RF PROTEIN

Immunoturbidimetric Method

DIATEK

CLINICAL SIGNIFICANCE :

Diagnosis of rheumatoid arthritis (RA) is based largely on clinical examination, but laboratory tests (e.g. RF Test) do support the clinical diagnosis. RF is a term used to describe a variety of auto-antibodies (in most cases of the IgM type) that will react with modified human IgG (e.g. IgG in circulating immune complexes, IgG adsorbed to latex, etc.) and IgG of animal origin. RF is highly associated with rheumatoid arthritis: About 90 % of patients with RA have RF titers of more than 40 IU/mL.

TEST PRINCIPLE :

This method utilizes the interaction of antigen and antibody to directly determine the RF in speicimen. Immunturbidimetric test for the determination of RF is based on photometric measurement of the Antibody - Antigen reaction between heat aggregated human IgG on RF of the sample. The amount Complex is measured as increase in absorbance at 340 nm with the use of multipoint calibration curve.

REAGENTS COMPOSITION :

Reagent 1 (R1) Buffer Reagent : Buffer (pH : 8.2)

Reagent 2 (R2) RF Antibody : Buffer, Heat aggregated human IgG, Stabilizers

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

KIT CONTENTS

CODE No.	*RFLW01*	*RFLW02*
Pack size :	(30 ml)	(120 ml)
Reagent 1 (R1)	2 x 12.5 ml	5 x 20 ml
Reagent 2 (R2)	1 x 5 ml	3 x 6.7 ml

REAGENT PREPARATION

All the Reagents are ready to use and unopened reagent is stable upto expiry. Once opened the onboard stability is 30 days in Fully Automated Analyser under on board cooling system.

SAMPLES : Use fresh Serum

MATERIALS REQUIRED BUT NOT PROVIDED :

MULTIPOINT CALIBRATION :

Use RF High Calibrator for preparing 6 calibrators ranging nearly 30 - 500 IU/mL and use normal saline as 0 (For Semi Auto input value as 0.01 for normal saline).

RF Immunoturbidimetry Control (Use of assayed QC sera is is recommended to validate test result).

QUALITY CONTROL :

Use RF Control Low and High for test validation.

Before the assay begins, bring all the reagents to room temperature.

For Semi-auto Analyser

ASSAY CONDITIONS for MULTI POINT ASSAY:

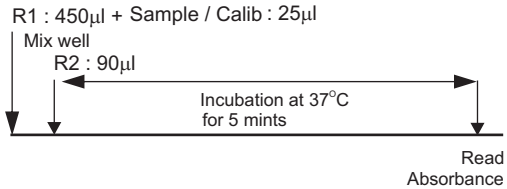
Wavelength (Primary) :..... 340 nm
Cuvette: ..... 1 cm light path
Constant temperature 37ºC
Reaction (Mode)..... End Point
Number of Standard..... 6
Standard Concentration..... As prepared
Curve..... Non linear
Linearity..... Upto highest Calib. conc.
Unit..... IU/mL
Reaction Slope..... Increasing
Blanking..... Reagent.

It is recommended to use RF High Calibrator nearly 500 IU/mL

	RF Calibrator preparation				
	C6	C5	C4	C3	C2
Dilution	Neat	1:2	1:4	1:8	1:16
Factor	1.0	0.5	0.25	0.125	0.0625

Calibrators concentration to be calculated by multiplying the factor correspond to dilution. Normal saline to be used as calibrator C1 value 0.01IU/mL

Assay Procedure summary Semi- Automated Analyser:



PROCEDURE MULTI POINT ASSAY:

Pipette into test tubes labeled as (B) Calibrators (1 to 6) and Test (T) as follows.

	B	C1	C2	C3	C4	C5	C6	T
Reagent R1	450µl	450µl	450µl	450µl	450µl	450µl	450µl	
Deionised water	25µl							
Normal Saline		25µl						
RF Calibs.			25µl	25µl	25µl	25µl	25µl	
Specimen								25µl
Mix well								
Reagent R2	90µl	90µl	90µl	90µl	90µl	90µl	90µl	90µl
Incubate for 5 mint at 37ºC								
Mix and Read **Absorbance** for each tube against Blank (B)								

1. Pipette into test tube Reagent1(R1) 450 µl and Add (Normal Saline / Calibrators / Samples) 25 µl as described above.
2. Mix well and Reagent 2 (R2) 90 µl
3. Incubate for 5 Min. at 37°C.
4. Read **Absorbance** of Calibrators (C1 to C6) and Test (T) at bicromatic mode Primary wavelength 340 nm and Secondary wave legth 680 nm against Blank (B).
5. Plot a non linear Point to point curve and calculate result from curve.

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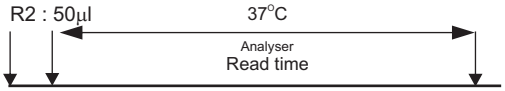
Immunoturbidimetric Method

Assay Procedure summary Fully Automated Analyser:

R1 : 250µl + Sample / Calib : 14µl
Mix well
R2 : 50µl 37°C



Analyser
Read time
Initial Abs. Bichromatic Primary 340 nm
Secondary 700 nm
300 Sec
Final Abs.

PROCEDURE MULTI POINT ASSAY FULLY AUTOMATED:

	B	C1	C2	C3	C4	C5	C6	T
Reagent R1	250µl	250µl	250µl	250µl	250µl	250µl	250µl	
Normal Saline		14µl						
RF Calibs.			14µl	14µl	14µl	14µl	14µl	
Specimen								14µl
Reagent R2	50µl	50µl	50µl	50µl	50µl	50µl	50µl	50µl
Read **Initial Absorbance A1** for each tube against Blank (B)								
Incubate for 5 mint at 37ºC								
Mix and Read **Final Absorbance (A2)** of Calibrators (C1 to C6) and Test (T) at Bichromatic mode with Primary wavelength 340 nm and Secondary wavelength 700 nm (670 - 700 nm) against Blank (B).								

1. Calculate the change of Absorbance ∆ **Abs (A2 - A1)** of each Calibrator and Test Sample.
2. Plot a Calibration Curve (Pont to Point) with **Calibrator Concentration** against corresponding ∆ **Abs.** from lower to higher sequentially.
3. Calculate the concentrations of Test samples / controls based on the calibration curve.
4. Do not attempt to extrapolate above or below the range of Calibrators
5. **Programmes for specific autoanalysers are available on request.**

PRECAUTIONS :

1. Storage conditions as mentioned on the kit to be adhered.
2. Use clean glassware and microtips while pipetting Reagents
3. Avoid contamination of the reagent during the assay process.
4. No interference for Intralipid (1000 mg/dL), Bilirubin (40 mg/dL), and Ascorbic acid (50mg/dl)
5. For accuracy of results, the assay procedure, reagent preparation and storage has to be meticulously followed.
6. No prozone Phenomenon observed till RF concentration of 1500 IU/mL
7. As with all the diagnostic procedures, the physician should evaluate data obtained by the use of this kit in light of other clinical information.

TEST SPECIFICATIONS:

Reproducibility Fully Automated system:

Sample	Within Run %CV	Between Run %CV
Level 1	2.68	3.07
Level 2	1.38	1.40
Level 3	1.55	1.78

TEST COMPARISON

This method (Y) was compared with another commercially available (X) and linear regression equation obtained Y = 0.9486X -n0.2587 with a correlation coefficient (r) of 0.9900.

REFERENCE RANGE :

0 - 20 IU/mL (WHO)

The above reference range is guideline and all the laboratories must establish their own normal reference range. Final diagnosis should be made with correlation of clinical factors.

Measuring range :

0.1 - 500 IU/mL (Multi point Calibration)

BIBLIOGRAPHY :

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