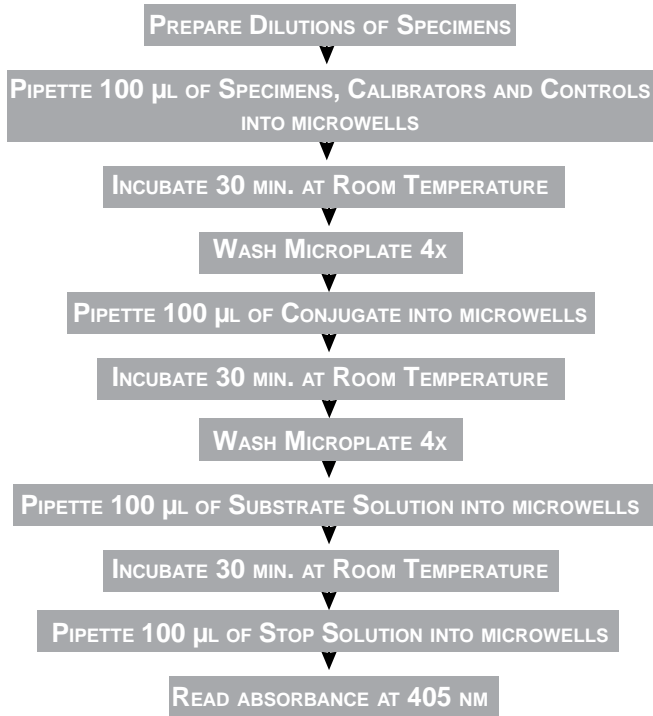


Immulin™ PROCEDURE AT A GLANCE



For technical assistance please contact:



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Immulin™ Anti-Ribonucleic Acid Antibody (RNA) ELISA

For Research Use only

PRODUCT INSERT

Catalog No. 1166

96 Determinations

INTENDED USE

An enzyme linked immunosorbent assay (ELISA) for the detection and semi-quantitation of antibodies to ribonucleic bands in serum of patients with collagen-vascular disorders.

SUMMARY AND EXPLANATION

Systemic lupus erythematosus (SLE) is a multi-faceted autoimmune disease characterized by the presence of a variety of circulating autoantibodies^{1,2}. Central nervous system (CNS) manifestations occur in a significant number of all SLE patients³ and elicit behavioral abnormalities resembling schizophrenia⁴. However, there is no single diagnostic test available, that can detect CNS manifestations of lupus with consistent sensitivity and specificity.

Bluestein et al⁵ have demonstrated an association between cerebral lupus and cytotoxic antibodies in the cerebrospinal fluid against a neuroblastoma cell line. In patients with psychotic SLE, a group of autoantibodies is targeted against the ribosomal phosphoproteins, called Po (38kD), P1 (19kD), and the P2 (17kD)⁶. Preceding the onset of psychotic episodes in these patients, there is a selective elevation of anti-ribosomal P antibodies⁷.

Similarly RNA antibodies also occur in SLE and the frequency may vary from 17-80%⁸. In addition, a correlation between anti-RNA antibodies and disease activity has also been reported⁹. In some patients anti-ribosomal P and anti-RNA antibodies may coexist¹⁰. Sera containing anti-ribosomal P and RNA antibodies invariably show a strong cytoplasmic immunofluorescence pattern on HEp-2 cells and mouse kidney substrate. sections due to the ribosomal and cytoplasmic distribution of these antigens. Anti-RNA antibodies occur in a significant number of patients with collagen-vascular disorders. These antibodies are difficult to recognize by indirect immunofluorescence. The Immulin™ anti-RNA test represents a significant technological advance and offers a sensitive and specific seriological method to assess these patients.

SUMMARY AND EXPLANATION

The ELISA is performed as a solid phase immunoassay. Microwells are coated with ribonucleic acid. Controls, calibrators and patient sera are incubated in the coated microwells, allowing specific antibodies present in the serum to bind to the antigen.

Unbound antibodies and other serum proteins are removed by washing the microwells. Bound antibodies are incubated with an enzyme labeled anti-human IgG conjugate. Unbound conjugate is removed by washing.

Specific enzyme substrate (pNPP) is then added to the wells and the presence of antibodies 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. Results are expressed in Enzyme 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 materials¹¹.

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.

REFERENCES

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8. Blanco F, Kalsi J and Isenberg DA. Analysis of antibodies to RNA in patients with systemic lupus erythematosus and other autoimmune rheumatic diseases. *Clin exp Immunol*; 1991, 86:66-70
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Recovery:

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

	RNA-Ab conc. added (EU/ml)	RNA-Ab conc. obtained (EU/ml)	% Recovery
Sample 1	135	147	109
Sample 2	113	117	104
Sample 3	56	61	108

Materials provided

ImmuLisa™ anti-RNA ELISA

Catalog No. 1166

Kit contains sufficient reagents to perform 96 determinations.

- 12 x 8** Ready to use **Microplate** with individual breakaway microwells coated with Ribonucleic acid antigen.
- 1 x 1.5 ml** *Ready to use **Positive Control** (*red cap*). Contains human serum positive for anti-RNA antibodies. The expected concentration range in EU/ml is printed on the label.
- 1 x 1.5 ml** *Ready to use **Negative Control** (*white cap*). Contains human serum.
- 4 x 1.5 ml** *Ready to use **set of 4 Calibrators**; Calibrator A (*green cap*), Calibrator B (*violet cap*), Calibrator C (*blue cap*) and Calibrator D (*yellow cap*). Human serum containing antibodies to RNA antigen. Concentrations in EU/ml are printed on the labels.
- 1 x 12 ml** *Ready to use **anti-human IgG 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 vials** Powder **Wash Buffer**. Reconstitute to one liter each.
- 1 x extra** Frame Holder
- 2 x** Protocol Sheets
- *CAUTION - Contains <0.1% NaN₃

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 towels
- 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 300 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 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.

PERFORMANCE CHARACTERISTICS

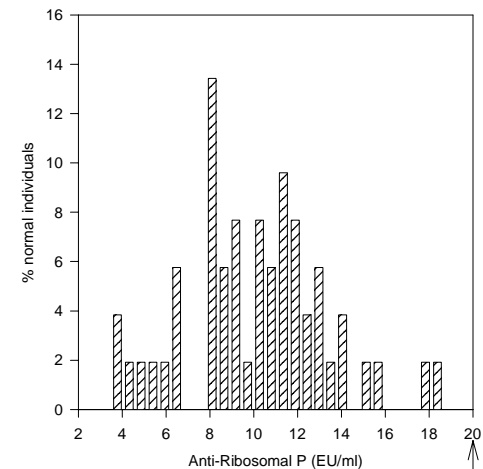
Precision:

Two anti-RNA positive sera were tested with the ImmuLisa™ Anti-RNA ELISA to determine inter- and intra-assay variability. The results are as follows:

	inter-assay %CV	intra-assay %CV
Sample 1	3.8	6.3
Sample 2	3.5	3.6

The following figure depicts the incidence of anti-RNA antibodies in normal population with the ImmuLisa™ anti-RNA.

Distribution of Normal Population with Anti-Ribosomal P ImmuLisa™



From Reference 12, 13 and 14.

cutoff

Interpretation

The following serves only as a guide in the interpretation of the laboratory results. The values depicted below were determined by testing 61 normal blood donors and represent the mean of the normals plus 3SD. Each laboratory must determine its own normal values.

Anti-RNA values	Interpretation
<20 EU/ml	Negative
20-25 EU/m	Borderline
>25 EU/ml	Positive

LIMITATIONS OF THE PROCEDURE

Test results obtained by this assay alone, are not diagnostic and should be considered in conjunction with the clinical presentation of the patient.

EXPECTED VALUES

Expected values in a normal population are negative. The incidence of anti-RNA antibodies vary and have been reported in 17-80% of patients with SLE and in a significant number of patients with scleroderma. The following table was abstracted from a study by Blanco et al⁸.

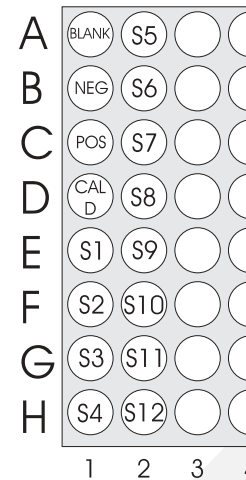
Incidence of anti-RNA Antibodies in Collagen-Vascular Disorders

Disease Group	n	No. Positive	% Positive
SLE	138	13	9.4
Rheumatoid Arthritis	25	0	0
Sjögren's Syndrome	25	0	0
Scleroderma	25	0	0
Normals	80	1	1.2

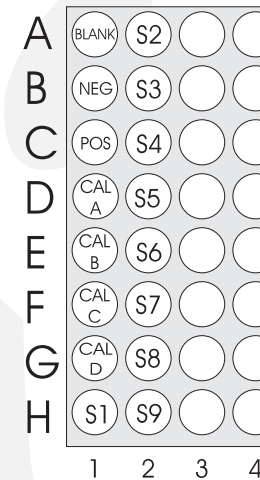
Test Method

- Step 1** Let all reagents and specimens 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** For a **qualitative determination** use only the Ready to Use Low Calibrator D (*vial with yellow cap*).
- or For a **semi-quantitative determination** use the Ready to Use Calibrators A through D as depicted in the sample layout below.

QUALITATIVE DETERMINATION



SEMI-QUANTITATIVE DETERMINATION



- Step 4** Prepare a **1:400** dilution of the patient samples by mixing **5 µl** of the patient sera with **1.0 ml** of Serum Diluent to get a **1:200** dilution.
- Step 5** In a separate tube, dilute (**1:2**) the **1:200** dilution of the sera by mixing **150 µl** of the **1:200** dilution with **150 µl** of the Serum Diluent to get a final dilution of **1:400**.
- Step 6** 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 7** Pipette **100 µl** of Ready to use Calibrators, Positive and Negative controls and diluted patient samples (**1:400**) 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.
- Step 8** Incubate **30 minutes** (± 5 min) at room temperature.

- Step 9** Wash **4x** with 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 towels. For automatic washers, program the washer as per manufacturer's instructions.
- Step 10** Pipette **100 µl** of Conjugate into microwells.
- Step 11** Incubate **30 minutes** (± 5 min) at room temperature.
- Step 12** Wash all microwells as in Step 8.
- Step 13** Pipette **100 µl** of Enzyme Substrate into each microwell in the same order and timing as for the Conjugate.
- Step 14** Incubate **30 minutes** (± 5 min) at room temperature.
- Step 15** 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 values within 1 hour from adding Stop Solution.
- Step 16** 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

Calibrators, 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. The Calibrator A 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. If the test is run in duplicate, the mean of the two readings should be taken for determining EU/ml. While performing Qualitative determinations, the optical density of the Calibrator D must be greater than that of the negative control and lesser than the absorbance of the positive control. For semi-quantitative determinations the positive control must give values in the range stated on the vial.

RESULTS

Calculations

The concentrations of the patient samples can be determined by either of two methods:

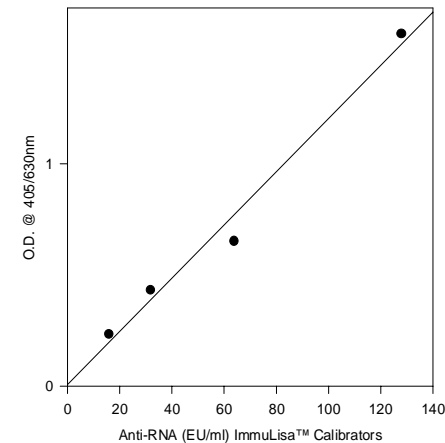
1. QUALITATIVE DETERMINATION

$$\frac{\text{Abs. of Test Sample}}{\text{Abs. of Calibrator D}} \times \text{EU/ml of Calibrator D} = \text{EU/ml Test Sample}$$

2. SEMI-QUANTITATIVE METHOD

Plot absorbance of Calibrator A through D against their respective concentration on a linear-linear graph paper. Plot the concentration in EU/ml on the X-axis against the absorbance on the Y-axis and draw the best fit curve. Determine the concentrations of the patient samples from the curve against its corresponding absorbance value.

Anti-RNA Immulisa™ Standard Curve



Calibrator

The Ready to Use Calibrators are included to provide semi-quantitation and must be used with each run. Patient samples containing higher antibody levels may give absorbance values greater than that of the Calibrator A. For determining accurate semi-quantitative values such serum sample should be further diluted so they fall within the range of the calibrator curve when retested. For determining EU/ml, multiply the units obtained by the dilution factor.