

## Quantitative determination of Rheumatoid Factors (RF) IVD DIAGNOSTIC 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 Sjogren'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)

### PRINCIPLE:

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 the patient sample that can be quantified by comparison from a calibrator of known RF concentration.

### PRESENTATION:

	No. of Bottles / Vials 1x50 ml
• R1 Diluent	1
• R2 Latex	1
• RF Calibrator (140 IU/mL)*	1

\* Value may vary from lot to lot.

### PREPARATION OF WORKING REAGENT:

Mix 0.9 ml of Diluent (R1) with 0.1 ml of latex (R2).

### PREPARATION OF WORKING CALIBRATOR:

Reconstitute with 2.0 ml of distilled water. Mix gently and incubate at room temperature for 10 minutes before use.

### STORAGE AND STABILITY:

All reagents are stable at 2-8° C till the expiry date mentioned on the label.

**WORKING REAGENT:** Stable for 30 days at 2-8°C.

**RF CALIBRATOR:** Stable for 1 month at 2-8°C or 3 months at -20°C.

Do not freeze, frozen Latex or Diluent could change the functionality of the test.

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

### SAMPLES:

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

### (A) FOR MULTIPOINT CALIBRATION :

#### Calibration curve (range from 20 to150 IU/mL):

Prepare the following RF calibrator dilutions in NaCl 9 gm/L. Multiply the concentration of 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)	-	10	25	50	75	100
• NaCl 9 g/L (µl)	100	90	75	50	25	-
• Factor	0	0.1	0.25	0.5	0.75	1.0
Concentration of diluted Calibrator (IU/mL)	0	14	35	70	105	140

### (B) FOR 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 diluted working calibrator which is equal to 42 IU/mL.

### 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

### REACTION PARAMETERS :

- Type of Reaction : End point / Multi Standard
- Wavelength : 650 nm (600-650)
- Flow cell temperature : 37°C
- Sample/Calibrator Volume : 7 µl
- Reagent Volume : 1 mL
- Zero setting with : Reagent Blank
- Light Path : 1 cm
- Incubation : 2 min
- Delay Time : 5 Sec

### PROCEDURE :

PIPETTE IN TEST TUBE	Blank	Calibrator or Sample
• NaCl 9 g/L (µl)	7	-
• Calibrator or sample (µl)	-	7
• Working Reagent (mL)	1.0	1.0

Mix and read the absorbance A for blank and (A2) after 2 minutes of the sample & Calibrator addition.

- **For Multipoint Calibration:** Use all the 6 Calibrators as stated above.
- **For Single point calibration:** Use diluted Calibrator as stated above.

### TEST RESULTS:

#### (A) BY MULTIPOINT CALIBRATION:

**Calibration curve (Note 1):** Calculate the absorbance difference (A2-A blank) of each point of the calibration curve and point the values obtained against the RF concentration of each calibrator dilution. Rheumatoid factor concentration in the sample is calculated by interpolation of its (A2-A blank) in the calibration curve.

#### (B) BY ONE POINT CALIBRATION:

$$\text{IU/ml RF Value} = \frac{(\text{A2-A Blank}) \text{ Sample}}{(\text{A2-A Blank}) \text{ Calibrator}} \times \text{Diluted working calibrator concentration}$$

### QUALITY CONTROL:

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

### NORMAL VALUES:

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

## PERFORMANCE CHARACTERISTICS:

- (a) **Linearity:** Up to 100 IU/mL for single point calibration & up to 150 for multipoint calibration under the described assay conditions.
- (b) **Detection limit:** Value less than 3 IU/mL give non-reproducible results.
- (c) **Measurement range (calibration curve):** 20-150 IU/mL under the described assay condition. 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 / 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.
- (d) **Sensitivity:**  $\Delta$  3.34 mAIU/mL

## INTERFERENCES:

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

## NOTES:

- 1. Multipoint calibration gives more accurate result 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.

## REFERENCE:

- 1. Frederick Wolfe et al. Arthritis and Rheumatism 1991; 34:951-960.
- 2. Robert W Dornier et al. Clinica Chimica Acta 1987;167:1-21.