patients tend to have a more severe disease course, more internal organ involvement and diffuse rather than limited skin involvement (14). Scl-70 antibodies are rarely found in other autoimmune diseases, and thus, their detection in a patient with the recent onset of Raynaud’s phenomenon is highly significant (15).

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, autoantibodies were tested 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.

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

### Table 1 (16)

<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>

* Highly Specific
* *Highly specific when present alone at high titer

### TEST PRINCIPLE

The DAI Sm ELISA test system is designed to detect IgG class antibodies to Sm in human sera. Wells of plastic microwell strips are sensitized by passive adsorption 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 specific antibodies immobilized on the solid phase in step 1. The wells are washed to remove unreacted Conjugate.
3. The microwells containing immobilized 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 (Current Edition).
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: All reactive reagents contain sodium azide as a preservative at a concentration of <0.1% (w/v):
- Calibrator, Controls, and Sample Diluent.
- 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.
- **Conjugate**: Conjugated (horseradish peroxidase) goat anti-human IgG (Fc chain specific). One, 15 mL, white-capped bottle. Ready to use.
- **Positive Control (Human Serum)**: One, 0.35 mL, red-cap vial.
- **Calibrator (Human Serum)**: One, 0.5mL blue-cap vial.
- **Negative Control (Human Serum)**: One, 0.35mL green-cap vial.
- **Sample Diluent**: One, 30mL, green-cap bottle containing Tween-20, bovine serum albumin and phosphate-buffered-saline (pH 7.2 ± 0.2). Ready to use. Note: The Sample Diluent will change color when combined with serum.
- **TMB**: One, 15 mL, amber-capped, amber bottle containing 3, 3’, 5, 5’ – tetramethylbenzidine (TMB). Ready to use.
- **Stop Solution**: One, 15 mL, red-capped, bottle containing 1M H₂SO₄ 0.7M HCl. Ready to use.
- **Wash Buffer Concentrate (10X)**: Dilute 1 part concentrate + 9 parts deionized or distilled water. One, 100mL, 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. Sample Diluent may be used interchangeably with any DAI ELISA Test System.

Note: Kit also contains
1. Component label 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 450nm.
2. Pipettes capable of accurately delivering 10 to 200µL.
3. Multichannel pipette capable of accurately delivering 50-200µL.
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. Disposal 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. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2-8°C.

<table>
<thead>
<tr>
<th>EXAMPLE PLATE SET-UP</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Blank</td>
<td>Patient 3</td>
</tr>
<tr>
<td>B</td>
<td>Neg. Control</td>
<td>Patient 4</td>
</tr>
<tr>
<td>C</td>
<td>Calibrator</td>
<td>Etc.</td>
</tr>
<tr>
<td>D</td>
<td>Calibrator</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Calibrator</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Pos. Control</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Patient 1</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>Patient 2</td>
<td></td>
</tr>
</tbody>
</table>

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of Sample Diluent. NOTE: Shake Well Before Use) 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 100µL of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100µL 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 0.5% sodium hypochlorite (bleach) at the end of the days run.

B. Automated Wash Procedure:
   If using an automated microwell wash system, set the dispensing volume to 300-350µL/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 treated with 0.5% sodium hypochlorite. It is highly recommended to wash the microwells by following the procedure as described in step 7.
6. Add 100µL of the Conjugate to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
7. Incubate the plate at room temperature (20-25°C) for 25 ± 5 minutes.
8. Wash the microwells by following the procedure as described in step 7.
9. Add 100µL of TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
10. Incubate the plate at room temperature (20-25°C) for 10 to 15 minutes.
11. Stop the reaction by adding 50µL of Stop Solution to each well, including
RESULTS

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.

\[
(CF \times \text{mean OD of Calibrator} = \text{cutoff OD value})
\]

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 value from step 2.

\[
\text{Index Value or OD Ratio} = \frac{\text{Specimen OD}}{\text{Cutoff OD}} = 0.093
\]

4. Conversion of Optical Density to AAU/mL
The conversion of OD to AAU/mL can be represented by the following equation:

\[
\text{Test Specimen AAU/mL} = (A \times B) / C
\]

Where: AAU/mL = Unknown Unit Value to be determined; 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:

<table>
<thead>
<tr>
<th>Test Specimen OD</th>
<th>Test Specimen AAU/mL</th>
<th>Calibration Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.946</td>
<td>0.946 \times 0.435</td>
<td>0.435</td>
</tr>
<tr>
<td>0.435</td>
<td>337 AAU/mL</td>
<td>3</td>
</tr>
<tr>
<td>155 AAU/mL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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>≤ 0.90</td>
</tr>
</tbody>
</table>

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

4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure and will not ensure precision at the assay cutoff.

5. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.


PERFORMANCE CHARACTERISTICS

1. Comparative Study
Technicians performed a comparative study to demonstrate the equivalence of Diagnostic Automation Inc. ELISA Sm 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.
2. Reproducibility

To assess the intra-assay and inter-assay variability of the test system technicians tested a strong positive, a low positive, and a negative sample 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 3: Reproducibility for DAI ELISA Sm Test System

<table>
<thead>
<tr>
<th>Intra-assay Reproducibility</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Inter-Assay Reproducibility: All Days Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen</td>
<td>Mean</td>
<td>Std</td>
<td>% CV</td>
<td>Mean</td>
</tr>
<tr>
<td>High Positive</td>
<td>576</td>
<td>71</td>
<td>12</td>
<td>690</td>
</tr>
<tr>
<td>Low Positive</td>
<td>480</td>
<td>45</td>
<td>9</td>
<td>587</td>
</tr>
<tr>
<td>Negative</td>
<td>12</td>
<td>3</td>
<td>N/A</td>
<td>8</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 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 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 reagents, 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, Table 1 in the Summary and Explanation section of this package insert shows the relative frequency of autoantibody activity for various rheumatic disorders.

### PRECAUTIONS

1. For In Vitro diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. 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, consider the strips potentially biohazardous materials and handle 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, handle these products 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).

### STORAGE

1. Store the unopened kit between 2 and 8°C.
2. Coated microwell strips: Store between 2 and 8°C. Extra strips should be immediately resealed with desiccant and returned to proper storage. Strips are stable for 60 days after the envelope has been opened and properly resealed, as long as the indicator strip on the desiccant pouch remains blue.
3. Conjugate: Store between 2 and 8°C. DO NOT FREEZE.
4. Calibrator, Positive Control and Negative Control: Store between 2 and 8°C. DO NOT FREEZE.
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, and Calibrator 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 upon hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
8. The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
9. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
11. Wipe the bottom of the 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. Do not use reagents from other sources or manufacturers.
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.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Collect the wash solution in a disposable basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. Do not allow the Conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing Sodium Azide as a preservative. Residual amounts of Sodium Azide may destroy the Conjugate’s enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.
REFERENCES


