

## Quantitative determination of Rheumatoid Factors (RF)

Store 2 - 8°C.

### PRINCIPLE OF THE METHOD

The RF-Turbilatex is a quantitative turbidimetric test for the measurement of RF in human serum or plasma.

Latex particles coated with human gammaglobulin are agglutinated when mixed with samples containing RF. The agglutination causes an absorbance change, dependent upon the RF contents of sample that can be quantified by comparison from a calibrator of known RF concentration.

### CLINICAL SIGNIFICANCE

Rheumatoid factors are a group of antibodies directed to determinants in the Fc portion of the immunoglobulin G molecule. Although rheumatoid factors are found in a number of rheumatoid disorders, such as systemic lupus erythematosus (SLE) and Sjögren's syndrome, as well as in nonrheumatic conditions, its central role in clinic lies its utility as an aid in the diagnosis of rheumatoid arthritis (RA).

A study of the "American College of Rheumatology" shows that the 80.4% of RA patients were RF positive.

### REAGENTS

<b>Diluent (R1)</b>	Tris buffer 20 mmol/L, pH 8.2. Sodium azide 0.95 g/L.
<b>Latex (R2)</b>	Latex particles coated with human gammaglobulin, pH 7.4. Sodium azide 0.95 g/L.
<b>RF-CAL</b>	Calibrator. Human serum. The RF concentration is stated on the vial label.

### PRECAUTIONS

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.

### CALIBRATION

The sensitivity of the assay and the target value of the calibrator have been standardized against the International Reference NIBSC 64/2 (Rheumatoid Arthritis Serum) WHO. Recalibrate when control results are out of specified tolerances, when using different lot of reagent and when the instrument is adjusted.

### PREPARATION

**RF Calibrator:** Reconstitute (→) with 2.0 mL of distilled water. Mix gently and bring to room temperature for about 10 minutes before use.

**Calibration Curve:** Prepare the following RF calibrator dilutions in NaCl 9 g/L. Multiply the concentration of the RF calibrator by the corresponding factor stated in table below to obtain the RF concentration of each dilution.

Calibrator dilution	1	2	3	4	5	6
Calibrator RF (µL)	--	25	50	100	200	400
NaCl 9 g/L (µL)	400	375	350	300	200	-
Factor	0	0,0625	0,125	0,25	0,5	1,0

**One point calibration (lineal range up to 100 IU/mL):** Prepare a RF Calibrator dilution:  
- 30 µL RF Calibrator + 70 µL NaCl 9 g/L

Multiply the RF calibrator concentration by 0.30 to obtain the RF concentration of the diluted calibrator.

### STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C and contaminations are prevented during their use. Do not use reagents over the expiration date.

**Reagent deterioration:** Presence of particles and turbidity.

**Reconstituted calibrator:** Stable for 1 month at 2-8°C or 3 months at -20°C. Do not freeze; frozen latex and diluent could change the functionality of the test.

### ADDITIONAL EQUIPMENT

- Thermostatic bath at 37°C.
- Spectrophotometer or photometer thermostatable at 37°C with a 650 nm filter (600 - 650 nm).

### SAMPLES

Fresh serum or plasma. Stable 7 days at 2-8°C or 3 months at -20°C. The samples with presence of fibrin should be centrifuged before testing. Do not use highly hemolized or lipemic samples.

### QUALITY CONTROL

Control Sera are recommended to monitor the performance of manual and automated assay procedures. It should be used GESAN Control ASO/CRP/RF Level L (Ref.: 704CTLL) and Level H (Ref.: 704CTLH). Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

### REFERENCE VALUES

Normal values up to 20 IU/mL. Each laboratory should establish its own reference range.

### PROCEDURE

1. Bring the reagents and the photometer (cuvette holder) to 37°C.
2. Assay conditions:

Wavelength : 650 nm (600-650 nm)

Temperature : 37 °C

Cuvette lighth path : 1 cm

3. Adjust the instrument to zero with distilled water.

4. Pipette into a cuvette:

	Blank
R1: Diluent (mL)	0.9
R2: Latex (mL)	0.1

5. Mix and read the absorbance ( Blank reagent ).

6. Add the sample/ calibrator.

	Blank	Calibrator /Sample
NaCl 9 g/L (µL)	7	--
Calibrator or sample (µL)	--	7

7. Mix and read the absorbance after 2 minutes ( $A_2$ ) of the sample addition.

### CALCULATIONS

**Calibration curve (Note 1):** Calculate the absorbance difference ( $A_2 - A_{\text{blank reagent}}$ ) of each point of the calibration curve and plot the values obtained against the RF concentration of each calibrator dilution. Rheumatoid factor concentration in the sample is calculated by interpolation of its ( $A_2 - A_{\text{blank reagent}}$ ) in the calibration curve.

### One point calibration:

$$(A_2 - A_{\text{blank reagent}})_{\text{sample}} \times \text{Diluted calibrator concentration} = \text{IU/mL RF}$$

$$(A_2 - A_{\text{blank reagent}})_{\text{calibrator}}$$

### PERFORMANCE CHARACTERISTICS

1. **Limit detection:** Values less than 6 IU/mL give non-reproducible results.

2. **Measurement range** (calibration curve): 6-160 IU/mL, under the described assay conditions. Samples with higher concentrations should be diluted 1/5 in NaCl 9 g/L and retested again. The linearity limit and measurement range depends on the sample to reagent/ratio, as well as the analyzer used. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.

3. **Prozone effect:** No prozone effect was detected upon 800 IU/mL.

4. **Sensitivity:**  $\Delta$  3.34 mA. IU/mL.

5. **Precision:**

Mean (IU/mL)	Intra-assay (n=10)		Inter-assay (n=10)	
	14.9	45.8	14.9	45.8
SD	0.96	1.32	1.2	2.54
CV	6.5	2.9	8.0	5.6

7. **Accuracy:** Results obtained using this reagent (y) were compared to those obtained using a commercial reagent (x) with similar characteristics. 86 samples ranging from 1 to 160 IU/mL of RF were assayed. The correlation coefficient (r) was 0.95 and the regression equation  $y = 0.797x - 1.075$ .

The results of the performance characteristics depend on the analyzer used.

### INTERFERENCES

Hemoglobin (10 g/L), bilirubin (20 mg/dL) and lipemia (10 g/L), do not interfere. Other substances may interfere.

### NOTES

1. Multipoint calibration gives more accurate results than one point calibration.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

### BIBLIOGRAPHY

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4. Vladimír Muié et al. Scand J Rheumatology 1972; 1: 181 - 187.
5. Paul R et al. Clin Chem 1979; 25/11: 1909 - 1914.
6. Young DS. Effects of drugs on clinical laboratory test, 4th ed. AACC Press, 1995.

### Symbols

	Biological risk
	CE Mark (98/79 CE regulation)
	in vitro medical device
	Batch Code
	Use by
	Storage temperature limits
	Read instruction for use
	Manufacturer in Spain