Instructions For Use

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RF IgA

REF 4027 Σ 96

REF 4027-5 Σ 5 x 96

Enzyme immunoassay for the determination of rheumatoid factor IgA in human serum

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IVD CE 2°C–8°C

1 Intended Purpose

The RF IgA is a quantitative immunoassay for the determination of rheumatoid factor (RF) IgA in human serum.

The RF IgA is intended as an aid in the diagnosis of rheumatoid arthritis (RA) in conjunction with other clinical and laboratory findings.

The immunoassay is designed for manual professional *in vitro* diagnostic use.

2 Diagnostic Relevance

Patients suffering from rheumatoid arthritis (RA) exhibit RF, autoantibodies recognizing the Fc part of IgG. RA or chronic polyarthritis has a yet unknown etiology and represents the most frequent rheumatic inflammatory disorder demonstrating a prevalence rate of up to 1%. One of the typical manifestations of RA is symmetric synovialitis of limb joints often accompanied by involvement of the cervical spinal column.

Beside clinical features one of the criteria of the American College of Rheumatology for the diagnosis of RA is the presence of RF. Up to 80 % of RA patients may demonstrate RF. RF can occur years prior to the onset of disease and RF positive apparently healthy individuals bear a 5 - 40 times higher risk to develop RA. However, patients suffering from other autoimmune, infectious or B-cell lymphoproliferative disorders as well as apparently healthy elderly individuals may develop RF.

High concentrations of RF are often associated with a more severe disease comprising a faster destruction of joints. In addition they are found in patients with extra-articular manifestations such as rheumatoid nodules, polyneuropathy, vasculitis or Sicca syndrome.

RF may belong to the IgG, IgM or IgA isotype whereas IgM RF is the most frequent isotype to be determined in RA patients. Extra-articular manifestations seem to be associated with IgA RF. Like RF of the IgM isotype high concentrations of IgG RF seems to appear with patients suffering from more progressive erosions of joints.

3 Test Principle

The ELISA (Enzyme Linked Immunosorbent Assay) is an immunoassay for the determination of specific antibodies. The strips of the microtiter plate are coated with test-specific antigens. If antibodies are present in the patient´s sample, they bind to the antigens. A secondary antibody conjugated with the enzyme peroxidase detects the generated immune complex. A colorless substrate is converted into the colored product. The signal intensity of the reaction product is proportional to the antibody activity in the sample. After stopping the signal intensity of the reaction product is measured photometrically.

4 Test Components

Component 96 (5x96) Determinations	Description
Microtiter plate **A** **Ag | 96** 1 piece (5 pieces)	12 (5x12) breakable microtiter strips (ready-to-use), 8 wells per strip, each well coated with rabbit IgG
Calibrator **0 – 4** **CAL** 5 x 1 mL (3.5 mL), white cap	Colored dilutions of human serum (ready-to-use; contains ProClin 950) The antibody activities are indicated on the quality control certificate.
Negative control **N** **CONTROL | -** 1 x 1 mL (3.5 mL), green cap	Colored dilution of human serum (ready-to-use; contains ProClin 950) The antibody activity is indicated on the quality control certificate.
Positive control **P** **CONTROL | +** 1 x 1 mL (3.5 mL), red cap	Colored dilution of human serum (ready-to-use; contains ProClin 950) The antibody activity is indicated on the quality control certificate.
Sample diluent **C** **DIL** 1 x 100 mL (2 x 250 mL), black cap	Colored solution (ready-to-use; contains ProClin 950)
Wash buffer **B** **BUF|WASH|10x** 1 x 100 mL (2 x 250 mL), white cap	Concentrated solution (10x; contains ProClin 950)
Conjugate IgA **D** **CONJ** 1 (5) x 15 mL, violet cap	Colored solution of polyclonal anti-human IgA antibody conjugated to horseradish peroxidase (ready-to-use; contains ProClin 950)
Substrate **E** **SOLN | TMB** 1 (5) x 15 mL, blue cap	3,3´,5,5´-Tetramethylbenzidin (ready-to-use)
Stop solution **F** **H2SO4 | 0.25M** 1 x 15 mL (75 mL), yellow cap	0.25 M Sulfuric acid (ready-to-use)
Adhesive Foil 2 (10) pieces	-

QC Certificate 1 piece	-
Instructions for Use 1 piece	-

5 Materials required but not provided

- Common laboratory equipment
- Precision pipettes (5 – 1000 µL), multi-channel pipettes (100 – 1000 µL) and disposable pipette tips
- Graduated cylinders (100 – 1000 mL)
- Sample tubes for the preparation of dilutions
- Vortex mixer or other rotators
- Microtiter plate washer or wash comb
- Microtiter plate reader with optical filters for 450 nm and 620 nm or 690 nm
- Adsorbent paper or paper towel
- Distilled or de-ionized water

6 Storage and Stability

Upon receipt, all test components must be stored at 2 °C to 8 °C, preferably in the original kit box. If stored properly in their original containers, all components are stable until their expiry date. All components are stable for at least 2 months after opening when stored properly at 2 °C to 8 °C.

7 General Information

This product is for *in vitro* diagnostic use only. The instructions for use must be carefully read before use. They are valid only for the present product with the given composition and must be strictly followed to ensure reliable test results. Deviations can lead to erroneous test results. Components must not be exchanged by test reagents of different lots or of other manufacturers.

Contamination of reagents must be avoided by use of aseptic techniques when removing aliquots from the vials. After use, reagent vials must be tightly closed with their corresponding caps.

Cross-contamination of samples or reagents can lead to inconsistent test results and must be avoided by use of consistent pipetting techniques.

Exposure of reagents to strong light must be avoided throughout the entire test procedure and storage.

Insufficient washing will result in poor precision and elevated measurement signals. After each washing step any residual fluid has to be removed completely.

8 Preparation

8.1 Preparation of Reagents

All components including the microtiter plate must be brought to room temperature (RT: 18 °C to 25 °C) before use for at least 30 min. All liquid components must be mixed gently to ensure homogeneity.

8.1.1 Microtiter Plate

The microtiter plate is sealed in an aluminium bag. Unused test strips should always be stored refrigerated and protected from moisture with the desiccant in the properly sealed aluminum bag. Carefully resealed, the test strips can be used for 8 weeks after opening.

8.1.2 Calibrators

The calibrators are ready-to-use and must not be diluted any further. Calibrators must be used in each test run.

8.1.3 Controls

The positive and the negative controls are ready-to-use and must not be diluted any further. Controls must be used in each test run. Laboratories can also validate their own control samples and use them alternatively.

8.1.4 Sample Diluent

The sample diluent is ready-to-use.

8.1.5 Wash Buffer

The wash buffer is concentrated and must be diluted 1:10 with distilled water before use (e. g. 100 mL + 900 mL). A sufficient amount of washing solution must be prepared. The diluted washing solution can be stored at 2 °C to 8 °C up to 30 days.

8.1.6 Conjugate

The conjugate is ready-to-use and stable up to 8 weeks after opening when stored at 2 °C to 8 °C.

8.1.7 Substrate

The substrate is ready-to-use. Exposure of the substrate solution to strong light should be avoided.

8.1.8 Stop Solution

The stop solution is ready-to-use.

8.2 Preparation of Samples

8.2.1 Sample Material

The use of freshly collected serum from blood taken by venipuncture is recommended. The use of icteric, lipemic, hemolytic or bacterially contaminated samples should be avoided. Insoluble substances must be removed from the sample by centrifugation. Samples must not be thermally inactivated.

8.2.2 Sample Dilution

The samples must be diluted 1:101 (e. g. 10 µL + 1000 µL) with sample diluent and mixed thoroughly. Building of foam should be avoided.

8.2.3 Sample Storage

Samples may be kept at 2 °C to 8 °C up to three days. Long-term storage requires -20 °C. Repeated freezing and thawing should be avoided. For multiple use, samples should be aliquoted and kept at -20 °C.

9 Test Performance

9.1 Pipetting Scheme

The following pipetting scheme is recommended:

	1	2	3	4
A	CAL 0	Sample 2		
B	CAL 1	Sample 3		
C	CAL 2	Sample 4		
D	CAL 3	Sample 5		
E	CAL 4	…		
F	N	…		
G	P	…		
H	Sample 1	…		

9.2 Procedure

The indicated incubation times and temperatures must be adhered to and significant time shifts during pipetting samples and reagents must be avoided. The microtiter plate should be shortly shaken after addition of reagents.

Step	Description
1. Addition of calibrators,	Add 100 µL ready-to-use calibrators, controls and diluted samples per well

controls and diluted samples	
2. Incubation	Cover the plate and incubate for 60 min. at RT
3. Wash cycle	Aspirate the solution and wash 3 times with 300 µL washing solution with at least 5 seconds soaking time each; dry by tapping the microtiter plate on a paper towel to remove any residual droplets
4. Addition of conjugate	Add 100 µL ready-to-use conjugate to each well
5. Incubation	Cover the plate and incubate for 30 min. at RT
6. Wash cycle	Aspirate the solution and wash 3 times with 300 µL washing solution with at least 5 seconds soaking time each; dry by tapping the microtiter plate on a paper towel to remove any residual droplets
7. Addition of substrate	Add 100 µL ready-to-use substrate to each well
8. Incubation	Cover the plate and incubate for 15 min. in the dark at RT
9. Addition of Stop Solution	Add 100 µL ready-to-use stop solution to each well
10. Analysis	Read optical density (OD) at 450 nm versus 620 or 690 nm within 30 min. after stopping the reaction

9.3 Automation

Automated processing of the immunoassays must be performed analogous to manual use and validated by the user.

10 Test Evaluation

10.1 Metrological Traceability

The immunoassay is calibrated using the International WHO reference preparation W1066 (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam). Quantitative results are expressed in U/mL.

10.2 Standard Curve

For generation of a standard curve, the optical signals (optical density, OD) of the calibrators are plotted against their antibody activities and correlated by a 4-parameter logistic (4 PL) fit. Antibody activities of unknown samples can be derived directly from their optical signals by use of the generated standard curve.

10.3 Criteria of Validity

Test runs are only valid if the following criteria of validity are fulfilled:

- OD CAL 0 < CAL 1 < CAL 2 < CAL 3 < CAL 4
- OD CAL 4 > 1.2
- The negative control must be evaluated negative.
- The positive control must be evaluated positive and present an antibody activity within the validity range indicated on the quality control certificate.

If these criteria are not met, the test is not valid and must be repeated.

10.4 Troubleshooting

In case of an invalid test run, the expiry dates and storage conditions, incubation times and temperatures, and precise calibration of all instruments used should be verified. If no reason for an invalid test run could be identified, please contact the supplier or manufacturer of the product.

10.5 Reference Ranges

The reference ranges are indicated below:

	Interpretation
Antibody activity < 25 U/mL	negative
Antibody activity 25 – 30 U/mL	borderline
Antibody activity > 30 U/mL	positive

As a result of different seroprevalences in individual regions, each laboratory should verify the reference ranges by own analysis and adapt, if necessary.

10.6 Interpretation of Test Results

A positive test result indicates the presence of specific antibodies. A negative result indicates the absence of specific antibodies, but does not exclude the possibility of an autoimmune reaction. In case of a borderline test result, a reliable evaluation is not possible.

10.7 Limitations of the Method

The interpretation of test results must always be considered in combination with the clinical picture of the patient. The diagnosis should not be based on the results of a sole diagnostic method. All clinical and laboratory findings should be evaluated to state a diagnosis. For confirmation, further investigations should be carried out.

11 Performance Characteristics

11.1 Analytical Performance Characteristics

11.1.1 Precision

The precision of test results was assessed by the determination of the intra- and interassay variation by the analysis of multiple samples with different antibody activities.

	Intraassay Precision		Interassay Precision	
	U/mL	CV (%)	U/mL	CV (%)
Sample 1	4.5	11.2	5.1	16.8
Sample 2	109.4	2.5	119.6	5.3
Sample 3	249.9	3.3	266.5	4.1

11.1.2 Measurement Range

Reliable accuracy, trueness, precision, linearity and recovery of test results have been observed within the measurement range of the assay from the LoQ to the upper calibrator in comprehensive studies. Samples with test results above the upper calibrator should be reported as >max. Samples with test results below the LoQ should be reported as <min. If test results above the upper calibrator are observed, the samples may be tested at a higher dilution. The resulting antibody activity must be multiplied with the additional dilution factor.

11.2 Diagnostic Performance Characteristics

11.2.1 Diagnostic Sensitivity and Specificity

The sensitivity and specificity of the immunoassay were assessed by the analysis of 41 serum samples from patients with rheumatoid arthritis in comparison to the results of the Elisa of a European manufacturer as a reference and 54 serum samples from unselected blood donors.

	Diagnostic Performance
Sensitivity	> 99 %
Specificity	94.4 %

12 Warnings and Precautions

The product is designed exclusively for *in vitro* diagnostic use by qualified, authorized and trained personnel. All test components

and human samples should be handled with care as potentially hazardous. Good laboratory practices (GLP) and all relevant regulations should be adhered to.

In case the product is damaged or product information including labelling is wrong or incorrect, please contact the manufacturer or supplier.

This product contains preparations of human and / or animal origin. Any material derived from human body fluids or organs used for the preparation of components were tested and found negative for HBsAg (Hepatitis B-Virus-surface Antigen) and anti-HIV as well as anti-HCV antibodies. However, all components and all patient samples should be handled as potentially hazardous in accordance with national laws and appropriate guidelines on biological safety.

As the product contains potentially hazardous materials, the following precautions should be followed: Do not smoke, eat or drink while handling kit material or samples. Avoid direct contact to kit material or samples by wearing protective gloves laboratory coat and safety glasses. Never pipette material by mouth. Wipe up spills promptly and wash the affected surface thoroughly with a decontaminant. Wash hands thoroughly after use.

Some of the reagents contain ProClin (< 1.0 %) as a preservative, may cause skin sensitization (H317) and must not be swallowed or allowed to come into contact with skin or mucosa (P280, P333+P313).

The information in the safety data sheet on possible hazards, first aid measures, measures in the event of the unintentional release of large quantities, handling and storage, personal protective equipment, information on disposal as well as information on toxicology must be observed.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the member state in which the user and/or the patient is established.

13 Disposal

For decontamination and disposal the recommendations of the CDC as well as the relevant local and national environmental guidelines and regulations should be adhered to. Samples, potentially contaminated materials and infectious waste must be decontaminated, e.g. by autoclaving for 20 min. at 121 °C.

14 References

- Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al.: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 1988, *31*, 315 – 24.
- MacGregor FJ, Silman AJ.: Rheumatoid factors as predictors of rheumatoid arthritis. J Rheumatol 1991, *18*, 1280 – 1.

15 Symbols

	Manufacturer
	CE marking of conformity
IVD	*In vitro* diagnostic medical device
REF	Catalogue number
UDI	Unique device identifier
LOT	Batch code
	Temperature limit
	Use-by date
	Consult instructions for use
	Contains sufficient for <n> tests
	Do not re-use
	Caution
	Warning
	Biological risk
	Keep away from sunlight
Ag 96	Microtiter plate
CAL	Calibrator
CONTROL -	Negative control
CONTROL +	Positive control
DIL	Sample diluent
CONJ	Conjugate
BUF WASH 10x	Wash buffer
SOLN TMB	Substrate
H2SO4 0.25M	Stop solution

16 Changes

Changes in current Instructions for Use	
Current Version	005/01.2023
Summary of Changes	Correction number of foils in chapter 4