



RHEUMATOID FACTOR (RF)

Turbilatex

Quantitative determination

Cat. No. 101-0465

Size 1x45 ml / 1x5 ml

PRINCIPLE:

This RF-Turbilatex agglutination assay is a quantitative turbidimetry assay for 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 the patient 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).

REAGENTS:

1. Reagent 1 (1x45 ml)

Diluent; TRIS buffer pH 8.3 20 mmol/L
Sodium azide 0.95 g/L

2. Reagent 2 (1x5 ml)

RF Latex
Sodium azide 0.95 g/L

3. Reagent 3 (1x2 ml)

RF Calibrator
Concentration see on the vial label

Optional: 101-0466 Control serum ASO/CRP/RF Level I
101-0467 Control serum ASO/CRP/RF Level II

Components of human serum origin have been tested and found to be negative for the presence of HBsAg, HCV and of antibody to HIV (1/2). However handle cautiously as potentially infectious.

CALIBRATION:

The assay is calibrated to the WHO 64/1 (Rheumatoid Arthritis Serum). The use of other commercially available RF calibrators is not recommended.

REAGENT PREPARATION AND STABILITY:

RF Calibrator: Reconstitute with 2.0 mL of distilled water. Stable for 1 month at 2-8°C or 3 months at -20°C.

Calibration Curve (range between 20 - 160 IU/mL): Prepare the following RF calibrator dilutions in NaCl, 9 g/L as diluent. Multiply the concentration of the RF calibrator by the corresponding factor stated in table below to obtain the RF concentration of each dilution.

Dilution	1	2	3	4	5
Calibrator	10	30	50	75	100
NaCl 9g/L	90	70	50	25	0
Factor	0.1	0.3	0.5	0.75	1.0

One point calibration (linear 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 0.3 to obtain the RF concentration of the dilution.

All the components of the kit are stable until the expiration date on the label when stored at 2-8°C. Do not use reagents over the expiration date.

Do not freeze: frozen reagents could change the functionality of the test.

SAMPLES:

Fresh serum. Stable 8 days at 2-8°C or 3 months at -20°C.

The samples with particles of fibrin should be centrifuged to eliminate them.

Do not use haemolized or lipemic samples.

PROCEDURE:

Wavelength: 650 nm (600-650)
Cuvette: 1 cm light path
Temperature: 37 °C
Zero: Distilled water

Pipette into a cuvette:	Blank	Calibrator	Sample
NaCl 9 g/L	5 µl	--	--
Calibrator	--	5 µl	--
Sample	--	--	5 µl
R1 Buffer	900 µl	900 µl	900 µl
R2 Latex	100 µl	100 µl	100 µl
Mix and read the absorbance against blank after 2 minutes (A) of the sample addition.			

CALCULATION:

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

One point calibration:

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

Chronolab has instruction sheets available for several automatic analyzers. Instructions for many of them are available on request.

QUALITY CONTROL:

Serum controls are recommended to monitor the performance of manual and automated assay procedures.

Chronolab Control Serum ASO/CRP/RF are available:

Level I (Cat.No. 101-0466) and Level II (Cat.No.101-0467)

REFERENCE VALUES:

Up to 20 IU/mL (Each laboratory should establish its own reference range)

PERFORMANCE CHARACTERISTICS:

- Linearity (one point calibration):** Up to 100 IU/mL, under the described assay conditions.
- Measurement range (calibration curve):** 20-160 IU/mL, under the described assay conditions. The linearity limit and measurement range depends on the sample to reagent ratio. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
- Prozone effect:** No prozone effect was detected upon 800 IU/mL.
- Sensitivity:** Values less than 2.5 IU/mL give non reproducible results.
- Precision:**

Mean (IU/mL)	Intra-assay		Inter-assay	
	14.9	45.74	14.9	45.74
SD	0.96	1.32	1.2	2.54
CV	6.50	2.86	8.05	5.56
N	10	10	10	10

- Accuracy:** Results obtained using this reagents (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 was $y = 0.7972x + 1.0752$.

INTERFERENCES:

Bilirubin: up to 20 mg/dL do not interfere.

Hemoglobin: up to 5 g/L do not interfere.

Lipids: up to 10 g/L do not interfere.

REFERENCES:

- Frederick Wolfe et al. Arthritis and Rheumatism 1991; 34: 951-960
- Robert W Dorner et al. Clinica Chimica Acta 1987; 167: 1-21
- Robert H Shmerling et al. The American Journal of Medicine 1991; 91: 528-534
- Vladimir Muié et al. Scand J Rheumatology 1972; 1: 181-187
- Paul R et al. Clin. Chem; 1979; 25/11: 1909-1914