CE

Quantia® 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.

Quantia®-RF is a turbidimetric immunoassay for quantitative detection of rheumatoid factors of the IgM class.

REAGENT

1. **Quantia®-RF** Activation buffer (R1): ready to use buffer.
2. **Quantia®-RF** Latex Reagent (R2): ready to use uniform suspension of polystyrene latex particles coated with suitably modified Fc fraction of human IgG.
3. **Quantia®-RF** Calibrator: lyophilized preparation of RF positive serum, which is equivalent to stated amount of RF on IU/ml basis, when hydrated appropriately. The **Quantia®-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 **Quantia®-RF** calibrator is stable for 7 days at 2-8°C and 48 hours at 25-30°C (RT.).
4. The working reagent for **Quantia®-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

Quantia®-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 **Quantia®-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 578-630 nm. The increase in turbidity corresponds to the concentration of RF in the test specimen.

NOTES

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 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 **Quantia®-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 **Quantia®-RF** reagents.
10. The calibration curve must be validated periodically with known controls.
11. The **Quantia®-RF** assay is recommended only for analyzers with cuvette mode. Though any semi automated analyzer with appropriate programming facility can be used, for best results it is recommended to use **Quantiamate** analyzer. Fully automated analyzers may be used, provided the reagent has been standardized on the system.
12. 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.

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.

ADDITIONAL MATERIAL REQUIRED

Spectrophotometer with 578-630 nm wavelength filters and cuvette mode, stopwatch, well calibrated micropipettes, disposable tips, isotonic saline, particulate free distilled water, test-tube rack, incubator/ waterbath set at 37°C, optically clean disposable cuvettes such as Quantiamate **semi micro cuvettes** / glass 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 reagents and specimen to room temperature before use.
Assay conditions:

Wavelength	630 nm
Reaction Temperature	37°C
Cuvette	1 cm path length

Method for preparation of RF Calibration Curve

The **Quantia®-RF** calibrator must be reconstituted exactly with 1.0 ml of distilled water, wait for 10 minutes, gently swirl the vial till the solution attains homogeneity. Once reconstituted it is ready to use for preparation of RF calibration curve. The Concentration (S) of RF in the reconstituted calibrator is as mentioned at the end of this package insert.
Dilute the calibrator serially as mentioned below for preparation of calibration curve.

Test Tube No.	1	2	3	4
Calibrator dilution No.	D1	D2	D3	D4
Vol. of Calibrator	100 µl	50 µl D1	50 µl D2	50 µl D3
Vol. of Isotonic Saline	-	50 µl	50 µl	50 µl
Conc. of RF in IU/ml	S	S/2	S/4	S/8

The above four dilutions of the calibrator including the highest 120 IU/ml (D1) and lowest 15 IU/ml (D4) concentrations of measuring range **must** be used for the preparation of the calibration curve.

NOTE: Increasing concentration of calibrators must be used for preparing the calibration curve.

Test procedure for preparation of calibration curve

1. Zero the instrument with distilled water.
2. Pipette 450 µl of **Quantia®-RF** activation buffer (R1) and 50 µl of **Quantia®-RF** latex reagent (R2) in the measuring cuvette. Mix well and incubate for five minutes at 37°C.
   OR

Pipette 500 µl of the **Quantia®-RF** working reagent in the measuring cuvette. Mix well and incubate for five minutes at 37°C.

3. Add 10 µl of calibrator (D1), mix gently and start the stopwatch simultaneously.
4. Read absorbance (A1), **exactly** at ten seconds, and absorbance (A2) again at the end of **exactly** two minutes.
5. Repeat steps No. 2-4 for each diluted calibrator (D2 to D4) for preparing calibration curve.
6. Calculate ΔA (A2-A1) for each calibrator (D1 to D4). Plot a graph of ΔA versus concentration of RF on the graph paper provided with the kit.

"The calibration curve" so obtained is valid only for the same lot of Quantia®-RF reagents.

Test procedure for specimen

For determination of RF concentration in the test specimen:

1. Follow steps 2 - 4 as mentioned in the above procedure for calibration curve using the test specimen in place of the calibrator.
2. Calculate ΔA (A2-A1) for the test specimen.

VALIDATION CRITERIA

If ΔA of the test specimen is less than ΔA obtained for the standard of highest concentration (D1) then the concentration of RF in the test specimen can be determined directly by interpolating ΔA of the test specimen from the calibration curve.
If the ΔA of the diluted test specimen is higher than ΔA of standard with highest concentration (D1) then the test has to be rerun by carrying out further dilution of test specimen such as 1:10, 1:20 etc., till the ΔA of the diluted test specimen is less than ΔA of D1. Then proceed for calculations.

CALCULATIONS

1. Interpolate ΔA of the diluted test specimen on the calibration curve and obtain the RF concentration 'C' of the diluted test specimen.
2. Multiply the RF concentration 'C' with the dilution factor (F) of the test specimen for obtaining the concentration of RF in the neat test specimen.

Concentration of RF in the neat test specimen in IU/ml = C x F
(Where 'F' is the dilution factor of the test specimen i.e. 10 for 1:10 dilution of test specimen and so on.)

SPECIFIC PERFORMANCE CHARACTERISTICS

Measuring range

The **Quantia®-RF** reagent has been designed to measure RF concentrations in the range of about 15 - 120 IU/ml and is linear between the measuring range. The linearity limit of the measuring range 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. The exact range is dependent on the calibrator value which is lot specific.

Detection limit / Analytical Sensitivity

Detection limit: 15 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

Intra-assay precision	n	Mean IU/ml	SD	CV(%)
Sample 1	20	43.25	2.83	6.54
Sample 2	20	85.00	5.43	6.39
Sample 3	20	106.35	4.98	4.68

Inter-assay precision	n	Mean IU/ml	SD	CV(%)
Sample 1	20	43.6	2.62	6.00
Sample 2	20	83.35	4.92	5.90
Sample 3	20	104.85	4.61	4.40

Interference
No interference was observed with:

Interference factor	No interference upto
Glucose	500 mg/dl
Albumin	10 g/dl
Bilirubin	50 mg/dl
Haemoglobin	500 mg/dl
Triglycerides	1000 mg/dl

REFERENCE VALUES
The reference value for RF was determined to be ≤ 20 IU/ml with **Quantia®-RF** on Quantiamate.
It is recommended that each laboratory must define its own reference range for relevant population taking into account all affecting factors.

REMARKS
1. Usage of well-calibrated equipment and accessories and procedures is critical for achieving correct results.
2. When ΔA obtained for the test specimen is greater than the ΔA of the standard with highest concentration then, it indicates that the concentration of RF in the test specimen is beyond the measuring range of the **Quantia®-RF** assay. Such specimens should be rerun with further dilutions.
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 tests should be correlated with clinical findings to arrive at final diagnosis.
8. **Quantia®-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.

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