Diamedix®
A Subsidiary of ERBA Diagnostics, Inc.

anti-Sm/RNP

Enzyme Immunoassay Test Kit
For *In Vitro* Diagnostic Use
Catalog No. 720-270 – 96 Tests

SUMMARY OF PROCEDURE

1. Prepare a 1:101 dilution of samples in Sample Diluent. Mix well.
2. Add 100 µl of diluted samples into the antigen wells. Reserve one well for reagent blank (100 µl of Sample Diluent).
3. Incubate at room temperature (18 - 30° C) for 30 ± 5 min.
4. Discard contents of the wells. Wash the wells 3 times with Wash Solution.
5. Add 100 µl of Conjugate to each well.
6. Incubate at room temperature for 30 ± 5 min.
7. Wash the wells as in #4 above.
8. Add 100 µl Substrate Solution to each well.
9. Incubate at room temperature for 30 ± 5 min.
10. Add 100 µl Stop Solution to each well.
11. Read the absorbance at 405 nm (reference at 600 - 630 nm) against the reagent blank.

INTENDED USE

For the detection and semi-quantitation of antibodies against the Sm/RNP antigen in serum as an aid in the diagnosis of autoimmune disease.

SUMMARY AND EXPLANATION

Systemic rheumatic disease is characterized by the presence of circulating autoantibodies that are widely reactive with both nuclear and cytoplasmic antigens. The RNP and Sm antigens consist of portions of the U_1 RNA and nine associated polypeptides.[1] Antibodies to Sm/RNP are detected in up to 40% of patients with systemic lupus erythematosus (SLE) either alone or in conjunction with Sm antibodies.[1,2]

The RNP antigen is very closely associated with the Sm antigen and is designated the Sm/RNP complex. In contrast to anti-Sm, anti-RNP is found in patients with a variety of rheumatic diseases including scleroderma, rheumatoid arthritis, discoid lupus, polymyositis and Sjogren's Syndrome.[1,3] High titers of anti-RNP, in the absence of anti-Sm, are correlated with mixed connective tissue disease (MCTD).[4]

Until recently, many laboratories used Immunodiffusion (ID), Counterimmuno-electro-phoresis, and hemagglutination to detect RNP antibodies. However, these methods are time- consuming and cumbersome to perform and are insensitive relative to newer methods. Enzyme immunoassay (EIA) has advantages over the ID method in sensitivity, specificity, ease of automation, and testing turnaround time.[5]

The Diamedix *Is*-anti-Sm/RNP Test Kit is an EIA procedure intended for the semi-quantitation of antibodies to RNP antigen. The results are reported in ELISA units (EU) per ml determined by comparison to a Calibrator.

PRINCIPLE OF THE PROCEDURE

Purified Sm/RNP antigen from calf and/or rabbit thymus is bound to microwells. Diluted patient sera, Calibrator, and controls are placed in the microwells and incubated. Anti-Sm/RNP antibodies, if present, will bind to the antigen in the microwells. After washing the microwells to remove unbound antibodies, a second incubation with anti-human IgG conjugated to alkaline phosphatase is carried out. The conjugate will bind to human anti-Sm/RNP antibodies, if present, forming an immunocomplex. The microwells are then washed again to remove unbound components and the enzyme substrate, para-nitrophenylphosphate is added. The enzyme, if bound, will catalyze the hydrolysis of the substrate to para-nitrophenol and result in formation of a yellow color. The reaction is then stopped and the color read with a photometer at 405 nm (reference at 600-630 nm). The intensity of the color developed is proportional to the concentration of anti-Sm/RNP IgG present in the sample.

REAGENTS

Each *Is*-anti-Sm/RNP Kit contains reagents for 96 tests.

Antigen Wells	Twelve, 8-well microwell breakapart strips, color-coded red, coated with Sm/RNP antigen.
Calibrator	One vial with blue cap containing 0.25 ml of human serum, 0.1% sodium azide. Assigned value printed on label.
Negative Control	One vial with black cap containing 0.25 ml of non-reactive human serum, 0.1% sodium azide.
Positive Control	One vial with white cap containing 0.25 ml of reactive human serum, 0.1% sodium azide. Assigned range printed on label.
Sample Diluent	One bottle with blue cap containing 60 ml Phosphate buffer with protein stabilizers. Contains Proclin® 300, 15 ppm active ingredient. Color-coded blue.
Wash Concentrate (20X)	Two bottles with clear caps containing 50 ml of Phosphate buffer with detergent and Proclin® 300, 15 ppm active ingredient. Each bottle is sufficient to make 1050 ml of wash solution.
Conjugate	One bottle with red cap containing 25 ml goat anti-human immunoglobulin G labeled with alkaline phosphatase. Also includes protein stabilizers and Proclin® 300, 30 ppm active ingredient. Color-coded pink.
Substrate	One amber bottle with brown cap containing 25 ml para-Nitrophenyl phos-phate in a buffered solution. *Substrate solution may develop a slight yellow color upon storage.*
Stop Solution	One bottle with white cap containing 25 ml. Sodium phosphate, tribasic. CAUTION: Solution is caustic. Avoid contact with skin. If contact is made, flush area with copious amounts of water.

Store these reagents at 2 to 8° C.

OTHER MATERIALS REQUIRED

Manual Users:

Wash bottle or automated microplate washer

Pipettors capable of dispensing appropriate volumes

Timer

One liter graduated cylinder

One liter wash solution reservoir

Deionized or distilled water

Absorbent toweling

Tubes or microwell plate for serum dilution

Reader capable of reading absorbance at 405 nm, reference at 600 or 630 nm.

Automated EIA Processor Users:

One liter graduated cylinder

Deionized or distilled water

Pre-dilution cups, strips or plates.

ProbeClean™ Concentrate, or tip washing detergent solution, if applicable.

PRECAUTIONS

1. Handle samples, calibrators, controls and the materials that contact them as potential biohazards. Each donor unit in the Calibrator and controls has been found negative for Hepatitis B surface antigen and HIV-I antibodies by FDA-approved third generation tests. However, because no method can offer complete assurance that HIV-1, Hepatitis B virus, or other infectious agents are absent, these materials should be handled at the Biosafety Level 2 as recommended for any potentially infectious serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbio-logical and Biomedical Laboratories", 1988.
2. Never pipette by mouth.
3. Avoid contact with open skin.
4. Certain of the test reagents contain Proclin® 300 as a preservative. When disposing of reagents containing Proclin® 300, flush drains with copious amounts of water to dilute the active components below active levels.
5. Reagents containing Sodium Azide:
   (a) **CAUTION:** Some reagents in this kit contain Sodium Azide as preservative. Sodium Azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts", in the Manual Guide – Safety Management No. CDC-

22, issued by the Centers for Disease Control and Prevention, Atlanta, GA, 1976.

European Communities Hazardous Substance Risk Phrases (Regulation (EC) No 1272/2008)

H300 –Fatal if swallowed.
H310 – Fatal if contact with skin.
EUH032 – Contact with acids liberates very toxic gas.
H410 – Very toxic to aquatic life with long lasting effect.
P264 – Wash all exposed external body areas thoroughly after handling.
P302+P352 – IF ON SKIN: Wash with plenty of water and soap.
P301+P310/P330 – IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician. Rinse mouth.
P270 – Do not eat, drink or smoke when using this product.
P501 – Dispose of contents/container as hazardous waste.
P391 – Collect spillage.
P273 – Avoid release to the environment. Refer to special instructions/ Safety Data Sheet.

(b) Sodium Azide inhibits horseradish peroxidase activity. Care must be taken to ensure that azide is not carried over from other reagents into conjugate and substrate steps.

RECOMMENDATIONS FOR LABORATORY QUALITY CONTROL

1. Do not mix or interchange wells, controls, or calibrators from different lots.
2. Do not use reagents beyond their expiration date. Expiration dates are printed on the reagent labels.
3. Incubations above or below the recommended temperatures or times may give erroneous results.
4. The ELISA method is a very sensitive technique. Maintain consistent pipetting technique, incubation times, and temperature conditions throughout the test procedure. Cross contamination between reagents can invalidate the test.
5. Antigen coated microwells should be stored with the desiccant in the resealable bag provided and returned to the refrigerator immediately after use.
6. *(Manual Procedure Only)* The washing procedure is very important and requires special attention. (Please refer to the Procedure section)

   NOTE: *Improperly washed wells may give erroneous results.*

SPECIMEN COLLECTION

Whole blood should be collected by accepted medical techniques. The serum is separated from the clot and refrigerated at 2 to 8°C for short-term storage (up to 7 days), or stored frozen at -20°C for long term storage. Avoid multiple freeze-thaw cycles. Specimens containing visible particulate matter should be clarified by centrifugation before testing. Grossly contaminated, hemolyzed, lipemic, or icteric specimens should not be used.

CAUTION: *Serum samples must not be heat-inactivated prior to use.*

PROCEDURE

Allow all test components and patient samples to warm to room temperature before use. Invert reagent bottles gently several times before use. Return promptly to the refrigerator after use.

Prepare Wash Solution by adding 50 ml of Wash Concentrate (20X) to one liter of deionized or distilled H_2O.

Manual Users:

1. Prepare 1:101 dilutions of the calibrator, controls, and patient samples in Sample Diluent. (e.g., by addition of 2 µl sample to 200 µl Sample Diluent or 5 µl sample to 500 µl Sample Diluent).
2. Mix sample dilutions gently by withdrawing and expelling in a pipette tip 2 or 3 times or by vortex mixing for 2 or 3 seconds. Transfer 100 µl of diluted calibrator, control, or patient sample, to the antigen wells. Avoid formation of bubbles when transferring diluted samples.

   NOTE: *Include one well which contains 100 µl of Sample Diluent only as the reagent blank. This will ultimately be used to "zero" the photometer before reading the test results.*
3. Allow the wells to incubate at room temperature (18 - 30°C) for 30 ± 5 minutes.
4. Aspirate or discard the contents of the wells. Remove any excess moisture in the wells by tapping on paper toweling if necessary. Wash the wells by rinsing 3 times with at least 300 µl per well of Wash Solution. Remove excess moisture from the wells after washing. When using an automated washer, follow the manufacturer's instructions.
5. Place 100 µl of Conjugate into each well, avoiding bubble formation.
6. Allow the wells to incubate uncovered at room temperature (18 - 30°C) for 30 ± 5 minutes.
7. Wash the wells as described in Step 4 above.
8. Place 100 µl of Substrate into each well, avoiding bubble formation.
9. Allow the wells to incubate uncovered at room temperature (18 - 30°C) for 30 ± 5 minutes.
10. Place 100 µl of Stop Solution into each well, avoiding bubble formation.
11. Read the absorbance of each well at 405 nm and zero against the reagent blank. A suitable reference wavelength (e.g., 600-630 nm) reading should be used. Read the plate within 60 minutes of adding Stop Solution.

Automated EIA Processor Users:

If using an Automated EIA Processor, refer to the Operator's Manual for the test setup and procedures.

NOTE: *Automated EIA Processor users must validate their equipment to demonstrate that the results obtained are equivalent to those obtained using manual assay.*

RESULTS

1. Calculation Results

Determine the EU/ml (ELISA Units/ml) for each patient specimen or control using the following formula:

$$\frac{\text{EU/ml Of Calibrator}}{\text{Absorbance of Calibrator}} \times \text{Absorbance of sample} = \text{EU/ml of sample}$$

2. Single Point Calibration

The *Is*-anti-Sm/RNP Test Kit has been developed using a single point calibrator. Patient values, which contain very high levels of antibody may produce absorbance values greater than the Calibrator absorbance. Patient sample results greater than the Calibrator value should be reported as "Greater than Calibrator value EU/ml". If numerical results are required for such samples, dilute the sample using Sample Diluent and re-assay. Several dilutions (for example 1/10, 1/50 and 1/100) of the pre-diluted sample may be re-assayed simultaneously. Select the dilution that has an absorbance reading about 50% of the absorbance reading of the Calibrator; calculate the EU/ml for this dilution and multiply by the dilution factor to obtain estimated values.

3. Test Validation Criteria

a. The Positive Control must be within its assigned range.
b. The Negative Control must be < 16 EU/ml.
c. The absorbance of the reagent blank must be < 0.30.

If any of these criteria are not met, the run is invalid and must be repeated.

4. Interpretation of Results

The following is a guide to interpretation of results. Each laboratory is encouraged to establish its own "normal" ranges based on populations encountered.

Is-anti-Sm/RNP Value	Index Value *	Interpretation
< 16 EU/ml	< 0.8	Negative for antibodies to Sm/RNP.
16-20 EU/ml	0.8 – 1.0	Equivocal for antibodies to Sm/RNP. Sample should be retested. If retest results are equivocal, the sample should be reported as equivocal, tested by another method, or a new sample should be tested. **
> 20 EU/ml	> 1.0	Positive for antibodies to Sm/RNP.

* The Index Value is calculated by dividing the EU/ml of the sample by 20.

** Equivocal samples that give positive results upon retest should be reported as positive. Equivocal samples that give negative results upon retest should be reported as negative.

NOTE: *This assay measures antibodies to Sm and RNP. To determine the approximate value for RNP alone, an anti-Sm determination must also be*

carried out using the Diamedix Is-anti-Sm test and the Index Value obtained for Sm should be subtracted from the Sm/RNP Index Value.

LIMITATIONS

1. The analysis of a single serum sample should not be used as the sole criterion for diagnosis of an autoimmune disease.
2. The results obtained with the *Is*-anti-Sm/RNP Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.
3. The test should be performed on serum. The use of whole blood or plasma has not been established.

EXPECTED VALUES

Antibodies to Sm/RNP are present in up to 40% of patients with Systemic Lupus Erythematosus (SLE) either alone or in conjunction with Sm antibodies.[1,2]

The expected values in the normal population were determined by assaying 100 normal donor sera collected in South Florida. Figures 1 and 3 show the distribution of Sm/RNP results in the normal population performed manually and on MAGO respectively.

The distribution of EU/ml values for 50 clinically characterized sera along with the 100 normal donor sera is shown in Figures 2 and 4 performed manually and on MAGO respectively.

FIGURE 1
Manual
***Is*-anti-Sm/RNP Normals**

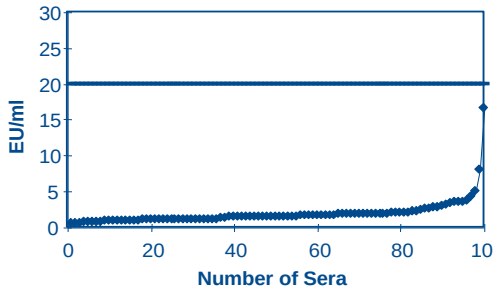


FIGURE 2
Manual
***Is*-anti-Sm/RNP Expected Values**

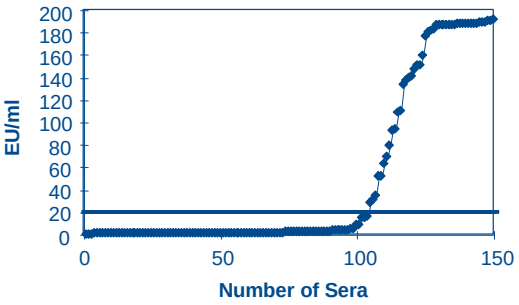


FIGURE 3
MAGO
***Is*-anti-Sm/RNP Normals**

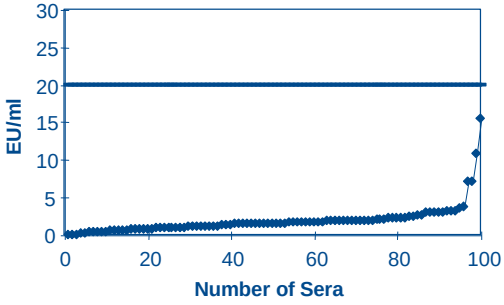
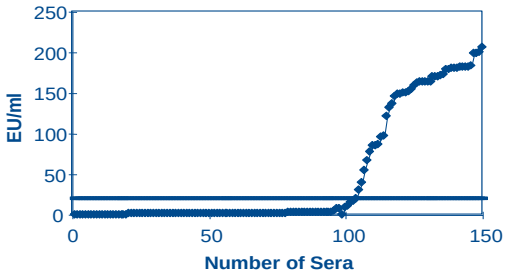


FIGURE 4
MAGO
***Is*-anti-Sm/RNP Expected Values**



PERFORMANCE CHARACTERISTICS

A. Comparison Testing

The Diamedix *Is*-anti-Sm/RNP Test Kit was evaluated relative to another commer-cially available anti-Sm/RNP ELISA test kit using clinically character-ized sera. One hundred sera from normal blood donors and 50 sera from autoimmune patients were tested by the *Is*-anti-Sm/RNP Test Kit and a commercially obtained anti-Sm/RNP ELISA test kit. Results are shown in Table 1.

TABLE 1

	MANUAL			MAGO		
	Number of Sera	%	95% Confidence	Number of Sera	%	95% Confidence
Relative Sensitivity	46/52	88	77-96	46/51	90	79-97
Relative Specificity	97/97	100	96-100	97/97	100	96-100
Agreement	143/149*	96	91-99	143/148**	97	91-99

* One borderline sample was excluded from the calculations.

** Two samples, one equivocal and one borderline, were excluded from the calculations.

Six sera negative by *Is*-anti-Sm/RNP (manual) and positive by the comparative method were negative when tested by a third method. Five sera negative by *Is*-anti-Sm/RNP (MAGO) and positive by the comparative method were negative by a third method.

B. Precision

The precision of the *Is*-anti-Sm/RNP Test Kit was determined by testing six different sera and kit Calibrator and controls in two runs on three different days. The intra- and interassay precision is shown in Table 2.

TABLE 2

	***Is*-anti-Sm/RNP Precision**				
	Overall	MANUAL		MAGO	
SERUM	MEAN EU/ml	INTRA-CV%	INTER-CV%	INTRA-CV%	INTER-CV%
1 (NEG)	1.9	8.8	13.3	15.4	13.0
2 (NEG)	1.8	7.3	6.3	10.9	10.5
3 (POS)	26.1	3.5	4.5	5.9	9.8
4 (POS)	47.0	5.6	6.0	7.8	9.8
5 (POS)	81.9	2.8	6.1	5.5	7.1
6 (POS)	108.6	3.1	5.4	3.7	7.1
CAL	100.4	3.5	5.8	4.3	4.6
POS CTRL	46.3	3.3	6.5	6.6	8.7
NEG CTRL	1.0	13.4	20.0	34.6	30.0

C. Linearity

Figures 5 and 6 show typical examples of *Is*-anti-Sm/RNP Test Kit linearity. The figures depict the results of the Calibrator tested by *Is*-anti- Sm/RNP after a serial two-fold manual dilution in Sample Diluent. Separate dilutions were tested both manually and with MAGO. The results demonstrate a high degree of linearity for the *Is*-anti- Sm/RNP Test Kit throughout the testing range.

FIGURE 5
Manual
***Is*-anti-Sm/RNP Calibrator Linearity**

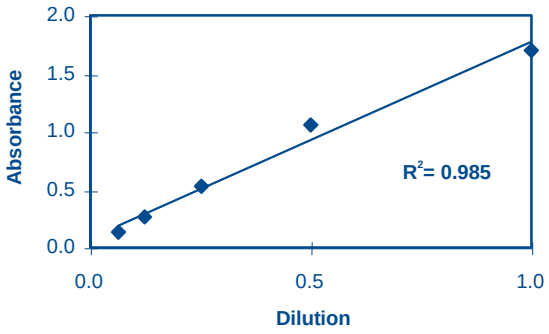
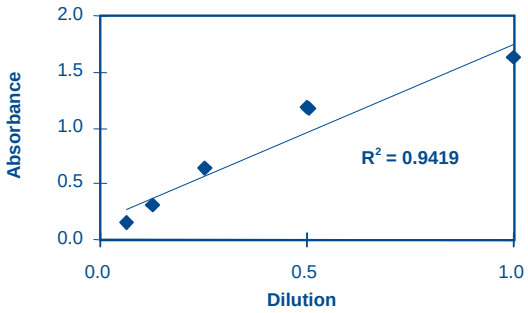


FIGURE 6
MAGO
***Is*-anti-Sm/RNP Calibrator Linearity**



D. Crossreactivity

Twenty-four sera positive for the six autoimmune specificities were tested in *Is*-anti-Sm/RNP Test Kit. The results are shown in Table 3. Since the antigen used in this test contains Sm and RNP determinants, it is not unexpected to find that the anti-Sm positive sera (9-12) are also positive in the Sm/RNP test.

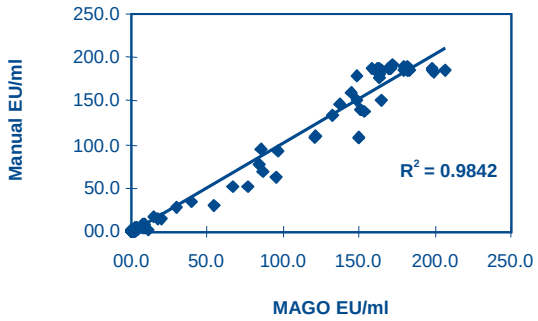
TABLE 3
Crossreactivity

Sample	*Is*-anti-Sm/RNP EU/ml	Interp	Specificity	Sample	*Is*-anti-Sm/RNP EU/ml	Interp	Specificity
1	5.5	NEG	SSA	13	156.2	POS	RNP
2	5.3	NEG	SSA	14	156.6	POS	RNP
3	2.3	NEG	SSA	15	156.8	POS	RNP
4	3.9	NEG	SSA	16	156.0	POS	RNP
5	7.9	NEG	SSB	17	2.2	NEG	Jo-1
6	6.2	NEG	SSB	18	3.4	NEG	Jo-1
7	3.9	NEG	SSB	19	1.9	NEG	Jo-1
8	5.0	NEG	SSB	20	2.5	NEG	Jo-1
9	155.9	POS	Sm	21	3.6	NEG	Scl-70
10	156.0	POS	Sm	22	2.0	NEG	Scl-70
11	129.0	POS	Sm	23	2.4	NEG	Scl-70
12	135.9	POS	Sm	24	1.2	NEG	Scl-70

E. Correlation of Manual and MAGO Results

Correlation of manual and MAGO EU/ml values for 150 samples tested in the *Is*-anti-Sm/RNP Test Kit is shown in Figure 7.

FIGURE 7
***Is*-Sm/RNP Correlation of**
Manual vs. MAGO



REFERENCES

1. Craft, J. and Hardin, J. A. 1993. Antinuclear Antibodies. In: Textbook of Rheumatology, Fourth Edition, W. B. Saunders Co., Philadelphia**.** p. 164 - 187.
2. Mattioli, M. and Reichlin, M. 1981. Characterization of a Soluble Nuclear Ribonucleoprotein Antigen Reactive with SLE Sera. J. Immunol. 107: 1281-1290.
3. Notman, D. D., Kurata, N. and Tan, E. M. 1975. Profiles of Antinuclear Antibodies in Systemic Rheumatic Diseases. Ann. Intern. Med. 83: 464-469.
4. Sharp, G. C., Irvin, W. S., Tan, E. M., *et. al.* 1972. Mixed Connective Tissue Disease-An Apparently Distinct Rheumatic Disease Syndrome Associated With a Specific Antibody to an Extractable Nuclear Antigen (ENA). Am. J. Med. 52: 148-159.
5. Engvall, E. and Perlmann, P. 1972. Enzyme-Linked Immunosorbent Assay (ELISA) III. Quantitation of Specific Antibodies by Enzyme-Labeled Anti-Immunoglobulin in Antigen-Coated Tubes. J. Immunol. 109:129-135.
6. Manual Guide – Safety Management No. CDC-22, "Decontamination of Laboratory Sink Drains to Remove Azide Salts", Centers for Disease Control and Prevention, Atlanta, GA, April 30, 1976.

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