The first quantitative assay for DNA is the Farr assay.\textsuperscript{4} The original procedure used precipitation, radio-labeled DNA and separation techniques. The difficulties in obtaining labeled DNA, the associated hazards and disposal problems of radioisotopes, and the expensive equipment required have led to the development of alternative methods.\textsuperscript{5} Anti-DNA can also be detected utilizing the Crithidia luciliae assay. This assay requires an experienced technician in interpreting fluorescence and becomes cumbersome for large numbers of samples.

The DAI ELISA test for dsDNA antibodies has many advantages over the immunofluorescent method including a sensitive, specific assay in an easy to use format providing quick and efficient results. The dsDNA antigen used in this kit is purified through modified immunoaffinity adsorption methods utilizing immobilized antigen-specific human immunoglobulins.

### TEST PRINCIPLE

The DAI dsDNA test is an Enzyme-Linked Imunosorbent Assay to detect antibodies to dsDNA antigen. Purified dsDNA antigen is attached to a solid phase microassay well. Enzyme-Linked Immunosorbant Assays (ELISA) rely on the ability of biological materials (i.e., antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid phase are brought into contact with a patient's serum, antigen specific antibody, if present, will bind to the antigen on the solid phase forming antigen-antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgG, M conjugated with horseradish peroxidase which then binds to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of Chromogen/Substrate, tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the patient’s serum, a blue color develops. When the enzymatic reaction is stopped with 1N H\textsubscript{2}SO\textsubscript{4}, the contents of the wells turn yellow. The color, which is indicative of the concentration of antibody in the serum, can be read on a suitable spectrophotometer or ELISA microwell plate reader.\textsuperscript{8,9,10,11}

### SPECIMEN COLLECTION AND PREPARATION

1. Handle all blood and serum as if capable of transmitting infectious agents.\textsuperscript{7}
2. Optimal performance of the DAI ELISA kit depends upon the use of fresh serum samples (clear, non-hemolyzed, nonlipemic, non-icteric). A minimum volume of 50 \textmu L is recommended, in case repeat testing is required. Specimens should be collected aseptically by venipuncture.\textsuperscript{12} Early separation from the clot prevents hemolysis of serum.
3. Store serum between 2 and 8 ºC if testing will take place within five days. If specimens are to be kept for longer periods, store at -20 to -70 ºC in a non-defrosting freezer. Do not use a frost free freezer because it may allow the specimens to go through freeze-thaw cycles and degrade antibody. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield erroneous results.
4. Serum containing visible particulate matter can be spun down utilizing slow speed centrifugation.
5. Do not use heat inactivate sera.
6. The NCCLS provides recommendations for storing blood specimens (Approved Standard - Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990).\textsuperscript{12}

### MATERIALS AND COMPONENTS

The EIA Anti-dsDNA Test Kit contains supplies for 96 Test. Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the package label:

**Materials provided with the test kits**

1. **Purified dsDNA antigen coated microassay plate**: 96 wells, configured in twelve 1x8 strips, stored in a foil pouch with desiccant. (96T: one plate)
2. **Serum Diluent Type II**: Ready to use. Contains proclin (0.1%) as a preservative. (96T: one bottle, 30 mL)
ASSAY PROCEDURE

1. Place the desired number of strips into a microwell frame. Allow five (5) Control/Calibrator determinations (one Negative Control, three Calibrators and one Positive Control) per run. A reagent blank (RB) should be run on each assay. Check software and reader requirements for the correct Control/Calibrator configuration. Return unused strips to the sealable bag with desiccant, seal and immediately refrigerate.

Plate Configuration:

<table>
<thead>
<tr>
<th>Plate Location</th>
<th>Sample Description</th>
<th>Plate Location</th>
<th>Sample Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>RB</td>
<td>2A</td>
<td>Patient #3</td>
</tr>
<tr>
<td>1B</td>
<td>NC</td>
<td>2B</td>
<td>Patient #4</td>
</tr>
<tr>
<td>1C</td>
<td>CAL</td>
<td>2C</td>
<td>Patient #5</td>
</tr>
<tr>
<td>1D</td>
<td>CAL</td>
<td>2D</td>
<td>Patient #6</td>
</tr>
<tr>
<td>1E</td>
<td>CAL</td>
<td>2E</td>
<td>Patient #7</td>
</tr>
<tr>
<td>1F</td>
<td>PC</td>
<td>2F</td>
<td>Patient #8</td>
</tr>
<tr>
<td>1G</td>
<td>Patient #1</td>
<td>2G</td>
<td>Patient #9</td>
</tr>
<tr>
<td>1H</td>
<td>Patient #2</td>
<td>2H</td>
<td>Patient #10</td>
</tr>
</tbody>
</table>

*Note: Serum vials may contain excess volume.

The following components are not kit lot # dependent and may be used interchangeably within the Diagnostic Automation Autoimmune Disease ELISA Kits: Chromogen/Substrate Solution Type I, and Stop Solution. Please check that the appropriate Diagnostic Automation, Inc. Reagent Type (Type I, Type II, etc.) is used for the assay.

Materials required but not provided

1. Wash bottle, automated or semi-automated microwell plate washing system.
2. Micropipettes, including multichannel, capable of accurately delivering 10-200 µL volumes (less than 3% CV).
3. One liter graduated cylinder.
5. Test tube for serum dilution.
6. Reagent reservoirs for multichannel pipettes.
7. Pipette tips.
8. Distilled or deionized water (dH₂O), CAP (College of American Pathology) Type 1 or equivalent.
9. Timer capable of measuring to an accuracy of +/- 1 second (0-60 minutes).
10. Disposal basins and 0.5% sodium hypochlorite (50 mL bleach in 950 mL dH₂O).
11. Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. Read the Operator's Manual or contact the instrument manufacturer to establish linearity performance specifications of the reader.

Note: Use only clean, dry glassware.

REAGENT PREPARATION

1. All reagents must be removed from refrigeration and allowed to come to room temperature (21 - 25 ºC) before use. Return all reagents to refrigerator promptly after use.
2. All samples and controls should be vortexted before use.
3. Dilute 50 mL of the 20X Wash Buffer Type I to 1 L with distilled and/or deionized H₂O. Mix well.

**IMPORTANT NOTE:** Regarding steps 5 and 8 - Insufficient or excessive washing will result in assay variation and will affect validity of results. Therefore, for best results the use of semi-automated or automated equipment set to deliver a volume to completely fill each well (250-300 µL) is recommended. A total of up to five (5) washes may be necessary with automated equipment. Complete removal of the Wash Buffer after the last wash is critical for the accurate performance of the test. Also, visually ensure that no bubbles are remaining in the wells.

6. Add 100 µL Conjugate to each well, including reagent blank well. Avoid bubbles upon addition as they may yield erroneous results.
7. Incubate each well at room temperature (21 to 25 °C) for 30 minutes +/- 1 minute.
8. Repeat wash as described in Step 5.
9. Add 100 µL Chromogen/Substrate Solution (TMB) to each well, including the reagent blank well, maintaining a constant rate of addition across the plate.
10. Incubate each well at room temperature (21 to 25 °C) for 5 – 15 minutes.

Diagnostic Automation/Cortez Diagnostics, Inc.
21250 Califa St, Suite 102/116, Woodland Hills, CA 91367 USA Phone: 818-591-3030, Fax : 818-591-6383 Email: onesrep@rapidtest.com Website: www.rapidtest.com

DAI CODE #1
RESULTS

1. Mean Calibrator O.D. - Calculate the mean value for the Calibrator from three Calibrator determinations. If any of the three Calibrator Values differ by more than 15% from the mean, discard that value and calculate the average of the two remaining values.

2. Correction Factor - To account for day-to-day fluctuations in assay activity due to room temperature and timing, a Correction Factor is determined by DAI for each lot of kits. The Correction Factor is printed on the Calibrator vial.

3. Cutoff Calibrator Value - The Cutoff Calibrator Value for each assay is determined by multiplying the Correction Factor by the mean Calibrator O.D. determined in Step 1.

4. Index Value - Calculate an Index Value for each specimen by dividing the specimen O.D. value by the Cutoff Calibrator Value determined in Step 3.

Example:

<table>
<thead>
<tr>
<th>O.D.s obtained for Calibrator</th>
<th>Mean O.D. for Calibrator</th>
<th>Correction Factor</th>
<th>Calibrator Value</th>
<th>O.D. obtained for patient sera</th>
<th>Index Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.38, 0.42, 0.40</td>
<td>0.40</td>
<td>0.50</td>
<td>0.50 x 0.40 = 0.20</td>
<td>0.60</td>
<td>0.60/0.20 = 3.00</td>
</tr>
</tbody>
</table>

Analysis

1. The patients’ Index Values are interpreted as follows:

<table>
<thead>
<tr>
<th>Index Value</th>
<th>Results</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 0.90</td>
<td>Negative</td>
<td>No detectable antibody to dsDNA by the ELISA test.</td>
</tr>
<tr>
<td>0.91-1.09</td>
<td>Equivocal</td>
<td>Samples should be re-tested. See number 2 below</td>
</tr>
<tr>
<td>≥ 1.10</td>
<td>Positive</td>
<td>Indicates presence of detectable antibody to dsDNA by the ELISA test.</td>
</tr>
</tbody>
</table>

2. Samples that remain equivocal after repeat testing should be retested on an alternate method or test a new sample.

International Unit conversion

International unit (IU) reactivity is determined relative to the Second Generation World Health Organization (WHO) derivative. Conversion of Index values to international units is accomplished by using an exponential regression analysis. Each lot is standardized versus international units and provided with a lot specific conversion table (Conversion of International Units (IU) per mL for dsDNA IgG, IgM).

For Example:

<table>
<thead>
<tr>
<th>Index Value</th>
<th>IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>43</td>
</tr>
<tr>
<td>1.5</td>
<td>64</td>
</tr>
<tr>
<td>2.0</td>
<td>96</td>
</tr>
<tr>
<td>2.5</td>
<td>145</td>
</tr>
<tr>
<td>3.0</td>
<td>&gt;150</td>
</tr>
</tbody>
</table>

Linear limits are 150 IU/mL. Greater than 150 IU/mL must be reported as >150 IU/mL. See included chart for the lot specific conversion table.

QUALITY CONTROL

For the assay to be considered valid the following conditions must be met.

1. Calibrators and controls must be run with each test run.
2. Reagent Blank must be < 0.150 O.D. (Optical density) at 450 nm (when read against Blank).
3. The mean O.D. for the Calibrator should be ≥ 0.250 at 450 nm (when read against Blank).
4. The Index Values for the Positive and Negative Controls should be in their respective ranges printed on the vials. If the control values are not within their respective ranges, the test should be considered invalid and should be repeated.
5. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. Refer to NCCLS C24A for guidance on appropriate Quality Control practices.13
7. If above criteria are not met on repeat, contact DAI Technical Service.

PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY

A study was performed using 197 patient sera obtained from outside clinical laboratories. These samples were tested using both the DAI dsDNA ELISA test and a commercially available anti-dsDNA ELISA test following the manufacturers’ package inserts. Fifty-one samples were found positive by the reference ELISA test, the remaining 146 samples were negative by the ELISA. Six samples were found to be false positive and one found false negative on the ELISA test as compared to the ELISA reference method. The seven discrepant samples were assayed by Cuthridia IFA as the referee method. Three samples were still false positive versus the IFA method, with the other four resolving correctly. Using the above data criteria, the DAI dsDNA ELISA test has a 100% sensitivity and 97.9% specificity as compared to the results obtained on both the ELISA and IFA methods. The following data were obtained:

<table>
<thead>
<tr>
<th>dsDNA</th>
<th>Corrected ELISA and IFA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>53</td>
</tr>
<tr>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

Agreement = 194/197 = 98.5%

CROSS REACTIVITY

A series of 72 patient samples each containing positive levels of antibodies to other common autoimmune markers (Scl-70, Sm, RNP, RF, Jo-1 and SS-B, SS-A, and ANA) were tested in the DAI dsDNA ELISA test. Three samples were positive on the reference ELISA test but negative on the DAI dsDNA ELISA and confirmed negative by IFA. Therefore, from the above data, the DAI dsDNA ELISA demonstrates no cross-reactivity to other common autoimmune markers.

INTERNATIONAL UNIT CONVERSION

The data in Table 1 illustrates dsDNA Index Values for the serially diluted Second Generation World Health Organization (WHO) derivative. The dsDNA Index Values are compared to serial dilutions of the standard serum by linear regression (exponential regression analysis). The data indicates that international units can be determined from the Index Value.
**Environmental causes.** Autoimmune rheumatic diseases are a group of chronic disorders, afflicting about 3% of the population. The etiology of these disorders is not elucidated but they may involve both genetic and environmental causes. Autoimmune rheumatic diseases have two common clinical and pathological features: A) a non-organ specific autoimmune condition is manifest; B) most patients exhibit some rheumatic symptoms throughout the course of their disease. It is apparent that certain systemic rheumatic diseases have distinct ANA profiles. Thus the ANA profile is helpful in establishing the diagnosis of systemic lupus erythematosus. Most patients with systemic lupus erythematosus (SLE) or systemic rheumatic diseases have two common clinical and pathological features: A) a non-organ specific autoimmune condition is manifest; B) most patients exhibit some rheumatic symptoms throughout the course of their disease.

The components in this kit have been quality control tested as a Master Lot unit. Do not mix components from different lot numbers except Chromogen/Substrate Solution Type I, Stop Solution, Wash Buffer Type I and Serum Diluent Type II. Do not mix with components from other manufacturers.

**PRECAUTIONS**

1. For in vitro diagnostic use.
2. The human serum components used in the preparation of the Controls and Calibrator in this kit have been tested by an FDA approved method for the presence of antibodies to human immunodeficiency virus 1 & 2 (HIV 1&2), hepatitis C (HCV) as well as hepatitis B surface antigen and found negative. Because no test method can offer complete assurance that HIV, HCV, hepatitis B virus, or other infectious agents are absent, specimens and human-based reagents should be handled as if capable of transmitting infectious agents.
3. The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2:

   1. Do not use reagents beyond the stated expiration date marked on the package label.
   2. All reagents must be at room temperature (21° to 25 °C) before running assay. Remove only the volume of reagents that is needed. **Do not pour reagents back into vials as reagent contamination may occur.**
   3. Before opening Control and Calibrator vials, tap firmly on the benchtop to ensure that all liquid is at the bottom of the vial.
   4. Use only distilled or deionized water and clean glassware.
   5. Do not let wells dry during assay; add reagents immediately after completing wash steps.
   6. Avoid cross-contamination of reagents. Avoid splashing or generation of aerosols. Wash hands before and after handling reagents. **Cross-contamination of reagents and/or samples could cause erroneous results.**
   7. If washing steps are performed manually, wells are to be washed three times. Up to five wash cycles may be necessary if a washing manifold or automated equipment is used.
   8. **Sodium azide inhibits Conjugate activity.** Clean pipette tips must be used for the Conjugate addition so that sodium azide is not carried over from other reagents.
   9. Certain reagents in this kit contain sodium azide for use as a preservative. It has been reported that sodium azide may react with lead and copper in plumbing to form explosive compounds. When disposing, flush drains with water to minimize build-up of metal azide compounds.
   10. **Never pipette by mouth or allow reagent or patient sample to come into contact with skin. Reagents containing proclin, sodium azide, and TMB may be irritating. Avoid contact with skin and eyes. In case of contact, immediately flush area with copious amounts of water.**
   11. If a sodium hypochlorite (bleach) solution is being used as a disinfectant, do not expose to work area during actual test procedure because of potential interference with enzyme activity.
   12. Avoid contact of Stop Solution (1N sulfuric acid) with skin or eyes. If contact occurs, immediately flush area with copious amounts of water.
   13. **Caution: Liquid waste at acid pH must be neutralized prior to adding sodium hypochlorite (bleach) solution to avoid formation of poisonous gas.** Recommend disposing of reacted, stopped plates in biohazard bags. See Precaution 3.
   14. Do not use Chromogen/Substrate Solution if it has begun to turn blue.
   15. The concentrations of anti-dsDNA in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity. The safety data sheet is available upon request.
WARNING
The safety data sheet is available upon request.

Serum Diluent, Conjugate, and Wash Buffer contain 0.1% ProClin 300R, a biocidal preservative that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

H317: May cause an allergic skin reaction.

STORAGE
Serum Diluent and Controls contain < 0.1% sodium azide.

REFERENCES