Presentation:
R1 45 ml/R2 5 ml/R3 2 ml Ref.: 41020
Store: 2 - 8 ºC.

RHEUMATOID FACTOR
Turbilatex

Diagnostic reagent for qualitative measurement of RF.

Only for *in vitro* use in clinical laboratory (IVD)

TEST SUMMARY
Latex particles coated with human anti-RF are mixed with a serum sample. Agglutination is formed when a sample containing RF is combined with the reagent. The degree of agglutination is directly proportional to the concentration of RF in the sample.

REAGENTS COMPOSITION

Diluent (R1)	Tris buffer 20 mmol/L, sodium azide 0.95 g/L. pH 8.2
Latex (R2)	Suspension of latex particles coated with human gamma globulin, sodium azide 0.95 g/L, pH: 7.4
Calibrator (R3)	Human serum: RF Concentration is stated on the vial label.
Optional	Ref.: 41012 Control ASO/CRP/RF Level L Ref.: 41022 Control ASO/CRP/RF Level H

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

Good laboratory safety practices should be followed when handling laboratory reagents or human samples.

REAGENT PREPARATION AND STABILITY
Latex reagent and diluent are ready to use and stable up to the end of the indicated month and year of expiry. Stored at tightly closed at 2-*8ºC.*
RF Calibrator: Reconstitute (→) with 2.0 mL of distilled water. Stable for 1 month at 2-8ºC or 3 months at –20ºC.
Do not freeze; frozen reagents could change the functionality of the test.
Signs of reagent deterioration:
- Particles and turbidity indicates contamination or reagents deterioration.

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Stored at tightly closed at 2-*8ºC*,. Do not use reagents over the expiration date.

CALIBRATION
The assay is calibrated against the international reference NIBSC 64/2 (Rheumatoid Arthritis Serum) WHO. The use of other commercially available RF calibrators is not recommended.

SPECIMEN
Fresh serum. Stable 7 days at 2-8ºC or 3 months at –20ºC.
The samples with particles or fibrin should be centrifuged to eliminate them.
Do not use haemolized or lipemic samples.

Discard contaminated specimen

MATERIAL REQUIRED BUT NOT PROVIDED
- Thermostatic bath at 37ºC.
- Spectrophotometer capable of accurate absorbance readings at 650 nm (600-650)
- Cuvettes with 1 cm light path.

General laboratory equipment

TEST PROCEDURE
CALIBRATION CURVE (range between 20 - 160 IU/mL):
Prepare the following RF calibrator dilutions in ClNa 9 g/L as diluent. 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)	--	10	25	50	75	100
ClNa 9 g/L (µL)	100	90	75	50	25	-
Factor	0	0.1	0.25	0.5	0.75	1.0

ONE POINT CALIBRATION (lineal range up to 100 IU/mL): Prepare a RF Calibrator dilution: 30 µL RF Calibrator + 70 µL ClNa 9 g/L. Multiply the RF calibrator concentration by 0.30 to obtain the RF concentration of the calibrator dilution.

MANUAL PROCEDURE
1- Bring the working reagent and the photometer to 37ºC.
2- Set spectrophotometer wavelength to 650 nm and adjust to zero absorbance against water.
3- Pipette into a cuvette:

	Blank	Calibrator	Sample
ClNa 9 g/L (µL)	7	--	--
Calibrator (µL)	--	7	--
Sample (µL)	--	--	7
R1: Diluent (µL)	900	900	900
R2. Latex µL)	100	100	100

4- 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-$A_{blank\ reagent}$) 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_2-$A_{blank\ reagent}$) in the calibration curve.

One point calibration:

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

QUALITY CONTROL
SERUM CONTROLS Ref.: 41012 and Ref.: 41022 are recommended to monitor the performance .

Serum Controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES
Up to 20 IU/ml.

It is suggested that each laboratory establish its own reference range.

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).

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENT PERFORMANCE
1. *Linearity (*one point calibration): Up to 100 IU/mL[note 2]. Under the described assay conditions.
2. *Measurement range* (calibration curve): 6-160 IU/mL. Under the described assay conditions.
3. *Limit detection*: Values less than 6 IU/mL give non-reproducible results.
4. *Prozone effect*: No prozone effect was detected upon 800 IU/mL.
5. *Sensitivity*: Δ 3.34 mA.IU/Ml
6. *Precision:*

	Intra-assay n = 10		Inter-assay n= 10	
Mean (IU/mL)	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) was 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 used analyzer.

INTERFERING SUBSTANCES
Do not interfere:
- Haemoglobin (10 g/L), Bilirubin (20 mg/dL), Lipemia (10 g/L).
May interfere:
- Other substances may interfere[6].

NOTES
1. Multipoint calibration gives more accurate results than one point calibration.
2. Samples with higher concentrations shoud be diluted 1/5 in NaCl 9 g/L. and retested again. The linearity limit depends on the sample / reagent ratio, as well the analyzer used. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.

BIBLIOGRAPHY
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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.