Scientists rarely find Scl-70 antibodies in other autoimmune diseases, and thus, their detection in a patient with the recent onset of Raynaud’s phenomenon is highly significant (15).

The following table summarizes the various autoantibodies noted above with respect to disease association:

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Disease State</th>
<th>Relative Frequency of Antibody Detection %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Jo-1</td>
<td>Myositis</td>
<td>25-44% (19)</td>
</tr>
<tr>
<td>Anti-Sm</td>
<td>SLE</td>
<td>30*</td>
</tr>
<tr>
<td>Anti-RNP</td>
<td>MCTD,SLE</td>
<td>100** and &gt;40, respectively</td>
</tr>
<tr>
<td>Anti-SSA (Ro)</td>
<td>SLE, Sjögren’s</td>
<td>15 and 30-40, respectively</td>
</tr>
<tr>
<td>Anti-SSB (La)</td>
<td>SLE, Sjögren’s</td>
<td>15 and 60-70, respectively</td>
</tr>
<tr>
<td>Anti-Scl-70</td>
<td>Systemic sclerosis</td>
<td>20-28*</td>
</tr>
</tbody>
</table>

The relative frequency of these autoantibodies in association with SLE and other connective tissue diseases either singularly, or as multiple autoantibodies, requires an autoantibody profile assessment of each patient’s serum in order to obtain the highest degree of clinical relevance in the laboratory workup of these types of patients. Until recently, testing of autoantibodies occurred individually by indirect immunofluorescence, Ouchterlony gel diffusion, hemagglutination, radioimmunoassay, or enzyme-linked immunosorbent assay (ELISA). The exact etiology of autoimmune diseases is unknown, and the specific role played by autoantibodies in the onset of various autoimmune connective tissue diseases is obscure.

**TEST PRINCIPLE**

The DAI. Sm/RNP ELISA test system is designed to detect IgG class antibodies to different autoantigens in human sera. Wells of plastic microwell strips are sensitized by passive absorption with immobilized antigens. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgG is added to the wells and the plate is incubated. The Conjugate will react with the antibodies immobilized on the solid phase in step 1. The wells are washed to remove unreacted Conjugate.
3. The microwells containing immoblized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

**SPECIMEN COLLECTION AND PREPARATION**

1. It is recommended that specimen collection be carried out in accordance with CLSI document M29: Protection of Laboratory Workers from Infectious Disease.
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
3. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay (17, 18). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2 and 8°C for no...
longer than 48 hours. If delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (21).

MATERIALS AND COMPONENTS

Materials provided with the test kits
Each kit contains the following components in sufficient quantities to perform the number of tests indicated on packaging label. Note: The following reagents contain sodium azide as a preservative at a concentration of <0.1% (w/v): Controls, Calibrators and Sample Diluent.

1. **Plate**: 96 wells configured in twelve, 1x 8-well, strips coated with inactivated antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
2. **Conjugate**: Conjugated (horseradish peroxidase) goat anti-human IgG (Fc chain specific). One, 15 mL, blue-cap.
3. **Positive Control (Human Serum)**: One, 0.35 mL, red-cap vial.
4. **Calibrator (Human Serum)**: One, 0.5 mL, blue-cap.
5. **Negative Control (Human Serum)**: One, 0.35 mL, green-cap vial.
6. **Sample Diluent**: One, 30 mL, green-cap, bottle containing Tween-20, bovine serum albumin and phosphate-buffered-saline, (pH 7.2 ± 0.2). Ready to use. Note: Shake well before use.
7. **TMB**: One, 15 mL, amber-capped, amber bottle containing 3, 3’, 5’, 5’- tetramethylbenzidine (TMB). Ready to use.
8. **Stop Solution**: One, 15 mL, red-capped, bottle containing 1M H$_2$SO$_4$, 0.7M HCl. Ready to use.
9. **Wash Buffer Concentrate** (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. One, 100 mL, clear capped, bottle containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (Blue solution). Note: 1X solution will have a pH of 7.2 ± 0.2.

The following components are not kit lot number dependent and may be used interchangeably with the ELISA assays: TMB, Stop Solution, and Wash Buffer.

**Note: Kit also contains**
1. Component list containing lot specific information is inside the kit box.
2. Package insert providing instructions for use.

Materials required but not provided
1. ELISA microwell reader capable of reading at a wavelength of 450 nm.
2. Pipettes capable of accurately delivering 10 to 200 μL.
3. Multichannel pipette capable of accurately delivering 50-200 μL.
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or microwell washing system.
6. Distilled or deionized water.
7. One liter graduated cylinder.
8. Serological pipettes.
9. Disposable pipette tips.
11. Laboratory timer to monitor incubation steps.
12. Disposable basin and disinfectant. (example: 10% household bleach - 0.5% sodium hypochlorite.)

ASSAY PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20-25°C).
2. Determine the number of microwells needed. Allow six Control/Calibrator determinations (one Blank, one Negative Control, three Calibrators and one Positive Control) per run. A Reagent Blank should be run on each assay.
3. Prepare a 1:21 dilution (e.g.: 10 μL of serum + 200 μL of Sample Diluent) of the Negative Control, Calibrator, Positive Control, and each patient serum. The Sample Diluent will undergo a color change confirming that the specimen has been combined with the diluent.
4. To individual wells, add 100 μL of each diluted control, calibrator and patient specimen. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100 μL of Sample Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration.
6. Incubate the plate at room temperature (20-25°C) for 25 ± 5 minutes.
7. Wash the microwell strips 5X.

**A. Manual Wash Procedure:**

a. Vigorously shake out the liquid from the wells.
b. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
c. Repeat steps a. and b. for a total of 5 washes.
d. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with disinfectant at the end of the day’s run.

**B. Automated Wash Procedure:**

If using an automated microwell wash system, set the dispensing volume to 300-350 μL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.

8. Add 100 μL of the Conjugate to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
9. Incubate the plate at room temperature (20-25°C) for 25 ± 5 minutes.
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100 μL of TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens.
12. Incubate the plate at room temperature (20-25°C) for 10 ± 15 minutes.
13. Stop the reaction by adding 50 μL of Stop Solution to each well, including reagent blank well, at the same rate and in the same order as the TMB was added. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450 nm and measure the
optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after the addition of the Stop Solution.

**ABREVIATED TEST PROCEDURE**
1. Dilute Serum 1:2
2. Add diluted sample to microwell - 100μL/well.
3. Incubate 25 ± 5 minutes.
4. Wash.
5. Add Conjugate - 100μL/well.
6. Incubate 25 ± 5 minutes.
7. Wash.
8. Add TMB - 100μL/well.
10. Add Stop Solution - 50μL/well - Mix.
11. READ within 30 minutes.

**RESULTS**

The Calibrator within this Test System has been assigned both a Correction Factor for the generation of Index Values and a Calibrator Value for the generation of Unit Values. Based upon testing of normal and disease-state specimens, a maximum normal Unit Value has been determined by the manufacturer and correlated to the Calibrator.

**A. Calculations:**

1. **Correction Factor**
   A cutoff OD value for positive samples has been determined by the manufacturer and correlated to the Calibrator. The correction factor (CF) will allow you to determine the cutoff value for positive samples and to correct for slight day-to-day variations in test results. The correction factor is determined for each lot of kit components and is printed on the Component List located in the kit box.

2. **Cutoff OD Value**
   To obtain the cutoff OD value, multiply the CF by the mean OD of the Calibrator determined above.
   
   \[
   \text{Cutoff OD Value} = \text{(CF x mean OD of Calibrator)}
   \]

3. **Index Values or OD Ratios**
   Calculate the Index Value or OD Ratio for each specimen by dividing its OD value by the cutoff OD from step 2.

   \[
   \text{Index Value or OD Ratio} = \frac{\text{OD}}{\text{Cutoff OD Value}}
   \]

4. **Conversion of Optical Density to Autoantibody Units (AAU)/mL**:
   The conversion of OD to Unit Value (AAU/mL) can be represented by the following equation:
   
   \[
   \text{Test Specimen AAU/mL} = \frac{(A \times B)}{C}
   \]
   Where: A = OD of the test specimen in question; B = Unit Value of the Positive Calibrator (AAU/mL) & C = The mean OD of the Calibrator.

   **Example:**
   Test Specimen OD = 0.946
   Test Specimen AAU/mL = (0.946 x 155) / 0.435
   Calibrator OD = 0.435
   Test Specimen = 337 AAU/mL
   Calibrator Unit Value = 155 AAU/mL

**B. Interpretations:**

Index Values or OD ratios are interpreted as follows:

<table>
<thead>
<tr>
<th>Unit Values</th>
<th>Index Value or OD Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Specimens</td>
<td>&lt;150 AAU/mL</td>
</tr>
<tr>
<td>Equivocal Specimens</td>
<td>150 to 180 AAU/mL</td>
</tr>
<tr>
<td>Positive Specimens</td>
<td>&gt;180 AAU/mL</td>
</tr>
</tbody>
</table>

Retest specimens with OD Ratio Values in the equivocal range (0.91 – 1.09) in duplicate. Report any two of the three results which agree. Evaluate repeatedly equivocal specimens using an alternate serological method and/or re-evaluate by drawing another sample one to three weeks later.

**PERFORMANCE CHARACTERISTICS**

**Comparative Study**

A comparative study was performed to demonstrate the equivalence of DAI ELISA Sm/RNP Test System to other commercially available autoantibody ELISA test systems, using 337 serum specimens; 152 normal donor samples from the northeastern and southeastern United States, and 185 disease-state repository samples previously characterized with respect to autoantibody activity. The results of the investigation have been summarized in Tables 1 and 2 below.

<table>
<thead>
<tr>
<th>Table 1: Relative Sensitivity, Disease-State Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAI ELISA Reactives</td>
</tr>
<tr>
<td>46</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2: Relative Specificity; Normal Donor Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAI ELISA Non-Reactives</td>
</tr>
<tr>
<td>141</td>
</tr>
</tbody>
</table>

2. **Reproducibility**

A reproducibility study was conducted to assess the intra-assay and inter-assay variability of the test system using a strong positive, a low positive, and a negative sample. Samples were tested eleven times on each of three days. The mean unit value, the standard deviation, and the percent CV were calculated for each sample. The results of this study are depicted below:

<table>
<thead>
<tr>
<th>Table 3: Reproducibility for DAI ELISA Sm/RNP Test System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-assay Reproducibility</td>
</tr>
<tr>
<td>Specimen</td>
</tr>
<tr>
<td>High Positivity</td>
</tr>
<tr>
<td>Low Positivity</td>
</tr>
<tr>
<td>Negative</td>
</tr>
</tbody>
</table>

3. **Cross Reactivity**

Specimens negative for ANA by HEp-2 IFA and positive for IgG antibody to various antigens such as EBV-VCA, EBNA, HSV-1, HSV-2, CMV, Rubella, and/or Toxo, were tested for potential cross reactivity using the DAI ELISA Sm/RNP Test System. All specimens tested were negative on the ELISA, indicating that the potential for cross reactivity with such antibodies is not likely, and therefore should not interfere with the results obtained.
LIMITATIONS OF PROCEDURE

1. Do not make a diagnosis solely on the basis of any of the DAI ELISA Sm/RNP Test System test results.
2. Interpret test results in conjunction with the clinical evaluation and the results of other diagnostic procedures.

EXPECTED VALUES

The expected value for a normal patient is a negative result. The number of reactives, and the degree of reactivity is dependent upon parameters such as population type being tested, treatment, etc. Each laboratory should establish their own expected values based upon the specimens typically being tested. With respect to disease-state and percent reactivity, the table in the Significance And Background section of this package insert shows the relative frequency of autoantibody activity for various rheumatic disorders.

QUALITY CONTROL

1. Each time the assay is run the Calibrator must be run in triplicate. A reagent blank, Negative Control, and Positive Control must also be included in each assay.
2. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.
3. The mean OD value for the Calibrator and the OD values for the Positive and Negative Controls should fall within the following ranges:

<table>
<thead>
<tr>
<th></th>
<th>OD Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>≤ 0.250</td>
</tr>
<tr>
<td>Calibrator</td>
<td>≥ 0.300</td>
</tr>
<tr>
<td>Positive Control</td>
<td>≥ 0.500</td>
</tr>
</tbody>
</table>

a. The OD of the Negative Control divided by the mean OD of the Calibrator should be < 0.9.

b. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥ 1.25.

c. If the above conditions are not met the test should be considered invalid and should be repeated.

4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off.
5. Additional controls may be tested according to guidelines or requirements of local, state, and federal laws or accrediting organizations.
6. Refer to CLSI document C24: Statistical Quality Control for Quantitative Measurements for guidance on appropriate QC practices.

PRECAUTIONS

1. For In Vitro Diagnostic Use.
2. Normal precautions exercised in handling laboratory reagents should be followed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, the strips should be considered POTENTIALLY BIOHAZARDOUS MATERIALS and handled accordingly.
4. The Controls are POTENTIALLY BIOHAZARDOUS MATERIALS. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg, and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual “Biosafety in Microbiological and Biomedical Laboratories”: current edition; and OSHA’s Standard for Bloodborne Pathogens (20).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. All reagents must be allowed to reach room temperature (20-25°C) before starting the assay. Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The Sample Diluent controls, wash buffer, and conjugate contain sodium azide at a concentration of 0.1% (w/v). Sodium azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing sodium azide.
8. The Stop Solution is TOXIC. Causes burns. Toxic by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
9. The TMB Solution is HARMFUL. Irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. Irritating to eyes, respiratory system and skin.
11. Wipe bottom of plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Reagents from other sources or manufacturers should not be used.
14. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation. Allow the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
21. Wash solution should be collected in a disposal basin. Treat the waste solution with 10% household bleach (0.5% sodium hypochlorite). Avoid exposure of reagents to bleach fumes.
22. Caution: Neutralize any liquid waste at acid pH should be neutralized before adding to bleach solution.

STORAGE

Coated Microwell Strips: Immediately reseal extra strips with desiccant and return to proper storage. After opening - strips are stable for 60 days, as long as the indicator strips on the desiccant pouch remains blue. 2-8°C
REFERENCES


