QUANTA Lite™ GPA (Gastric Parietal Cell Antibody) ELISA 708765
For In Vitro Diagnostic Use
CLIA Complexity: High

Intended Use
QUANTA Lite™ GPA (Gastric Parietal Cell Antibody) kit is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of gastric parietal cell (H+/K+) ATPase antibodies of the IgG class in human serum. The presence of gastric parietal cell (H+/K+) ATPase antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of conditions with elevated levels of anti-gastric parietal cell (H+/K+) ATPase antibodies including pernicious anemia.

Summary and Explanation of the test
Pernicious anemia is a chronic disease and is the end stage of type A (autoimmune) chronic atrophic gastritis. Type A chronic atrophic gastritis affects the fundus and body of the stomach, while Type B (nonimmune) affects the antrum as well as the fundus and body. Type A is associated with pernicious anemia and type B with H. pylori infection. During the progression of type A chronic atrophic gastritis to gastritis, gastric parietal cells, which produce intrinsic factor and HCl, are destroyed and production of intrinsic factor and HCl is eliminated. Intrinsic factor is essential for the absorption of vitamin B12 from the intestine and its absence leads to vitamin B12 deficiency and megaloblastic anemia. The onset of pernicious anemia is typically slow, with the progression from chronic atrophic gastritis to gastritis and chronic anemia taking 20-30 years. The median age at diagnosis is 60 years old. Although the disease is often not recognized until the anemia or other symptoms become significant, the underlying gastric lesions can be recognized years before the anemia develops. An increased frequency of autoimmune diseases including autoimmune thyroiditis (Hashimoto’s thyroiditis), type 1 diabetes, Addison’s disease, Grave’s disease, and myasthenia gravis is seen in patients with pernicious anemia. Patients with pernicious anemia have been reported to have a 3x increased risk of gastric carcinoma and a 13x increased risk of gastric carcinoid.

Autoantibodies to gastric parietal cells are found in approximately 90% of patients with pernicious anemia and in about 30% of first-degree relatives of patients with pernicious anemia. Gastric parietal cell antibodies (GPA) are usually detected by indirect immunofluorescence (IFA) using tissue sections of rodent gastric mucosa. Gastric parietal cell antibodies are directed toward membrane bound antigen in the secretary canalliculi and the tubulovesicles of the gastric parietal cells. The antigen target of the GPA has been identified as the gastric H+/K+ ATPase (gastric proton pump), which is responsible for acidification of the stomach lumen. GPA bind to both the α and β subunits of the H+/K+ ATPase. GPA prevalence increases with age and have been reported in 2.5% of a normal population 30-39 years old and in 9.6% of a population in their 8th decade. A recent study reported that a high prevalence of GPA and associated autoimmune gastritis is present in type 1 diabetics and strongly suggested that diabetic patient’s with anemia and/or gastrointestinal symptoms should be tested for GPA. Increased levels of GPA have been found in patients with thyroid disease, iron deficiency anemia, alopecia areata, and vitiligo. Detection of GPA by IFA is labor-intensive, requires subjective interpretation of results by highly trained personnel, and is subject to variability in the quality and reproducibility of the tissue sections used to manufacture the IFA slides. With the identification of the gastric H+/K+ ATPase α and β subunits as the major molecular targets of gastric parietal cell autoantibodies, sensitive and specific ELISA assays for the detection of GPA antibodies have been constructed. Utilization of ELISA assays allows for standardized, reproducible, and objective test results for GPA.

Principles of the Procedure
Purified H+/K+ ATPase antigen, isolated from pig gastric mucosa, 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 H+/K+ ATPase 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.

Reagents
1. Polystyrene microwell ELISA plate coated with a purified H+/K+ ATPase 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 H+/K+ ATPase, prediluted, 1.2mL
3. GPA ELISA Low Positive, 1 vial of buffer containing preservative and human serum antibodies to H+/K+ ATPase, prediluted, 1.2mL
4. GPA ELISA High Positive, 1 vial of buffer containing preservative and human serum antibodies to H+/K+ ATPase, prediluted, 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 IgG Conjugate, (goat), anti-human IgG, 1 vial – colored blue containing buffer, protein stabilizers and preservative, 10mL

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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 GPA ELISA Low Positive, GPA ELISA High Positive and ELISA Negative Control should be handled in the same manner as potentially infectious material.16
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. Microbiologically 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. 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.

Procedure
Materials provided
1. GPA ELISA microwell plate (12-1 x 8 wells), with holder
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.
4. Determination of the presence or absence of H+/K+ ATPase antibody 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 GPA ELISA Low Positive, the GPA 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 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.
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 GPA ELISA Low Positive, the GPA 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 GPA ELISA Low Positive, the GPA 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 GPA ELISA High Positive must be greater than the absorbance of the prediluted GPA ELISA Low Positive, which must be greater than the absorbance of the prediluted ELISA Negative Control.
b. The prediluted GPA 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 GPA ELISA Low Positive absorbance must be more than twice the ELISA Negative Control or over 0.25.
d. The ELISA Negative Control and GPA ELISA High Positive are intended to monitor for substantial reagent failure. The GPA ELISA High Positive will not ensure precision at the assay cutoff.
e. The user should refer to NCCLS Document C24-A for additional guidance on appropriate QC practices.17

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 GPA ELISA Low Positive. The result is multiplied by the number of units assigned to the GPA ELISA Low Positive found on the label.

\[
\text{Sample OD} = \left( \frac{\text{Sample Value}}{\text{GPA ELISA Low Positive OD}} \right) \times \text{GPA ELISA Low Positive (units)}
\]

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). If a more accurate quantitation of patient antibody is required, serial dilutions of the patient sample should be run and the last dilution to measure positive in the assay should be reported as the patient’s antibody titer.

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 (IgG antibody to H+/K+ ATPase detected) according to the table below.

<table>
<thead>
<tr>
<th>Units</th>
<th>Negative</th>
<th>Equivocal</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0 - 20.0</td>
<td>20.1 – 24.9</td>
<td>≥ 25</td>
</tr>
</tbody>
</table>

Equivocal specimens should be retested before results are reported.

1. A positive result indicates the presence of H+/K+ ATPase antibodies and suggests the possibility of pernicious anemia or related diseases.
2. A negative result indicates no H+/K+ ATPase antibody or levels below the negative cut-off of the assay.
3. A specimen with equivocal levels of GPA cannot be assessed for antibody status. If the results remain equivocal after repeat testing, the result should be reported as equivocal and/or an additional sample should be taken.
4. It is suggested that the results reported by the laboratory should include the statement: “The following results were obtained with the INOVA QUANTA Lite™ GPA ELISA. GPA 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.”

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. A negative Gastric Parietal Cell Antibody result does not rule out the presence of pernicious anemia.
3. A negative GPA result does not rule out the presence of GPA, because the concentration of antibody may be below the detection limit of the assay.
4. A positive test result only indicates the presence of antibody to H+/K+ ATPase and does not necessarily indicate the presence of autoimmune or other disease.
5. The assay performance has not been established for pediatric patients.
6. Results of this assay should be used in conjunction with clinical findings and other serological tests.
7. The assay performance characteristics have not been established for matrices other than serum.

Expected Values
The ability of the QUANTA Lite™ GPA ELISA to detect H+/K+ ATPase antibodies was evaluated by comparison to a commercially available H+/K+ ATPase ELISA kit. A comparison of the results obtained by the QUANTA Lite™ GPA ELISA and by an indirect immunofluorescence assay using mouse kidney/stomach tissue sections was also completed.

The prevalence of GPA in healthy normal populations increases with age from approximately 2.5% in the third decade to 9.6% in the eighth decade. The frequency of GPA is generally higher in women than in men. For men under 55 years old 5.5% were positive for GPA compared to 14.7% of the women. For individuals over 55 years old, 9.2% men and 22.3% women had GPA"
Normal Range
A combined panel of 210 specimens from asymptomatic, healthy individuals was tested with the QUANTA Lite™ GPA ELISA kit. Ages ranged from 17-77 (median 32). Of the 210 specimens, 154 (73.3%) were from male and 56 (26.7%) from female individuals. The mean value was 7.3 units with a range of 1.3 to 84.1 units. Two of the 7 GPA positive specimens were GPA IFA positive. Excluding 4 equivocal results resulted in a specificity of 96.6% (199/206).

Relative Sensitivity and Specificity
The results of testing 20 specimens from pernicious anemia patients by the QUANTA Lite™ GPA ELISA and a predicate GPA ELISA kit are shown in Table 1. Comparison of results obtained by IFA using Mouse kidney/stomach slides on this panel is shown in Table 2. Table 3 shows comparative results of the QUANTA Lite™ GPA ELISA and the predicate GPA ELISA kit results on a panel of 41 specimens.

Table 1: QUANTA Lite™ GPA (Gastric Parietal Antibody) and Predicate GPA ELISA Results on Pernicious Anemia Patient Panel

<table>
<thead>
<tr>
<th>Predicate GPA ELISA</th>
<th>QUANTA Lite™ GPA ELISA RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=20</td>
<td>Pos (15)</td>
</tr>
<tr>
<td>Positive</td>
<td>14</td>
</tr>
<tr>
<td>Equivocal</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
</tbody>
</table>

Agreement (excluding equivocal results): 100% (15/15)

Table 2: QUANTA Lite™ GPA ELISA and ANA (Mouse kidney/stomach) IFA results on Pernicious Anemia Patient Panel

<table>
<thead>
<tr>
<th>GPA IFA</th>
<th>N=20</th>
<th>QUANTA Lite™ GPA ELISA RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Pos (15)</td>
<td>EQ (0)</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Equivocal</td>
<td>EQ (0)</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>Neg (5)</td>
<td>0</td>
</tr>
</tbody>
</table>

Agreement: 100% (20/20)

Table 3: QUANTA Lite™ GPA (Gastric Parietal Antibody) and Predicate GPA ELISA Results on Specimens submitted for GPA testing

<table>
<thead>
<tr>
<th>Predicate GPA ELISA</th>
<th>QUANTA Lite™ GPA ELISA RESULTS</th>
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<tbody>
<tr>
<td>N=41</td>
<td>Pos (19)</td>
</tr>
<tr>
<td>Positive</td>
<td>17</td>
</tr>
<tr>
<td>Equivocal</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
</tr>
</tbody>
</table>

Agreement (excluding equivocal results): 89.7% (35/39)

Cross-reactivity Study
Sera from patients with autoimmune or infectious disease antibodies or various clinical conditions including H. pylori (7), thyroid peroxidase (5), thyroid-M (5), thyroid-T (4), beta-2 glycoprotein (5), LKM-1 (5), autoimmune hepatitis,type1 (14), mitochondria M2 (4) were tested for cross-reactivity with the QUANTA Lite™ GPA ELISA. One H. pylori and 2 TPO specimens were positive by the QUANTA Lite™ GPA ELISA. These 3 specimens were also positive for GPA by IFA.

Precision and Reproducibility
Intra-assay performance for the QUANTA Lite™ GPA ELISA was evaluated by testing 6 specimens a total of 9 times each. The results, summarized in Table 4, show the intra-assay precision.

Table 4: Intra-assay Performance for QUANTA Lite™ GPA ELISA

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Mean units</td>
<td>40.7</td>
<td>93.4</td>
<td>35.4</td>
<td>58.3</td>
<td>47.0</td>
</tr>
<tr>
<td>SD</td>
<td>2.3</td>
<td>5.7</td>
<td>3.2</td>
<td>5.4</td>
<td>5.1</td>
</tr>
<tr>
<td>CV %</td>
<td>5.6</td>
<td>6.1</td>
<td>9.0</td>
<td>9.2</td>
<td>10.8</td>
</tr>
</tbody>
</table>

Inter-assay performance was assessed by testing, in duplicate, a panel of 5 specimens twice daily for 3 days.

Table 5: Inter-assay Performance for QUANTA Lite™ GPA ELISA

<table>
<thead>
<tr>
<th>Spec. 1</th>
<th>Spec. 2</th>
<th>Spec. 3</th>
<th>Spec. 4</th>
<th>Spec. 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean units</td>
<td>32.7</td>
<td>95.2</td>
<td>11.4</td>
<td>32.9</td>
</tr>
<tr>
<td>SD</td>
<td>1.61</td>
<td>2.25</td>
<td>0.37</td>
<td>1.77</td>
</tr>
<tr>
<td>CV %</td>
<td>4.9</td>
<td>2.4</td>
<td>3.2</td>
<td>5.4</td>
</tr>
</tbody>
</table>
References