, if longer storage is required samples should be frozen at -20C. Care should be taken that frozen samples are thawed and mixed completely prior to testing.

PROCEDURE
Qualitative method
1. Hold the pipette between the thumb and forefinger. Squeeze whilst inserting the tip into the specimen. Then release finger pressure to withdraw the sample taking care not to transfer any cellular elements.
2. Hold the dropper over a test card circle and squeeze the teat to allow one drop (50µl) to fall onto the card. It is important to maintain the dropper in a vertical position whilst dispensing the sample to be tested.
3. Using the flat end of the stirrer, spread the sample to cover the test circle.
4. Attach the dispensing needle to the plastic bottle. Withdraw sufficient antigen (*WELL SHAKEN*) for the number of tests performed. Maintaining the needle in a vertical position, allow one drop to fall on each test sample. Do not restir.
5. Rotate the RPR test card manually or using an automatic rotator for 8 minutes at 100 revolutions/minute.

Semi-Quantitative method

1. Dispense one drop of 0.85% saline on circles 1 to 5, of the test card using disposable pipette. Do not spread the saline.
2. Using an accurate volumetric pipette dispense 50µl of sample onto circle number 1.
3. Using the pipette prepare two-fold dilutions by drawing the mixture up and down the pipette 5 or 6 times. Avoid the formation of bubbles. Transfer 50ul from circle numbers 1 to 5 which now represent the following dilutions:

Circle	1	2	3	4	5
Dilution	1:2	1:4	1:8	1:16	1:32

4. Repeat steps 3-5 as for the qualitative method above.

INTERPRETATION OF RESULTS

At the end of 8 minutes of rotation Positive results will display characteristic agglutination ranging from slight (Weak-reactive) to intense (Strong Reactive).

Very weak reactive results are characterised by small agglutinates around the periphery of the test area.

Negatives results do not exhibit this reaction and display a macroscopically smooth and even appearance.

Positive test specimens should be subject to further serological studies (i.e. TPHA, FTA, and ABS) since, as with any serological testing procedure, the diagnosis of syphilis should not be made on a single reactive result.

For the semi quantitative method, read the test results and note the last circle that has a positive result.

If the highest dilution tested (1:32) still shows strong reactivity, proceed with further doubling dilution series until end point titre can be determined.

PERFORMANCE CHARACTERISTICS

The product has been independently tested against known standards and samples from CDC Atlanta. All specimens showed 100% correlation to all reagents tested.

LIMITATIONS OF THE METHOD

In common with all Reagin tests, VDRL Carbon Antigen may give a small proportion of false positive results. Such reactions can be caused by diseases such as infectious mononucleosis, leprosy, lupus erythematosus, vaccinia and virus pneumonia. In common with other serological tests VDRL Carbon Antigen test cannot distinguish between syphilis and other pathogenic treponemal infections, egg. Yaws.

Clinical evidence should always be considered when making a diagnosis of treponemal infections.

QUALITY CONTROL

Positive and negative control sera should be used routinely to verify the test. International standards are commercially available.

TABLE OF SYMBOLS

LOT	Batch Number	IVD	*In-vitro* Diagnostic
REF	Catalogue Reference		Store At
	Expiry Date		Manufacturer
	Read Pack Insert		

REFERENCES

1. *McGrew, B.E. et al., Amer. J. Med Tech. , **34**, 634 (1968a)*
2. *McGrew, B.E. et al., Amer. J. Clin. Path., **50**, 52 (1968b)*
3. *Norins, L.C. Automation in Clinical Chemistry, 1, 157New York Mediad (1968)*
4. *Portnoy, J. et al., U.S. Publ. Health Report, **77**, 645 (1962)*
5. *Schroeter, A.L. et al.,Adv. In Automated Analysis **1**, 256 N.Y. Mediad*
6. *Stevens, R.W. and Stroebel, E., Amer. J. Clin. Path., **53**, 32 (1970)*
7. *Stout, G.W. et al., J. Conf. Pub. Health Lab. Directors, **26**, 7, (1968)*
8. *Manual Test for Syphilis PHS Publications No 411, (1969*

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