Rheumatoid Factor IgA ELISA
Enzyme Immunoassay for quantitative determination of IgA Rheumatoid Factors in human serum or plasma

DE7630
96 Tests
PRINCIPLE OF THE TEST

Fc fragments of highly purified human Immunoglobulin G are coated on to microwells. Antibodies against the coated antigen, if present in diluted patient sample, bind to the respective antigen. Washing of the microwells removes unbound unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human antibodies immunologically detect 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 colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of antibodies present in the original sample.

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. In this definition we do not include 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 an-tiglobulin 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, hyperglobulinemia, 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 immobilised 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 manifestations. 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.
CONTENTS OF THE KIT

Sufficient for 96 determinations

1 One divisible microplate consisting of 12 modules of 8 wells each. Ready to use.

5x 1.5 ml Calibrator A-E (0, 15, 50, 150, 500 U/ml), containing rheumatoid factor in a serum/buffer matrix (PBS, BSA, detergent, NaN₃ 0.09%), yellow. Ready to use.

2x 1.5 ml Control positive (1) and negative (2), containing rheumatoid factor in a serum/buffer matrix (PBS, BSA, detergent, NaN₃ 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.

20 ml Sample Buffer P, containing PBS, BSA, detergent, preservative NaN₃ 0.09%, yellow, concentrate (5 x).

15 ml Enzyme Conjugate; light red, containing anti-human IgA antibodies, HRP labelled; PBS, BSA, detergent, preservative ProClin 300 0.05%. Ready to use.

15 ml TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.

15 ml Stop Solution; contains acid. Ready to use.

20 ml Wash Solution, containing Tris, detergent, preservative NaN₃ 0.09%; 50 x conc.

1 Instruction for Use

1 Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

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

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and desiccated in the clip bag provided.
- Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash solution and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.
PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, perform the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash solution.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- 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 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3’,5,5’-Tetramethyl-benzidine).
- Stop solution contains acid, classification is non-hazardous. Avoid contact with skin.
- Control, calibrator, sample buffer and wash solution contain sodium azide (NaN₃) 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.
- During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:
  - First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
  - Personal precautions, protective equipment and emergency procedures:
    - Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.
    - Exposure controls / personal protection: Wear protective gloves of nitrile rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
    - Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
    - For disposal of laboratory waste the national or regional legislation has to be observed.
    - Observe the guidelines for performing quality control in medical laboratories by assaying control sera.
PREPARATION OF REAGENTS

Wash Solution
Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

Sample Buffer P
Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples
Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.
1. Pipette 100 µl of calibrators, controls and prediluted patient samples into the wells.
2. Incubate for 30 minutes at room temperature (20-28 °C).
3. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
4. Dispense 100 µl of enzyme conjugate into each well.
5. Incubate for 15 minutes at room temperature.
6. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
7. Dispense 100 µl of TMB substrate solution into each well.
8. Incubate for 15 minutes at room temperature
9. Add 100 µl of stop solution to each well of the modules
10. Incubate for 5 minutes at room temperature
11. Read the optical density at 450 nm (reference 600-690nm) and calculate the results. The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

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<th>B</th>
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<td>... patient sample</td>
<td>A-E calibrators</td>
<td>C+, C- controls</td>
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VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit. If these quality control criteria are not met the assay run is invalid and should be repeated.

CALCULATION OF RESULTS

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation. Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.
PERFORMANCE CHARACTERISTICS

CALIBRATION
This assay system is calibrated in relative arbitrary units. Calibration is related to 1st British Standard Preparation 64/2 for Rheumatoid Factor.

Measuring range
The calculation range of this ELISA assay is 0 - 500 U/ml

Expected values
In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 20 U/ml

Interpretation of results
Negative: < 20 U/ml
Positive: ≥ 20 U/ml

Linearity
Patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper/ lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

<table>
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<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Observed U/ml</th>
<th>Expected U/ml</th>
<th>O/E [%]</th>
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<td>1:100</td>
<td>350.0</td>
<td>463.1</td>
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<td></td>
<td>1:200</td>
<td>298.8</td>
<td>231.6</td>
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<td>1:400</td>
<td>112.3</td>
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<td>1:800</td>
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<td>1:1600</td>
<td>25.6</td>
<td>30.1</td>
<td>85</td>
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Limit of detection
Functional sensitivity was determined to be: 1 U/ml

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

*Intra-assay precision:* Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.

*Inter-assay precision:* Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

![Intra-Assay and Inter-Assay tables]

Study results

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<th>Study population</th>
<th>n</th>
<th>n Pos</th>
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<tr>
<td>Rheumatoid Arthritis</td>
<td>302</td>
<td>235</td>
<td>77.8</td>
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<tr>
<td>Normal human sera</td>
<td>169</td>
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<td>7.1</td>
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**Clinical Diagnosis**

<table>
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<tr>
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<tr>
<td>Pos</td>
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<td>Neg</td>
<td>67</td>
<td>157</td>
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<td>Total</td>
<td>302</td>
<td>169</td>
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</table>

Sensitivity: 77.8 %
Specificity: 92.9 %
Overall agreement: 83.2 %

**LIMITATIONS OF THE PROCEDURE**

This assay 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 concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually. The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.

**REFERENCES**


SYMBOLS USED WITH DEMEDITEC ASSAYS

<table>
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<tr>
<th>Symbol</th>
<th>English</th>
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<th>Español</th>
<th>Italiano</th>
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