QUANTA Lite® TPO ELISA  
708725
For In Vitro Diagnostic Use  
CLIA Complexity: High

Principles of the Procedure
Purified human TPO antigen is bound to the wells of a polystyrene microwell plate under conditions that will preserve the antigen in its native state. Pre-diluted controls and diluted patient sera are added to separate wells, allowing any microsomal antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgG conjugate is added to each well. A second incubation allows the enzyme labeled anti-human IgG to bind to any patient antibodies, which have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgG, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. The assay can be evaluated spectrophotometrically by measuring and comparing the color intensity that develops in the patient wells with the color in the control wells.

Circulating thyroid autoantibodies have been widely implicated in the etiology of autoimmune thyroid disease and both thyroglobulin and microsomal antibodies are measured routinely in clinical practice. Serum autoantibodies to thyroid microsomal antigen(s) are commonly found in patients with thyroid autoimmune diseases and their presence correlates well with histological changes in Hashimoto's thyroiditis. Antibodies to thyroid microsomal antigens are positive in 70-90% of patients with chronic thyroiditis. These antibodies are also found in 64% of patients with primary hypothyroidism, 50% with thyrotoxicosis, 10% with simple goiters and 17% with thyroid tumors. Thyroglobulin autoantibodies are detected at high titers, mainly in autoimmune thyroiditis and Graves' disease. Serum autoantibodies to thyroglobulin/colloid have been found in 40-70% of patients with chronic thyroiditis and in smaller percentages of patients with thyrotoxicosis and nontoxic goiters.

It has become apparent that the main antigen found in thyroid microsomes is the enzyme thyroid peroxidase or TPO. Assays constructed with purified TPO have several performance advantages over assays that use the relatively cruder whole microsome preparations that may contain thyroglobulin as well as other yet unidentified antigens. Because autoantibody binding to TPO has been shown to be at conformational epitopes, possibly at or near the active enzymatic site of the molecule, native, affinity purified antigens may have some performance advantages over recombinant or synthetic antigens.

Thyroid autoantibodies have been investigated classically by means of precipitation reactions, latex fixation and by immunofluorescence. Recently newer ELISA techniques have been developed. These ELISA procedures have been found to be more sensitive than either hemagglutination or immunofluorescence and at the same time are more objective and not subject to interference by agglutination inhibition factors or heterophile antibodies.

The ELISA technique employed in this test is sensitive, specific and objective. It can be conveniently used to test both large and small numbers of samples.

QUANTA Lite® TPO is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of thyroid peroxidase (TPO) autoantibodies in human serum. The presence of antibodies to human peroxidase antigen can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of autoimmune thyroid diseases such as Hashimoto's Thyroiditis and Graves' Disease.
Reagents
1. Polystyrene microwell ELISA plate coated with a purified TPO antigen (12-1 x 8 wells), with holder in foil package containing desiccants
2. ELISA Negative Control, 1 vial of buffer containing preservative and human serum with no human antibodies to thyroid peroxidase, prediluted, 1.2mL
3. TPO ELISA Control, 1 vial of buffer containing preservative and human serum human antibodies to thyroid peroxidase, prediluted, 1.2mL
4. TPO ELISA Calibrator A, 1 vial of buffer containing preservative and human serum antibodies to thyroid peroxidase, prediluted, 1.2mL
5. TPO ELISA Calibrator B, 1 vial of buffer containing preservative and human serum antibodies to thyroid peroxidase, prediluted, 1.2mL
6. TPO ELISA Calibrator C, 1 vial of buffer containing preservative and human serum antibodies to thyroid peroxidase, prediluted, 1.2mL
7. TPO ELISA Calibrator D, 1 vial of buffer containing preservative and human serum antibodies to thyroid peroxidase, prediluted, 1.2mL
8. TPO ELISA Calibrator E, 1 vial of buffer containing preservative and human serum antibodies to thyroid peroxidase, prediluted, 1.2mL
9. HRP Sample Diluent, 1 vial – colored pink containing Tris-buffered saline, Tween 20, protein stabilizers and preservative, 50mL
10. HRP Wash Concentrate, 1 vial of 40x concentrate - colored red containing Tris-buffered saline and Tween 20, 25mL. Refer to the Methods Section for dilution instructions.
11. HRP IgG Conjugate, (goat), anti-human IgG, 1 vial – colored blue containing buffer, protein stabilizers and preservative, 10mL
12. TMB Chromogen, 1 vial containing stabilizers, 10mL
13. HRP Stop Solution, 0.344M Sulfuric Acid, 1 vial – colorless, 10mL

Materials Provided
1. TPO ELISA microwell plate (12-1 x 8 wells), with holder
2. 1.2mL prediluted ELISA Negative Control
3. 1.2mL prediluted TPO ELISA Control
4. 1.2mL prediluted TPO ELISA Calibrator A
5. 1.2mL prediluted TPO ELISA Calibrator B
6. 1.2mL prediluted TPO ELISA Calibrator C
7. 1.2mL prediluted TPO ELISA Calibrator D
8. 1.2mL prediluted TPO ELISA Calibrator E
9. 50mL HRP Sample Diluent
10. 25mL HRP Wash Concentrate, 40x concentrate
11. 10mL HRP IgG Conjugate, (goat), anti-human IgG
12. 10mL TMB Chromogen
13. 10mL HRP Stop Solution, 0.344M Sulfuric Acid

Additional Materials Required But Not Provided
Micropipets to deliver 5, 100, 200-300 and 500µL
Disposable micropipet tips
Test tubes for patient sample dilutions, 4mL volume
Distilled or deionized water
1L container for diluted HRP Wash Concentrate
Microwell plate reader capable of measuring OD at 450nm (and 620nm for dual wavelength readings)

Specimen
Specimen Collection
This procedure should be performed with a serum specimen. Addition of azide or other preservatives to the test samples may adversely affect the results. Microbially contaminated, heat-treated, or specimens containing visible particulate should not be used. Grossly hemolyzed or lipemic serum or specimens should be avoided.

Following collection, the serum should be separated from the clot. CLSI (formerly NCCLS) Document H18-A2 recommends the following storage conditions for samples: 1) Store samples at room temperature no longer than 8 hours. 2) If the assay will not be completed within 8 hours, refrigerate the sample at 2-8°C. 3) If the assay will not be completed within 48 hrs., or for shipment of the sample, freeze at -20°C or lower. Frozen specimens must be mixed well after thawing and prior to testing.
Special Safety Precautions/Storage Conditions
1. Store all the kit reagents at 2-8°C. Do not freeze. Reagents are stable until the expiration date when stored and handled as directed.
2. Unused antigen coated microwell strips should be resealed securely in the foil pouch containing desiccants and stored at 2-8°C.
3. Diluted wash buffer is stable for 1 week at 2-8°C.

Procedural Notes:
Warnings
1. WARNING: This product contains a chemical (0.02% chloramphenicol) in the sample diluent, controls, and conjugate known to the State of California to cause cancer.
2. All human source material used in the preparation of controls for this product has been tested and found negative for antibody to HIV, HBsAg, and HCV by FDA cleared methods. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the TPO ELISA Control, TPO ELISA Calibrators and ELISA Negative Control should be handled in the same manner as potentially infectious material.¹⁹
3. Sodium Azide is used as a preservative. Sodium Azide is a poison and may be toxic if ingested or absorbed through the skin or eyes. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. Flush sinks, if used for reagent disposal, with large volumes of water to prevent azide build-up.
4. The HRP conjugate contains a dilute poisonous/corrosive chemical, which may be toxic if ingested in large amounts. To prevent possible chemical burns, avoid contact with skin and eyes.
5. TMB Chromogen contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
6. The HRP Stop Solution consists of a dilute sulfuric acid solution. Avoid exposure to bases, metals, or other compounds, which may react with acids. Sulfuric acid is a poison and corrosive, which may be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
7. Use appropriate personal protective equipment while working with the reagents provided.
8. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

Precautions
1. This product is for In Vitro Diagnostic Use.
2. Substitution of components other than those provided in this system may lead to inconsistent results.
3. Incomplete or inefficient washing and insufficient liquid removal from the ELISA well strips will cause poor precision and/or high background.
4. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
5. A variety of factors influence the assay performance. These include the starting temperature of the reagents, the ambient temperature, the accuracy and reproducibility of the pipetting technique, the thoroughness of washing and liquid removal from the wells of the ELISA strips, the photometer used to measure the results, and the length of the incubation times during the assay. Careful attention to consistency is required to obtain accurate and reproducible results.
6. Strict adherence to the protocol is recommended.
7. Incomplete resealing of the zip-lock pouch containing microwell strips and desiccants will result in antigen degradation and poor precision.
8. Unacceptably low absorbencies may be observed following two or more uses from a single bottle of HRP conjugate over a period of time. It is important to follow all recommended HRP conjugate handling procedures to prevent this occurrence.
9. Chemical contamination of the HRP conjugate can result from improper cleaning or rinsing of equipment or instruments. Residues from common laboratory chemicals such as formalin, bleach, ethanol or detergent will cause degradation of the HRP conjugate over time. Thoroughly rinse all equipment or instruments after the use of chemical cleaners/disinfectants.
Quality Control
1. The TPO ELISA Control, the TPO ELISA Calibrators A through E and the ELISA Negative Control should be run with every batch of samples to ensure that all reagents and procedures perform properly.
2. Note that since the TPO ELISA Control, the TPO ELISA Calibrators A through E and the ELISA Negative Control are prediluted, they do not control for procedural methods associated with dilution of specimens.
3. Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations. Additional suitable control sera may be prepared by aliquoting pooled human serum specimens and storing at < -20°C.
4. In order for the test results to be considered valid, all of the criteria listed below must be met. If any of these are not met, the test should be considered invalid and the assay repeated.
   a. The absorbance of the prediluted TPO ELISA Calibrator A must be greater than the absorbance of the prediluted TPO ELISA Control, which must be greater than the absorbance of the prediluted ELISA Negative Control.
   b. The prediluted TPO ELISA Calibrator A must have an absorbance greater than 1.0 while the prediluted ELISA Negative Control absorbance cannot be over 0.2.
   c. The TPO ELISA Control absorbance must be more than twice the ELISA Negative Control or over 0.25.
   d. The TPO ELISA Control concentration must be within the range stated on its label.
   e. The user should refer to CLSI (formerly NCCLS) Document C24-A3 for additional guidance on appropriate QC practices.

Procedure:
Method Before you start
1. Bring all reagents and samples to room temperature (20-26°C) and mix well.
2. Dilute the HRP Wash Concentrate 1:40 by adding the contents of the HRP Wash Concentrate bottle to 975mL of distilled or deionized water. If the entire plate will not be run within this period, a smaller quantity can be prepared by adding 2.0mL of the concentrate to 78mL of distilled or deionized water for every 16 wells that will be used. The diluted buffer is stable for 1 week at 2-8°C.
3. Prepare a 1:101 dilution of each patient sample by adding 5µL of sample to 500µL of HRP Sample Diluent. Diluted samples must be used within 8 hours of preparation. DO NOT DILUTE the TPO ELISA Control, TPO ELISA Calibrators and ELISA Negative Control.
4. Determination of the presence or absence of TPO antibodies requires two wells for each of the calibrators and controls and one or two wells for each patient sample. It is recommended that samples be run in duplicate.
5. Preparation of standard curve. For a 5 point standard curve, use PREDILUTED TPO ELISA Calibrators A through E directly from the vial. The five point standard curve has the following values:
<table>
<thead>
<tr>
<th>Point</th>
<th>TPO WHO Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1000.0</td>
</tr>
<tr>
<td>B</td>
<td>500.0</td>
</tr>
<tr>
<td>C</td>
<td>250.0</td>
</tr>
<tr>
<td>D</td>
<td>125.0</td>
</tr>
<tr>
<td>E</td>
<td>62.5</td>
</tr>
</tbody>
</table>

Assay Procedure
1. **ALL REAGENTS MUST BE BROUGHT TO ROOM TEMPERATURE (20-26°C) PRIOR TO BEGINNING THE ASSAY.** Place the required number of microwells/strips in the holder. Immediately return unused strips to the pouch containing desiccants and seal securely to minimize exposure to water vapor.
2. Add 100µL of the prediluted TPO ELISA Control, the TPO ELISA Calibrators A through E, the ELISA Negative Control and the diluted patient samples to the wells. Cover the wells and incubate for 30 minutes at room temperature on a level surface. The incubation time begins after the last sample addition.
3. Wash step: Thoroughly aspirate the contents of each well. Add 200-300µL of the diluted HRP Wash buffer to all wells then aspirate. Repeat this sequence twice more for a total of three washes. Invert the plate and tap it on absorbent material to remove any residual fluid after the last wash. It is important to completely empty each well after each washing step. Maintain the same sequence for the aspiration as was used for the sample addition.
4. Add 100µL of the HRP IgG Conjugate to each well. Conjugate should be removed from the bottles using standard aseptic conditions and good laboratory techniques. Remove only the amount of conjugate from the bottle necessary for the assay. **TO AVOID POTENTIAL MICROBIAL AND/OR CHEMICAL CONTAMINATION, NEVER RETURN UNUSED CONJUGATE TO THE BOTTLE.** Incubate the wells for 30 minutes as in step 2.
5. Wash step: Repeat step 3.
6. Add 100µL of TMB Chromogen to each well and incubate in the dark for 30 minutes at room temperature.
7. Add 100µL of HRP Stop Solution to each well. Maintain the same sequence and timing of HRP Stop Solution addition as was used for the TMB Chromogen. Gently tap the plate with a finger to thoroughly mix the wells.
8. Read the absorbance (OD) of each well at 450nm within one hour of stopping the reaction. If bichromatic measurements are desired, 620nm can be used as a reference wavelength.

Reporting Results:
Interpretation of Results
The ELISA assay is very sensitive to technique and is capable of detecting even small differences in patient populations. The values shown below are suggested values only. Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.
1. A positive result indicates the presence of thyroid peroxidase antibodies and suggests the possibility of Hashimoto's Thyroiditis and/or Graves' Disease.
2. A negative result indicates no thyroid peroxidase antibody or levels below the negative cut-off of the assay.
3. It is suggested that the results reported by the laboratory should include the statement: "The following results were obtained with the INOVA QUANTA Lite® TPO ELISA. Thyroid peroxidase values obtained with different manufacturers' assay methods may not be used interchangeably. The magnitude of the reported IgG levels cannot be correlated to an endpoint titer."

Calculations
Calculation of Results
1. Determine a mean value for all duplicate readings.
2. Plot the mean absorbance (OD) of the Calibrator curve against the log of their concentrations. Use a line of best fit or a point-to-point plot. Alternatively a log/log plot may be used.
3. Determine the unknown TPO concentration in units (WHO) from the "X" axis by reading corresponding absorbance on the "Y" axis. Calibrators and Control for this kit are referenced to the WHO reference standards available from WHO, Reference Preparation MRC 66/387.
4. Negative values range from 0-100 units. Positive results are greater than 100 units.
5. Over-range samples with ODs above the S1 standard can be reported out as > 1000 units. Alternatively, the sample can be diluted further (i.e., 1:2 and 1:4 in HRP Sample Diluent) and retested to bring the OD into the assay range spanned by the standard curve.

Below is an example of a typical standard curve.

<table>
<thead>
<tr>
<th>Point</th>
<th>Mean OD</th>
<th>Concentration (WHO units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.577</td>
<td>1000.0</td>
</tr>
<tr>
<td>B</td>
<td>1.172</td>
<td>500.0</td>
</tr>
<tr>
<td>C</td>
<td>0.790</td>
<td>250.0</td>
</tr>
<tr>
<td>D</td>
<td>0.472</td>
<td>125.0</td>
</tr>
<tr>
<td>E</td>
<td>0.306</td>
<td>62.5</td>
</tr>
</tbody>
</table>

Expected Values
Normal Range
A total of 198 random normal samples were run. This group included 104 males ranging in age from 20-72, and 94 females ranging in age from 15-63. Eleven samples or 5.5% were found to be positive. Six of the 11 positive samples were from females and the remaining 5 were from males. Seven of these 11 positive normal samples were also found to be positive by an ELISA method for thyroid microsomal antibodies and/or another commercial TPO ELISA. The mean value for the normal male population was 17 units and a value of 36 units was obtained for the normal female population.

Relative Sensitivity and Specificity
The ability of the QUANTA Lite® TPO ELISA to detect autoantibodies to thyroid peroxidase (TPO) was evaluated by comparison to another commercially available TPO ELISA test. A total of 88 samples were tested. Sixty-nine samples submitted to a reference lab for thyroid autoantibody testing as well as 19 additional normal samples were tested. The results are summarized below:
Relative Sensitivity 90.8%
Relative Specificity 100%
Relative Efficiency 92.6%

* One of these samples was from a normal population and 4 were found to be negative by both immunofluorescence and by ELISA against thyroid microsomes.

In another study, the QUANTA Lite® TPO test was also compared against another ELISA method for detecting autoantibodies against whole human thyroid microsome. The results are summarized below:

INOVA TPO
+ -
+ 67 7* Relative Sensitivity 80.5%
Reference TPO
- 0 19 Relative Specificity 92.7%
- 17** 200 Relative Efficiency 89.7%

* 13 of these 15 had high levels of thyroglobulin antibody
** 16 of these 17 were also positive using a reference TPO ELISA

A third study compared the QUANTA Lite® TPO ELISA against an indirect immunofluorescent procedure, which uses primate thyroid sections as substrate. The results are summarized below:

INOVA TPO
+ -
+ 19 0 Relative Sensitivity 100%
Indirect Immunofluorescence
- 5* 17 Relative Specificity 81.5%
Relative Efficiency 89%

* All 5 of these samples were also positive by a reference TPO ELISA.

** Precision and Reproducibility**

The precision and reproducibility of the assay was measured by running six replicates each of a negative, moderate positive and strong positive sample in six separate assays. The mean value of the strong positive was 815 units, the moderate positive was 294 units and the value for the negative was 53 units. The standard deviation and coefficient of variation for each sample are summarized below.

<table>
<thead>
<tr>
<th></th>
<th>Negative</th>
<th>Strong Positive</th>
<th>Moderate Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
<td>CV</td>
<td>SD</td>
</tr>
<tr>
<td>Overall</td>
<td>6.0</td>
<td>11.1%</td>
<td>44.4</td>
</tr>
<tr>
<td>Within-Run</td>
<td>4.6</td>
<td>8.5%</td>
<td>33.5</td>
</tr>
<tr>
<td>Between-Run</td>
<td>6.6</td>
<td>12.5%</td>
<td>45.2</td>
</tr>
</tbody>
</table>

**Limitations of the Procedure**

1. The presence of immune complexes or other immunoglobulin aggregates in the patient sample may cause an increased level of non-specific binding and produce false positives in this assay.
2. Not all Hashimoto’s thyroiditis patients are positive for microsomal or TPO antibodies.
3. Results of this assay should be used in conjunction with clinical findings and other serological tests.
4. The assay performance characteristics have not been established for matrices other than serum.
References