REF NB012
NB013

NewBio RPR

CE

2797

1. Intended Use

Intended for the qualitative detection of reagin antibodies in human serum and EDTA plasma as an aid in the diagnosis of syphilis. The intended use population is patients with a suspected syphilis infection or at elevated risk of syphilis infection who attend STI clinics or other healthcare settings.This assay is not intended for automated use. This assay is not intended for blood screening or as a confirmatory assay on donor samples.

2. Principle of assay

NewBio RPR utilises carbon particles coated with cardiolipin antigen to detect reagin antibodies present in serum or plasma of syphilitic persons.

NewBio RPR measures IgM & IgG antibodies to lipoidal material released from damaged host cells as well as possibly cardiolipin released from treponemes. If antibodies are present, they combine with lipid particles of the antigen, causing them to aggregate. The carbon particles appear as dark clumps against a white background. The aggregation can be read macroscopically. Non-reactive samples typically appear as a smooth non-aggregated pattern which may form buttons in the centre of the test area.

3. Components

Name	Description	100 tests NB012	500 tests NB013
RPR antigen	Carbon particles coated with cardiolipin antigen in buffer with preservative.	2mL	5mL x 2
Positive control	Human antiserum in buffer with preservative.	1mL	2mL
Negative control	Rabbit serum in buffer with preservative.	1mL	2mL
Dispensing bottle			
Dispensing needle			
Test cards		10	**50**
Pipstirs		100	**500**
Instructions For Use			

4. Additional required materials

Micro-pipettes capable of delivering 50µL
Card rotator to deliver 90-110 rpm

5. Reagent preparation

Bring all reagents and samples to room temperature before use.

6. Storage and shelf life after first opening

1. Antigen and controls should be stored at 2–8°C. Do not freeze.
2. After opening Antigen and Controls are stable for up to 3 months when stored at 2–8°C.
3. Do not use after the expiration date.

7. Warnings and precautions

1. NewBio RPR is for *in vitro* diagnostic use only. For professional use only.
2. Antigen and Controls contain sodium azide (< 0.1% w/v) as a preservative, which can accumulate in lead or copper pipes to form potentially explosive azides. To prevent azide build-up, flush with large volumes of water after disposing of solutions containing azide into the drains.
3. Refer to NewBio RPR Safety Data Sheet for detailed information on reagent chemicals. Safety Data Sheet is available on the Newmarket Biomedical website - www.new-bio.com or requested electronically through info@new-bio.com.
4. **Caution:** Controls contain material of human or animal origin. All human origin material in the NewBio RPR has been tested and found negative or nonreactive for HBsAG, HIV 1 Ag [or HIV PCR(NAT)], HIV 1/2 antibody, HCV antibody, and HCV PCR (NAT) as required at the time of collection using FDA licensed test kits. No known test methods can offer total assurance that products derived from human origin will not transmit HIV, hepatitis or other potentially infectious agents. Therefore, the Controls and all specimens should be handled as potentially infectious.
5. This device contains material of animal origin. All bovine material is origin certified from approved sources.
6. Do not freeze Antigen and Controls.
7. Reagents from the same lot may be pooled using good laboratory practices.
8. Reagents showing visible signs of microbial growth or gross turbidity may indicate degradation and should be discarded according to local rules.
9. The effects of microbial contamination in specimens cannot be predicted.
10. Do not use reagents after the expiration date.
11. Do not interchange caps between the Positive and Negative Control vials. Controls are differentiated by colour coded caps and the vial label. If caps are inadvertently switched, the Control tubes should be discarded.
12. The reaction areas on the Test Cards should not be touched as this may invalidate results.
13. Samples exhibiting gross lipemia, hemolysis or icterus may be compromised and may require alternative testing.
14. Deviations from the NewBio RPR Instructions for Use can lead to erroneous results.
15. Dispose of leftover reagents in a safe manner, in accordance with local regulations.

8. Sample collection, handling and storage

NewBio RPR may be used for testing with either human serum or EDTA plasma specimens for up to 7 days after collection. Specimens should be free of particulate matter to prevent interference with the assay result. If erythrocytes or other visible components are present in the specimen, remove by centrifugation to prevent interference with the test results. Store EDTA plasma and serum specimens at 2-8°C up to 7 days. EDTA plasma and serum specimens can be frozen at less than -20°C for up to one month, thawed and mixed thoroughly prior to testing. Specimens may be frozen and thawed up to 5 times.

Allow all specimens to equilibrate to room temperature before use.

9. Assay procedure

1) Place 50µl of sample into a circle marked on the test card.

2) Spread the sample evenly over the test circle area. The flat end of the pipsters can be used to spread the sample over the test circle.

3) Shake the vial of RPR antigen to ensure even mixing.

4) Attach the dropping needle to the plastic dropping bottle and take up the RPR antigen by suction.

5) Invert the dropper bottle containing antigen and gently squeeze to expel air from the needle.

6) Holding the dropper bottle vertically over the test sample dispense a single drop,17.5 µl, of antigen.

7) Place test card on a card rotator and rotate at 100 RPM for 8 minutes.

8) Read and interpret results visually in good light. See interpretation.

9) It is recommended that the kit positive and negative controls are run with each batch of test samples.

10) Return unused antigen from dropper bottle to glass vial.

11) Clean out dropper bottle and needle with distilled water and allow to dry before re-using.

Sample titration assay procedure

1) Make doubling dilutions from Undiluted to 1:16 in normal saline.

2) Place 50µl of each dilution in to a separate circle on the test card.

3) Spread each dilution evenly over the test circle.

4) Continue as from Assay procedure section (3).

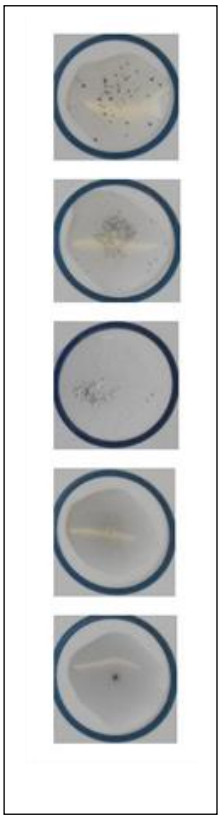
The titre of the sample is expressed as the final dilution which shows aggregation of the carbon particles.

10. Control procedure

The Positive and Negative Controls must be run with each assay. Additional QC testing may be performed by the operator by the inclusion of other characterised specimens or reference material.

The Positive Control should produce a positive result and the Negative Control should produce a negative result with the test. If the appropriate results are not obtained with the controls, the assay is considered invalid and all samples within that assay should be retested.

11. Interpretation of results



Strong Reactive: Large clumps of carbon particles with a clear background.

Reactive: Large clumps of carbon particles somewhat more disperse than strong Reactive pattern.

Weak Reactive: Small clumps of carbon particles with light grey background.

Trace Reactive: Slight clumping of carbon particles typically seen as a button of aggregates in the centre of the test circle or dispersed around the edge of the test circle.

Non-Reactive: Typically a smooth grey pattern or a button of non-aggregated carbon particles in the centre of the test circle.

12. Performance characteristics

Reproducibility

A panel of syphilis-negative samples and syphilis-positive samples of varying reactivity were tested twice per day for 5 days over a 7 day period using 3 reagent lots.

Samples	Agreement N=	Total N=	Rate of Agreement	95% CI
Syphilis positive	250	250	100.00%	98.54 – 100%
Syphilis negative	50	50	100.00%	92.89 – 100%
Overall	300	300	100.00%	98.78 – 100%

Cross reactivity and interference

At least 9 syphilis positive samples and 9 syphilis negative samples from patients with a variety of potentially interfering diseases and conditions were tested using 3 different lots of RPR reagents in order to determine whether these diseases or conditions cause positive or negative analytical interference. Cross reactivity and interference of Rubella, Toxoplasma, Borrelia, EBV, HCV, HBV, HAV, HIV, HTLV, Herpes, Chlamydia), ANA antibodies, Rheumatoid Factor antibodies and samples from pregnant (multiparous) subjects were tested.
All samples tested (151 syphilis positives and 140 syphilis negatives) showed concordance with the clinical status of the sample.

Diagnostic sensitivity

The diagnostic sensitivity for NewBio RPR was calculated for 158 samples (37 EDTA plasma and 121 sera) which had been confirmed as RPR positive by two other CE marked assays for non-treponemal antibodies.

Sample	**Agreement measure**	**Agreement N=**	**Total N=**	**ROA (%)**	**95% CI (%)**
EDTA Plasma	Sensitivity	37	37	100%	90.51-100.00
Sera	Sensitivity	119	121	98.35%	94.16-99.80
All Samples	Sensitivity	156	158	98.73%	95.50-99.85

Diagnostic specificity

The false positive rate of NewBio RPR was compared with another CE-marked assay for non-treponemal antibodies associated with syphilis infection using known syphilis-negative samples.

		NewBio RPR	
		R	NR
CE-marked RPR	R	0	1
	NR	1	1246

R: Reactive

NR: Non-Reactive

NPA agreement for NewBio RPR and alternative RPR product

Sample	**Agreement measure**	**Agreement N=**	**Total N=**	**ROA (%)**	**95% CI (%)**
EDTA plasma	NPA	1246	1247	99.92	99.55-100.0

13. Limitations

Pinta, yaws, bejel and other treponemal diseases may produce reactive results with non-treponemal tests.

NewBio RPR is intended for use as an aid to diagnosis. Results should be interpreted in combination with other serological test results and clinical evaluation.

14. Key to symbols

REF	Catalogue number
IVD	IVD In Vitro Diagnostic Medical Device
	Manufactured by
EC REP	EU Authorised Representative
	EU importer
	Distributor
	Temperature limitation
	Use by
LOT	Batch code
	Consult instructions for use

15. Post Market Surveillance

Should this IVD be implicated in any serious incident a report shall be made to the manufacturer and competent authority of the Member State in which the user and/or the patient is established.

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16. Summary of Safety and Performance

SSP can be obtained from the EUDAMED website https://ec.europa.eu/tools/eudamed.

17. Bibliography

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4. Portnoy J, Brewer JH, Harris A. Rapid plasma reagin card test for syphilis and other treponematoses. Public Health Rep 1962;77:645-52.
5. Portnoy J. Modifications of the rapid plasma reagin (RPR) card test for syphilis, for use in large scale testing. Am J Clin Pathol 1963;40:473-9.
6. Ratnam S. The laboratory diagnosis of syphilis. Can J Infect Dis Med Microbiol 2005 16(1)45-51

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