Enzyme Immunoassay

Anti-dsDNA

Cat # 2553Z

For In Vitro Diagnostic Use Only

<table>
<thead>
<tr>
<th>Test</th>
<th>Anti –ds DNA ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>ELISA: Enzyme Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>Principle</td>
<td>ELISA - Indirect; Antigen Coated Plate</td>
</tr>
<tr>
<td>Detection Range</td>
<td>Quantitative 10-300IU/mL</td>
</tr>
<tr>
<td>Sample</td>
<td>5 µl serum</td>
</tr>
<tr>
<td>Specificity</td>
<td>N/A</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>N/A</td>
</tr>
<tr>
<td>Total Time</td>
<td>~ 75min</td>
</tr>
<tr>
<td>Shelf Life</td>
<td>12 months</td>
</tr>
</tbody>
</table>

IVD  See external label  2°C-8°C  Σ=96 tests  REF Cat # 2553Z
NAME AND INTENDED USE

The Diagnostic Automation Inc. Anti-ds-DNA Quantitative AD-201 is a solid phase enzyme linked immunosorbent assay. This test provides quantitative measurement of human antibody to native double stranded DNA to aid in the management of patients with systemic lupus erythematosus and other autoimmune diseases in the human serum. (For Professional Use Only)

SUMMARY AND EXPLANATION OF TEST

Antibodies reactive with double-stranded DNA (ds-DNA) are of primary importance for diagnosis of systemic lupus erythematosus (SLE), appear to play a central role in the pathogenesis of tissue injury and are closely correlated with clinical activity\(^1\). Their presence is also associated with active lupus and usually with immuno-complex glomerulonephritis\(^2,3,4\) procedure for autoimmune diseases\(^1\). A wide variety of techniques are available for measuring the presence and concentration of anti-DNA. These techniques include hemagglutination, complement fixation, immunofluorescence and radioimmunoassay\(^5,6,7,8\). The enzyme immunoassay (EIA) offers advantages over other assays in area of sensitivity, reproducibility, and efficiency without sacrificing specificity\(^9,10\).

DAI Anti-dsDNA is an ELISA test utilizing purified DNA immobilized solid phase to provide a simple, reliable and rapid quantitative measurement of anti-ds-DNA in human serum.

PRINCIPLE OF THE ASSAY

The DAI Anti-ds-DNA -quantitative is a solid phase enzyme linked immunosorbent assay (ELISA). The wells are coated with purified double stranded DNA. The samples, controls and calibrator are incubated in the wells first. After incubation, the serum anti-ds-DNA antibodies will bind with coated DNA. The enzyme conjugate, goat anti-human IgG, which is chemically conjugated with horseradish peroxidase, is then added to bind immunologically to the captured anti-ds-DNA to form a sandwich complex on the well. Unbound enzyme conjugate is washed off by washing buffer. Upon addition of the TMB Substrate, the intensity of color developed is proportional to the concentration of serum Anti-ds-DNA antibody in the samples.

WARNINGS AND PRECAUTIONS

1. DAI Anti- ds-DNA -quantitative is designed for in vitro diagnostic use only
2. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
3. Warning potential bio-hazardous material: The matrix of Negative and Positive controls is human serum. The serum found negative for HBsAg, HIV and HCV antibodies when tested with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HBsAg, HIV, HCV, or other infectious agents are absent, these reagents should be handled at Bio-safety level 2, as recommended for any potentially infectious human serum or blood specimen in the Center for Disease Control/National Institutes of Health Manual, “Bio-safety in Microbiological and Biomedical Laboratories” 1984.
STORAGE AND STABILITY

1. Store the kit at 2-8°C in a refrigerator. Keep micro-wells sealed in dry bag with desiccants.
2. The unopened reagents are stable until expiration of the kit.
3. TMB Solution should be colorless; if the solution turns blue, it must be replaced. Do not expose test reagents to strong light during storage or usage.

SAFETY INSTRUCTIONS

1. Positive Control is made from human origin and found to be negative from HBsAg, HIV and HCV antibodies. However, for safety, it must be treated as infectious materials.
2. Do not smoke or eat in areas where specimens or reagent kits are handled.
3. Do not mouth pipette. Wear PVC gloves when handling reagent kits or specimens, and wash hands thoroughly afterwards.
4. Infectious specimens and non-acid-containing spills should be wiped up thoroughly with 5% sodium hypochlorite solution.
5. All waste material should be properly disinfected before disposal. Both liquid and solid waste can be autoclaved for at least one hour at 121.5°C. Solid waste can also be incinerated. Non-acidic liquid waste requires neutralization before similar treatment and should stand for 30 minutes to obtain effective disinfection.
6. Avoid contact of hydrochloric acid with skin and mucous membranes.

MATERIALS PROVIDED

1. Micro-wells strips (96 wells): Native, double-stranded DNA coated wells. 8x12 strips
2. Sample Diluent (50 mL): 1 bottle
3. Washing Buffer (10 mL): (For Anti-ds-DNA only) 1 vial, prepare working washing solution by adding 990mL of distilled water to 10 mL of the above concentrated washing buffer.
4. Enzyme conjugate (11mL): Goat anti-human IgG antibody conjugated with horseradish peroxidase
5. Positive Control (1 mL), (Ready to use, do not dilute)
6. Reference Standard Set (1 mL each vial): Calibrated to 10, 50, 150, 300 IU/mL (Ready to Use, Do not dilute)
7. TMB Solution (11 mL): Buffer solution containing hydrogen peroxide and TMB.
8. Stop Solution: 2 N HCl.
9. Well holder: For securing individual wells

MATERIALS REQUIRED BUT NOT PROVIDED

1. Micro-well reader at 450 nm.
2. Pipetor with tips for 5, 50 & 100 uL.

SAMPLE COLLECTION AND HANDLING

Collect blood aseptically by venipuncture, allow to clot. Separate the serum by centrifugation at room temperature, and store in sterile tubes. If sera cannot be assayed immediately, they can be stored at 2-8°C for a week or frozen at -20°C for up to 6 months. Repeated freezing and thawing is not recommended.
Do not store in self-defrosting freezer. Do not use hyperlipemic, hemolyzed, contaminated or heat inactivated sample as they may cause erroneous results.

PREPARATION FOR ASSAY

1. Before beginning the test, bring all samples and reagents to room temperature (24±3°C) and mix each gently.
2. Have all reagents and samples ready before the start of the assay. Once the test has begun it must be performed without any interruption to get the most reliable and consistent results
3. Use new disposable tips for each sample.

ASSAY PROCEDURE (30/30/15)

1. Secure the desired number of coated wells in holder.
2. Prepare 1:100 dilutions of test samples by adding 5 uL of sample to 0.5 mL sample diluent in the separate tubes. Do not dilute Reference Standard and Control
3. Dispense 100 uL of reference standard, control, and diluted Test samples as well as sample diluent (as the zero point) into each well.
4. Incubate 30 minutes at room temperature.
5. Rinse the wells 5 times with washing solution (300 uL washing solution/well/each rinse).
6. Dispense 100 uL of enzyme conjugate into each well and mix for 5 seconds and incubated at room temperature for 30 minutes.
7. Remove mixture and rinse the wells 5 times with washing solution (300 uL washing solution/well/each rinse). (Be sure to wash the wells thoroughly and completely dry the wells. Improper wash may cause false positive results).
8. Dispense 100 uL TMB Solution into each well including the blank. Mix for 5 seconds and incubated in the dark for 15 minutes.
9. Stop reaction by adding 50 uL of stop solution to each well and read O.D. at 450 nm with microwell reader against Blank well.

QUALITY CONTROL

Each laboratory should utilize internal controls several levels to monitor assay performance. The DAI DNA kit includes 1 internal control. The control should be treated as unknown. Results obtained should be in agreement with the assigned values of the Control.

CALCULATION OF RESULTS

Any microwell reader capable of determining at 450 nm may be used. The ds-DNA value of patient is obtained as follows:
1. Plot the concentration (X) of each Reference Standards against its absorbance (Y) on full logarithmic graph paper.
2. Obtain the value of patient ds-DNA by reference to the Standard Curve. This data is for demonstration purposes only. It must not be used in place of data generated for each assay.
<table>
<thead>
<tr>
<th>Well No.</th>
<th>Description (IU/mL)</th>
<th>Absorbance (450 nm)</th>
<th>DS-DNA (IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>0</td>
<td>0.072</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>10</td>
<td>0.374</td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>50</td>
<td>1.297</td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td>150</td>
<td>2.238</td>
<td></td>
</tr>
<tr>
<td>E1</td>
<td>300</td>
<td>3.100</td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>Patient 1</td>
<td>1.603</td>
<td>91.75</td>
</tr>
<tr>
<td>G1</td>
<td>Patient 2</td>
<td>2.270</td>
<td>161.7</td>
</tr>
</tbody>
</table>

**INTERPRETATION**

Microtiter strip must be read with an ELISA reader set at 450 nm. Results should be read after stopping solution.

*Negative:* Less than 25 IU/mL
*Borderline Positive:* 25-30 IU/mL
*Low Positive:* 31-60 IU/mL
*Positive:* 61-200 IU/mL
*High Positive:* >200 IU/mL.
Reading of the positive control should be within the range indicated on the vial.

SLE patients tend to have high levels of anti-ds-DNA antibodies, while patients with other autoimmune diseases, such as sjogren’s syndrome or rheumatoid arthritis, may be low positive for anti-ds-DNA.

**LIMITATIONS**

For diagnostic purpose, the Anti-ds-DNA values should be used as an adjunct to other data available to the physician. A positive test suggests certain diseases, but is not diagnostic and should be confirmed by clinical findings.

**COMPARISON STUDY**

The comparison study was carried out on 93 serum specimens with a commercial anti-ds-DNA quantitative EIA test kit and DAI anti-ds-DNA ELISA. The specimens were obtained from a local laboratory. The correlation coefficient of these two tests is 0.85. The results are summarized as following table:

<table>
<thead>
<tr>
<th></th>
<th>Commercial ELISA</th>
<th>DAI ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>5</td>
<td>42</td>
</tr>
<tr>
<td>Negative</td>
<td>35</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>53</td>
</tr>
</tbody>
</table>

**CROSS-REACTIVITY**

Serum samples with positive for anti-extractable nuclear antibody (ENA), single stranded DNA, rheumatoid factor (RF), anti-Toxoplasma gondii IgG, IgM and Cytomegalovirus (CMV) IgG and IgM have shown negative results in DAI anti-ds-DNA test.

**REFERENCES**


<table>
<thead>
<tr>
<th>Date Adopted</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005-07-01</td>
<td>DA-dsDNA-2009</td>
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