QUANTA Lite™ Intrinsic Factor ELISA  
For In Vitro Diagnostic Use  
CLIA Complexity: High

Principles of the Procedure
Purified full-length recombinant human Intrinsic Factor 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 Intrinsic Factor 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.

Pernicious anemia (Biermer’s anemia) is a chronic disease and the end stage of type A (autoimmune) chronic atrophic gastritis. Type A is autoimmune in nature and associated with pernicious anemia. Type B (non-autoimmune) is associated with H. pylori infection.1,2,3 During the progression of type A chronic atrophic gastritis, gastric parietal cells, which produce intrinsic factor and HCl, and zymogenic cells, which produce pepsinogen, are destroyed and production of Intrinsic Factor (IF) 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. Diagnosis of pernicious anemia is important for treatment of the anemia itself and prevention of irreversible neurological damage.1,4 Patients with pernicious anemia have been reported to have a 3 times increased risk of gastric carcinoma, a 13 times increased risk of gastric carcinoid, and an increased risk of esophageal squamous cell cancer.5,6,7 Circulating antibodies to Intrinsic Factor are highly specific and can be detected in >50% of patients with pernicious anemia.1,3 These antibodies are of 2 types: Type 1, blocking antibodies which prevent the binding of vitamin B₁₂ to the IF molecule and Type 2 antibodies which may interfere with the binding of the IF-vitamin B₁₂ complex to the ileal receptor.1,8 The QUANTA Lite™ Intrinsic Factor ELISA, unlike radioimmunoassay-based methods, detects both Type 1 and 2 antibodies. Together with other clinical and laboratory data, a positive Intrinsic Factor antibody result can help distinguish autoimmune pernicious anemia from other megaloblastic anemias as well as distinguish type A atrophic gastritis from other forms of nonspecific histological gastritis.1,3,9

The QUANTA Lite™ Intrinsic Factor ELISA is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of Intrinsic Factor antibodies in human serum. The presence of Intrinsic Factor antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of pernicious anemia.

Reagents
1. Polystyrene microwell ELISA plate coated with recombinant human Intrinsic Factor (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 Intrinsic Factor antigen, prediluted, ready-to-use, 1.2mL
3. Intrinsic Factor ELISA Low Positive, 1 vial of buffer containing preservative and human serum antibodies to Intrinsic Factor antigen, prediluted, ready-to-use, 1.2mL
4. Intrinsic Factor ELISA High Positive, 1 vial of buffer containing preservative and human serum antibodies to Intrinsic Factor 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 IgG Conjugate, (goat), anti-human IgG 1 vial – colored blue, 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
Materials provided
1  Intrinsic Factor ELISA microwell plate, (12-1 x 8 wells), with holder
1  1.2mL prediluted ELISA Negative Control
1  1.2mL prediluted Intrinsic Factor ELISA Low Positive
1  1.2mL prediluted Intrinsic Factor ELISA High Positive
1  50mL HRP Sample Diluent
1  25mL HRP Wash Concentrate, 40x concentrate
1  10mL HRP IgG Conjugate, (goat)
1  10mL TMB Chromogen
1  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. Microbiocally 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 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.11

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 Intrinsic Factor ELISA Low Positive, Intrinsic Factor ELISA High Positive and ELISA Negative Control should be handled in the same manner as potentially infectious material.10
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.

Quality Control
1. The Intrinsic Factor ELISA Low Positive, the Intrinsic Factor 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 Intrinsic Factor ELISA Low Positive, the Intrinsic Factor 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 Intrinsic Factor ELISA High Positive must be greater than the absorbance of the prediluted Intrinsic Factor ELISA Low Positive, which must be greater than the absorbance of the prediluted ELISA Negative Control.
   b. The prediluted Intrinsic Factor 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 Intrinsic Factor ELISA Low Positive absorbance must be more than twice the ELISA Negative Control or over 0.25.
   d. The ELISA Negative Control and Intrinsic Factor ELISA High Positive are intended to monitor for substantial reagent failure. The Intrinsic Factor 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.  

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 Intrinsic Factor ELISA Low Positive, Intrinsic Factor ELISA High Positive and ELISA Negative Control.
4. Determination of the presence or absence of antibodies to Intrinsic Factor 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.
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** Intrinsic Factor ELISA Low Positive, the Intrinsic Factor 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.** 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.

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

<table>
<thead>
<tr>
<th>Units</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>≤ 20</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 Intrinsic Factor and suggests the possibility of pernicious anemia.
2. A negative result indicates the absence of antibodies to Intrinsic Factor or levels below the negative cut-off of the assay.
3. A negative Intrinsic Factor result does not exclude the diagnosis of pernicious anemia since only 60% of patients with pernicious anemia have this antibody.⁹
4. A specimen with equivocal levels of Intrinsic Factor antibody 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 at a later time.
5. The presence of anti-gastric parietal cell antibodies may or may not be concordant with that of Intrinsic Factor antibodies and their measurement, in addition to, or in conjunction with measurement of Intrinsic Factor antibodies, may aid in the evaluation of patients with suspected pernicious anemia.⁹
6. It is suggested that the results reported by the laboratory should include the statement: “The following results were obtained with the INOVA QUANTA Lite™ Intrinsic Factor ELISA. Intrinsic Factor ELISA 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
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 Intrinsic Factor ELISA Low Positive. The result is multiplied by the number of units assigned to the Intrinsic Factor ELISA Low Positive found on the label.

\[
\text{Sample Value} = \frac{\text{Sample OD}}{\text{Intrinsic Factor ELISA Low Positive OD}} \times \text{Intrinsic Factor ELISA Low Positive (units)}
\]

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.

Expected Values
Normal Range
A panel of 476 specimens collected from asymptomatic, healthy individuals was tested with the QUANTA Lite™ Intrinsic Factor ELISA kit. Ages ranged from 14-78 (median 43). Of the 276 specimens with age and sex information, 150 were from males and 126 from females. Of the 476 specimens, one was a strong positive at 103.2 units, 1 was a borderline positive at 25.5 units and one was borderline equivocal at 20.2 units. The specificity of the assay was 99.4% (473/476). The frequency of pernicious anemia has been estimated at 0.1-0.2%. Excluding the one strong positive result outlier, the mean and median values for the healthy control group were 5.7 units and 5.0 units.

Specific Performance Characteristics
A total of 177 specimens from patients with suspected, presumed, or confirmed pernicious anemia as described below in “Clinical Studies” and 499 specimens from healthy (476) and disease controls (23) were analyzed by the QUANTA Lite™ Intrinsic Factor ELISA to assess the sensitivity and specificity of the test. The mean and the median values of the non-pernicious anemia group were 5.9 and 4.9 units respectively.

Clinical Studies
Specimens were classified into 3 categories to reflect the degree of confidence in a diagnosis of pernicious anemia: 1) “Suspected pernicious anemia” - gastric parietal cell antibody positive and/or low vitamin B₁₂, but Intrinsic Factor antibody results were discrepant; 2) “Presumed pernicious Anemia” – laboratory measures consistent with pernicious anemia including gastric parietal cell antibody positive, low vitamin B₁₂ levels and mean corpuscular volume increased (typical of pernicious anemia); 3) “Confirmed pernicious anemia” – laboratory measures consistent with pernicious anemia including gastric parietal cell antibody positive, low vitamin B₁₂ levels and increased mean corpuscular volume (typical of pernicious anemia) and hematological confirmation of megaloblastic anemia.

Sensitivity was calculated: 1) counting the “confirmed or presumed” pernicious anemia specimens as disease positive and the “suspected” pernicious anemia as disease negative and 2) counting the “confirmed, presumed and suspected” pernicious anemia specimens as disease positive. None of the “suspected” anemia specimens were found to be positive for Intrinsic Factor antibodies.

Table 1: Sensitivity and Specificity of QUANTA Lite™ Intrinsic Factor ELISA

<table>
<thead>
<tr>
<th>Category</th>
<th>n</th>
<th>pos</th>
<th>eq</th>
<th>neg</th>
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</thead>
<tbody>
<tr>
<td>Confirmed (n=43) and presumed (n=55) pernicious anemia*</td>
<td>98</td>
<td>92</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>All pernicious anemia specimens**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspected (n=79), Presumed (n=55) &amp; Confirmed Pernicious Anemia (n=43)</td>
<td>177</td>
<td>92</td>
<td>2</td>
<td>83</td>
</tr>
<tr>
<td>Non-pernicious anemia (healthy controls and disease controls)</td>
<td>499</td>
<td>2</td>
<td>1</td>
<td>496</td>
</tr>
</tbody>
</table>

* suspected pernicious anemia specimens counted as disease negative
** suspected pernicious anemia specimens counted as disease positive
Sensitivity
1) Presumed & confirmed pernicious anemia groups included as disease positive: 93.9% (92/98); 95% Confidence Interval (CI): 87.1%- 97.7%
   Note: Equivocal results are considered negative in the analysis
2) Suspected, presumed or confirmed pernicious anemia groups included as disease positive: 52.0% (92/177); 95% Confidence Interval (CI): 44.4%- 59.5%
   Note: Equivocal results are considered negative in the analysis

Specificity
99.6% (497/499); 95% Confidence Interval (CI): 98.8-100%
Note: Equivocal results are considered negative in the analysis

Efficiency
1) Presumed & confirmed pernicious anemia groups included as disease positive: 98.7%
2) Suspected, presumed or confirmed pernicious anemia included as disease positive: 87.1%

Comparison to Predicate Devices
The QUANTA Lite™ Intrinsic Factor ELISA was compared to a FDA- cleared gastric parietal cell antibody (GPA) ELISA and to an Intrinsic Factor (IF) antibody RIA assay. Negative percent agreement was between 97.5 to 100% for all three assays. The positive percent agreement between the QUANTA Lite™ Intrinsic Factor ELISA and the IF RIA performed at site 1 was 93.3%, but 30.3% site 2. All positive RIA results at site 1 were from individuals with hematological evidence of pernicious anemia. Clinical information was not available for specimens at site 2.

<table>
<thead>
<tr>
<th>QUANTA Lite™ Intrinsic Factor Antibody ELISA</th>
<th>Predicate GPA ELISA</th>
<th>Predicate Intrinsic Factor RIA Site 1</th>
<th>Predicate Intrinsic Factor RIA Site 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive % Agreement</td>
<td>29.7% (22/74)</td>
<td>93.2% (41/44)</td>
<td>30.3% (24/79)</td>
</tr>
<tr>
<td>Negative % Agreement</td>
<td>97.5% (193/198)</td>
<td>100% (25/25)</td>
<td>100% (26/26)</td>
</tr>
<tr>
<td>Overall Agreement</td>
<td>49.1% (220/278)</td>
<td>95.6% (66/69)</td>
<td>63.3% (50/79)</td>
</tr>
</tbody>
</table>

Cross-Reactivity
Sera from 23 patients with autoimmune or infectious disease antibodies including *H. pylori*, mitochondrial M2, cytomegalovirus, herpes simplex virus, ASCA, RNP, SS-A, SS-B, Scl-70, dsDNA, tissue transglutaminase and glomerular basement membrane were tested for cross-reactivity with the QUANTA Lite™ Intrinsic Factor ELISA. None of the specimens were found to be positive by the QUANTA Lite™ Intrinsic Factor ELISA.

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

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</thead>
<tbody>
<tr>
<td>Mean units</td>
<td>108.1</td>
<td>45.4</td>
<td>115.2</td>
<td>108.7</td>
<td>21.3</td>
<td>17.1</td>
<td>10.6</td>
<td>13.4</td>
</tr>
<tr>
<td>SD</td>
<td>2.1</td>
<td>0.8</td>
<td>2.5</td>
<td>3.4</td>
<td>1.7</td>
<td>0.8</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>CV %</td>
<td>1.9</td>
<td>1.8</td>
<td>2.2</td>
<td>3.2</td>
<td>2.0</td>
<td>4.0</td>
<td>5.0</td>
<td>5.3</td>
</tr>
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</table>

Inter-assay performance was assessed by testing in duplicate, 5 specimens, the kit high positive control (HPC) and negative control (NC) twice daily (once in the morning and once in afternoon) for 3 days.

<table>
<thead>
<tr>
<th>QUANTA Lite™ Intrinsic Factor ELISA</th>
<th>HPC</th>
<th>NC</th>
<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>109.1</td>
<td>1.1</td>
<td>107.9</td>
<td>47.4</td>
<td>13.9</td>
<td>108.0</td>
<td>29.2</td>
</tr>
<tr>
<td>SD</td>
<td>6.0</td>
<td>0.2</td>
<td>4.8</td>
<td>1.7</td>
<td>0.7</td>
<td>5.4</td>
<td>2.4</td>
</tr>
<tr>
<td>CV %</td>
<td>5.5</td>
<td>14.4</td>
<td>4.5</td>
<td>3.6</td>
<td>5.0</td>
<td>5.0</td>
<td>8.2</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. Results of this assay should be used in conjunction with clinical findings and other serological tests. Patients with pernicious anemia may have antibodies to Intrinsic Factor and/or gastric parietal cell antibodies. While the presence of both antibodies adds to the confidence of the diagnosis of pernicious anemia, patients with pernicious anemia may have only one of the two antibodies. 1,9

3. The assay performance characteristics have not been established for matrices other than serum.

References