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NAME AND INTENDED USE

Rheumatoid Factor IgM is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of IgM class rheumatoid factor antibodies in human serum or plasma.

The assay is intended for in vitro diagnostic use only as an aid in the determination of rheumatoid arthritis (RA). In the United States, this kit is intended for Research Use Only.

SUMMARY AND EXPLANATION OF THE TEST

The presence of IgM Rheumatoid Factor (RF) in the serum is the sole serological indicator included in the ACR list of criteria for the diagnosis of RA. RFs are a subset of antiglobulins directed against the Fc region of IgG. We do not include in these definition antibodies to the IgG Fab region and pepsin agglutinators, directed against neoantigens on IgG exposed by pepsin cleavage. It is claimed that the majority of antiglobulin activity in normal serum is Fab-specific, whereas antiglobulin from RA patients is mostly Fc-specific. RFs are present in the serum of 75-80% of patients with RA at some time during the disease course. However, RFs are also found in the serum of patients with infectious and autoimmune diseases, hyperglobulinemias, B-cell lymphoproliferative disorders and in the aged population. This suggests that RF may be a finding associated with B-cell hyperactivity.

Rheumatoid Factors which have been found among the IgM, IgG and IgA classes of immunoglobulins, reacting only with xenogeneic Fc are not autoantibodies and are unlikely to be of pathological significance. However, RFs can bind IgG from many species, including autologous IgG, when immobilized on surfaces. Autologous binding is of higher affinity than xenogeneic binding. The here presented test systems for the determination of rheumatoid factors uses only human Fc-fragments as coated antigen.

It is generally considered that high titer RFs are associated with more severe disease and the presence of extra-articular features and rheumatoid nodules.This conclusion may depend on the disease duration. Serum IgM RF may precede the onset of RA by several years. A high titer of RF in non-RA individuals is associated with increased risk of developing RA. In the first 2 years of RA (early RA), serum levels of IgM, IgG and IgA RF do not correlate with disease activity. Serum IgG and IgA RF in these years are prognostic of erosive joint disease.

In established RA, high titer serum IgM RF correlates with the presence of articular disease and nodules but not with systemic disease activity. The presence of either IgG or IgA RF in patients with long-standing RA may be a good prognostic indicator of systemic manifesta-ions. IgG and IgM RF are associated with extra-articular RA including rheumatoid vasculitis and nodules. The presence of IgM RF - containing immune complexes with bound complement (C1q) -is also associated with extra-articular RA.

PRINCIPLE OF THE TEST

Fc fragments of highly purified human Immunoglobulin G is bound to microwells. Antibodies against this antigen, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgM immunologically detects the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product.

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The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgM antibodies present in the original sample.

WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for in vitro use only. In the United States, this kit is intended for Research Use Only.

2. Do not interchange kit components from different lots.

3. Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 and HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.

4. Avoid contact with the TMB (3,3´,5,5´-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.

5. Avoid contact with the Stop Solution which is acid. If it comes into contact with skin, wash thoroughly with water and seek medical attention.

6. Some kit components (i.e. Controls, Sample buffer and Buffered Wash Solution) contain Sodium Azide as preservative. Sodium Azide (NaN_3) is highly toxic and reactive in pure form. At the product concentrations (0.09%), though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 8., 9., 10.).

7. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.

8. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.

9. Do not pipette by mouth.

10. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.

11. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

CONTENTS OF THE KIT

Package size	96 determ.
Qty.1	Divisible **microplate** consisting of 12 modules of 8 wells each, coated Fc fragments of highly purified human Immunoglobulin G. Ready to use.
5 vials, 1.5 ml each	combined **Calibrators** with IgM class rheumatoid factor antibodies (AE) in a serum/buffer matrix (PBS, BSA, NaN_3 <0,1% (w/w)) containing: IgM: 0; 15;50; 150; 500 IU/ml. Ready to use.
2 vials, 1,5 ml each	Rheumatoid Factor **Controls** in a serum/buffer matrix (PBS, BSA, NaN_3 <0.1% (w/w)) positive (1) and negative (2), for the respective concentrations see the enclosed package insert. Ready to use.

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1 vial, 20 ml	**Sample buffer** (Tris, NaN$_3$ <0,1% (w/w)), yellow, concentrate (5x).
1 vial, 15 ml	**Enzyme conjugate** solution (PBS, Proclin 300 <0.5% (v/v)), (light red) containing polyclonal rabbit anti-human IgM; labelled with horseradish peroxidase. Ready to use.
1 vial, 15 ml	**TMB substrate solution**. Ready to use.
1 vial, 15 ml	**Stop solution** (contains acid). Ready to use.
1 vial, 20 ml	**Wash solution** (PBS, NaN$_3$ <0.1% (w/w)), concentrate (50x).

STORAGE AND STABILITY

1. Store the kit at 2-8 °C.
2. Keep microplate wells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage and usage.
5. Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2-8 °C.

MATERIALS REQUIRED

Equipment

- Microplate reader capable of endpoint measurements at 450 nm
- Multi-Channel Dispenser or repeatable pipet for 100 µl
- Vortex mixer
- Pipets for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Data reduction software

Preparation of reagents

- Distilled or deionized water
- Graduated cylinder for 100 and 1000 ml
- Plastic container for storage of the wash solution

SPECIMEN COLLECTION, STORAGE AND HANDLING

1. Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8 °C for up to five days or stored at -20 °C up to six months.
5. Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.
6. Testing of heat-inactivated sera is not recommended.

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PROCEDURAL NOTES

1. Do not use kit components beyond their expiration dates.
2. Do not interchange kit components from different lots.
3. All materials must be at room temperature (20-28 °C).
4. Have all reagents and samples ready before start of the assay. Once started, the test must be performed without interruption to get the most reliable and consistent results.
5. Perform the assay steps only in the order indicated.
6. Always use fresh sample dilutions.
7. Pipette all reagents and samples into the bottom of the wells.
8. To avoid carryover contamination change the tip between samples and different kit controls.
9. It is important to wash microwells thoroughly and remove the last droplets of wash buffer to achieve best results.
10. All incubation steps must be accurately timed.
11. Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.
12. Do not re-use microplate wells.

For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the patient results semi-quantitatively.

PREPARATION OF REAGENTS

Preparation of sample buffer
Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled or deionized water to a final volume of 100 ml prior to use. Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Preparation of wash solution
Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Sample preparation
Dilute all patient samples **1:100** with sample buffer before assay.
Therefore combine 10 µl of sample with 990 µl of sample buffer in a polystyrene tube. Mix well.

Controls are ready to use and need not be diluted.

TEST PROCEDURE

1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted patient samples.
2. Pipet 100 µl of calibrators, controls and prediluted patient samples in duplicate into the wells.

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	1	2	3	4	5	6	
A	SA	SE	P2	P..			
B	SA	SE	P2	P..			
C	SB	C1	P3				SA - SE: standards A to E
D	SB	C1	P3				P1, P2... patient sample 1, 2 ...
E	SC	C2	P4				C1: positive control
F	SC	C2	P4				C2: negative control
G	SD	P1	P5				
H	SD	P1	P5				

3. Incubate for 30 minutes at room temperature (20-28 °C).

4. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.

5. Dispense 100 µl of enzyme conjugate into each well.

6. Incubate for 15 minutes at room temperature.

7. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.

8. Dispense 100 µl of TMB substrate solution into each well.

9. Incubate for 15 minutes at room temperature.

10. Add 100 µl of stop solution to each well of the modules and incubate for 5 minutes at room temperature.

11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.
    The developed colour is stable for at least 30 minutes. Read optical densities during this time.

Automation
The Rheumatoid Factor IgM ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.

DRG® RF IgM   (EIA-3585)

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INTERPRETATION OF RESULTS

Quality Control
This test is only valid if the optical density at 450 nm for Positive Control (1) and Negative Control (2) as well as for the Calibrator A and E complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit ! If any of these criteria is not fulfil-led, the results are invalid and the test should be repeated.

Calculation of results
For Rheumatoid Factor IgM a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.
Recommended Lin-Log Plot
First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

Calculation example
The figures below show typical results for Rheumatoid Factor IgM ELISA. These data are intended for illustration only and should not be used to calculate results from another run.

Calibrators									
No.	Position	OD 1	OD 2	Mean	Conc. 1	Conc. 2	Mean	decl. Conc.	CV %
STA	A 1/B 1	0,029	0,031	0,030	0	0	0	0	1
STB	C 1/D 1	0,232	0,214	0,223	16,4	14,9	15,6	15	4
STC	E 1/F 1	0,563	0,536	0,550	50,2	47,2	48,7	50	3
STD	G 1/H 1	1,263	1,181	1,222	160,6	143,8	152,2	150	5
STE	A 2/B 2	2,190	2,118	2,154	522,3	474,3	498,4	500	3

Interpretation of results
In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Rheumatoid Factor IgM test:

Rheumatoid Factor IgM [IU/ml]
normal: < 20
elevated: > 20

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually.
It is recommended that each laboratory establishes its own normal and pathological ranges of serum Rheumatoid Factors.

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PERFORMANCE CHARACTERISTICS

Parallelism

In dilution experiments sera with high IgM-antibody concentrations were diluted with sample buffer and assayed in the Rheumatoid Factor IgM kit.

Rheumatoid Factor	Sample No.	Dilution	Observed [IU/ml]	Expected [IU/ml]	O/E
IgM	1	1:100	206.2		
		1:200	103.1	103.1	100 %
		1:400	52.3	51.6	101 %
		1:800	27.7	25.8	107 %
		1:1600	13.9	12.9	108 %
IgM	2	1:200	150.4		
		1:400	73.7	75.2	98 %
		1:800	39.2	37.6	104 %
		1:1600	20.0	18.8	106 %
		1:3200	10.5	9.4	112 %
		1:6400	5.3	4.7	113 %

Precision (Reproducibility)

Statistics for coefficients of variation (CV) were calculated for each of three samples from the results of 24 determinations in a single run for Intra-Assay precision. Run-to-run precision was calculated from the results of 5 different runs with 6 determinations of each sample:

Intra-Assay			Inter-Assay		
Sample No	Mean [IU/ml]	CV [%]	Sample No	Mean [IU/ml]	CV [%]
1	29.2	2.4	1	26.7	4.0
2	116.3	3.6	2	102.0	3.6
3	294.4	4.9	3	275.1	3.2

Sensitivity

The lower detection limit for Rheumatoid Factor IgM was determined at 1.0 IU/ml.

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Specificity

The microplate is coated with the FC fragment of highly purified human Immunoglobulin G. The test kit is specific for all classes of rheumatoid factors.

Calibration

The quantitative test system for Rheumatoid Factor IgM is calibrated in relative arbitrary units. The calibration is related to the 1st British Standard Preparation 64/2. This material tests positive for IgM Rheumatoid Factors.

LIMITATIONS OF PROCEDURE

The absence of Rheumatoid Factor does not rule out rheumatoid arthritis. Rheumatoid Factor may appear transiently during various infections.
The Rheumatoid Factor IgM ELISA is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.
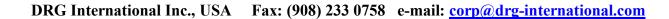
INTERFERING SUBSTANCES

No interference has been observed with haemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL triglycerides) or bilirubin (up to 40 mg/dL) containing sera. Nor have any interfering effects been observed with the use of anticoagulants .However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

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