Audit Diagnostics

CE

RF-TURBI

RF-turbilatex

Latex turbidimetry

Quantitative determination of Rheumatoid Factors (RF)
IVD

Store 2 - 8ºC.

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

Diluent (R1)	Tris buffer 20 mmol/L, pH 8.2. Preservative.
Latex (R2)	Latex particles coated with human gammaglobulin, pH 7.4. Preservative.
RF-CAL	Calibrator. Human serum. The RF concentration is stated on the vial label.
Optional	Ref.:1102114 Control serum ASO/CRP/RF Level L Ref.:1102115 Control serum ASO/CRP/RF Level H.

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

Use RF Calibrator Reference 1107007.
The sensitivity of the assay and the target value of the calibrator have been standardized against the International Reference Standard from NIBSC 64/002.
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 bellow 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

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC 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 2-8ºC or 3 months at –20ºC. Do not freeze; frozen latex and diluent could change the functionality of the test.

ADDITIONAL EQUIPMENT

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

SAMPLES

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

PROCEDURE

1. Bring the reagents and the photometer (cuvette holder) to 37ºC.
2. Assay conditions:
   Wavelength : 650 nm (600-650 nm)
   Temperature : 37 ºC
   Cuvette ligth path : 1cm
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:

	Blank
R1: Diluent (mL)	0.8
R2. Latex (mL)	0.2

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.

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

CALCULATIONS

Calculate the absorbance difference (A_2-$A_{blank\ reagent}$) 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-$A_{blank\ reagent}$) in the calibration curve.

QUALITY CONTROL

Control Sera are recommended to monitor the performance of manual and automated assay procedures. It should be used Audit Diagnostics Control ASO/CRP/RF Level L and Level H .
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.

PERFORMANCE CHARACTERISTICS

1. **Limit detection:** Values less than 6 IU/mL give non-reproducible results.
2. **Measurement range**: 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:** Δ 3.34 mA. IU/mL.
5. **Precision:** The reagent has been tested for 20 days, using three different FR concentrations in a EP5-based study.

EP5	CV (%)		
	35.8 IU/mL	78.05 IU/mL	123.26 IU/mL
Total	4.5%	4.1%	5.9%
Within Run	3.3%	2.6%	3.2%
Between Run	1.7%	2.3%	3.4%
Between Day	2.5%	2.1%	3.6%

6. **Accuracy:** Results obtained using this reagent (y) were compared to those obtained using a commercial reagent (x) with similar characteristics. 41 samples of different concentrations of FR were assayed. The correlation coefficient (r) was 0.91 and the regression equation y = 1.2042x +3.1344.
   The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Hemoglobin (10 g/L), bilirrubin (20 mg/dL) and lipemia (10 g/L), do not interfere. Other substances may interfere[6].

NOTES

1. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

BIBLIOGRAPHY

1. Frederick Wolfe et al. Arthritis and Rheumatism 1991; 34: 951- 960.
2. Robert W Dorner et al. Clinica Chimica Acta 1987; 167: 1-21.
3. Robert H Shmerling et al. The American Journal of Medicine 1991; 91: 528 – 534.
4. Vladimir 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.

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