WAALER-ROSE

Qualitative and semiquantitative determination of rheumatoid factors by passive haemagglutination

TEST SUMMARY
Rheumatoid factors contained in the serum produce an agglutination of erythrocytes covered by IgG of rabbit.

SAMPLES
Fresh serum. Stability 7 days at 2-8°C. For longer time freeze at –20°C, and bring to room temperature before testing.
Turbid samples must be centrifuged.

REAGENTS
Suspension
Erythrocytes covered by IgG of rabbit, conservative and stabilizer.

Positive control
Human base stabilized solution of rheumatoid factors with a titre that gives a clear agglutination.

Negative control
Proteic solution not reactive with erytrocytes suspension.

All reagents contain 0.095% of sodium azide.

REAGENTS PREPARATION
Reagents are ready for the use.
Erythrocytes suspension must be resuspended carefully. When the suspension becomes homogeneous by sweet inversion, it is necessary to fill and to empty the dosage's pipette many times.
Stability: The reagents are stable until expiration date on the label at 2-8°C.
Do not freeze.

MATERIALS REQUIRED BUT NOT SUPPLIED
Physiologic solution.
COD. AK00250 Slide and disposable stirrers.

PRECAUTIONS
Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.
Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

QUALITATIVE PROCEDURE

Reagents	Sample	Positive control	Negative control
Sample	50 µl (1 gt)	--	--
Control +	--	50 µl (1 gt)	--
Control -	--	--	50 µl (1 gt)
Suspension	50 µl (1 gt)	50 µl (1 gt)	50 µl (1 gt)

Mix using disposable stirrers and spreading homogeneously the mixture on the slide, then, shake slide for 2 minutes by a sweet rotating motion or by a stirrer at 100 r.p.m., after this time incline of 30° about the slide and wait one minute again; observe eventual agglutination using artificial light.

RESULTS INTERPRETATION
POSITIVE: A clear agglutination within 2 minutes.
NEGATIVE: No agglutination within 2 minutes.
In case of positivity it is opportune to titre semiquantitatively the serum.

SEMIQUANTITATIVE PROCEDURE
Prearrange serial dilution of the serum, pipetting in six slide areas, 50 µl of physiologic solution and 50 µl of sample in the first area. Using the same pipette (inspiring and discharging many times) mix carefully contents of first area and transfer 50 µl in the following area etc. Discharge 50 µl from last area. Dispense erytrocytes suspension, shake for 2 minutes, and after 3 minutes observe agglutination. The titre is given by last clear agglutination. Procedure is in the scheme below:

Reagents	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6
Physiologic	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl
Sample	50 µl	50 µl from 1	50 µl from 2	50 µl from 3	50 µl from 4	50 µl from 5
Discharge 50 µl from last area						
Suspens.	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl
Titre	16 UI/ml	32 UI/ml	64 UI UI/ml	128 UI/ml	256 UI/ml	512 UI/ml

EXPECTED VALUES
Approximately 70-80% of patients with a clinical diagnosis of rheumatoid arthritis are seropositive for rheumatoid factor. Positive results were shown for nearly all patients with variants of rheumatoid arthritis such as Felty's or Sjogren's syndrome. A positive result can be expected in less than 5% of healthy individuals, while in the population aged 60 years and older, 30% may be seropositive using latex tests for the detection of rheumatoid factor.

CLINICAL SIGNIFICANCE
Rheumatoid factors found in the sera of most patients with rheumatoid arthritis as well as in a variety of other diseases, are a group of antibodies most belonging to the IgM class directed against determinants on the Fc fragment of the patients' IgG immunoglobulin.

NOTE
- Turbid or lipemic sera can give false positivity.
- If reaction's time are bigger than 3 minutes, they may cause a supervalutation of samples concentrations.
- All the reagents used, have been found negative in the reaction with HIV and HBsAg. However, they should be handled with care.
- If the results are incompatible with clinical presentation, they have to be evaluated within a total clinical study.

CALIBRATION
Positive and Negative control sera should be always used to distinguish an eventual background's agglutination of reactive.

TEST PERFORMANCE
Interferences
Any interferences are produced with :

Haemoglobin	≤ 1000 mg/dl
Bilirrubin	≤ 20 mg/dl
Lipids	≤ 1000 mg/dl

Sensitivity
Test gives positive results as from concentrations of 8 UI/ml.

Not happened phenomenon of prozone in RF concentrations studied until 624 UI/ml.

Specificity
A comparison with an available commercial method gave following results on 84 samples compared, giving a specificity = 93.6%:

		LTA srl		
		+	-	TOT.
COMPETITORS	+	37	0	37
		100%	0%	
	-	3	44	47
		6.4%	93.6%	
	TOT.	40	44	84

WASTE DISPOSAL
Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

PACKAGING

CODE AK00250	**(50 TESTS)**
Suspension	1 x 2.5 ml
Positive control	1 x 0.5 ml
Negative control	1 x 0.5 ml

CODE AK00252	**(50 TESTS)**
Suspension	1 x 2.5 ml
Positive control	1 x 0.5 ml
Negative control	1 x 0.5 ml
Slide white spot	9
Stirrers	25

REFERENCES

Singer. J.M. et al., Am.J.Med. 21:888-892 (1956).
Waaler. M. et al., Arthrtis Rheum., 4:47-57 (1961).
Jones. W.L. et al., Amer.J.Clin. Path., 60:603-608 (1973).

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SYMBOLS
IVD Only for IVD use
LOT Lot of manufacturing
REF Code number
Storage temperature interval
Expiration date
Warning, read enclosed documents
Read the directions
Biological risk

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