**PRINCIPLE**

A Sequential CLIA Method (TYPE 1): 
The reagents required for the sequential CLIA assay include immobilized antigen, circulating antibody and enzyme-linked species-specific antibody. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and the exogenous added biotinylated H. pylori antibody. 

Upon mixing biotinylated antibody and a serum containing the antibody, reaction results between the antigen and the antibody to form an immune-complex. The interaction is illustrated by the following equation:

\[
\text{Antigen} + \text{Antibody} \rightarrow \text{Immune complex}
\]

\[\begin{align*}
\text{Ag} & \quad + \quad \text{Ab} \\
\text{Ag-Ab} & \quad + \quad \text{Streptavidin} \\
\text{Ag-Ab-Streptavidin} & \quad \rightarrow \quad \text{Immunocomplex (IC)}
\end{align*}\]

The employment of several serum references of known antibody activity that binds the anti-H. pylori IgG or IgM enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing several different serum references of known antibody activity, a reference curve can be generated from which the antibody activity of an unknown can be ascertained.

**MATERIALS PROVIDED FOR 96-well MICROPLATE**

A. Anti-H.Pylori Calibrators — 1ml/vial - Icons A-E 
Five (5) vials of references for anti-H-Pylori at levels of 0(A), 10(B), 25(C), 50(D), and 100(E) Units/ml of the IgG, IgM, or IgA have been added. 

B. *H. Pylori Biotin Reagent – 13ml/vial - Icon B*
One (1) vial of biotinylated inactivated H.*Pylori* (IgG, IgM or IgA) in a buffing matrix. A preservative has been added. Store at 2-8°C.

C. *H. Pylori Tracer Reagent – 13ml/vial - Icon C*
One (1) vial of anti-human IgG, IgM or IgA-horseradish peroxidase labelled H.*Pylori* in a buffing matrix. A preservative has been added. Store at 2-8°C.

D. Light Reaction Wells – 96 wells - Icon D
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Serum Diluent - - 20ml
One (1) vial of serum diluent containing buffer salts and a dye. Store at 2-8°C.

F. Wash Solution – 20ml - Icon E
One (1) vial containing a surfactant in buffered saline. A preservative has been added.

G. Signal Reagent A – 7.0ml/vial - Icon F
One (1) bottle containing luminol in buffer. Store at 2-8°C. 

H. Signal Reagent B – 7.0ml/vial - Icon G
One (1) bottle containing hydrogen peroxide (H₂O₂) in buffer. Store at 2-8°C. (see Reagent Preparation Section).

I. Product Insert.
Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Pre-diluted reagents are stable for sixty (60) days when stored at 2-8°C.

Required But Not Provided:
1. Pipette capable of delivering 10, 25 & 50µl volumes with a precision of better than 1.5%.
2. Dispensers for repetitive deliveries of 0.100ml and 0.300ml volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate luminometer.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.

**PRECAUTIONS**

For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for anti-H*. Pylori* Surface Antibigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human products can be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosecurity in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8596.

**SPECIMEN COLLECTION AND PREPARATION**

The specimens shall be blood, serum or plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain red-top vacutainer tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin,. Allow the blood to clot for serum samples. Centrifuge the specimens to separate the serum or plasma from the cells. The samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid refrigerative freezing and thawing. When assayed in duplicate, 0.100ml (IgG & IgM) or 0.050ml (IgG) of the diluted specimen is required.

**REAGENT PREPARATION:**

1. Serum Diluent
Dilute the serum diluent to 200ml in a suitable container with distilled or deionized water. Store at room temperature 20-27°C for up to 60 days.

2. Wash Buffer
Dilute solution of wash solution to 1000 ml with distilled or deionized water in a suitable storage container. Store at room temperature 20-27°C for up to 60 days.

3. Signal Reagent Solution - Store at 2 - 8°C.
Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1ml of B per 2 (eight well) strip. A preservative (solution of sucrose is made). Discard the unused portion if not used within 36 hours after mixing. If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

4. Patient Sample Dilution (1/100)
Dispense 0.050ml (10µl) of each patient specimen into 1 ml of serum diluent. Cover and vortex or mix thoroughly by inversion. Store at 2-8°C for up to forty-eight (48) hours.
QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and high ranges of the dose response curve for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

RESULTS

A reference curve is used to ascertain the concentration of anti-H. Pylori unknown samples. A control curve is also used for the determination of the presence of specific antibodies to the target antigen.

1. Record the RLU’s obtained from the printout of the microplate luminometer as outlined in Example 1.
2. Plot the RLU’s for each duplicate serum reference versus the corresponding anti-H. Pylori activity in U/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To determine the level of anti-H. Pylori activity for an unknown, locate the average RLU’s for each unknown on the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLU’s (70400) of the unknown intersects the calibration curve at (69.3 U/ml) anti-H. Pylori concentration (See Figure 1)*.

Note 1: Computer data reduction software designed for chemiluminescence assay may also be used for the data reduction. Duplicates of the unknown may be averaged as indicated (See Figure 1).

Note 2: Monobind can assist the laboratory in the purchase and implementation of equipment/software to measure and interpret chemiluminescence data.

EXAMPLE 1 (Typical results for IgG, M or A)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean RLU (U/ml)</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1 1427</td>
<td>1479</td>
<td>0</td>
</tr>
<tr>
<td>Cal B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1 11289</td>
<td>11231</td>
<td>10</td>
</tr>
<tr>
<td>Cal C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1 26584</td>
<td>26825</td>
<td>25</td>
</tr>
<tr>
<td>Cal D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1 49362</td>
<td>51570</td>
<td>40</td>
</tr>
<tr>
<td>Cal E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2 99903</td>
<td>10000</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2 7857</td>
<td>7756</td>
<td>6.5</td>
</tr>
<tr>
<td>D2 7653</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3 71366</td>
<td>70400</td>
<td>69.3</td>
</tr>
<tr>
<td>B3 69434</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:
1. The Dose Response Curve (80%: 50% & 20% intercepts) should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

LIMITATIONS OF Procedure

A. Assay Performance

It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift. If more than one (1) plate is used, it is recommended to repeat the dose response curve.

Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and triplicate results.

Very high concentration of anti-H. Pylori in patient specimens can contaminate samples immediately following these extreme levels. Bad duplicates are indicative of cross contamination. Repeat any sample, which follows any patient specimen with over twice the RLU’s of the 100 U/ml calibrator.

Samples, which are contaminated microbiologically, should not be used.

B. Interpretation

1. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
2. The clinical significance of the result should be used in evaluating the possible presence of gastrointestinal disease. However, clinical inference should not be solely based on this test but rather as an adjunct to the clinical manifestations of the patient and other relevant tests such as Histology, Urease and Culture. A positive result does not indicate gastrointestinal disease and does not distinguish between the colonization and infection of H. Pylori. Similarly, a negative result does not eliminate the absence of H. Pylori infection but rather a very low titer of antibody that may be related to the early stages of colonization.

EXPECTED RANGES OF VALUES

A study of apparently healthy population (n=118) and patients suffering from gastric abnormalities (n=154) was undertaken to determine expected values for the Anti-H. Pylori Acculite™ CLIA test system. Based on the data following cut-off points were established.

The presence of IgG and IgA antibodies to H. Pylori is confirmed when the serum level exceeds 20 U/ml.

The presence of IgM antibodies to H. Pylori is confirmed when the serum level exceeds 40 U/ml.

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

PERFORMANCE CHARACTERISTICS

A. Precision Anti-H. Pylori - IgG

The within and between assay precision of the Anti-H. Pylori (IgG) Microplate Acculite™ CLIA were determined by analyses on two different levels of pool control sera. The number, mean value, standard deviation (σ) and coefficient of variation for each of these control sera are presented in Table 1 & 2.

B. Precision Anti-H. Pylori - IgM

The within and between assay precision of the Anti-H. Pylori (IgM) Microplate Acculite™ CLIA were determined by analyses on two different levels of pool control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 1 and 2.

C. Precision Anti-H. Pylori - IgA

The within and between assay precision of the Anti-H. Pylori (IgA) Microplate Acculite™ CLIA were determined by analyses on two different levels of pool control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 5 and 6.

REFERENCES

9. Cat #: 1475-300 (IgG)
10. Cat #: 1757-300 (IgA)

For Orders and Inquiries, please contact

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Please visit our website to learn more about our other interesting products and services.

TABLE 1

Within Assay Precision (Values in U/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>20</td>
<td>4.6</td>
<td>0.28</td>
<td>6.4%</td>
</tr>
<tr>
<td>Positive</td>
<td>20</td>
<td>46.4</td>
<td>2.23</td>
<td>4.8%</td>
</tr>
</tbody>
</table>

TABLE 2

Between Assay Precision (Values in U/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>10</td>
<td>5.2</td>
<td>0.43</td>
<td>8.2%</td>
</tr>
<tr>
<td>Positive</td>
<td>10</td>
<td>49.2</td>
<td>3.13</td>
<td>6.4%</td>
</tr>
</tbody>
</table>

TABLE 3

Within Assay Precision (Values in U/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>10</td>
<td>3.4</td>
<td>0.22</td>
<td>6.4%</td>
</tr>
<tr>
<td>Positive</td>
<td>20</td>
<td>59.2</td>
<td>4.30</td>
<td>7.3%</td>
</tr>
</tbody>
</table>

TABLE 4

Between Assay Precision (Values in U/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>10</td>
<td>3.7</td>
<td>0.31</td>
<td>6.4%</td>
</tr>
<tr>
<td>Positive</td>
<td>10</td>
<td>61.3</td>
<td>3.70</td>
<td>6.0%</td>
</tr>
</tbody>
</table>

TABLE 5

Within Assay Precision (Values in U/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>20</td>
<td>2.8</td>
<td>0.19</td>
<td>6.8%</td>
</tr>
<tr>
<td>Positive</td>
<td>20</td>
<td>44.2</td>
<td>2.35</td>
<td>5.3%</td>
</tr>
</tbody>
</table>

TABLE 6

Between Assay Precision (Values in U/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>10</td>
<td>2.8</td>
<td>0.25</td>
<td>8.9%</td>
</tr>
<tr>
<td>Positive</td>
<td>10</td>
<td>45.1</td>
<td>3.31</td>
<td>7.3%</td>
</tr>
</tbody>
</table>

D. Sensitivity, Specificity & Accuracy

The sensitivity, specificity and accuracy for the Microplate Acculite™ CLIA were determined using the following definitions on a population of diseased and normal patients. The total number of specimens was 245.

<table>
<thead>
<tr>
<th>IgG</th>
<th>IgM</th>
<th>IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>95%</td>
<td>91%</td>
</tr>
<tr>
<td>Specificity</td>
<td>98%</td>
<td>88%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>97%</td>
<td>91%</td>
</tr>
</tbody>
</table>