INSTRUCTIONS FOR USE

WAALER ROSE TEST

RAPID AGGLUTINATION SLIDE TEST FOR QUALITATIVE AND SEMIQUANTITATIVE DETERMINATION OF RHEUMATOID FACTORS (RF)

1 – CLINICAL SIGNIFICANCE AND INTENDED USE

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

Rheumatoid Factor is an antibody directed against an organism's own tissues; it is defined as an antibody against the Fc portion of IgG. Although rheumatoid factors are found in a number of rheumatoid disorders with "alteration" of immune system, its central role lies its utility as an aid in the diagnosis of rheumatoid arthritis (RA). An study of the "American College of Rheumatology" shows that the 80,4% of RA patients were RF positive.
The Waaler Rose Test is a hemagglutination test on sensitized red blood cells for the qualitative and semiquantitative determination of rheumatoid factor (RF) in human serum.

2 - PRINCIPLE OF THE METHOD

Stabilized sheep erythrocytes sensitized with rabbit IgG anti-sheep erythrocyte are agglutinated when mixed with samples containing RF. The WAALER ROSE TEST sensitivity is calibrated against the WHO Rheumatoid Arthritis Serum for a minimum concentration of 8 IU/mL.

3 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
WAALER ROSE TEST CND: W0102160699 EDMA: 12.11.01.90; RDM: 1555762/R	Latex agglutination test	**UA80255** (62 tests)	1 glass bottle containing suspension for WR, with preservative. pH 8.2 (3,1 mL = 62 tests) 1 glass bottle containing Positive Control: human serum with a RF concentration > 30 IU/mL. (0.5 mL) 1 glass bottle containing Negative Control: stabilized liquid control, contain Sodium azide <0.1% (0,5 mL) Slide, 6 test areas: plastic waterproof sheets for reaction (11 items) Sticks (1x25): plastic sticks for mixing (3 items) Secondary packaging: cardboard box.
WAALER ROSE CONTROLLI CND: W0102160801 EDMA: 12.50.01.13; RDM: 1555764/R	Controls for latex agglutination test	**UD80252** (2x0,5 mL)	1 glass bottle with Positive Control: human serum with a RF concentration > 30 IU/mL. Preservative. (0.5 mL) 1 glass bottle containing Negative Control: stabilized liquid control, contain Sodium azide <0.1% (0,5 mL) Secondary packaging: cardboard box.

Suspension and Positive Control

Warning
H317
P261; P272; P501
(2-methyl-2H-isothiazol-3-one (Proclin 950)

4 - MATERIALS REQUIRED BUT NOT PROVIDED

Timer or clock. *Pipettes 50 µL*

5 - PRECAUTIONS AND WARNINGS

- WAALER ROSE 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 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*.
Always keep vials in vertical position. If the position is changed, gently mix to dissolve aggregates that may be present.
Reagents deterioration: Presence of particles and turbidity

7 – SPECIMENS COLLECTION

Fresh serum. 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 suspension particles.
2. Always use positive and negative controls as references.
3. *Place 50 µL of serum and one drop or 50 µL of each Positive and Negative control in different area of the slide.*
4. Add 1 drop *or 50 µL* of Suspension reagent at the drop of serum.
5. Mix 2 drops of the stretching over the entire surface of the circle with a stick. *Use different stick for each sample*
6. Let the slide undisturbed on a flat surface for 2 minutes.
7. After this time, twist very carefully the slide once to about 45º from the horizontal and let the slide again to stay on a flat surface for 1 minute more.

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
Let the slide undisturbed on a flat surface for 2 minutes. After this time, twist very carefully the slide once to about 45º from the horizontal and let the slide again to stay on a flat surface for 1 minute more.				

9 – READING, INTERPRETATION AND CALCULATION

Examine macroscopically the presence or absence of visible agglutination immediately avoiding any movement or lifting the slide during the observation. The presence of visible agglutination indicates a RF concentration equal or greater than 8 IU/mL (Note 1). The titer, in the semi-quantitative method, is defined as the highest dilution showing a positive result.The approximate RF concentration in the patient sample is calculated as follows

8 x RF titer = IU/mL

10 – EXPECTED VALUES

≤ 8 IU/mL. Each laboratory should establish its own reference range.

11 – CHARACTERISTICS

- **Analytical sensitivity:** 8 (6-16) IU/mL, under the described assay conditions.
- **Prozone effect:** no prozone effect was detected up to 800 IU/mL.
- **Diagnostic sensitivity:** 100%
- **Diagnostic specificity:** 93,6%
- **Interferences:** the follow substances not interfere: bilirubin (20 mg/L), hemoglobin (10 g/L), and lipemia (10 g/L). Other substances may interfere[6].

12 – NOTES

1. Results obtained with the Waaler Rose test do not compare with a latex method. Differences in results between methods do not reflect differences in the ability to detect Rheumatoid Factors. Rheumatoid Factors are immunoglobulins (in most cases IgM) with antibody activity. These factors are present in most individuals with Rheumatoid Arthritis. There are several Rheumatoid Factors, and there is no test that can determine all of them, as some of them react with human IgG, some with animal IgG, and some with both.

13 – LIMITATIONS OF THE METHOD

- The incidence of false positive results is about 3-5%. Individuals suffering from infectious mononucleosis, hepatitis, syphilis as well as elderly people may give positive results.
- As with all diagnostic tests, a final diagnosis cannot rely on the outcome of a single test and must be supported by other clinical parameters.
- 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. Robert W Dorner et al. Clinica Chimica Acta 1987; 167: 1 – 21.
2. Frederick Wolfe et al. Arthritis and Rheumatism 1991; 34: 951- 960.
3. Robert H Shmerling et al. The American Journal of Medicine 1991; 91: 528 – 534.
4. Koritz T N et al. Journal of Inmmunological Methods. 1980; 32; 1 – 9.
5. Assameh S N et al. Journal of Immunological Methods 1980; 34: 205 – 215.
6. 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 5	Updated layout and content	2022/03
Instructions for Use (IFU) – Revision 6	Update number of slides on REF. UA80255	2022/10

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