INSTRUCTIONS FOR USE

RPR TEST

QUICK TEST FOR THE QUALITATIVE AND SEMI-QUANTITATIVE DETERMINATION OF REAGINS ANTIBODIES IN SERUM OR PLASMA

1 – CLINICAL SIGNIFICANCE AND INTENDED USE

For ***in Vitro*** diagnostic use only

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.
RPR TEST is a non-treponemal slide agglutination test for the qualitative and semi-quantitative detection of plasma reagins in human serum or plasma.

2 - PRINCIPLE OF THE METHOD

A suspension of cardiolipidic antigen gives a flocculation reaction with reagin in serum. The antigen used in the kit is a modification of V.D.R.L. antigen which contains microparticulate charcoal to enhance the visual difference between a positive and negative result.

3 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
RPR TEST	Slide agglutination test	**UC80600** (100 tests)	1 glass bottle containing carbon particles coated with a lipid complex, cardiolipin, lecithin and cholesterol in phosphate buffer 20 mmol/L. Preservative. pH, 7,0. (2,2 mL = 100 tests) 1 glass bottle containing Positive Control: stabilized liquid control, ready to use, artificial serum with reagin titer 1/4 (0.5 mL) 1 glass bottle containing Negative Control: stabilized liquid control, non-reactive with RPR antigen, contain Sodium azide <0.1% (0,5 mL) Slide, 6 test areas: plastic waterproof sheets for reaction (17 items) Sticks (1x25): plastic sticks for mixing (4 items) Secondary packaging: cardboard box.
RPR TEST	Slide agglutination test	**UC80610A** (300 tests)	3 glass bottles containing carbon particles coated with a lipid complex, cardiolipin, lecithin and cholesterol in phosphate buffer 20 mmol/L. Preservative. pH, 7,0. (3x2,2 mL = 300 tests) 1 glass bottle containing Positive Control: stabilized liquid control, ready to use, artificial serum with reagin titer 1/4 (0.5 mL) 1 glass bottle containing Negative Control: stabilized liquid control, non-reactive with RPR antigen, contain Sodium azide <0.1% (0,5 mL) Slide, 6 test areas: plastic waterproof sheets for reaction (50 items) Sticks (1x25): plastic sticks for mixing (12 items) Secondary packaging: cardboard box.

Positive control

Warning

H317- May cause an allergic skin reaction
P261; P272; P501
2-methyisothiazol-3(2H)-one (Proclin 950)

4 - MATERIALS REQUIRED BUT NOT PROVIDED

Mechanical rotator with adjustable speed at 80-100 r.p.m. Timer or clock. Pipettes 50 µL

5 - PRECAUTIONS AND WARNINGS

- RPR TEST is a kit for *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel.
- Components from human origin, if any, have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). However handle cautiously as potentially infectious.
- The sensitivity of the test may be reduced at low temperatures. Allow the reagents and samples to reach room temperature (15-30ºC/59-86ºF) before use.
- Do not use after expiration date or if the packaging is damaged. The quality of the reagent cannot be guaranteed beyond their shelf-life date or if the reagents are stored under inappropriate conditions.
- Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state, provincial or national regulations. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information.
- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- This product is classified as dangerous according to current European legislation (view above table and consulting the MSDS).
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.masciabrunelli.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.
- Notify Mascia Brunelli Spa and the Relevant Authorities of any serious incidents occurring in connection with the in vitro diagnostic device. complaint@masciabrunelli.it

6 - STORAGE CONDITIONS AND SHELF LIFE

All the kit components will remain stable until the expiration date printed on the label, when stored tightly closed at 2-8ºC and contaminations are prevented during their use. Do not freeze: frozen reagents could change the functionality of the test.
Mix reagents gently before use.
Reagents deterioration: Presence of particles and turbidity

7 – Specimens Collection

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

8 - Test Procedure

Allow the components of the kit to reach to room temperature (15-30ºC/59-86ºF) prior to testing.

Qualitative test

1. Gently shake the suspension for homogenization of the carbon particles.
2. Always use positive and negative controls as references.
3. Place 50 µL of sample and one drop or 50 µL of each Positive and Negative control in different area of the slide.
4. Add 20 µL of Carbon RPR suspension at the drop of sample and controls.
5. Mix 2 drops of the stretching over the entire surface of the circle with a stick. Use different stick for each sample
6. Spinning the slide, either manually or with a mechanical stirrer 80 to 100 rpm for 8 minutes. Read the presence or absence of visible agglutination within 8 minutes. Non-specific agglutination may appear if the test is read after 8 minutes.

Semiquantitative test

Runs in the same way as the qualitative test, but by making a dilution of the serum sample with saline (NaCl 9 g/L):

Dilutions	**1:2**	**1:4**	**1:8**	**1:16**
Serum / Sample	100 µl	...	...	...
Saline	100 µl	100 µl	100 µl	100 µl
	—	→ 100 µl		
		—	→ 100 µl	
			—	→ 100 µl
Sample Volume	50 µl	50 µl	50 µl	50 µl
Spinning the slide, either manually or with a mechanical stirrer 80 to 100 rpm for 8 minutes. Read the result within 8 minutes.				

9 – Reading, Interpretation and Calculation

Qualitative test: examine macroscopically the presence or absence of visible agglutination immediately after removing the slide test from the rotator. Rotate the slide twice by hand before reading.

Agglutination	**Reading**	**Report**
Medium or large clumps	R	Reactive
Small clumps	W	Weakly Reactive
No clumping or very slight “roughness”	N	Non Reactive

Semiquantitative tests: the titer is defined as the highest dilution showing a positive result.

10 – Quality Control

Positive and Negative controls are recommended to monitor the performance of procedure, as well as a comparative pattern for a better result interpretation. All result different from the negative control result, will be considered as a positive.

11 – Characteristics

- **Analytical sensitivity:** the sensitivity is calibrated against the International Reference WHO (1st Standard Human Syphilitic Serum, ref. 05/132). Accurate titer determination of the Reference Material, under the described assay conditions.
- **Prozone effect:** no prozone effect was detected up to titers ≥ 1/128.
- **Diagnostic sensitivity:** 100%
- **Diagnostic specificity:** 100%
- **Interferences:** the follow substances not interfere: bilirubin (20 mg/L), hemoglobin (10 g/L), and lipemia (10 g/L). Rheumatoid factors (300 IU/mL), interfere. Other substances may interfere[5].

12 – Notes

1. During the 8 minutes of reaction time do not expose the slide to a source of heat or intense light in order to reduce evaporation. Such evaporation could cause a false agglutination and therefore false positive results.

13 – Limitations of the Method

- False positive results have been reported in diseases such as infectious mononucleosis, viral pneumonia, toxoplasmosis, pregnancy and autoimmune diseases.
- All Reactive samples should be retested with treponemic methods such as TPHA (REF. UC80500) and FTA-Abs to confirm the results.
- 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.
- The components of this I.v.D. were always tested together without compatibility with components from other manufacturers. While not excluding the possibility that these components can be used with components of the same formulation but produced by other companies, there is no experimental evidence of such compatibility.

14 – References

1. George P. Schimid. Current Opinion in Infectious Diseases 1994; 7: 34-40
2. Sandra A Larsen et al. Clinical Microbiology Reviews 1995; 8 (1): 1-21.
3. Sandra Larsen et al. A manual of Test for Syphilis American Public Health Association 1990: 1-192.
4. Joseph Earle Moore et al. Gastrointestinal Haemorrhage 1952; 150(5): 467-473.
5. Young DS. Effects of drugs on clinical laboratory test, 4th ed. AACC Press, 1995.

TABLE OF APPLICABLE SYMBOLS

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (DXXX)		Manufacturer		Keep dry	UDI	Unique device identifier
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 7	Updated layout and content	2022/07
Instructions for Use (IFU) – Revision 8	Update number of slides on REF. UC80600	2022/10
Instructions for Use (IFU) – Revision 9	Elimination Controls kit	2024/05
Instructions for Use (IFU) – Revision 10	Elimination CND, EDMA and RDM codes; change of H and P phrases	2024/11

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.