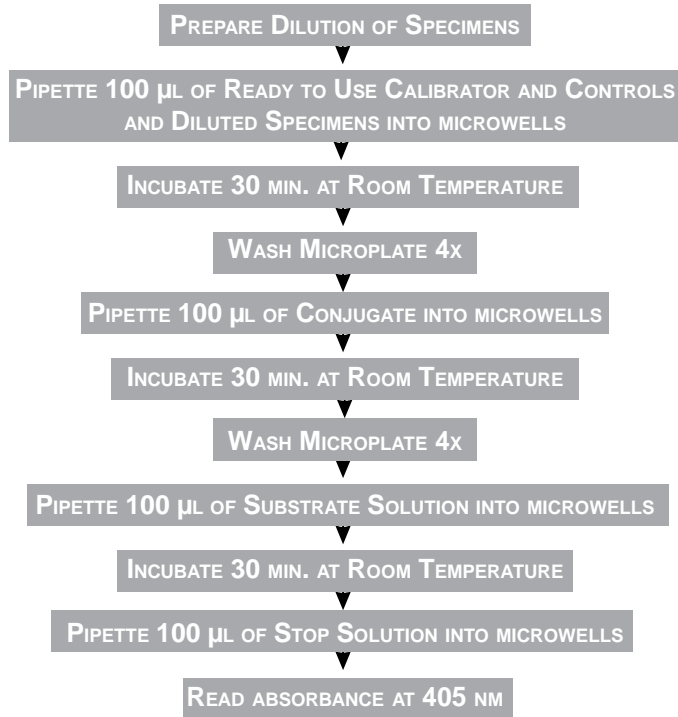


ImmuLisa™ PROCEDURE AT A GLANCE



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ImmuLisa™ Rheumatoid Factor (RF) Screen ELISA

For Investigational Use Only

PRODUCT INSERT

Catalog No. 1139S

96 Determinations

INTENDED USE

An enzyme linked immunosorbant assay (ELISA) for the detection of IgA/IgG/IgM class Rheumatoid Factor (RF) in human serum.

SUMMARY AND EXPLANATION

The measurement of RF is important in the diagnosis and prognosis of rheumatoid arthritis, as high titers of RF occur in sera of patients who tend to develop extra-articular complications^{1,2}. The majority of routine laboratory tests measure IgM RF by its ability to agglutinate sheep red blood cells, latex or similar particles coated with IgG¹⁻⁴. However, recent studies suggest that RF of other immunoglobulin isotypes is also present in rheumatoid arthritis and other disorders⁵⁻¹⁰.

RF is present in 70-90% of patients with rheumatoid arthritis and is included in the classification criteria¹¹. According to the revised ARA criteria, if RF is positive in patients with arthritis of three or more joints then the patient has rheumatoid arthritis. Arthritis of less than three joints with RF negative excludes rheumatoid arthritis. Such a classification affords 93.5% sensitivity and 89.3% specificity for rheumatoid arthritis¹¹.

RF as detected by agglutination is of the IgM isotype. Other methods such as ELISA have improved specificity, sensitivity and reliability over existing and routinely used agglutination methods³⁻⁵. The ELISA method described herein can detect RF of various immunoglobulin isotypes.

PRINCIPLE OF PROCEDURE

The test is performed as a solid phase immunoassay (ELISA). Microwells are coated with IgG followed by blocking the unreacted sites to reduce nonspecific binding. Controls, calibrator and patient serum samples are incubated in the antigen coated wells which allows RF present in the serum to bind. Unbound serum proteins are removed by washing the microwells. Bound antibodies are detected by adding an enzyme labeled anti-human immunoglobulin conjugate to the wells. Unbound conjugate is removed by washing. Specific enzyme substrate (pNPP) is then added to the wells and the presence of RF is detected by a color change produced by the conversion of pNPP substrate to a colored reaction product. The reaction is stopped and the intensity of the color change, which is proportional to the concentration of antibody, is read by a spectrophotometer at 405 nm. The results are expressed in ELISA units per milliliter (EU/ml).

REAGENTS

Storage and Preparation

Store all reagents at 2°-8°C. **Do not freeze.** Do not use if reagent is not clear or if a precipitate is present. All reagents must be brought to room temperature (20°-25°C) prior to use. When stored at 2°-8°C, the reconstituted wash buffer is stable until the kit expiration date. Reconstitute the wash buffer to 1 liter with distilled or deionized water. Coated microwell strips are for one time use only.

Precautions

All human derived components used have been tested for HBsAg, HCV, HIV-1 and 2 and HTLV-I and found negative by FDA required tests. However human blood derivatives and patient specimens should be considered potentially infectious. Follow good laboratory practices in storing, dispensing and disposing of these materials⁷.

WARNING - Sodium azide (NaN₃) may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal of liquids, flush with large volumes of water to prevent azide buildup. Sodium azide may be toxic if ingested. If ingested, report incident immediately to laboratory director or poison control center.

Instructions should be followed exactly as they appear in this kit insert to ensure valid results. Do not interchange kit components with those from other sources other than the same catalog number from IMMCO DIAGNOSTICS. Follow good laboratory practices to minimize microbial and cross contamination of reagents when handling. Do not use beyond expiration date on the label.

Materials provided

ImmuLisa™ RF Screen ELISA Catalog No. 1139S

Kit contains sufficient reagents to perform 96 determinations.

- 12 x 8** Ready to use **Microplate** with individual breakaway microwells. Coated with Rabbit IgG.
 - 1 x 1.5 ml** *Ready to use **Positive Control**. Contains human serum positive for RF. The expected concentration range is printed on the label in EU/ml.
 - 1 x 1.5 ml** *Ready to use **Negative Control** (*white cap*). Contains human serum.
 - 1 x 1.5 ml** *Ready to use **Calibrator** (*green cap*). Contains human serum positive for RF. The expected concentration in EU/ml is printed on the label.
 - 1 x 12 ml** *Ready to use **anti-human IgA/G/M Alk. Phos. Conjugate**. Color coded pink.
 - 2 x 60 ml** *Ready to use **Serum Diluent**. Color coded blue.
 - 1 x 12 ml** *Ready to use **Enzyme substrate**. Contains pNPP. **Protect from light.**
 - 1 x 12 ml** Ready to use **Stop Solution**.
 - 2 x vials** **Powder Wash Buffer**. Reconstitute to one liter each.
 - 1 extra** Frame Holder
 - 2** Protocol Sheets
- *Contains <0.1% NaN₃

Results obtained with the ImmuLisa™ RF Screen ELISA were also compared to the ImmuLisa™ RF individual methods. The results obtained are tabulated below and indicate a high degree of correlation:

ImmuLisa™ RF Screen

	Positive	Negative	Total
RF-Individual Methods			
Positive	33	3	36
Negative	0	73	73
Total	33	76	109

Relative Agreement: 97%
 Relative Sensitivity: 92%
 Relative Specificity: 100%

REFERENCES

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11. Arnett FC. Revised criteria for the classification of rheumatoid arthritis. *Bull Rheum Dis.* 1989; 38(5):1-6.
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LIMITATIONS OF THE PROCEDURE

The Immulisa™ RF Test should not be performed on grossly hemolyzed, microbially contaminated or lipemic samples. This method should be used for testing human serum samples only.

EXPECTED VALUES

RF is present in 70-90% of patients with rheumatoid arthritis¹¹, 74-86% in patients with *Sjögren's Syndrome*⁶⁻⁸, and in over 50% of patients with *Hennoch Schönlein purpura*. As compiled from the cited literature, the incidence of RF in rheumatoid arthritis or other rheumatological disorders is summarized in the following table:

Incidence of RF in Patients with Rheumatic Diseases	
Rheumatoid Arthritis	70-90%
Sjögren's Syndrome	58%
Systemic lupus erythematosus	18%
Dermatomyositis	16%
Mixed connective tissue disease	13%
Cranial arteritis	10%
Polymyalgia rheumatica	10%
Polyarthritis	7%
Scleroderma	6%
Juvenile rheumatoid arthritis	6%

Precision:

Two RF positive sera were tested with the Immulisa™ RF Screen ELISA to determine inter- and intra-assay variability. The results are as follows:

	inter-assay %CV	intra-assay %CV
Sample 1	5.06	4.7
Sample 2	8.35	4.4

Recovery:

Samples with known RF concentrations were mixed with appropriate dilutions of another positive sample with known amounts of RF. RF levels of the mixed samples were determined and from the values obtained the percent recovery calculated. The results are as follows:

	RF conc. added (EU/ml)	RF conc. obtained (EU/ml)	% Recovery
Sample 1	88.5	95	107
Sample 2	34	32	93.2
Sample 3	11.1	12	108

Materials Required But Not Provided

- Deionized or distilled water
- Squeeze bottle to hold diluted wash buffer
- Pipettes capable of delivering 5 µl to 1000 µl
- Disposable pipette tips
- Clean test tubes 12 x 75 mm and test tube rack
- Timer
- Absorbent paper
- Microplate reader capable of reading absorbance values at 405 nm. If dual wavelength microplate reader is available, the reference filter should be set at 600-650 nm.
- Automatic microplate washer capable of dispensing 200 µl

SPECIMEN COLLECTION AND HANDLING

Only serum specimens should be used in this procedure. Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used. Store specimens at 2°- 8°C for no longer than one week.. For longer storage, serum specimens should be frozen. Avoid repeated freezing and thawing of samples.

PROCEDURE

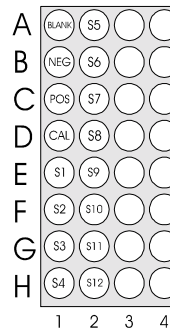
Procedural Notes

- Before starting with the assay read carefully the product insert.
- Let serum specimens and test reagents equilibrate at room temperature for at least 30 minutes before starting with the test procedure. Return all unused specimens and reagents to refrigerator immediately after use.
- All dilutions of the patient samples should be prepared prior to starting with the assay.
- Good washing technique is critical. If washing is performed manually, adequate washing is accomplished by directing a forceful stream of wash buffer with a wide tip wash bottle across the entire microplate. **An automated microplate washer is recommended.**
- Use a multichannel pipette capable of delivering 8 wells simultaneously. This speeds the process and provides for a more uniform incubation time.
- For all steps, careful control of timing is important. The start of all incubation periods begins with the completion of reagent addition.
- Addition of all samples and reagents should be performed at the same rate and in the same sequence.
- Remove required microwell strips from the pouch and carefully reseal the pouch to prevent condensation in the unused wells. Return pouch immediately to refrigerator.

Test Method

- Step 1** Let all reagents equilibrate at room temperature.
- Step 2** Label protocol sheet to indicate sample placement in the wells. It is good laboratory practice to run samples in duplicate.
- Step 3** Prepare a **1:201** dilution of patient specimen by pipetting **5µl** of serum into **1.0 ml** of Serum Diluent. **Mix well.**

Sample Layout



- Step 4** Remove the required microwells from pouch and return unused strips in the sealed pouch to refrigerator. Securely place the microwells into the extra provided holder.
- Step 5** Pipette **100 µl** of the Ready to Use Calibrator, Positive and Negative controls and the diluted patient samples to the appropriate microwells as per protocol sheet.
- Note:** Include one well which contains **100 µl** of the Serum Diluent as a reagent blank. Zero the ELISA reader against the reagent blank. The absorbance of the reagent blank should not be more than 0.3 when read against air.
- Step 6** Incubate **30 minutes** (± 5 min) at room temperature.
- Step 7** Wash **4x** with the wash buffer. For manual washing, fill each microwell with reconstituted wash buffer. Discard the fluid by inverting and tapping out the contents of each well or by aspirating the liquid from each well. To blot at the end of the last wash, invert strips and tap the wells vigorously on absorbent paper. For automatic washers, program the washer as per manufacturer's instructions.
- Step 8** Pipette **100 µl** of Conjugate into microwells.
- Step 9** Incubate **30 minutes** (± 5 min) at room temperature.
- Step 10** Wash all microwells as in Step 7.
- Step 11** Pipette **100 µl** of Enzyme Substrate to each well in the same order and timing as for the conjugate.
- Step 12** Incubate **30 minutes** (± 5 min) at room temperature.
- Step 13** Pipette **100 µl** of Stop Solution into each microwell using the same order and timing as for the addition of the enzyme substrate. Read absorbance within 1 hour of adding Stop Solution.
- Step 14** Read absorbance of each microwell at **405 nm** using a single or 405/630nm dual wavelength microplate reader against the reagent blank set at zero absorbance.

Quality Control

Calibrator, Positive and Negative Controls and a reagent blank must be run with each assay to verify the integrity and accuracy of the test. The absorbance reading of the reagent blank should be <0.3 OD. The calibrator should have an absorbance reading of not less than 1.0, otherwise the test must be repeated. The Negative Control must be <20 EU/ml. The absorbance of the Positive Control should be greater than the Negative Control and lesser than that of the Calibrator. If the test is run in duplicate, take the mean of the two readings to determine the concentration of RF.

RESULTS

Calculations

The qualitative method should be used to determine if a specimen is negative or positive.

1. QUALITATIVE DETERMINATION

Abs. of Test Sample

$$\times \text{EU/ml of Calibrator} = \text{EU/ml Test Sample}$$

Abs. of Calibrator

Interpretation

The following serves only as a guide in the interpretation of the laboratory results. Each laboratory must determine its own normal values.

RF value	Interpretation
< 20 EU/ml	Negative
> 20 EU/ml	Positive

Frequency distribution of RF in normal blood donors with Immulisa™ RF Screen ELISA

