CE

TURBILYTE® RF

TURBIDIMETRIC IMMUNOASSAY FOR DETERMINATION OF RHEUMATOID FACTORS

SUMMARY

In Rheumatoid arthritis (RA), diagnostically useful autoantibodies termed as Rheumatoid Factors (RF) can be detected which are immunoglobulins of the class IgG, IgM, IgA, and IgE. IgM class RF with specificity to human IgG Fc is the most useful prognostic marker for RA.
RF play a role in perpetuating the rheumatoid inflammatory process, the severity of joint damage could be predicted according to the strength of RF reactivity. A significant decline of RF with the remission of disease activity has also been demonstrated. Therefore, quantified serial determinations of RF are more meaningful for the diagnosis, prognosis, and assessment of therapeutic efficacy of rheumatoid arthritis.
Initial RF positivity has been a sensitive predictor for later joint destruction. Quantified measurement of initial RF level and especially repeated measurements of RF at regular intervals seems to add significantly to the prognostic value of RF in distinguishing between progressive and non-progressive disease in early RA.
TURBILYTE®-RF is a turbidimetric immunoassay for quantitative detection of rheumatoid factors of the IgM class.

REAGENT:

1. **TURBILYTE®-RF** Activation buffer (R1): ready to use.
2. **TURBILYTE®-RF** Latex Reagent (R2): Ready to use uniform suspension of polystyrene latex particles coated with suitably modified Fc fraction of human IgG.
3. **TURBILYTE®-RF** Calibrator (S): lyophilized preparation of RF positive serum that is equivalent to stated amount of RF on IU/ml basis, when hydrated appropriately. The **TURBILYTE®-RF** calibrator is traceable to the W.H.O., International Reference Preparation of Rheumatoid Arthritis Serum.

Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity, sensitivity, and performance.

REAGENT STORAGE AND STABILITY

1. Store the reagents at 2-8°C. DO NOT FREEZE.
2. The shelf life of the reagent, activation buffer and the calibrator is as per the expiry date mentioned on the respective vial labels.
3. The reconstituted **TURBILYTE®-RF** calibrator is stable for 7 days at 2-8°C and 48 hours at 25-30°C (RT.).
4. The working reagent for **TURBILYTE®-RF** can be prepared by mixing R2 and R1 in the ratio 1:10.
5. The mixed stability of the working reagent (R1+ R2) is 7 days when stored at 2-8°C.

PRINCIPLE

TURBILYTE®-RF is a turbidimetric immunoassay for the determination of rheumatoid factors and is based on the principle of agglutination reaction. The test specimen is mixed with **TURBILYTE®-RF** latex reagent (R2) and activation buffer (R1) and allowed to react. Presence of RF in the test specimen results in formation of an insoluble complex resulting in an increase in turbidity, which is measured at wavelength 505-578 nm. The increase in turbidity corresponds to the concentration of RF in the test specimen.

NOTE

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. The reagents that are derived from human source have been tested for HBsAg and Anti-HIV antibodies and are found to be non-reactive. However handle the material as if infectious.
3. Reagents contain 0.095% Sodium Azide as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
4. The reagents can be damaged due to microbial contamination or on exposure to extreme temperatures. It is recommended that the performance of the reagents be verified using known controls periodically.
5. Gently mix the **TURBILYTE®-RF** latex reagent well before use to disperse the latex particles uniformly to improve test performance.
6. The working reagent should be mixed gently.
7. Do not use vortex mixers for mixing. Gently mix the reagents and samples during test procedures.
8. As the reagents within lots have been matched, reagents from different lots must not be interchanged.
9. Calibrators of different manufacturers must not be used with **TURBILYTE®-RF** reagents.
10. **TURBILYTE®-RF** assay can be used on any semi automated analyzer with appropriate programming facility . Fully automated analyzers may be used, provided the reagent has been standardized on the system.
11. The procedures mentioned in this pack insert are based on a minimum reading volume of 500 µl (0.5 ml). In case of instruments where minimum volume required for reading absorbance is 1.0 ml, use double the quantity of reagents and samples mentioned in the test procedure.
12. Do not use damaged or leaking reagents.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to specimen collection by approved techniques.
Only serum should be used for testing. Should a delay in testing occur, store the samples at 2 - 8°C. Samples can be stored for upto three days at 2 - 8°C, provided they are not contaminated. Do not use hemolysed, icteric, or highly turbid sera. Turbid or particulate serum samples must be clarified by centrifugation at 2000 rpm for 15 minutes. Use the clear supernatant for testing.

SAMPLE WASTE AND DISPOSAL

Do not reuse the reagent containers, bottles, caps or plugs due to the risks of contamination and the potential to compromise reagent performance.
This product requires the handling of human specimens. It is recommended that all human sourced material are considered potentially hazardous and are handled in accordance with the OSHA standard on blood borne pathogens.
Appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
Handle specimens, solid and liquid waste and test components in accordance with local regulations and NCCLS guidelines M29, or other published biohazard safety guidelines.

ADDITIONAL MATERIAL REQUIRED

Spectrophotometer with 578 to 630 nm wavelength filters, stopwatch, well calibrated micropipettes, disposable tips, isotonic saline, particulate free distilled water, test-tubes, test-tube rack, incubator/ waterbath set at 37°C, optically clean disposable/glass **semi micro cuvettes**.
Note: Though any filter between the wavelengths 578-630 can be used, optimum results are obtained with 630 nm wavelength filter.

TEST PROCEDURE

Bring reagent and sample to room temperature before use.

Assay conditions;

Wavelength	630 nm
Reaction Temperature	37°C
Path Length	1 cm

The **TURBILYTE®-RF** calibrator must be reconstituted exactly with the stated amount of distilled water (refer vial label), wait for 10 minutes, gently swirl the vial till the solution attains homogeneity. Once reconstituted it is ready to use for RF calibration. The Concentration (S) of RF in the reconstituted calibrator is as mentioned on the calibrator vial label.

Pipette into the cuvette:

	For calibration	For sample
R1	450 µl	450 µl
R2	50 µl	50 µl
Mix well and incubate for 5 minutes		
Calibrator	5 µl	-
Sample	-	5 µl
Mix well and read absorbance A1 at 10 seconds and A2 at 2 minutes.		

CALCULATIONS

1. Calculate ΔA:
   ΔA = (A2-A1).
2. Concentration of RF (IU/ml) in sample = $\frac{\Delta A \text{ Sample}}{\Delta A \text{ Calibrator}}$ x Concentration (S) of calibrator

SPECIFIC PERFORMANCE CHARACTERISTICS

Linearity

The Linearity of **TURBILYTE®-RF** is upto 100 IU/ml. The linearity limit depends on the sample to reagent ratio as well as the analyzer used. It will be higher by decreasing the sample volume, though the detection limit of the assay will be proportionately decreased.

Detection limit / Analytical Sensitivity

Detection limit: 10 IU/ml

The detection limit represents the lowest measurable RF concentrations that can be distinguished from zero.

Prozone limit

No prozone effect was observed upto a concentration of 1250 IU/ml of RF.

Precision

Within run	n	Mean IU/ml	SD	CV(%)
Sample 1	10	6.50	1.29	19.94
Sample 2	10	15.37	1.27	8.24
Sample 3	10	41.07	1.30	3.16

Between run	n	Mean IU/ml	SD	CV(%)
Sample 1	10	5.83	1.25	21.32
Sample 2	10	14.69	1.22	8.32
Sample 3	10	42.06	1.38	3.32

Interference

No interference was observed by Glucose upto 500 mg/dl, Bilirubin upto 50 mg/dl, Haemoglobin upto 500 mg/dl and Intralipid upto 1000 mg/dl.

REFERENCE VALUES

The reference values of RF in normal population are ≤ 20 IU/ml.

Each laboratory should define its own reference range for relevant population.

REMARKS

(1) Usage of well-calibrated equipment and accessories and procedures is critical for achieving correct results. (2) Samples with values beyond the linearity limit have to be diluted with isotonic saline and retested. The values obtained must be multiplied with the dilution factor for calculating the result. (3) Markedly lipemic, hemolysed, and contaminated serum samples could produce erroneous RF values. (4) Use of plasma rather than serum can result in erroneous RF values. (5) Do not read results beyond two minutes. (6) Rheumatoid factors are not exclusively found in rheumatoid arthritis but sometimes in syphilis, systemic lupus erythromatosus, hepatitis, and hypergammaglobulinemia also. (7) It is recommended that results of the test should be correlated with clinical findings to arrive at final diagnosis. (8) **TURBILYTE®-RF** assay is sensitive to the presence of IgM RF with heterogeneous specificity.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

(1) Paimela, T.Palosuo, M. Leirisalo-repo, T. Helve and K. Aho, British Journal of Rheumatology., 1995: 34: 1146 1150. (2) Cecil R. L., Nichols E.E., Am. J. Med. Sci., 181: 12 (1931). (3) Heller G., Jacobson S.A., Koloday M. H., Kammerer M.H., J. Immunol., 72 : 66 (1954). (4) Singer J. M., Bull. Rheum. Dis., 24: 762 (1974). (5) Clinical Laboratory Diagnostics, Edited by Lothar Thomas, M.D., 1st Ed., 1998, TH-Books Verlagsgesellschaft mbH, Frankfurt, Germany. (6) Data on file: Coral Clinical Systems

TRF1/ 0522/ VER- 01

Manufactured by:
Coral Clinical Systems
A Division of Tulip Diagnostics (P) Ltd.
BUILDING E, PLOT NO. M-46/47, PHASE III B, VERNA INDUSTRIAL ESTATE, VERNA, GOA-403 722, INDIA.
REGD. OFFICE : GITANJALI, TULIP BLOCK, DR. ANTONIO DO REGO BAGH, ALTO SANTACRUZ, BAMBOLIM COMPLEX P.O., GOA-403 202, INDIA.
EC REP
CMC Medical Devices & Drugs S.L.,
C/ Horacio Lengo No. 18, CP 29006, Malaga, Spain.