NewBio RPR

REF NB012 NB013

CE 2797

IVD

1. Intended Use

1.1. 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

2.1. Syphilis is caused by the spirochaete Treponema pallidum, and is usually acquired by sexual contact, although the disease may be transmitted by transfusion of infected blood. Intrauterine infection also occurs. The infection is a chronic condition that typically progresses through distinct primary, secondary, tertiary, and quaternary stages of infection. These stages produce diverse clinical symptoms, typically producing initial sores known as chancres, then syphilitic rash followed by long periods of dormancy. Untreated infection may eventually result in cardiovascular problems and neurosyphilis.

2.2. The organism cannot be routinely cultured in artificial media, and diagnosis of the infection usually depends on the demonstration of antibodies in the blood, which appear soon after initial infection.

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

2.4. 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 circle.

3. Components

Name	Description	100 tests NB012	500 tests NB013
Carbon 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
Dropper Bottle			
Dispensing Needle			
Test Cards		10	50
Pipstirs		100	500
Instructions for Use			

4. Additional Required Materials

4.1. Micro-pipettes capable of delivering 50µL

4.2. Card rotator to deliver 90 – 110 rpm

5. Reagent Preparation

5.1. Bring all reagents and samples to room temperature before use.
5.2. Kit controls must be run with each assay.
5.3. Ensure Carbon Antigen and Controls are thoroughly re-suspended.

6. Storage and Shelf-life after First Opening

6.1. Kit reagents and controls should be stored at 2 – 8°C. Do not freeze.
6.2. After opening, kit reagents and controls are stable for up to 3 months when stored at 2 – 8°C.
6.3. Do not use after the expiration date.

7. Warnings and Precautions

7.1. NewBio RPR is for *in vitro* diagnostic use only. For professional use only.
7.2. Inspect the kit box and contents before use. Do not use if damaged.
7.3. Read these Instructions for Use carefully. Deviations can lead to erroneous results.
7.4. Do not use kit reagents or controls after expiration date.
7.5. Do not freeze kit reagents and controls.
7.6. Kit reagents and controls contain sodium azide (<0.1% w/w) 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 when disposing into the drains.
7.7. Refer to NewBio RPR Safety Data Sheet (SDS) for detailed information on reagent chemicals. SDS is available on the Newmarket Biomedical website – www.new-bio.com or requested electronically through email at info@new-bio.com.
7.8. Kit reagent and controls contain material of animal origin. All bovine material is origin certified from approved sources.
7.9. All human origin material in the NewBio RPR has been tested and found negative or non-reactive for HSsAG, HIV 1Ag [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, all controls and patient samples should be handled, stored, and treated as potentially infectious. All laboratory personnel must be trained in the correct handling of human samples according to good laboratory practices.
7.10. Reagents and controls must be thoroughly re-suspended prior to use. Failure to do so could result in an inadequate dilution and erroneous results.
7.11. The reaction areas on the Test Cards should not be touched as this may invalidate results.
7.12. Reagents from the same lot may be pooled using good laboratory practices.
7.13. 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.
7.14. Reagents showing visible signs of microbial growth or gross turbidity may indicate degradation and should be discarded according to local rules.
7.15. Samples exhibiting gross lipemia, haemolysis, or icterus may be compromised and may require alternative testing.
7.16. The effects of microbial contamination in samples cannot be predicted.
7.17. Handle, store, and dispose of patient samples and test components in accordance with appropriate national laboratory safety guidelines or regulations.

8. Sample Collection, Handling, and Storage

8.1. NewBio RPR may be used for testing with either human serum or EDTA plasma samples for up to 7 days after collection.

8.2. Samples should be free of particulate matter to prevent interference with the assay result. If erythrocytes or other visible components are present in the sample, remove by centrifugation to prevent interference with the test results.

8.3. Store EDTA plasma and serum samples at 2 – 8°C for up to 7 days. EDTA plasma and serum samples can be frozen at less than -20°C for up to 8 months, thawed and mixed thoroughly prior to testing. Samples may be frozen and thawed up to 5 times.

8.4. Allow all patient samples to equilibrate to room temperature prior to use.

9. Assay Procedure

Note: The kit Positive and Negative Controls must be run with each batch of test samples.

9.1. Place 50µL of Positive and Negative Controls into each of two circles marked on the Test Card.

9.2. Place 50µL of each patient sample into a separate circle marked on the Test Card.

9.3. Spread each sample evenly over the test circle using the flat end of a pipstir.

9.4. Use a new pipstir for each sample to avoid cross contamination.

9.5. Shake the vial of Carbon Antigen to ensure even mixing.

9.6. Attach the Dispensing Needle to the plastic Dropper Bottle and take up the Carbon Antigen by suction.

9.7. Invert the Dropper Bottle containing Carbon Antigen and gently squeeze to expel air from the Dispensing Needle.

9.8. Holding the Dropper Bottle vertically over the test sample, dispense a single drop, ~17.5µL, of Carbon Antigen.

9.9. Place Test Card on a card rotator and rotate at 100 rpm for 8 minutes.

9.10. Read and interpret the results visually in good light. See interpretation.

9.11. Return unused Carbon Antigen from Dropper Bottle to glass vial.

9.12. Clean out Dropper Bottle and Dispensing Needle with distilled water and allow to dry before re-using.

9.13. Sample Titration Procedure (*up to and including 1:16)*

- Note: Care must be taken to avoid carryover of sample between serial dilutions.
- Make doubling dilutions from Undiluted to 1:16 in normal saline.
- Place 50µL of each dilution into a separate circle on the Test Card.
- Continue as from Assay procedure section 9.3.
- The titre of the sample is expressed as the final dilution which shows aggregation of the carbon particles.

9.14. Sample Titration Procedure *(for dilutions higher than 1:16*)

- Prepare a 1:50 dilution of non-reactive serum or EDTA plasma in 0.9% saline (**High titre diluent**). This is to be used for 1:32 and higher dilutions of samples.
- Prepare a 1:16 dilution of the test sample by adding 0.1mL of sample to 1.5mL of 0.9% saline. Mix thoroughly.
- Place 50µL of 1:50 **High titre diluent** in assigned circles on the Test Card (e.g. circles 2, 3, 4, and 5).
- Place 50µL of the 1:16 dilution of test sample in circles 1 and 2 on the Test card.
- Mix the solution in circle 2 by drawing the solution up and down in a pipette tip several times, avoiding bubble formation.
- Make 2-fold serial dilutions of the test sample by transferring 50µL to circle 3 and repeat mixing. Continue the serial dilution as required and discard 50µL from the last circle after mixing.
- Continue as from Assay procedure section 9.5.

10. Control Procedure

10.1. 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.

10.2. The Positive Control should produce a reactive result, and the Negative Control should produce a non-reactive 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

11.1. A sample where the reaction appears as large clumps of carbon particles with a clear background should be considered as a Strong Reactive result.

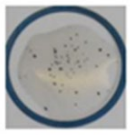
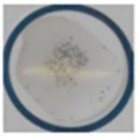
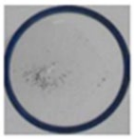
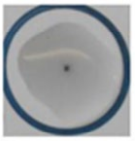
11.2. A sample where the reaction appears as large clumps of carbon particles somewhat more disperse should be considered a Reactive result.

11.3. A sample where the reaction appears as small clumps of carbon particles with a light grey background should be considered as a Weak Reactive result.

11.4. A sample where the reactions appear as 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 should be considered a Trace Reactive result.

11.5. A sample showing a smooth grey pattern or a button of non-aggregated carbon particles in the centre of the test circle should be considered a Non-reactive result.

11.6. Examples of the result interpretation are shown in the figure below:

				
Strong Reactive	Reactive	Weak Reactive	Trace Reactive	Non-reactive

12. Performance Characteristics

12.1. Reproducibility Study 1

12.1.1. 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%

12.2. Reproducibility Study 2

12.2.1. Assay reproducibility was assessed using a characterised panel of 20 plasma samples to determine variability across the concentration range of the assay.

12.2.2. Testing was completed in triplicate on 3 different manufactured lots over 3 days, with 3 different operators at 3 testing sites.

12.2.3. Testing sites included Newmarket Biomedical and two additional clinical laboratories in Australia.

Samples	Agreement N=	Total N=	Rate of Agreement	95% CI
RPR Lot 1	540	540	100.00%	99.32 – 100.00%
RPR Lot 2	540	540	100.00%	99.32 – 100.00%
RPR Lot 3	540	540	100.00%	99.32 – 100.00%
Site 1	540	540	100.00%	99.32 – 100.00%
Site 2	540	540	100.00%	99.32 – 100.00%
Site 3	540	540	100.00%	99.32 – 100.00%
Day 1 testing	540	540	100.00%	99.32 – 100.00%
Day 2 testing	540	540	100.00%	99.32 – 100.00%
Day 3 testing	540	540	100.00%	99.32 – 100.00%
Syphilis Positive	1215	1215	100.00%	99.70 – 100.00%
Syphilis Negative	405	405	100.00%	99.09 – 100.00%
Overall	1620	1620	100.00%	99.77 - 100.00%

12.3. **Repeatability**

12.3.1. Assay reproducibility was assessed using a characterised panel of 20 plasma samples to determine variability across the concentration range of the assay.

12.3.2. Testing was completed using 1 lot of NewBio TPHA in 3 testing runs on 1 day by a single operator. Each sample was tested 4 times per assay.

Samples	Agreement N=	Total N=	Rate of Agreement	95% CI
Syphilis Positive	180	180	100.00%	99.70 – 100%
Syphilis Negative	60	60	100.00%	99.09 – 100%
Overall	240	240	100.00%	99.77 – 100%

12.4. **Cross reactivity and interference**

12.4.1. 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.

12.4.2. All samples tested (151 syphilis positives and 140 syphilis negatives) showed concordance with the clinical status of the sample.

12.5. **Drug interference**

12.5.1. Five common therapeutic drugs were tested for potential interference. Each drug was spiked into 3 anti-treponemal samples and 4 non-reactive samples at levels recommended in CLSI guidelines. Results were compared to unspiked reference samples. Substances tested were selected based on their relevance to the intended use population.

12.5.2. Each drug was found to be non-interfering at the claimed concentration.

Substance	Tested concentration (µM)	Tested Concentration (mg/L)
Benzathine benzylpenicillin	200	182
Doxycycline hydrochloride	65	31.2
Ibuprofen	2425	500
Paracetamol	4340	656
Tenofovir disproxil	3.41	1.78

12.6. **Prozone**

12.6.1. Prozone effects may be seen at very high antibody levels for agglutination assays. In studies for NewBio RPR, no negative results were obtained for samples with high levels of reagin antibodies up to 1:2048 titre.

12.7. **Diagnostic sensitivity**

12.7.1. 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

12.8. **Diagnostic specificity**

12.8.1. 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

13.1. NewBio RPR may be used for human serum and EDTA plasma samples.

13.2. In early primary syphilis, specific antibodies may not be detected.

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

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

14. Australian Sponsor Information

Southern Cross Diagnostics Pty Ltd.
Unit 7, 17 Green Street, Banksmeadow, NSW 2019, Australia
Tel: 1-800-146-900 www.scdiagnostics.com.au

15. Key to Symbols

REF	Catalogue Number	CE	CE Mark of Conformity
IVD	In vitro diagnostic medical device	Σ	Contains sufficient for <n> tests
	Manufacturer		Temperature Limit
EU REP	EU Authorised Representative		Use by date
	EU Importer	LOT	Batch code
	Distributor		Consult Instructions for Use

16. Post Market Surveillance

16.1. 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.

www.new-bio.com info@new-bio.com

17. Summary of Safety and Performance

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

18. Literature References

18.1. Wasley G.D. & Wong H.H.Y. Syphilis Serology Principles and Practice. Oxford Medical Publications

18.2. Larsen S.A., Pope A. et al. A Manual of Tests for Syphilis. American Public Health Association.

18.3. Pope V. Rapid Plasma Reagin 18-mm Circle Card Test : Syphilis Serology Reference Laboratory Sexually Transmitted Infections Branch Division of AIDS, STD, and TB Laboratory Research NCHSTP

18.4. Portnoy J, Brewer JH, Harris A. Rapid plasma reagin card test for syphilis and other treponematoses. Public Health Rep 1962;77:645-52.

18.5. 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.

18.6. Ratnam S. The laboratory diagnosis of syphilis. Can J Infect Dis Med Microbiol 2005 16(1)45-51

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