Monobind Inc.
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AccuLite
CLIA Microwells

H. Pylori Ab IgG, IgM, IgA Test Systems

Product Codes: 1475-300 IgG
1575-300 IgM & 1675-300IgA

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Anti-H.Pylori Specific Antibodies of the IgG, IgA or IgM type in Human Serum or Plasma by Microplate Enzyme Immunoassay, Chemiluminescence

2.0 SUMMARY AND EXPLANATION OF THE TEST

Helicobacter pylori has been shown to be the unidentified curved bacillus that was observed by Warren[1] and Marshall[2] in close contact with gastric epithelium in biopsy studies of patients suffering from chronic gastritis. Although the source of *H. pylori* infection is not known, the evidence is quite convincing that the bacillus can cause acute gastritis and may lead to chronic gasteritis[3, 4]. Sethi et al[5] has reported that *H. pylori* was present in ninety-one (91) percent of patients with chronic superficial gastritis. Marshall[6] determined that *H. pylori were* present in ninety (90) percent of duodenal ulcer and seventy (70) percent of gastric ulcer patients.

The use of serological testing to ascertain the immunologically produced antibody caused by *H. pylori* infection has been suggested as the method of choice to screen large populations. Measurements of the antibodies to *H. pylori* have been done by hemagglutination, serum complement fixation and bacterial agglutination. These tests do not have the sensitivity of enzyme immunoassay and are limited by subjective interpretation. This procedure, with the enhanced sensitivity of EIA, permits easy detectability of antibodies to *H.Pylori*. In addition, the results are quantitated by a spectrophotometer, which eliminates subjective interpretation.

Monobind's microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, diluted patient specimen, or control is first added to a microplate well. Biotinylated *H. Pylori* is added, and then the reactants are mixed. A reaction result between the autoantibodies to *H.Pylori* and the biotinylated *H.Pylori* to form an immune complex, which is deposited to the surface of streptavidin coated wells through the high affinity reaction of biotin and streptavidin.

After the completion of the required incubation period, aspiration or decantation separates the reactants that are not attached to the wells. An enzyme anti-human IgG, M or A conjugate is then added to permit quantitation of reaction through interacting with human IgG, M or A of the immune complex. After washing, the enzyme activity is determined by reaction with substrate to produce light. The employment of several serum references of known antibody activity permits construction of a graph of enzyme and antibody activities. From comparison to the dose response curve, an unknown specimen's enzyme activity can be correlated with autoimmune antibody level.

3.0 PRINCIPLE

A Sequential CLIA Method (TYPE 1):
The reagents required for the sequential CLIA assay include immobilized antigen, circulating autoantibody 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 exogenous added biotinylated H. Pylori antigen.

Upon mixing biotinylated antigen 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:

$$h\text{-}Ab_{(X\text{-}H.Pylori)} + {}^{Btn}Ag_{(H.Pylori)} \underset{k_a}{\overset{k_a}{\rightleftharpoons}} h\text{-}Ab_{(X\text{-}H.Pylori)} - {}^{Btn}Ag_{(H.Pylori)}$$

$^{Btn}Ag_{(H.Pylori)}$ = Biotinylated Antigen (Constant Quantity)
$h\text{-}Ab_{(X\text{-}H.Pylori)}$= Human Auto-Antibody (Variable Quantity)
$Ab_{(X\text{-}H.Pylori)} - {}^{Btn}Ag_{(H.Pylori)}$ = Immune Complex (Variable Quantity)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antigen. This interaction is illustrated below:
$h\text{-}Ab_{(X\text{-}H.Pylori)} - {}^{Btn}Ag_{(H.Pylori)} + Streptavidin_{CW} \Rightarrow$ immobilized complex (IC)
$Streptavidin_{CW}$ = 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 conjugates binds to the immune complex that formed.
$IC_{(h\text{-}IgG,M\text{ or }A)} + {}^{Enz}Ab_{(X\text{-}h\text{-}IgG,M\text{ or }A)} \Rightarrow {}^{Enz}Ab_{(X\text{-}h\text{-}IgG,M\text{ or }A)} - IC_{(h\text{-}IgG,M\text{ or }A)}$
$IC_{(h\text{-}IgG,M\text{ or }A)}$ = Immobilized Immune complex (Variable Quantity)
$^{Enz}Ab_{(X\text{-}h\text{-}IgG,M\text{ or }A)}$ = Enzyme-antibody Conjugate (Constant Quantity)
$^{Enz}Ab_{(X\text{-}h\text{-}IgG,M\text{ or }A)}$ - I.C. $_{(h\text{-}IgG,M\text{ or }A)}$ = Ag-Ab Complex (Variable)

The anti-h-IgG, IgM or IgA 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

4.0 REAGENTS

Materials Provided:

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)** U/ml* of the IgG, IgM or IgA type. Store at 2-8°C. A preservative has been added. *Manufacturers' Reference Value

B. H.Pylori Biotin Reagent – 13ml/vial – Icon ∇
One (1) vial of 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 Tracer Reagent – 13ml/vial – Icon Ⓔ
One (1) vial of anti-human IgG, IgM or IgA-horseradish peroxides (HRP) conjugate in a buffering matrix. A preservative has been added. Store at 2-8°C**.**

D. Light Reaction Wells – 96 wells – Icon ⇓
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/vial
One (1) vial of serum diluent containing buffer salts and a dye. Store at 2-8°C.

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

G. Signal Reagent A – 7.0ml/vial - Icon C^{A}
One (1) vial containing luminol in buffer. Store at 2-8°C. (see Reagent Preparation Section).

H. Signal Reagent B – 7.0ml/vial - Icon C^{B}
One (1) vial containing hydrogen peroxide (H_2O_2) 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: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.010, 0.025, and 0.050ml (10, 25 & 50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 and 0.350ml (100 & 350µl) 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.
9. Quality control materials.

5.0 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 Hepatitis B Surface Antigen, 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 serum products should 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, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395**.**

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 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 redtop venipuncture 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 specimen to separate the serum or plasma from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

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 use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100µl for IgM & IgA) or 0.050ml (50µl for IgG) of the diluted specimen is required.

7.0 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.

8.0 REAGENT PREPARATION

1. Serum Diluent
Dilute the serum diluent to 200ml in a suitable container with distilled or deionized water. Store at 2-8°C.

2. Wash Buffer
Dilute contents of wash solution to 1000 ml with distilled or deionized water in a suitable storage container. Store at 2-30°C for up to 60 days.

3. Working 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 two (2) eight well strips (A slight excess of solution 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 0.010ml (10µl) of each patient specimen into 1ml of serum diluent. Cover and vortex or mix thoroughly by inversion. Store at 2-8°C for up to forty-eight (48) hours

Note: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference calibrator, control or diluted patient specimen into the assigned well for IgG determination. **For IgM or IgA, pipette 0.050ml (50µl) of the appropriate serum reference calibrator, control or diluted patient specimen into the assigned well.**
3. Add 0.100 ml (100µl) of H.Pylori Biotin Reagent.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 45 minutes at room temperature**.**
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times.**
8. Add 0.100 ml (100µl) of Anti-Ig (x-IgG, IgM or IgA) Tracer Reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
   DO NOT SHAKE THE PLATE AFTER TRACER ADDITION
9. Cover and incubate for thirty (30) minutes at room temperature.
10. Repeat steps (6 & 7) as explained above.
11. Add 0.100 ml (100µl) of Working Signal Reagent to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
    DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION
12. Incubate at room temperature for five (5) minutes in the dark.
13. Read the RLUs (*Relative Light Units*) in each well in a microplate luminometer for at least 0.2 seconds/ well. **The results can be read within 30 minutes of adding the signal solution.**

Note: For re-assaying specimens with concentrations greater than 100 U/ml, dilute the sample an additional 1:5 or 1:10 using the original diluted material in the serum diluent. Multiply by the dilution factor to obtain the concentration of the specimen.

10.0 CALCULATION OF RESULTS

A reference curve is used to ascertain the concentration of anti-*H. Pylori* in unknown specimens.
1. Record the RLUs obtained from the printout of the microplate luminometer as outlined in Example 1.

2. Plot the RLUs 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 RLUs 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 RLUs (70400) of the unknown intersects the calibration curve at (69.3U/ml) anti-*H.Pylori* concentration (See Figure 1)*.

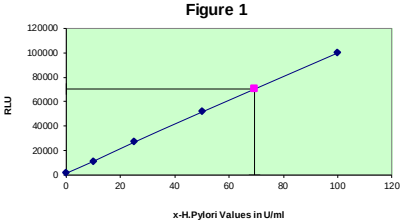
Note: Computer data reduction software designed for chemiluminescence assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

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

Sample I.D.	Well Number	RLU (A)	Mean RLU (B)	Value (U/ml)
Cal A	A1	1427	1479	0
	B1	1529		
Cal B	C1	11288	11231	10
	D1	11172		
Cal C	E1	26584	26825	25
	F1	27604		
Cal D	G1	49362	51570	50
	H1	53776		
Cal E	A2	99903	100000	100
	B2	100097		
Control	C2	7857	7756	***6.5***
	D2	7653		
Patient	A3	71366	70400	***69.3***
	B3	69434		

* The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay. In addition, the RLUs of the calibrators have been normalized to 100,000 RLUs for the E calibrator (greatest light output). This conversion eliminates differences cause by efficiency of the various instruments that can be used to measure light output.

Figure 1



11.0 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.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Formfor this product is available upon request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. 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 RLUs of the 100 U/ml calibrator.
9. Patient specimens with concentrations greater than 100 U/mL may be diluted (1/5 or 1/10) further than the initial 1/100 dilution using the serum diluent. The sample's concentration is obtained by multiplying the result by the dilution factor.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 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 specimens 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 MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. 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.
7. The clinical significance of the result should be used in evaluating the possible presence 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, 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.

13.0 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 H. Pylori AccuLite® CLIA test system. Based on the data following cut-off points were established.

Presence of H.Pylori antibodies Confirmed	(CONC)
IgG	> 20 U/mL
IgA	> 20 U/mL
IgM	> 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

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the H.Pylori AccuLite® CLIA Test System 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 below.

14.1.1 Precision Anti-H. pylori - IgG

TABLE 1
Within Assay Precision (Values in U/ml)

Sample	N	X	σ	C.V.
Negative	20	4.6	0.28	6.1%
Positive	20	46.4	2.23	4.8%

TABLE 2*
Between Assay Precision (Values in U/ml)

Sample	N	X	σ	C.V.
Negative	10	5.2	0.43	8.2%
Positive	10	48.2	3.13	6.4%

*As measured in ten experiments in duplicate.

14.1.2 Precision Anti-H. Pylori - IgM

TABLE 3
Within Assay Precision (Values in U/ml)

Sample	N	X	σ	C.V.
Negative	20	3.4	0.22	6.4%
Positive	20	59.2	4.30	7.3%

TABLE 4*
Between Assay Precision (Values in U/ml)

Sample	N	X	σ	C.V.
Negative	10	3.7	0.31	8.4%
Positive	10	61.3	3.70	6.0%

*As measured in ten experiments in duplicate.

14.1.3 Precision Anti-H. Pylori - IgA

TABLE 5
Within Assay Precision (Values in U/ml)

Sample	N	X	σ	C.V.
Negative	20	2.8	0.19	6.8%
Positive	20	44.2	2.35	5.3%

TABLE 6*
Between Assay Precision (Values in U/ml)

Sample	N	X	σ	C.V.
Negative	10	2.8	0.25	8.9%
Positive	10	45.1	3.31	7.3%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the '0 U/ml' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose:

The IgG AccuLite® CLIA test system has a sensitivity of 0.0956U/ml.
The IgM AccuLite® CLIA test system has a sensitivity of 0.1430U/ml.
The IgA AccuLite® CLIA test system has a sensitivity of 0.2100U/ml.

14.3 Specificity, & Accuracy

The 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 spciemens was 245.

	IgG	IgM	IgA
Specificity	98%	88%	91%
Accuracy	97%	91%	93%

15.0 REFERENCES

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MP1475 Product Code: *1475-300 (IgG)*
MP1575 Product Code: *1575-300 (IgM)*
MP1675 Product Code: *1675-300 (IgA)*

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

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2°-8° — Temperature Limitation Storage Condition (2-8°C)
Consult Instructions for Use
REF — Catalogue Number
Σ — Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity