AccuDiag™
TPO IgG
ELISA Kit

INTENDED USE
The DAI ELISA Thyroid Peroxidase (TPO) IgG Test System is intended for the qualitative and semi-quantitative detection of IgG-class antibodies to thyroid peroxidase (TPO) in human serum. The test system is intended to be used as an aid in the diagnosis of thyroid diseases. This test is for In Vitro diagnostic use.

SUMMARY AND EXPLANATION
Thyroid antibodies are a characteristic finding in patients with Hashimoto’s and Graves’ diseases (1). The presence of thyroid antibodies in the sera of 80% of patients with these two diseases led to the recommendation that some type of thyroid antibody testing be a feature of the work-up of any patient with a goiter (1). Although thyroid antibodies are predominantly associated with Hashimoto’s or Graves’ diseases, they may be found in the sera of patients with other diseases such as myxedema, granulomatous thyroiditis, nontoxic nodular goiter, and thyroid carcinoma (1). Thyroid antibodies are also found in most cases of lymphocytic thyroiditis in children (2), and rarely in patients with pernicious anemia and Sjögren’s Syndrome (3-4).

TEST PRINCIPLE
The Diagnostic Automation Inc. (TPO) ELISA test system is designed to detect IgG class antibodies to TPO in human sera. Wells of plastic microwell strips are sensitized by passive absorption with TPO antigen. 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 antibody 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. DACD recommends that the user carry out specimen collection 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, consider all blood derivatives potentially infectious.
3. Use only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures in this assay (6, 7). Do not use if there are any added anticoagulants or preservatives. 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 - 8°C, for no longer than 48 hours. If a delay in testing is anticipated, store sera at ~20°C or lower. Avoid multiple freeze/thaw cycles which 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 (8).

MATERIALS AND COMPONENTS
Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on packaging label.

NOTE: The following components contain sodium azide as a preservative at a concentration of <0.1 % (w/v): Controls, Calibrators and Sample Diluent.

Materials provided with the test kits
1. Plate: 96 wells configured in twelve, 1x8-well, strips coated with human thyroid peroxidase (~98% pure). The strips are packaged in a strip holder and sealed in an envelope desiccant.
2. Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgG (Fc chain specific), One, 15 mL, white-capped bottle. Ready to use.
3. Positive Control (Human Serum): One, 0.35 mL vial with a red-cap.
4. Calibrator A (Human Serum): One, 0.5mL vial with a white cap.
5. Calibrator B (Human Serum): One, 0.5mL vial with a yellow cap.
6. Calibrator C (Human Serum): One, 0.5mL vial with an orange cap.
7. Calibrator D (Human Serum): One, 0.5mL vial with a blue cap.
8. Negative Control (Human Serum): One, 0.35 mL vial with a green-cap.
9. 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 in the presence of serum.
11. Stop Solution: One, 15 mL, red-capped, bottle containing 1M H2SO4, 0.7M HCl. Ready to use.
12. 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.

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.)

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 MATERIAL and handled accordingly.
4. The human serum controls are POTENTIALLY BIOHAZARDOUS MATERIAL. 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 Blood borne Pathogens (5).
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, control, wash buffer, and conjugate contain sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate’s enzymatic activity.
8. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this kit.

ASSAY PROCEDURE

1. Remove the individual component from storage and allow them to warm to room temperature (20-25°C).
2. Determine the number of microwells needed. Allow seven Control/Calibrator determinations (one Blank, one Negative Control, four Calibrators and one Positive Control) per run. A Reagent Blank should be run on each assay. Check software and reader requirements for the correct Control/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2°C and 8°C.

**EXAMPLE PLATE SET-UP**

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

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL 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 100µL 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 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.
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 store thoroughly.

Incubate the plate at room temperature (20° to 25°C) for 10 to 15 minutes.

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 moved from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.

1. Summary of the comparative investigation, DAI ELISA TPO IgG Test System versus a commercially available ELISA test system.

2. The mean OD value for the calibrator, positive control, and negative control should fall within the following ranges:
   - Positive Control: Must be > 30 IU/mL
   - Negative Control: Must be < 15 IU/mL
   - TPO IgG ELISA: Must be > 30 IU/mL

3. The positive control and negative control are intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off.

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

**Performance Characteristics**

A. **Comparative Study**

A comparative study was performed to determine the equivalence of the DAI ELISA TPO IgG Test System to another commercially available TPO IgG ELISA test system. Evaluation of the performance occurred using 248 specimens. Table 1 below summarizes the results.

<table>
<thead>
<tr>
<th>Commercial</th>
<th>TPO IgG ELISA</th>
<th>TPO IgG ELISA Test System</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Equivocal*</td>
</tr>
<tr>
<td>TPO IgG ELISA</td>
<td>Negative</td>
<td>7</td>
</tr>
<tr>
<td>Test System</td>
<td>Totals</td>
<td>111</td>
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</tbody>
</table>

**Confidence Interval of: 91.0 to 97.1%**

**Relative Sensitivity = 104/111 = 93.7% ± 95%**

**Confidence Interval of: 89.1 to 98.2%**

**Relative Specificity = 117/124 = 94.4% ± 95%**

**Confidence Interval of: 90.3 to 98.4%**

**Relative Agreement = 221/235 = 94.0% ± 95%**

**Confidence Interval of: 91.0 to 97.1%**

**Equivocal specimens were excluded from calculations below**

*Please be advised that the term 'relative' refers to the comparison of this assay’s results to that of a similar assay. There was not an attempt to correlate the assay’s results with disease presence or absence. No judgement can be made on the comparison assay’s accuracy to predict disease.*
B. Precision and Reproducibility
A study was conducted in house to determine reproducibility. Briefly, six specimens were tested; two negative, two strong positive, and two positive specimens that were near the assay cut off. Each specimen was tested in eight replicate wells on each day, for a total of three days. The resulting data was used to determine both intra and inter assay reproducibility. A summary of the study appears in Table 2 below.

Table 2. Results of three day reproducibility study:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean(IU/mL) Day One % CV</th>
<th>Mean(IU/mL) Day Two % CV</th>
<th>Mean(IU/mL) Day Three % CV</th>
<th>Three Days Combined Mean(IU/mL) % CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>77</td>
<td>3.1</td>
<td>55</td>
<td>3.9</td>
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<td>2</td>
<td>112</td>
<td>5.8</td>
<td>88</td>
<td>16.4</td>
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<td>3</td>
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<td>9.2</td>
<td>37</td>
<td>3.8</td>
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<td>4</td>
<td>34</td>
<td>4.9</td>
<td>40</td>
<td>1.2</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>2.4</td>
<td>12</td>
<td>1.9</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>6.0</td>
<td>6</td>
<td>1.3</td>
</tr>
</tbody>
</table>

C. Cross Reactivity
To evaluate the system for potential cross reactivity to other autoantibodies, seventeen specimens which were positive for antibodies to nuclear antigens (ANA) on Hep-2 cells were tested. Of the specimens tested, none were positive on the TPO IgG ELISA. This study indicates that the potential for interference due to cross reactive autoantibodies is unlikely.

Correlation to the World Health Standard (NIBSC 66/387)
The World Health Standard (NIBSC 66/387) was tested on the DAI assay to determine the correlation of the result obtained to the expected result. The data from this study is presented in Table 3 below.

Table 3. Correlation to the World Health Standard; (NIBSC 66/387)

<table>
<thead>
<tr>
<th>Dilution of the Standard</th>
<th>IU/mL as Tested</th>
<th>OD (450 nm)</th>
<th>Result: (IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>neat</td>
<td>1000</td>
<td>&gt; 3.000</td>
<td>278</td>
</tr>
<tr>
<td>1:2</td>
<td>500</td>
<td>&gt; 3.000</td>
<td>275</td>
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<tr>
<td>1:4</td>
<td>250</td>
<td>2.710</td>
<td>243</td>
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<tr>
<td>1:8</td>
<td>125</td>
<td>1.740</td>
<td>144</td>
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<tr>
<td>1:16</td>
<td>62</td>
<td>0.890</td>
<td>61</td>
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<tr>
<td>1:32</td>
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<td>1:64</td>
<td>16</td>
<td>0.202</td>
<td>14</td>
</tr>
<tr>
<td>1:128</td>
<td>8</td>
<td>0.112</td>
<td>8</td>
</tr>
</tbody>
</table>

LIMITATIONS OF PROCEDURE
1. Do not make a diagnosis solely based on the ELISA result. Interpret test results for anti-thyroglobulin antibodies in conjunction with the clinical evaluation and the results of other diagnostic procedures.
2. Reproducible results with an ELISA system require careful pipetting, strict adherence to incubation periods and temperatures requirements, as well as thorough washing of the test wells and thorough mixing of all solutions.

EXPECTED VALUES
The clinical investigation included 80 random normal donor specimens. With respect to this group, four (5%) were positive and 76 (95%) were negative.

STORAGE
1. 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.
2. Conjugate: Store between 2-8°C. DO NOT FREEZE.
3. Unopened Test System, Calibrators, Positive Control, Negative Control, TMB, Sample Diluent: 2-8°C.
4. Stop Solution: Store between 2-25°C.
5. Wash Buffer concentrate (10X): Store between 2-25°C. Diluted wash buffer (1X) is stable at room temperature (20 to 25 °C) for up to 7 days or for 30 days between 2-8°C.

REFERENCES