Primary biliary cirrhosis (PBC) is a chronic liver disease characterized by the destruction of the small intrahepatic bile ducts. Progressive duct destruction leads to increasing functional impairment of the liver and, over time, can lead to liver failure and the necessity of liver transplantation. The etiology of PBC is unknown, although a genetic component as well as other factors may be important for the development of the disease. PBC typically occurs between the ages of 30 and 65 and affects women more frequently than men (estimated female : male ratio of 9:1). The prevalence of PBC in first-degree relatives of PBC patients ranges from 1.3 to 6.4%. PBC is found in all races and has a worldwide distribution. Wide variations in geographic prevalence of PBC have been reported, from estimates of 2 per 100,000 in Japan and Australia to 40 per 100,000 in the United States.

In patients with suspected autoimmune liver disease, but not satisfying all the classical criteria, can be clinically challenging. Individuals with “AMA-negative” PBC fall into this group. New assays for detection of antibodies to gp210, sp100 and AMA utilizing the enhanced sensitivity MIT3 antigen, have reduced the number of PBC patients without serological evidence of PBC. The QUANTA Lite™ PBC Screen IgG/IgA detects antibodies to the MIT3, sp100 and gp210 PBC-specific antigens. The dual specificity conjugate (IgG/IgA) offers increased sensitivity for detection of samples that may be weak or negative on testing with a monospecific (IgG only) conjugate. The combined testing for IgG and IgA AMA antibodies are present in the serum, bile, saliva and urine of individuals with PBC. It has been suggested that this secretory anti-PBC IgA may play a role in the pathogenesis of PBC. Patients with suspected autoimmune liver disease, but not satisfying all the classical criteria, can be clinically challenging. Individuals with “AMA-negative” PBC fall into this group. New assays for detection of antibodies to gp210, sp100 and AMA utilizing the enhanced sensitivity MIT3 antigen, have reduced the number of PBC patients without serological evidence of PBC.

Reagents
1. Polystyrene microwell ELISA plate coated with a PBC Screen antigen (mixture of purified MIT3, sp100 and gp210 antigens) (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 PBC Screen antigens, prediluted, ready to use 1.2mL
3. PBC Screen IgG/IgA ELISA Low Positive, 1 vial of buffer containing preservative and human serum antibodies to PBC Screen antigen, prediluted, ready to use 1.2mL
4. PBC Screen IgG/IgA ELISA High Positive, 1 vial of buffer containing preservative and human serum antibodies to PBC Screen antigen prediluted, ready to use 1.2mL
5. HRP Sample Diluent, 1 vial – colored pink containing Tris-buffered saline, Tween 20, protein stabilizers and preservative, 50mL
6. 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.
7. HRP PBC Screen IgG/IgA Conjugate, (goat), anti-human IgG/IgA, 1 vial – colorless containing buffer, protein stabilizers and preservative, 10mL
8. TMB Chromogen, 1 vial containing stabilizers, 10mL
9. HRP Stop Solution, 0.344M Sulfuric Acid, 1 vial – colorless, 10mL

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 PBC Screen IgG/IgA ELISA Low Positive, PBC Screen IgG/IgA ELISA High Positive and ELISA Negative Control should be handled in the same manner as potentially infectious material.15
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.

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.
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 (NCCLS) Document H18-A3 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 hours, or for shipment of the sample, freeze at -20°C or lower. Frozen specimens must be mixed well after thawing and prior to testing.16

Procedure

Materials provided
1. PBC Screen IgG/IgA ELISA microwell plate (12-1 x 8 wells), with holder
2. 1.2mL prediluted ELISA Negative Control
3. 1.2mL prediluted PBC Screen IgG/IgA ELISA Low Positive
4. 1.2mL prediluted PBC Screen IgG/IgA ELISA High Positive
5. 50mL HRP Sample Diluent
6. 25mL HRP Wash Concentrate, 40x concentrate
7. 10mL HRP PBC Screen IgG/IgA Conjugate, (goat), anti-human IgG/IgA
8. 10mL TMB Chromogen
9. 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)

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 PBC Screen IgG/IgA ELISA Low Positive, PBC Screen IgG/IgA ELISA High Positive and ELISA Negative Control.
4. Determination of the presence or absence of antibodies to PBC Screen IgG/IgA antigens using arbitrary units requires two wells for each of the three controls and one or two wells for each patient sample. It is recommended that samples be run in duplicate.

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 PBC Screen IgG/IgA ELISA Low Positive, the PBC Screen IgG/IgA ELISA High Positive, 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 PBC Screen IgG/IgA 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.

**Quality Control**

1. The PBC Screen IgG/IgA ELISA Low Positive, the PBC Screen IgG/IgA ELISA High Positive 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 PBC Screen IgG/IgA ELISA High Positive, the PBC Screen IgG/IgA ELISA High Positive 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 PBC Screen IgG/IgA ELISA High Positive must be greater than the absorbance of the prediluted PBC Screen IgG/IgA ELISA Low Positive, which must be greater than the absorbance of the prediluted ELISA Negative Control.
   b. The prediluted PBC Screen IgG/IgA ELISA High Positive must have an absorbance greater than 1.0 while the prediluted ELISA Negative Control absorbance cannot be over 0.2.
   c. The PBC Screen IgG/IgA ELISA Low Positive absorbance must be more than twice the ELISA Negative Control or over 0.25.
   d. The ELISA Negative Control and PBC Screen IgG/IgA ELISA High Positive are intended to monitor for substantial reagent failure. The PBC Screen IgG/IgA ELISA High Positive will not ensure precision at the assay cutoff.
   e. The user should refer to CLSI (NCCLS) Document C24-A2 for additional guidance on appropriate QC practices.

**Calculation of Results**

The average OD for each set of duplicates is first determined. The reactivity for each sample can then be calculated by dividing the average OD of the sample by the average OD of the PBC Screen IgG/IgA ELISA Low Positive. The result is multiplied by the number of units assigned to the PBC Screen IgG/IgA ELISA Low Positive found on the label.

\[
\text{Sample Value} = \frac{\text{Sample OD}}{\text{PBC Screen IgG/IgA ELISA Low Positive OD}} \times \text{PBC Screen IgG/IgA ELISA Low Positive (units)}
\]

Reactivity is related to the quantity of antibody present in a non-linear fashion. While increases and decreases in patient antibody concentrations will be reflected in a corresponding rise or fall in reactivity, the change is not proportional (i.e. a doubling of the antibody concentration will not double the reactivity).

**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.

The sample can then be classified as negative, equivocal or positive according to the table below.

<table>
<thead>
<tr>
<th>Units</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>≤ 20.0</td>
</tr>
<tr>
<td>Equivocal</td>
<td>20.1 – 24.9</td>
</tr>
<tr>
<td>Positive</td>
<td>≥ 25</td>
</tr>
</tbody>
</table>

1. A positive result indicates the presence of antibodies to one or all of the antigen in the PBC Screen and suggests the possibility of primary biliary cirrhosis.

2. A negative result indicates the absence of antibodies to Mitochondria, gp210 and sp100 or levels below the negative cut-off of the assay.

3. Specimens with positive results may be tested for the presence of individual MIT3, gp210 and sp100 antibodies with MIT3, gp210 and sp100 specific ELISA assays.

4. A specimen with equivocal levels of antibody cannot be assessed for antibody status. It is recommended that testing be repeated on a fresh sample or at a later time.

5. It is suggested that the results reported by the laboratory should include the statement: “The following results were obtained with the INOVA QUANTA Lite™ PBC Screen IgG/IgA ELISA. PBC Screen values obtained with different manufacturers’ assay methods may not be used interchangeably. The magnitude of the reported IgG/IgA levels cannot be correlated to an endpoint titer.”
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 primary biliary cirrhosis patients are positive for antibodies to the antigens used in the PBC Screen IgG/IgA ELISA.
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.

Expected Values
Normal Range
A panel of 520 asymptomatic, healthy individuals residing in the United States was tested with the QUANTA Lite™ PBC Screen IgG/IgA ELISA. Age and sex data was available for 307 specimens. The ages ranged from 18-78 years old and included 150 male and 157 female individuals. The average value for this population was 6.6 units, the median value was 4.6 units. The specificity of the assay was 98.1% (510/520) for the normal subjects. Of the 10 positive samples, 7 showed reactivity on the M2 EP(MIT3) IgG ELISA and 1 specimen was reactive when tested for M2 EP(MIT3) IgA antibodies.

Specific Performance Characteristics
Clinical Studies
A total of 440 PBC patients and 769 non-PBC patients, which included 520 healthy normals, were analyzed by the QUANTA Lite™ PBC Screen IgG/IgA ELISA to assess the specificity and sensitivity of the test. Specimens with known AMA-negative PBC or patients with suspected, but unconfirmed disease and AIH patients were excluded. The PBC group (440) included specimens from 426 individuals with definite PBC and 14 with AIH/PBC overlap. The non-PBC group included specimens from 520 healthy controls and specimens from individuals with viral hepatitis (HBV or HCV) (149), PSC (48), non-PBC liver disease (23) and infectious or other autoimmune diseases (29). The mean and the median values of the non-PBC group were 8.1 and 5.3 units respectively. The results are summarized in Table 1.

Table 1: Specificity and Sensitivity of QUANTA Lite™ PBC Screen IgG/IgA ELISA

<table>
<thead>
<tr>
<th>N=1209</th>
<th>n</th>
<th>pos</th>
<th>equiv</th>
<th>neg</th>
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</thead>
<tbody>
<tr>
<td>PBC Group</td>
<td>440</td>
<td>422</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Non-PBC Group</td>
<td>769</td>
<td>30</td>
<td>20</td>
<td>719</td>
</tr>
</tbody>
</table>

Sensitivity: 95.9% (422/440); 95% Confidence Interval (CI): 93.6% to 97.6%
Specificity: 96.1% (739/769); 95% CI: 94.5% to 97.4%
Overall agreement (equivocal results considered negative): 96.0% (1161/1209)

Comparison to Predicate Devices
The QUANTA Lite™ PBC Screen IgG/IgA ELISA was compared with the individual QUANTA Lite™ M2 EP (MIT3) ELISA, the QUANTA Lite™ gp210 ELISA and the QUANTA Lite™ sp100 ELISAs. A total of 1209 specimens (440 clinical PBC, 249 non-PBC and 520 asymptomatic healthy individuals) were tested on the PBC Screen and the individual QUANTA Lite™ MIT3, sp100 and gp210 ELISAs.

N=1209

<table>
<thead>
<tr>
<th>Individual QUANTA Lite™ Test</th>
<th>Pos</th>
<th>Eq*</th>
<th>Neg</th>
</tr>
</thead>
<tbody>
<tr>
<td>QUANTA Lite™ PBC Screen IgG/IgA ELISA</td>
<td>437</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Eq*</td>
<td>10</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Neg</td>
<td>2</td>
<td>3</td>
<td>730</td>
</tr>
</tbody>
</table>

*Equivocal results are excluded in the analysis

The positive agreement and negative agreement were calculated by comparing the results obtained on the PBC Screen with the results obtained on any of the individual assays.

Positive agreement (excluding equivocals) = 99.5% (437/439)
Negative agreement (excluding equivocals) = 98.9% (730/738)
Overall Agreement (excluding equivocals) = 99.2% (1167/1177)

Cross-reactivity
Sera from 463 patients with non-PBC liver disease (213 AIH-1, 48 PSC, 10 AIH/PSC, 8 AIC, 9 cryptogenic hepatitis, 11 VBD5, 3 AIH-2, 11 alcoholic liver disease, 1 drug induced hepatitis, 71 HBV and 78 HCV) and 29 patients with autoimmune or infectious disease (7 H. pylori, 3 ASCA, 4 ANA, 3 tTG, 2 GPA, 2 GBM and 8 CMV) were tested with the QUANTA Lite™ PBC Screen IgG/IgA ELISA to assess the assay’s specificity. From the autoimmune/infectious disease group (29 patients) only one CMV positive sample was interpreted as positive (34.4 units) on the PBC screen. This patient had weak nuclear and cytoplasmic staining on IFA, but not specific to any of the PBC antigen patterns. In the non-PBC liver group comprising of 463 patients, 53 patients were positive on the PBC screen. 42 of the 53 positive patients showed some reactivity on one or more of the individual tests, while 9 patients were negative for all the markers. It is possible that the 53 positive patients from the non-PBC liver group may have some undiagnosed or evolving PBC overlap disease.
Precision and Reproducibility

Intra-assay performance was assessed by running 7 specimens, the kit high positive control (HPC) and negative control (NC) a total of 5 times each.

Table 2: Intra-assay Performance of QUANTA Lite™ PBC Screen IgG/IgA ELISA

<table>
<thead>
<tr>
<th></th>
<th>HPC</th>
<th>NC</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
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<tbody>
<tr>
<td>Mean</td>
<td>100.6</td>
<td>1.1</td>
<td>61.6</td>
<td>39.8</td>
<td>27.9</td>
<td>13.6</td>
<td>27.5</td>
<td>26.5</td>
<td>23.6</td>
</tr>
<tr>
<td>SD</td>
<td>2.2</td>
<td>0.1</td>
<td>2.3</td>
<td>1.0</td>
<td>0.8</td>
<td>0.3</td>
<td>0.9</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>CV %</td>
<td>2.2</td>
<td>6.5</td>
<td>3.7</td>
<td>2.4</td>
<td>3.0</td>
<td>2.4</td>
<td>3.2</td>
<td>2.2</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Table 3: Inter-assay Performance for QUANTA Lite™ PBC Screen IgG/IgA ELISA

<table>
<thead>
<tr>
<th></th>
<th>HPC</th>
<th>NC</th>
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<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
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<th>H</th>
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</thead>
<tbody>
<tr>
<td>Mean</td>
<td>104.8</td>
<td>1.6</td>
<td>67.5</td>
<td>8.4</td>
<td>29.7</td>
<td>35.2</td>
<td>6.3</td>
<td>31.5</td>
<td>29.8</td>
<td>25.2</td>
</tr>
<tr>
<td>SD</td>
<td>1.9</td>
<td>0.3</td>
<td>1.7</td>
<td>0.3</td>
<td>1.2</td>
<td>0.6</td>
<td>0.6</td>
<td>1.9</td>
<td>1.3</td>
<td>0.25</td>
</tr>
<tr>
<td>CV %</td>
<td>1.8</td>
<td>21.9</td>
<td>2.6</td>
<td>3.6</td>
<td>4.0</td>
<td>1.6</td>
<td>9.2</td>
<td>6.1</td>
<td>4.5</td>
<td>1.0</td>
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References

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