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IVD



See external label



2°C-8°C



Σ=96 tests

REF

#2550

Rheumatoid Factor IgM ELISA

Cat. No. 2550

INTENDED USE

The Diagnostic Automation, Inc. (DAI) Rheumatoid Factor (RF) Enzyme-Linked Immunosorbent Assay (ELISA) is intended for the detection of IgM antibodies in human serum to RF antigen and as an aid in the diagnosis of rheumatoid arthritis. For *in vitro* diagnostic use.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown etiology (1). Rheumatoid arthritis is a systemic disease characterized by chronic proliferation and inflammation of joint cartilage and supporting structures. RA is mainly defined by clinical criteria, in which systematic pathogenetic studies have been hampered by doubts about the presence of common pathogenetic mechanisms and the relative lack of unique laboratory findings (2). IgG rheumatoid factor has been reported to be present in sera of patients with rheumatoid arthritis both with and without IgM rheumatoid factor activity (3,4).

RFs are immunoglobulins of any isotype with antibody activity directed against antigenic sites on the Fc portion of IgG molecules. Because of its pentavalent structure and ability to cross-link immunoglobulin G antigen, IgM-RF is the main isotype identified by clinically available diagnostic assays for RF detection (5). Rheumatoid factors may exist as the mu, gamma, alpha, and epsilon isotypes (6).

Rheumatoid factors are found in 1 to 4% of the general population. RFs are present in 75% of adult patients with the highest incidence of rheumatoid factors occurs in persons over 65 years of age and nearly all patients with Felty and Sjogren syndrome. The clinical correlation of an elevated rheumatoid factor should be interpreted cautiously. Increased titers may accompany a variety of acute immune responses, particularly viral infections and a number of other diseases (e.g., infectious mononucleosis, tuberculosis, leprosy, various parasitic diseases, liver disease, sarcoidosis, and lymphoproliferative syndromes) (6).

The earliest tests and those still most widely used rely on the agglutinating properties of the IgM class of rheumatoid factors. Sensitized sheep red blood cell and latex agglutination tests have been

developed and routinely employed. These assays are most sensitive for the detection of RF that is of the IgM isotype because of its multivalent structure. These tests provide a dilution which is difficult to standardize and have laborious processing and poor reproducibility. Enzyme immunoassays are more sensitive than agglutination and very specific using purified antigen (5).

PRINCIPLE OF THE TEST

The DAI Rheumatoid Factor test uses the ELISA technique for the detection of IgM antibodies to IgG antigen. The purified antigen is bound to a solid phase microassay well. Patient serum samples to be assayed for antibody are first diluted and are added to each well. If antibody is present in the patient's serum, antigen-antibody complexes are formed. After washing the unbound serum from the well, horseradish peroxidase conjugated anti-human IgM is added to the wells and allowed to incubate. The conjugate will bind to human antibody which is present. After washing the unbound conjugate from the wells, TMB substrate solution is added and incubated. The enzyme conjugate present will react with the H_2O_2 substrate and tetramethylbenzidine (TMB) chromogen, resulting in blue color development. The addition of 1N H_2SO_4 stops the enzymatic reaction and turns the blue color to yellow. The absorbance of the solution, measured at 450 nm, is directly related to the concentration of IgM antibody bound to the well.

MATERIALS SUPPLIED

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the package label.

1. Purified IgG antigen coated microassay plate: 96 well, provided with a strip holder and stored in a foil bag with desiccant and humidity indicator card.
2. Positive Control Serum: one vial, 0.4 mL, human serum containing 0.1% sodium azide and 0.01% pen/strep as preservatives, with range printed on vial label.
3. Calibrator Serum: one vial, 0.4 mL, human serum containing 0.1% sodium azide and 0.01% pen/strep added as preservatives, with factor printed on vial label.
4. Negative Control Serum: one vial, 0.4 mL, human serum containing 0.1% sodium azide and 0.01% pen/strep as preservatives, with range printed on vial label.
5. Conjugate, Goat anti-human IgM, peroxidase, affinity purified: one bottle, 16 mL. Ready to use, containing proclin (0.1%) as preservative.
6. Serum Diluent Type II: one bottle, 30 mL. Ready to use, containing proclin (0.1%) as preservative.
7. Wash Buffer (20X concentrate): one bottle, 60 mL. The buffer contains TBS, Tween-20 and proclin (0.1%) as preservative. Dilute the buffer 1 + 19 by pouring the contents of the bottle into a container and add dH_2O to 1200 mL final volume. Mix thoroughly.
8. Chromogen/Substrate Solution: one bottle, 15 mL, Tetramethylbenzidine (TMB). Ready to use.
9. Stop Solution: one bottle, 15 mL. Ready to use, contains a H_2SO_4 solution.

REAGENT STORAGE CONDITION

1. All kit components that are stored at their recommended storage conditions are stable until the expiration date on their label. Do not use past their expiration date.
2. Antigen coated wells. Unused strips should be immediately resealed in the foil bags with desiccant and humidity indicator card and returned to storage at 2-8° C.

3. All other reagents are stored at 2-8° C in their original containers.
4. Store 1X (diluted) Wash Buffer at room temperature (21° to 25° C) for up to 5 days, or 1 week between 2° and 8° C.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Test tubes (12 X 75 mm) and rack for sample dilution.
2. Distilled or deionized water.
3. Precision micropipettes or micropipette tips to deliver 10, 100, and 200 µL.
4. Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. Read the operators' manual or contact the instrument manufacturer to establish linearity performance specifications of the reader.
5. Wash bottle, ELISA handwasher or automatic washing device.
6. Timer (30 minutes minimum).
7. Measuring cylinder of 1200 mL.
8. Absorbent paper towels.
9. Disinfectant solution (e.g., dilute household bleach 1:10 with distilled water) or disposal basin. Do not use bleach while running test, as it may interfere with conjugate activity.

PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. Patient specimens may contain infectious agents and should be handled and disposed of as potential biohazards. Do not mouth pipette (use precision pipettors). Wear disposable gloves while handling specimens and performing test procedure.
3. CAUTION: Source material from which the human Calibrator and Controls were derived were tested for HBsAg and HIV-1 and found negative. However no test method can offer complete assurance that human blood will not transmit HIV, Hepatitis or other potentially infectious agents, these reagents should be handled at Biosafety Level 2 as recommended in the CDC/NIH Manual *Biosafety in Microbiology and Biomedical Laboratories*, 1993, or latest edition.
4. While carrying out the test, smoking, eating, and drinking is not permitted.
5. Stop solution should be handled carefully as it can cause burns or irritation to the skin.
6. Positive and Negative Controls, as well as the Calibrator, must be run with each assay.
7. After taking out the required test strips, reclose the unused wells in the original pouch. Store them together with the other reagents at 2-8° C immediately after removing wells to be used. Numbering each strip used with permanent marking pen is recommended.
8. Do not use reagents past their expiration date.
9. Do not mix conjugates and reagents from other kits. Reagents have been optimized to perform properly and provide correct results.
10. Incubation times and temperatures other than those specified may give erroneous results.
11. Use separate pipette tips for each sample, control and reagent. Cross contamination of samples could cause false results.
12. Do not reuse microwells.
13. No assurance is given that these reagents are free of microbial or fungal contamination.
14. Some components of this kit contain sodium azide (as preservative) which in reaction with lead or copper plumbing may result in the formation of highly explosive metal azides. As a

precaution, on disposal in laboratory sinks, flush with large volumes of water to prevent azide build-up.

SPECIMEN COLLECTION AND PREPARATION

A blood sample should be collected by qualified personnel using approved aseptic venipuncture techniques. Clarify serum samples containing visible particulate matter by centrifugation. The samples may be stored at 2-8° C for short term testing (within a week). For longer storage, the samples should be frozen at -20° C to -70° C in a non-defrosting freezer. Avoid freeze-thaw cycles of patient samples. Do not use hyperlipemic, hemolytic, heat treated or contaminated samples.

PREPARATION OF REAGENTS

1. All reagents must be removed from refrigeration and allowed to come to room temperature (21 - 25° C) before use. Return all reagents to refrigerator promptly after use.
2. All samples and controls should be vortexed before use.
3. Dilute 60 mL of the 20X Wash Buffer to 1200 mL with distilled and/or deionized H₂O. Mix well.

TEST PROCEDURE

1. Determine the number of patients to be assayed. For each assay the Calibrator should be run in duplicate. Also the Positive Control, Negative Control, and a reagent blank (RB) should be run on each assay. Check software and reader requirements for the correct Calibrator/Control configurations.

Example Configuration:

1A	RB	2A	Patient #4
1B	NC	2B	Patient #5
1C	Cal	2C	Patient #6
1D	Cal	2D	Patient #7
1E	PC	2E	Patient #8
1F	Patient #1	2F	Patient #9
1G	Patient #2	2G	Patient #10
1H	Patient #3	2H	Patient #11

RB= Reagent blank – well without serum addition runrun with all reagents.

NC= Negative control

Cal= calibrator

PC= Positive control

2. For each test serum, Calibrator and Control to be assayed prepare a 1:21 serum dilution. Add 10 µL of each serum sample to 200 µL of Serum Diluent . Mix well.
3. Remove the number of wells needed from the plate bag and arrange in a strip holder. The remaining strips should be resealed in the plate bag with desiccant and humidity card. The bag should be reheat sealed or rolled over and the end taped. If the color of the humidity card changes from blue to pink the strips should not be used.
4. Transfer 100 µL of the prediluted samples to the reaction wells, using a multichannel pipette. Withdraw and expel each sample at least three times to ensure proper mixing of the sample before transferring to the reaction plate. Use new fresh pipettes tips for each sample. Add 100 µL of Serum Diluent to the reagent blank.

5. Incubate the plate at room temperature (21-25° C) for **30 minutes ±1 minute**.
6. Wash the reaction plate three times with 1x Wash Buffer. Shake all of the liquid out of the wells. With a wash bottle, automated or semi-automated wash system fill each well with 250-300 µL Wash Buffer making sure no air bubbles are trapped in the wells. Shake all of the Wash Buffer out of the wells. Repeat the wash two more times. A total of up to 5 washes may be necessary with automated equipment. After the last wash shake out the Wash Buffer and remove residual Wash Buffer by tapping the plate firmly on a paper towel. The Wash Buffer can be collected in a basin and treated with 0.5% sodium hypochlorite (bleach) at the end of the days run.
7. Add 100 µL of the Conjugate to each well of the reaction plate, including reagent blank.
8. Incubate each well of the reaction plate at room temperature (21-25° C) for **30 minutes ± 1 minute**.
9. Repeat wash as described in Step 6.
10. Add 100 µL of the TMB Substrate to each well of the reaction plate, including reagent blank.
11. Incubate each well of the reaction plate at room temperature (21-25° C) for **5 minutes ± 1 minute**.
12. Add 100 µL of the Stop Solution to each well, including reagent blank, at the same rate as the TMB Substrate was added. Positive samples will turn from blue to yellow. Tap plate to ensure mixing.
13. Read the plate using a spectrophotometer at a wavelength of 450 nm. If dual wavelength is used, set the reference filter to 600-650 nm. Measure each optical density (OD) against the reagent blank. The plate should be read within 30 minutes of assay completion.

CALCULATION OF RESULTS

Absorbance must be converted into Index Values by using the following formula:

$$\text{Index} = \frac{\text{Sample Absorbance (450 nm)}}{\text{Mean Calibrator Absorbance (450 nm) X Factor}^*}$$

- * The Factor is empirically derived by the manufacturer by analyzing cut-off data. Factors must not be interchanged between kit lot numbers.

INTERPRETATION OF RESULTS

1. Sample Index Values \leq 0.90 are considered **NEGATIVE**.
2. Sample Index Values between 0.91 - 1.09 are considered **EQUIVOCAL** and should be retested in this assay or analyzed in a different assay system.
3. Sample Index Values \geq 1.10 are considered **POSITIVE**.

INTERNATIONAL UNIT CONVERSION

International unit (IU) reactivity is determined relative to the IU standard. Conversion of Index values to international units is accomplished by using an exponential regression analysis. Each lot is standardized versus international units and provided with a lot specific conversion table (Conversion of International Units (IU) per mL for RF IgM) For example:

Index Value	IU	Index Value	IU
1.0	7.7	3.0	189.8
1.5	16.6	3.5	368.7
2.0	36.0	4.0	800.5
2.5	78.2	4.5	1738.0

See attached addendum for the lot specific conversion table.

QUALITY CONTROL

1. Calibrator and Controls must be run with each test run.
2. The Index Value of the Negative Control must be ≤ 0.90 .
3. The Index Value of the Positive Control must be ≥ 1.10 .
4. The Absorbance of the Calibrator must be between 0.250 - 1.200 A.
5. If above criteria are not met on repeat, contact DAI Technical Service.

LIMITATIONS

1. Only if test instructions are rigidly followed will optimum results be achieved.
2. Reproducible results depend on careful pipetting, observation of incubation periods and temperature, as well as washing the test strips and thorough mixing of all prepared solutions.
3. If comparisons with other methods are required, always perform both tests simultaneously.
4. Do not scratch coated wells during washing and aspiration. Wash and fill all reagents without interruption. While washing, check that all wells are filled evenly with washing solution, and that there are no residues in the wells.
5. Instructions for using appropriate photometers are to be observed; check adjustment of proper wave length (450 nm) and reference wave length (620 nm, optional) respectively.
6. The values obtained from this assay are intended to be an aid for diagnosis only. Each physician must interpret the results in conjunction with the patient's history, physical findings and other diagnostic procedures.

EXPECTED VALUES

Rheumatoid arthritis is a chronic inflammatory disease afflicting about 1-4% of the population. The highest incidence of rheumatoid factors occurs in persons over 65 years of age, reaching approximately 20% when latex fixation is used. The clinical correlation of an elevated rheumatoid factor should be interpreted cautiously. Increased titers may accompany a variety of acute immune responses, particularly viral infections and a number of other diseases (e.g., infectious mononucleosis, tuberculosis, leprosy, various parasitic diseases, liver disease, sarcoidosis, and lymphoproliferative syndromes) (6).

PERFORMANCE CHARACTERISTICS

REPRODUCIBILITY

Studies were performed to assess the precision of the test using five patient sera run in 10 wells each in one assay. The Intra-assay results are as follows:

	Serum 1	Serum 2	Serum 3	Serum 4	Serum 5
Mean	0.23	0.57	1.53	2.46	2.85
S.D.	0.02	0.03	0.06	0.05	0.08
C.V%	10.4%	6.4%	4.2%	2.1%	2.8%

Another run was performed to evaluate the Inter-assay precision using five patient sera run over five days each. The following results were obtained:

	Serum 1	Serum 2	Serum 3	Serum 4	Serum 5
Mean	0.11	0.64	1.47	2.25	2.68
S.D.	0.01	0.03	0.09	0.08	0.11
C.V.	14.2%	4.6%	6.1%	3.6%	4.2%

SENSITIVITY AND SPECIFICITY

A study was performed using 204 patient sera obtained from outside clinical laboratories. These samples were tested using both the DAI RF ELISA test and a commercially available RF ELISA test following the manufacturers' package inserts. Forty-five samples were found positive by the ELISA test, the remaining 157 samples were negative by the reference ELISA test. Two samples were found to be false positive and none found false negative on the DAI test as compared to the ELISA reference method. Using the above data criteria, the DAI RF ELISA test has a 100% sensitivity and 98.7% specificity as compared to the results obtained on the reference ELISA method. The following data was obtained:

	Reference +	ELISA -	Relative Sensitivity	Relative Specificity
DAI Rheumatoid Factor	+ 45	2	100% (45/45)	98.7% (157/159)
	- 0	157		

Agreement = 99.0%

INTERNATIONAL UNIT CONVERSION

The data in Table 1 illustrates RF Index values for the serially diluted international unit standard, obtained from the World Health Organization. The RF Index values are compared to serial dilutions of the international unit standard serum by linear regression (exponential regression analysis). The data indicates that international units can be determined from the Index value.

Table 1

International Unit Conversion

International Unit Standard Units / mL	Index Value
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25	1.8
12.5	1.2
6.30	0.8
3.25	0.5

Linear regression compared Index Value versus International Units

$$r^2 = 0.979 \quad a = 0.633 \quad b = 0.311 \quad Y = \text{Index} \quad X = \text{IU} / \text{mL}$$

Exponential Regression Equation Calculation

$$X = \frac{(y+b)}{a} \quad e^X = \text{derived IU} / \text{mL}$$

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Addendum

Conversion of International Units (IU) per mL for Rheumatoid Factor IgM

Rheumatoid Factor IgM Kit Lot Number: Sample

Index	IU
1.0	7.9
1.2	10.9
1.4	14.9
1.6	20.5
1.8	28.1
2.0	38.5
2.2	52.8
2.4	72.4
2.6	99.4
2.8	136.3
3.0	186.9
3.2	256.4
3.4	351.6
3.6	482.3
3.8	661.5
4.0	907.3
4.2	1244.4
4.4	1706.7