ImmunoWELL ™

CE

TPO IgG QUANTITATIVE TEST

Product No. 3020

IVD **For *In Vitro* Diagnostic Use**

INTENDED USE

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

SUMMARY AND EXPLANATION[1,2]

Autoimmune thyroid gland disorders are characterized by detection of anti-thyroid antibodies, primarily against thyroglobulin and microsomal thyroid antigens. Recently it has been shown that thyroid peroxidase (TPO) is the protein responsible for microsomal antigenicity[3]. TPO autoantibodies occur in sera of most autoimmune thyroid disease patients and predict raised serum TSH levels in random populations, but do not necessarily imply tissue destruction.

TPO antibody level correlates with the degree of lymphoid infiltration of the thyroid gland[4]. It is reported that low levels of autoimmune antibodies predict at-risk pregnancy[5,6]. Furthermore, a report using 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[7].

Thyroid autoantibodies are detected using immunoassays such as passive hemagglutination (HAd), indirect fluorescent antibody (IFA), enzyme immunoassay (EIA), and radioimmunoassay (RIA) techniques. ImmunoWELL TPO IgG Quantitative Test uses recombinant human thyroid peroxidase which does not contain contaminating thyroglobulin and/or mitochondria found in other microsome antigen preparations 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, recombinant human thyroid peroxidase (TPO)

Specimen Diluent - 0.01M phosphate buffered saline (PBS, pH 6.2-7.6) and carrier protein and <0.1% NaN_3

Calibrators (5) - human anti-TPO prediluted, ready for use

Positive Control - human anti-TPO serum containing <0.1% NaN_3

Negative Control - nonreactive human serum containing <0.1% NaN_3

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

Warnings and Precautions

For *In Vitro* Diagnostic Use: ImmunoWELL reagents have been optimized for use as a system. Do not substitute other manufacturers' reagents or other ImmunoWELL Test reagents. Dilution or adulteration of these reagents may also affect the performance of the test. Do not use any kits beyond the stated expiration date. Close adherence to the test procedure will assure optimal performance. Do not shorten or lengthen stated incubation times since this may result in poor assay performance.

Some reagents contain sodium azide that may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

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.[8]

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.

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)	

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.

Positive and Negative Control Sera (undiluted) are used to assure test performance.

Calibrators (prediluted) are used to construct a standard curve.

Substrate Blank - All reagents, except serum, are added to the substrate blank well. This blank well is intended to baseline (zero) the microwell spectrophotometer.

Assay 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).

CAUTION: Calibrators are prediluted. Do not dilute further.

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.
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:

	IU/mL	Interpretation
Negative	<45	Specific Antibody not detected
Equivocal	45 – 65	Report as negative or retest. If retested, the second result is considered final. If the repeat test is also equivocal, report as equivocal.
Positive	>65	Specific antibody detected.

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

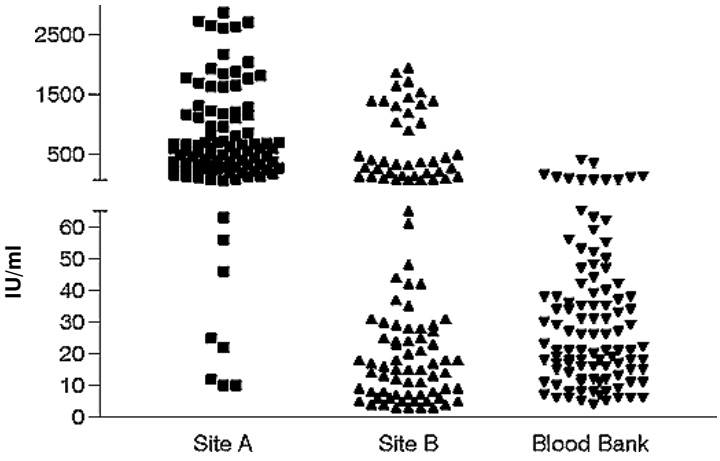
Epidemiologic factors, clinical findings, and other laboratory results should be considered in addition to autoantibody laboratory results for diagnosis of the patient.

Thyroid autoantibodies may be present in non-thyroid disorders such as Sjögren's syndrome, pernicious anemia, Addison's disease, myasthenia gravis and diabetes mellitus[2,9,10] and in apparently healthy subjects[3].

EXPECTED RESULTS

Asymptomatic subjects may have low anti-TPO levels[2,4,9,10]. The significance of low-level anti-TPO 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-TPO 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



PERFORMANCE CHARACTERISTICS

ImmunoWELL TPO IgG Quantitative Test was tested in presumptive normal samples and microsome positive (HAd) samples. The results for samples from asymptomatic, normal subjects (n=98) predict assay specificity. These data predict the TPO test to be 99% (97/98) specific and are consistent with previous reports of low level anti-TPO in the populations[2,4,9,10]. Three additional specimens were indicated as borderline reactions.

One hundred one (101) passive hemagglutination (HAd) positive samples were evaluated in the ImmunoWELL test to assess test sensitivity compared to the classic hemagglutination test method. These results are shown in Table 1

Table 1: Microsome (TPO) Assay Sensitivity

	ImmunoWELL Result		
HA Titer	Nonreactive	Borderline	Reactive
100	1	4	0
400	4	8	20
1600	0	0	40
6400	0	0	16
25600	0	0	8

The microsome hemagglutination test kit package insert classifies less than 1:1600 HAd titer as low and greater than or equal to 1:6400 HAd 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-TPO reactions are possible using an EIA assay. Based on observations of others using an EIA assay with recombinant TPO antigen[7,11], assay improvement is expected.

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

Table 2: Microsome (TPO) Assay Reproducibility

MEAN	WITHIN ASSAY VARIATION	BETWEEN ASSAY VARIATION
0.74	0.05 (7%)	0.03 (4%)
0.72	0.02 (3%)	0.05 (7%)
0.43	0.03 (7%)	0.03 (7%)
0.90	0.04 (4%)	0.05 (6%)
0.92	0.04 (4%)	0.03 (3%)
0.85	0.02 (2%)	0.02 (2%)
0.05	0.01 (20%)	0.02 (40%)
0.06	0.01 (17%)	0.01 (17%)

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

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QUICK REFERENCE PROCEDURE

ImmunoWELL

TPO 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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