RF (Rheumatoid Factors)

TURBIDIMETRY

Intended use:
Immunoturbidimetric assay for the in vitro quantitative determination of rheumatoid factors in human serum and plasma. Measurements may be used as an aid in the diagnosis of rheumatoid arthritis.

Summary:
Rheumatoid factors are a heterogeneous group of autoantibodies directed against the antigenic determinants on the Fc-region of IgG molecules. They are important in the diagnosis of rheumatoid arthritis, but can also be found in other inflammatory- rheumatic diseases and in various non-rheumatic diseases. They are also found in clinically healthy persons over 60 years of age. Despite these restrictions, the detection of rheumatoid factors is a diagnostic criterion of the American College of Rheumatology for classifying rheumatoid arthritis. These autoantibodies occur in all the immunoglobulin classes, although the usual analytical methods are limited to the detection of rheumatoid factors of the IgM type.
The classic procedure for the quantisation of rheumatoid factors is by agglutination with IgG-sensitized sheep erythrocytes or latex particles. Particular problems of these semi-quantitative methods are the poor between-laboratory precision and reproducibility, together with standardization difficulties. For these reasons, new assay methods such as nephelometry, turbidimetry, enzyme-immuno-assays and radioimmunoassays have been developed. The Analyticon RF assay is based on the immunological agglutination principle with enhancement of the reaction by latex particles.

Test principle:
Particle enhanced immunoturbidimetric assay.
Sample and addition of R1 (buffer)
Addition of R2 (latex-bound IgG/buffer) and start of reaction.
Latex-bound heat-inactivated IgG (antigen) reacts with the RF-antibodies in the sample to form antigen/antibody complexes. Following agglutination is measured turbidimetrically.

Reagent concentration:
R1: Tris buffer 20 mmol/L, sodium azide 0.95 g/L, pH 8.2.
R2: Suspension of latex particles coated with human gamma-globulin, sodium azide 0.95 g/L.

Preparation and stability
R1: Ready for use.
R2: Ready for use. Mix the reagent well once weekly.
Store at 2-8ºC. Reagents and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use. Indications of deterioration:
On board stability: R1: 28 days
R2: 28 days

Specimen
Collect serum using standard sampling tubes
Li-/Na-heparin, EDTA plasma
Stability:
24 hours at +20°C to +25°C
3 days at +2°C to +8°C
4 weeks (do not refreeze) at -20°C
Centrifuge samples containing precipitate before performing the assay

Limitations - interference:
Criterion: Recovery within 10% of initial value.
Icterus: No significant interference up to an index I of 60 (approximate conjugated and unconjugated bilirubin concentration: 60 mg/dl).
Hemolysis: No significant interference up to an index H of 1000 (approximate haemoglobin concentration: 1000 mg/dl).
Lipemia (Intralipid): Elevated levels of triglycerides may interfere. There is poor correlation between turbidity and triglycerides concentration.
No high-dose hook-effect up to RF-activities of 6000 IU/ml.
Thirty one commonly used pharmaceuticals were tested in vitro.
No interference with the assay was found.

Testing procedure:
Applications for automated systems are available on request.
Materials provided
Working solutions as described above Additional materials required
Calibrators and controls as indicated below
0.9% NaCl

Manual procedure: Wavelength: Temperature: Cuvette: Zero adjustment:	 660 nm +37°C 1 cm against reagent blank
	Sample/Calibrator
R1	800ul
Sample	10ul
R2	200ul
Mix, read absorbance A1 after 10sec. Incubate 2 min. and read absorbance A2.	
Calculation:	
A = [(A2 - Aı) sample or calibrator] - [(A2 - A1) blank]	

Measuring/reportable range:
7-160 IU/ml
Determine samples having higher activities via the rerun function. On instruments without rerun function, manually dilute the samples with 0.9% NaCl or distilled/ deionized water (e.g. 1 + 1). Multiply the result by the appropriate dilution factor (e.g. factor 2).

Expected values:
< 30 IU/ml
Each laboratory should investigate the transferability of the expected values to its own patients' population and if necessary determine its own reference range. For diagnostic purposes the rheumatoid factor results should always be assayed in conjunction with the patient's medical history, clinical examinations and other findings.

Analytical sensitivity (lower detection limit)
Detection limit: 7 IU/ml
The low detection limit represents the lowest measurable RF concentration that can be distinguished from zero.

Imprecision:
Reproducibility within run was determined using controls (n = 21). The following results were obtained:

Within run			
Sample	Mean (IU/ml)	SD (IU/ml)	CV (%)
Sample 1	24.7	0.57	2.30
Sample 2	60.5	0.52	0.85

Reproducibility was determined using human samples and controls in an internal protocol (n = 20). The following results were obtained:

Between day			
Sample	Mean (IU/ml)	SD (IU/ml)	CV (%)
Sample 1	17.8	0.44	2.49
Sample 2	52.4	0.83	1.59
Sample 3	94.7	1.13	1.19
Sample 4	134.8	1.82	1.35

Method comparison:
A comparison of the BIOANALYTIC RF (y) with a commercial obtainable assay (x) gave with 55 samples the following result:
y = 0.972 x - 0.951; r = 0.998

Quality Control:

RHEUMATOID CONTROL LEVEL 1	5 x 1 ml	#B10846
RHEUMATOID CONTROL LEVEL 2	5 x 1 ml	#B10849

The control intervals and limits must be adapted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

RF (Rheumatoid Factors)

TURBIDIMETRY

Calibration:

Standardization: This RF method was calibrated against an international standard defined for RF.

S1: BIOANALYTIC RF CAL. SET 5 x 3 ml #B11937

Calibration frequency:

It is suggested to use Calibrator products produced by Bioanaliytic. It is suggested to use supplementary calibrator (pure water or 0.9% NaCl) to conduct 5-point calibration with spline method. The calibration curve is formed automatically. When lot number is changed or QC is invalid, calibration shall be conducted again. Recalibrate the assay every 30 days under ideal conditions, or when the following occur:

Change in reagent lot or significant shift in control values;

Major preventative maintenance was performed on the analyser or a critical part was replaced(Halogen Lamp)

Literature:

Arnett FC, Edworthy SM, Bloch DA et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-324

Bablok W et al. General Regression Procedure for Method Transformation. J Clin Chem Biochem 1988; 26:783-790

Bampton JL, Cawston TE, Kyle MV, Hateleman BL. Ann Rheum Dis 1985;44:379-383

Bartfied H. Distribution of rheumatoid factor activity in non-rheumatoid states. Ann NY Acad Sci 1969;168:30-40

Borque L, Barozzi D, Ferrari L et al. The determination of Rheumatoid Factors by an immunoturbidimetric assay on Boehringer Mannheim/Hitachi analysis Systems. Klin Lab 1994;40:445-453.

Borque L, Yago M, Mar C, Rodriques C. Clin Chem 1986;32:24-129

Glinck MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-474

Guder WG, Narayanan S, Wisser H, Zawta B. List of Analytes Preanalytical Variabled. Broschüre in: Samples: From the Patient to the Laboratory. Darmstadt: GIT-Verlag, 1996

Heil W, Koberstein R, Zawta B. Reference Ranges for Adults and Children, Pre-Analytical Considerations (published by Roche 1997/98)

Jaspers JP, Van Oers RJM, Leerkes B. Nine Rheumatoid Factor Assays Compared. J Clin Chem Clin Biochem 1988;26:863-871

Koopmann WJ, Schrohenloker RE. Arthritis Rheum 1980;23:302-308

Moore TL, Dorner RN. Rheumatoid factors. Clin Biochem 1993;26:75-84

Passing H, Bablok W. A New Biometrical Procedure for Testing the Equality of Measurements from Two different Analytical Methods. J Clin Chem Clin Biochem 1983;21: 709-720

Roberts-Thomson PJ, Mc Evoy R, Langhans T, Bradley J. Ann Rheum Dis 1985;44:379-3383

Waaler E. On the occurrence of a factor in human serum activating the specific agglutination of sleep blood corpuscles. Acta Pathol Microbiol Scand 1940;17:172-178

Order information(Cat No.):

CC465	CR465-466	B22265	B27265	B30265	B32267	B35266	B80266
CC466	BASO500	B24265	B27266	B30266	B33265	B36265	B80267
OL465	BASO250	B24266	B27267	B30267	B33266	B36266	B80268
OL466	BASO125	B24267	B27268	B30268	B33267	B36267	
KL465	B21265	B25265	B28265	B31265	B33268	B37265	
KL466	B21266	B25266	B28266	B31266	B34265	B37266	
AB465	B21267	B25267	B28267	B32265	B34266	B37267	
AB466	B21268	B25268	B28268	B32266	B35265	B80265	

Manufacturer

Diaclinica Diagnostik Kimya.San.Tic.Ltd.Şti

Adress : İkitelli O.S.B Mutsan San.Sit. M4 Blok No:17-19 Başakşehir/İSTANBUL

Tel:+90(212) 549 33 88- Fax:+90 (212) 549 55 50

Web :www.diaclinica.com

SYMBOLS

IVD	for in vitro diagnostic use only
LOT	lot of manufacturing
REF	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	Read the directions