THYROGLOBULIN IgG QUANTITATIVE TEST

INTENDED USE

The ImmunoWELL Thyroglobulin IgG Quantitative Test is a quantitative enzyme immunoassay (EIA) for screening and detection of autoantibodies against human thyroglobulin in serum and is used as an aid in the diagnosis of thyroid disorders.

SUMMARY AND EXPLANATION

Autoimmune thyroid gland disorders are characterized by detection of anti-thyroid antibodies, primarily against thyroglobulin and microsomal thyroid antigens. Thyroglobulin autoantibodies occur in sera of most autoimmune thyroid disease patients.

Thyroglobulin antibody level correlates with the degree of lymphoid infiltration of the thyroid gland. It is reported that levels of autoimmune antibodies predict at-risk pregnancy. Furthermore, an EIA test for anti-thyroglobulin and anti-recombinant TPO demonstrated a 100% increase in the rate of spontaneous miscarriage in women who had detectable serum thyroid autoantibodies in their first trimester of pregnancy.

Thyroid autoantibodies are detected using immunoassays such as passive hemagglutination (HAd), indirect fluorescent antibody (IFA), and radioimmunoassay (RIA) techniques. ImmunoWELL Thyroglobulin IgG Quantitative Test uses human thyroglobulin in an EIA test format.

ASSAY PRINCIPLE

The ImmunoWELL Test uses an EIA microtiter plate technique for the detection of antibodies. Serum is added to antigen coated microtiter wells and allowed to react. After removal of unbound antibodies, horseradish peroxidase-conjugated antihuman IgG antibodies are allowed to react with bound antibodies. The bound peroxidase reacts with tetramethylbenzidine (TMB), the chromogenic substrate, developing a color. Finally, the substrate reaction is stopped and the optical density is read with a microwell spectrophotometer.

REAGENTS

Reaction Wells coated with purified, human thyroglobulin
Specimen Diluent - 0.01M phosphate buffered saline (PBS, pH 6.2-7.6) and carrier protein and <0.1% NaN₃
Calibrators (5) - human anti-thyroglobulin prediluted, ready for use
Positive Control - human anti-thyroglobulin serum containing <0.1% NaN₃
Negative Control - nonreactive human serum containing <0.1% NaN₃
Wash Buffer Concentrate - a 20X concentrate of 0.01 M PBS (pH 6.2-7.6) and 0.05% Tween
Conjugate - peroxidase-conjugated goat antihuman IgG in PBS (pH 6.2-7.6) and carrier protein containing preservatives
Substrate - tetramethylbenzidine (TMB).
Stop Solution - 0.5 N hydrochloric acid

Performance Considerations

Reproducibility in the assay is largely dependent upon the consistency with which the microwells are washed. Carefully follow the recommended washing sequence as outlined in the assay procedure.

Materials Provided

Microtiter Wells in Carrier SPECIMEN DILUENT
Calibrators Positive Control
Negative Control Wash Buffer Concentrate (20X)
Conjugate Substrate
Stop Solution Package Insert

Materials Required But Not Provided

Distilled or deionized water Pipets
Microwell washer Test tubes
Microwell spectrophotometer (450 nm)

PROCEDURE

1. Allow all components including diluted Wash Buffer to warm to room temperature (22-27°C).
2. Determine the total number of specimens to be run. Include one blank and duplicates of calibrators and controls in each run.
3. For each control and specimen, pipet 10 µL serum into a clean tube containing 1 mL Specimen Diluent and mix (1:100 dilution).
4. Determine the total number of wells to be run including blank, calibrators, controls, and specimens. Well strips can be broken to the exact number needed to conserve reagent wells. Strips need to be completed with used wells to facilitate washing procedures.
5. Add 100 µL of Specimen Diluent into the first well as a substrate blank.

Warning - Potential Biohazardous Material: Human sera used in the preparation of this product were tested using FDA approved procedures and found non-reactive for hepatitis B surface antigen and for antibodies to HIV-1, HIV-2, and hepatitis C virus. Because no test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting disease.

Reconstitution and Storage

Kit is stored at 2-8°C until stated expiration date.

Reaction wells are removed from the foil pouch and unused wells are resealed in the pouch using the integral zip-lock.

Wash Buffer (pH 6.2-7.6) is prepared by adding the contents of the Wash Buffer Concentrate (20X) bottle into 1 liter of distilled/deionized water. After reconstitution, the 1X solution is stored at 2-8°C. Discard when visibly turbid.

Note: In some instances the Wash Buffer Concentrate (20X) may develop crystals upon storage at 2-8°C. It is important that these crystals are completely redissolved before dilution of the Concentrate. This can be accomplished by warming the Concentrate to 37°C in a water bath with occasional mixing.

SPECIMEN COLLECTION AND HANDLING

ImmunoWELL Test is performed on serum. The test requires 10 µL of serum. Lipemic or hemolyzed serum has not been shown an acceptable specimen.

Store samples at room temperature for no longer than eight hours. If the assay will not be completed within eight hours, refrigerate the sample at 2-10°C. If the assay or shipment of the samples will not be completed within 48 hours, freeze at –20°C.
6. Pipet 100 µL of the prediluted calibrators and diluted controls and specimens (step 3) into each assigned well.
7. Incubate at room temperature (22–27°C) for 30±2 minutes.
8. Aspirate the samples out of the wells.
9. Wash the wells three times by completely filling the wells with Wash Buffer (see Reconstitution and Storage) and aspirating the wells completely after washes.
10. Pipet 100 µL Conjugate into all wells.
11. Incubate the wells at room temperature (22–27°C) for 30±2 minutes.
12. Aspirate the conjugate out of the wells.
13. Wash the wells three times as described in step 9.
14. Pipet 100 µL of Substrate into each well.
15. Incubate at room temperature (22–27°C) for 30±2 minutes.
16. Add 100 µL of Stop Solution to each well.
17. Inspect the outside bottom surface of the microwells for the presence of condensation, dried buffer salts or wash solution which might interfere with the spectrophotometric reading. Carefully clean the well bottoms with a soft tissue.
18. Using the substrate blank to zero the spectrophotometer, read the optical density of each well at 450 nm within 30 minutes of completion of step 16.

Quality Control
GenBio furnishes positive and negative controls with defined ranges provided in a Supplement included in the kit. The positive control value is approximately five standard deviations (absorbance) above the upper cutoff. Interpretations should not be made unless the control results fall within the defined limits. NCCLS C24-A should be consulted for guidance on appropriate quality control practices. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

INTERPRETATION

Procedure for Calculating Activity of Specimen

ImmuNOWell calibrators are assigned values of antibody concentration (IU/mL) which may vary by lot number. Please verify that the lot numbers on the vials match the lot numbers on the Package Insert Supplement to assure the proper values are used in the calculation.

Construct a point-to-point standard curve using the absorbance values you observe and their corresponding assigned values. Use this curve to calculate antibody concentration of controls and specimens.

The interpretive ranges are:

<table>
<thead>
<tr>
<th>IU/mL</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;85</td>
<td>Specific Antibody not detected</td>
</tr>
<tr>
<td>85 – 120</td>
<td>Report as negative or retest. If retested, the second result is considered final. If the repeat test is also equivocal, report as equivocal.</td>
</tr>
<tr>
<td>&gt;120</td>
<td>Specific antibody detected.</td>
</tr>
</tbody>
</table>

These ranges are determined using normal sera. The borderline range is defined as lying between two and three standard deviation units above the mean of normals. It is recommended that laboratories confirm these ranges.

LIMITATIONS

Epidemiologic factors, clinical findings, and other laboratory results should be considered in addition to autoantibody laboratory results for diagnosis of the patient.

Thyroid autoantibodies may be present in non-thyroid disorders such as Sjögren’s syndrome, pernicious anemia, Addison’s disease, myasthenia gravis and diabetes mellitus and in apparently healthy subjects.

EXPECTED RESULTS

Asymptomatic subjects may have low anti-thyroglobulin levels. The significance of low level anti-thyroglobulin is not established. Figure 1 illustrates various types of results that were observed in three study populations. Sites A and B are reference laboratories and the results are those of samples submitted for anti-thyroglobulin testing. It should be noted that samples submitted to Site A contained significantly more positives than did samples submitted to reference laboratory B. The Blood Bank samples are sera collected from asymptomatic blood donors. Note that a small percentage of asymptomatic normals report a low positive result.

![Figure 1](image)

**Performance Characteristics**

<table>
<thead>
<tr>
<th>HA Titer</th>
<th>Nonreactive</th>
<th>Borderline</th>
<th>Reactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>2</td>
<td>1</td>
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</tr>
<tr>
<td>40</td>
<td>5</td>
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<td>80</td>
<td>2</td>
<td>2</td>
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<td>160</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>320</td>
<td>1</td>
<td>0</td>
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<td>640</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>1280</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>5120</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

The thyroglobulin hemagglutination test kit package insert classifies less than 1:160 HA titer as low and greater than or equal to 1:640 HA titer as moderate to high reactivity. Based on this classification, the ImmunoWELL test is 100% sensitive with respect to the hemagglutination test method. Table 1 shows that further discrimination of specific anti-thyroglobulin reactions is possible using an EIA assay. Based on observations of others using an EIA assay with purified thyroglobulin antigen, assay improvement is expected.

The reproducibility of the assay was evaluated by testing a variety of samples in four assay runs. Each sample was repeatedly tested six times within each assay run. The within assay and between assay variation is presented in Table 2.
Table 2: Thyroglobulin Assay Reproducibility

<table>
<thead>
<tr>
<th>MEAN</th>
<th>WITHIN ASSAY VARIATION</th>
<th>BETWEEN ASSAY VARIATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.08</td>
<td>0.04 (4%)</td>
<td>0.04 (4%)</td>
</tr>
<tr>
<td>0.76</td>
<td>0.03 (4%)</td>
<td>0.05 (7%)</td>
</tr>
<tr>
<td>0.34</td>
<td>0.02 (6%)</td>
<td>0.02 (6%)</td>
</tr>
<tr>
<td>0.51</td>
<td>0.02 (4%)</td>
<td>0.05 (10%)</td>
</tr>
<tr>
<td>0.79</td>
<td>0.03 (4%)</td>
<td>0.17 (22%)</td>
</tr>
<tr>
<td>1.05</td>
<td>0.03 (3%)</td>
<td>0.04 (4%)</td>
</tr>
<tr>
<td>0.06</td>
<td>0.01 (17%)</td>
<td>0.00 (NA)</td>
</tr>
<tr>
<td>0.04</td>
<td>0.01 (25%)</td>
<td>0.01 (25%)</td>
</tr>
</tbody>
</table>

Variation is presented as Standard Deviation (Coefficient of Variation).
NA = not applicable

BIBLIOGRAPHY

7. Centers for Disease Control/National Institutes of Health manual Biosafety in Microbiological and Biomedical Laboratories (1983)

QUICK REFERENCE PROCEDURE

**ImmunoWELL Thyroglobulin IgG Quantitative Test**

- Prepare Wash Buffer from Wash Concentrate
- Dilute each control and specimen 1:100 in Specimen Diluent
- Add 100 µL of Specimen Diluent into the first well as a substrate blank
- Pipet 100 µL of the prediluted calibrators and diluted controls and specimens into coated microwells and incubate 30 minutes at room temperature
- Aspirate microwells and wash microwells three times with Wash Buffer
- Pipet 100 µL of Conjugate into microwells and incubate 30 minutes at room temperature
- Aspirate microwells and wash microwells three times with Wash Buffer
- Pipet 100 µL of Substrate into microwells and incubate 30 minutes at room temperature
- Pipet 100 µL Stop Solution into microwells and read results at 450 nm

To place an order for ImmunoWELL products, contact your local distributor or call GenBio directly for the distributor nearest you and for additional product information.
For assistance, please call toll-free 800-288-4368.

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