h-Ab(X-H. Pylori) - BtnAg(H. Pylori) = Immune Complex (Variable Quantity)

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

h-Ab(X-H. Pylori) - Streptavidin > Immobilized complex
Streptavidin = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

After the incubation time, the well is washed to separate the unbound components by aspiration and/or decantation. The enzyme linked species-specific antibody (anti-h-IgG, M or A) is then added to the microwells. This conjugate binds to the immobilized complex. The enzyme activity in this reaction 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.

4.0 REAGENTS

Materials provided:

A. Anti-H. Pylori Calibrators – 1ml/vial - Icos A-E
Five (5) wells of references for anti-H. Pylori at levels of (A) 1000, 500 (B) 250, 100 (C) 50, (D) 25, (E) 10 ng/ml. Each well is pre-labeled with a number. Store at 2-8 °C. A preservative has been added. Store at 2-8 °C.

B. H. Pylori Biotin Reagent – 12ml/vial – Icon V
One (1) vial containing biotinylated inactivated H. Pylori (IgG, IgM, or IgA) in a buffering matrix. A preservative has been added. Store at 2-8 °C.

C. Anti-H. Pylori Enzyme Reagent – 13ml/vial – Icon V
One (1) vial containing anti-human IgG, IgM or IgA- horseradish peroxidase (HRP) conjugate in a buffering matrix. A preservative has been added. Store at 2-8 °C.

D. Streptavidin Biotinylated – Icon M
One (1) vial containing streptavidin biotinylated (TM) in a buffering matrix. Store at 2-8 °C.

E. Substrate B – 7ml/vial – Icon S
One (1) vial containing hydrogen peroxide (H₂O₂) in buffer. Store at 2-8 °C.

F. Wash Solution Concentrate – 20ml/vial – Icon L
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8 °C.

G. Substrate A – 31ml/vial – Icon L
One (1) vial containing tetramethylbenzidine (TMB) in buffer, Store at 2-8 °C.

H. Substrate B – 7ml/vial – Icon S
One (1) vial containing hydrogen peroxide (H₂O₂) in buffer. Store at 2-8 °C.
1.0 CALCULATION OF RESULTS
A reference curve is used to ascertain the concentration of anti-H. Pylori in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding anti-H. Pylori activity in U/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
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 absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in U/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example the average absorbance 1.603 intersects the dose response curve at 64.0 U/ml anti-H. Pylori concentration (See Figure 1). *

Note: computer data reduction software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Well</th>
<th>Mean Abs (A)</th>
<th>Value (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal A</td>
<td>A1</td>
<td>0.042</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A1</td>
<td>0.042</td>
<td></td>
</tr>
<tr>
<td>Cal B</td>
<td>C1</td>
<td>0.424</td>
<td>0.040</td>
</tr>
<tr>
<td></td>
<td>D1</td>
<td>0.388</td>
<td></td>
</tr>
<tr>
<td>Cal C</td>
<td>F1</td>
<td>0.722</td>
<td>0.791</td>
</tr>
<tr>
<td></td>
<td>H1</td>
<td>1.312</td>
<td>5.0</td>
</tr>
<tr>
<td>Cal D</td>
<td>G1</td>
<td>1.351</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>1.273</td>
<td></td>
</tr>
<tr>
<td>Cal E</td>
<td>A2</td>
<td>2.377</td>
<td>2.328</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>2.277</td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>C1</td>
<td>0.163</td>
<td>0.172</td>
</tr>
<tr>
<td></td>
<td>D2</td>
<td>0.182</td>
<td></td>
</tr>
<tr>
<td>Patient 2</td>
<td>A1</td>
<td>1.203</td>
<td>1.603</td>
</tr>
<tr>
<td></td>
<td>B1</td>
<td>1.671</td>
<td>64.0</td>
</tr>
</tbody>
</table>

*The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a standard curve prepared with each assay.

1.1.5. C. PARAMETERS
In order for the assay results to be considered valid the following criteria must be met:
1. Maximum Absorbance (100 U/ml) should be greater than 1.5.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS
The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1.2. Interpretation
1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimen and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart NC. Heterophile antibodies: a problem for all immunoassays. Clin. Chem. 1988;34(2):33-37). For diagnostic purposes, the results from this assay should be in comparison with clinical correlation, patient history and all other clinical findings. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incongruously interpreted, Monobind shall have the right to refuse the test.
5. 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.
6. The clinical significance of the result should be used in evaluating the possible significance of gastrointestinal disease. However, clinical inferences 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, Ureaase 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 level of antibody that may be related to the early stages of colonization.

13.0 EXPECTED RANGES OF VALUES
A study of apparently healthy population (n=118) and patients suffering from gastric abnormalities (n=194) was undertaken to determine expected values for the Anti-H. Pylori IgA AccuBind® ELISA test system. Based on the data, the following cut-off points were established:

<table>
<thead>
<tr>
<th>Presence of H.Pylori antibodies Confirmed</th>
<th>IgG &gt; 20 U/ml</th>
<th>IgA &gt; 20 U/ml</th>
<th>IgM &gt; 40 U/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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. Particularly if 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.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

14.1.1 Accuracy

14.1.2 Precision Antigen Specificity

14.1.3 Precision Anti-body Specificity

14.1.4 Precision Antigen/Ab Specificity

14.1.5 Precision Assay Specificity

14.1.6 Precision Assay Reagent Specificity

14.2 Sensitivity

14.3 Accuracy

15.0 REFERENCES


Revision: 5 Date: 2019-Jul-16  DCO: 1353 MP1425 Product Code: 1425-300 (IgG) MP1525 Product Code: 1525-300 (IgM) MP1625 Product Code: 1625-300 (IgA)