

Rheumatoid Factors II**Order information**

REF	CONTENT	Analyzer(s) on which cobas c pack(s) can be used
20764574 322	Rheumatoid Factors II (100 tests)	System-ID 07 6457 4 COBAS INTEGRA 400 plus COBAS INTEGRA 800
12172828 322	Preciset RF (5 × 1 mL)	System-ID 07 7995 4
03005496 122	RF Control Set Level I (2 × 1 mL) Level II (2 × 1 mL)	System-ID 07 8040 5 System-ID 07 9007 9
20756350 322	NaCl Diluent 9 % (6 × 22 mL)	System-ID 07 5635 0

English**System information**

Test RF-II, test ID 0-757

Intended use

In vitro test for the quantitative immunological determination of human rheumatoid factors in serum and plasma on COBAS INTEGRA systems.

Summary^{1,2,3,4,5,6,7,8,9,10}

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. The 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 quantitation of rheumatoid factors is by agglutination with IgG-sensitized sheep erythrocytes or latex particles. Particular problems of these semiquantitative methods are the poor between-laboratory precision and reproducibility, together with standardization difficulties. For these reasons, new assay methods such as nephelometry, turbidimetry, enzyme-immunoassays and radioimmunoassays have been developed. The Roche RF assay is based on the immunological agglutination principle with enhancement of the reaction by latex.

Test principle^{4,5,6}

Immunoturbidimetric assay

Latex-bound heat-inactivated IgG (antigen) reacts with the RF-antibodies in the sample to form antigen/antibody complexes which, following agglutination, are measured turbidimetrically.

Reagents - working solutions

- R1** Reaction buffer
Glycine buffer: 170 mmol/L, pH 8.0; polyethylene glycol 0.05 %; bovine serum albumin; preservatives and stabilizers
- SR** Latex particles coated with human IgG; glycine buffer: 170 mmol/L, pH 7.3; preservatives and stabilizers

R1 is in position B and SR is in position C.

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

For USA: For prescription use only.

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV.

The testing methods applied were FDA-approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A. However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.^{11,12}

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C See expiration date on **cobas c** pack label

COBAS INTEGRA 400 plus systems

On-board in use at 10-15 °C 8 weeks

COBAS INTEGRA 800 systems

On-board in use at 8 °C 8 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum

Plasma: Heparin (Li-, Na-) or EDTA (Na₂-, K₂-) plasma.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:¹³ 24 hours at 15-25 °C
3 days at 2-8 °C
4 weeks at (-15)-(-25) °C (freeze only once)

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- NaCl Diluent 9 %, Cat. No. 20756350 322, system-ID 07 5635 0 for automatic postdilution. NaCl Diluent 9 % is placed in its predefined rack position and is stable for 4 weeks on-board COBAS INTEGRA 400 plus/800 analyzers.
- NaCl 0.9 % (isotonic saline solution) for calibration.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Application for serum and plasma**COBAS INTEGRA 400 plus test definition**

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A	583 nm
Calc. first/last	35/48
Typical prozone effect	> 600 IU/mL
Antigen excess check	Yes*

Unit IU/mL

Pipetting parameters

		Diluent (H ₂ O)
R1	90 µL	
SR	30 µL	10 µL
Sample	3 µL	10 µL
Total volume	143 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A	583 nm
Calc. first/last	47/67
Typical prozone effect	> 600 IU/mL
Antigen excess check	Yes*
Unit	IU/mL

Pipetting parameters

		Diluent (H ₂ O)
R1	90 µL	
SR	30 µL	10 µL
Sample	3 µL	10 µL
Total volume	143 µL	

* Samples with concentrations > 130 IU/mL are flagged either >TEST RNG or "HIGH ACT". Rerun the sample with postdilution, or, if the sample has already been postdiluted, rerun the sample with a higher postdilution factor.

Calibration

Calibrator	Preciset RF
RF concentration	Lot-specific values. For calibration values, please refer to the assigned value sheet/barcode sheet obtained with Preciset RF.
Calibration mode	Logit/log 5
Calibration replicate	Duplicate recommended
Calibration interval	Each lot, every 180 days, and as required following quality control procedures.

Calibrators must be placed from the highest concentration first, to the lowest last, on the CAL/QC rack. 0 IU/mL calibrator is not provided with Preciset RF. Please use 0.9 % NaCl.

Traceability: This method has been standardized against the World Health Organization Reference Preparation for Rheumatoid Factors (1st preparation, 1970).¹⁴

Quality control

Quality control	RF Control Set
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined

limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Icterus:¹⁵ No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:¹⁵ No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 0.621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):¹⁵ No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

γ-Globulin: Due to the binding of RF to the Fc portion of IgG, pathologically high levels of γ-globulin (25 g/L) decrease the apparent RF concentration significantly.

Therapeutic drug interference was tested according to the recommendations of the VDGH^{a)}. No interferences were found.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.¹⁶

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

a) Verband der Diagnostica und Diagnostica Geräte Hersteller. Refer to section 1 / Introduction of this Method Manual for a list of drugs tested and their concentrations.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges**Measuring range**

10-130 IU/mL

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

Lower limits of measurement

Lower detection limit of the test:

10.00 IU/mL

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of a zero sample (zero sample + 3 SD, repeatability, n = 30).

Expected values¹⁷

In a study of 541 apparently healthy individuals, the expected reference range for RF was found to be less than 14 IU/mL.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (1 aliquot per run, 1 run per day, 21 days). The following results were obtained:

	Level 1	Level 2
Mean	17 IU/mL	56 IU/mL
CV repeatability	2.3 %	1.3 %
CV intermediate precision	4.4 %	2.1 %

Method comparison

RF values for human serum samples obtained on a COBAS INTEGRA 400 analyzer with the COBAS INTEGRA Rheumatoid Factors II reagent (y) were compared with those determined using the same reagent for rheumatoid factors on a COBAS INTEGRA 700 analyzer (x) and a Roche/Hitachi 917 analyzer (x).

COBAS INTEGRA 700 analyzer

Sample size (n) = 50

Passing/Bablok¹⁸ Linear regression $y = 1.04x + 0.96$ $y = 1.02x + 1.69$ $\tau = 0.953$ $r = 0.996$ SD (md 95) = 3.601 $Sy.x = 1.878$

The sample concentrations were between 2.10 and 109.60 IU/mL.

Roche/Hitachi 917 analyzer

Sample size (n) = 50

Passing/Bablok¹⁸ Linear regression $y = 1.09x - 2.69$ $y = 1.08x - 2.45$ $\tau = 0.958$ $r = 0.998$ SD (md 95) = 3.009 $Sy.x = 1.577$

The sample concentrations were between 2.10 and 115.90 IU/mL.

References

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
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Volume after reconstitution or mixing

GTIN	Global Trade Item Number
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