IMMUNOTURBIDIMETRIC ASSAY FOR DETERMINATION OF SERUM RF ON TURBDYNE™ SC

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. Turbdynne™ RF is a turbidimetric immunocassay for quantitative detection of rheumatoid factors of the IgM class in human serum.

PRESENTATION

<table>
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<th>REF</th>
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<tbody>
<tr>
<td>V</td>
<td>20 tests</td>
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</tr>
<tr>
<td>R1</td>
<td>4.5 ml</td>
<td>3 x 4.5 ml</td>
</tr>
<tr>
<td>R2</td>
<td>0.5 ml</td>
<td>3 x 0.5 ml</td>
</tr>
<tr>
<td>SC</td>
<td>1 No.</td>
<td>1 No.</td>
</tr>
<tr>
<td>CT</td>
<td>20 Nos.</td>
<td>60 Nos.</td>
</tr>
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</table>

REAGENT

1. R1 Turbdynne™ RF Activation buffer: Ready to use.
2. R2 Turbdynne™ RF Latex Reagent: Ready to use uniform suspension of polystyrene latex particles coated with suitably modified Fc fraction of human IgG.
3. SC Turbdynne™ RF SMART CARD: Card with Master calibration curve calibrated with a standard 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 and activation buffer is as per the expiry date mentioned on the respective vial labels.
3. Once opened the reagents are stable for 75 days when stored at 2-8°C provide the reagents are not contaminated.
4. Store the Turbdynne™ RF Smart card at a clean dry place. The Turbdynne™ SC smart card is valid upto the use of labelled number of tests within 75 days from the first insertion in Turbdynne™ SC analyzer.

PRINCIPLE

Turbdynne™ RF is a quantitative immunocassay for the determination of rheumatoid factors and is based on the principle of agglutination reaction. The test specimen is mixed with Turbdynne™ RF activation buffer (R1) and latex reagent (R2) 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 ~ 650 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 0.09% Sodium Azide as preservative. Avoid contact with skin and mucousa. On disposal flush with large quantities of water.
4. Gently mix the Turbdynne™ RF latex reagent well before use to disperse the latex particles uniformly to improve test performance.
5. Do not use vortex mixers for mixing. Gently mix the reagents and samples during test procedures.
6. As the reagents and Smart card within lots have been matched, different lots reagents or Smart cards must not be interchanged.
7. It is recommended that the reagent performance and Smart card calibration must be validated periodically with known controls such as Turbodyne™ RF (Ref:108540005).
8. Do not use damaged or leaking reagents.
9. The reagents can be damaged due to microbial contamination or on exposure to extreme temperatures.
10. Always use fresh clean disposable micropipette tips to aspirate the reagents to prevent contamination.

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 up to 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.

ADDITIONAL MATERIAL REQUIRED
Turbodyne™ SC analyser, stopwatch, well calibrated micropipettes, disposable tips, incubator.

TEST PROCEDURE
1. Bring reagents and samples to room temperature before use.
2. Insert the Turbodyne™ RF smart card in the card reader slot of the Turbodyne™ SC as described in the user manual.
3. The instrument will indicate to place cuvettes with R1 + sample in the reading chamber.
4. Take a disposable Turbodyne™ cuvette (Provided in the kit) and add 225 µl R1 using clean disposable micropipette tip. Then add 5 µl sample and incubate the cuvettes for 5 minutes.
5. Place the cuvette with R1 + sample in the Turbodyne™ SC reading chamber.
6. Press “Teasing”: The instrument will mix the sample and then indicate to add R2.
7. Pipette 25 µl R2 reagent with the Turbodyne™ SC Electronic Pipette to the cuvette with R1 + sample.
8. The reaction will start and the counter will start in the display. Results will be displayed on completion of reaction.

SPECIFIC PERFORMANCE CHARACTERISTICS
Measuring Range
The Measuring Range of Turbodyne™ RF is 15-120 IU/ml.
Detection limit / Analytical Sensitivity
Detection limit: 15 IU/ml
The detection limit represents the lowest measurable RF concentrations that can be distinguished from zero.

Precision

<table>
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<tr>
<th>Precision</th>
<th>n</th>
<th>Mean IU/ml</th>
<th>SD</th>
<th>CV [%]</th>
<th>Precision</th>
<th>n</th>
<th>Mean IU/ml</th>
<th>SD</th>
<th>CV [%]</th>
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Interference
No interference was observed by Glucose up to 500 mg/dl, Albumin up to 50 g/dl, Bilirubin up to 50 mg/dl, Haemoglobin up to 500 mg/dl and Triglycerides up to 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 pipette and correct procedure 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 icemic, hemolysed, and contaminated serum samples could produce erroneous RF values.
4. Use of plasma rather than serum can result in erroneous RF values.
5. Rheumatoid factors are not exclusively found in rheumatoid arthritis but sometimes in syphilis, systemic lupus erythematosus, hepatitis, and hypergammaglobulinemia also.
6. It is recommended that results of the test should be correlated with clinical findings to arrive at final diagnosis.
7. Turbodyne™ RF assay is sensitive to the presence of IgM RF with heterogeneous specificity.

8. Values above linearity will be flagged “Exceed”. Such samples must be diluted 1:4 with normal saline and retested. The result obtained must be multiplied with the dilution factor.

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. Amy M. Wasserman et al., Diagnosis and Management of Rheumatoid Arthritis, American Family Physician, Vol.84, No.11, Dec 1, 2011, pgs 1245-1252.
3. Data on file; Tulip Diagnostics (P) Ltd.