GAP®-IgG

(Gastritis and Peptic Ulcer Test)

Catalog # 7004D

ELISA test for the Detection of *Helicobacter pylori* Specific IgG Antibodies

August 2018

For in vitro diagnostic use

BIOMERICA

I. INTENDED USE

The GAP-IgG is an ELISA test for the qualitative detection of IgG specific antibodies to *Helicobacter pylori* (*H. pylori*) in human serum. It is a serum test which, when used with other clinical information, can be used as an aid in the diagnosis of infection caused by *H. pylori*.

II. SUMMARY AND EXPLANATION

In 1983, *Helicobacter pylori* (previously known as *Campylobacter pylori*) was found in close contact with gastric epithelium in biopsy samples showing active type B or chronic gastritis (6,7,8,9,10). Although the source of *H. pylori* infection is unknown, reports (3,12,14) have shown quite convincingly that *H. pylori* infection can be associated with chronic gastritis. A positive correlation has been found between the presence of *H. pylori* and gastric lesions in some cases of duodenal ulcers (15,16). Complete resolution of gastritis after eradication of the organism has also been reported (17,18).

H. pylori is a gram negative, curved, spiral shaped rod (0.2 - 0.8 µm in width by 0.5 - 5.0 µm in length). *H. pylori* colonization is found in the deep portions of the mucous gel layer that coats the gastric mucosa, and between the mucous gel layer and apical surface of the gastric mucosal epithelial cells in some patients infected by *H. pylori* (1). *H. pylori* may also be located in the regions of the junctions between adjacent mucosal epithelial cells. It produces three enzymes in large amounts; urease, superoxide dismutase and catalases (14,17,18). Urease splits urea to produce ammonia, which provides conditions needed for the multiplication and sustenance of the organism in the gastric environment. The colonization may induce the host's local and systemic immune response and may cause clinical signs and symptoms including neutrophil infiltration and the production of specific antibodies.

The presence of *H. pylori* has been detected by using both invasive and non-invasive methods. Invasive methods include culture, histology, and the rapid urease test done on biopsy samples. The gold standard for the detection of *H. pylori* in tissue, is a combination of culture and histologic staining of mucosal biopsy specimens obtained by endoscopy (2). Due to *H. pylori*'s patchy distribution and difficulties to culture under controlled conditions, false negatives are common. Histological staining by Giemsa or hematoxylin can detect the bacterium. Urease activity of the biopsy sample can be used as an indicator to the presence of the organism. Non-invasive procedures include the urea breath test, which utilizes a radioisotope of carbon labeled urea, and the detection of antibodies to *H. pylori* in the sera. Antibody production against *H. pylori* has been studied by several different groups. In 1986, Goodwin et al. (21) used serum complement fixation, hemagglutination and bacterial agglutination. Rathbone et al. (22) used ELISA techniques to show a significant rise of IgG specific antibodies to *H. pylori* in patients with histological evidence of the organism. A serological determination of antibodies specific to *H. pylori* is simple and can be performed in any clinical laboratory.

The understanding of how *H. pylori* causes disease is in its infancy. It can survive in the gastric mucosa and stay there for years. *H. pylori* is also frequent in individuals considered to be otherwise normal with no clinical signs or symptoms of the disease. Histologic gastritis has long been recognized as common in healthy asymptomatic individuals. *H. pylori* infection has been reported in the gastric biopsy samples of healthy individuals who were found to be normal with the endoscopic examination(1).

The GAP-IgG is an ELISA test to be used for the qualitative detection of IgG specific antibodies to *H. pylori*. However, the presence the IgG specific antibodies does not preclude the diagnosis of chronic gastritis. The serological findings should be confirmed by histological examination, urease activity, and culture tests on the biopsy samples of individuals suspected of having *H. pylori* infection. All the clinical signs and symptoms, and serological and histological results should be examined before making any decision regarding the disease state of the patient.

III. PRINCIPLE OF THE TEST

The GAP-IgG test is a qualitative ELISA test to detect the presence of IgG antibodies specific to *H. pylori* in human serum. Partially purified *H. pylori* antigens are immobilized on the wells of a microwell plate. Diluted patient serum is added to the wells. IgG antibodies specific to *H. pylori*, if present, bind to the antigen on the microwells. Excess IgG antibodies are washed away with buffer. Anti-Human IgG Enzyme Conjugate is added to the wells. The conjugate binds with the antigen-antibody complex, if present, on the plate. Excess enzyme conjugate is washed away and a color is developed by the addition of an enzyme substrate. The intensity of the color corresponds directly to the amount of antibody present. The color intensity read on the spectrophotometer is a direct interpretation of *H. pylori* specific antibodies in the patient serum.

IV. KIT COMPONENTS

1. 12 x 8 microwell strips coated with inactivated *H. pylori* antigens. The strips are packaged in twelve-strip holders, sealed in a zip lock bag with desiccant. Carefully reseal unused strips in the zip lock bag.
2. GAP *H. pylori* IgG Calibrators & Controls, human serum base, 7 x 1.0 ml vials:

   GAP IgG Calibrator 0 (0 Units/ml)
   GAP IgG Calibrator 1 (12.5 Units/ml)
   GAP IgG Calibrator 2 (25 Units/ml)
   GAP IgG Calibrator 3 (50 Units/ml)
   GAP IgG Calibrator 4 (100 Units/ml)

3. GAP IgG Negative Control (1 x 1.0 ml).
4. GAP IgG Positive Control (1 x 1.0 ml).
5. Anti Human IgG-HRP Conjugate (1 x 10.0 ml).
6. Substrate Solution A (TMB) (1 x 12 ml).
7. Substrate Solution B (H_2O_2) (1 x 12 ml).
8. Serum Sample Diluent (concentrate 25x) (1 x 20 ml).
9. Washing Buffer (concentrate 50x) (1 x 20 ml).
10. Stopping Solution (1N H_2SO_4) (1 x 6 ml).

Serum diluent, calibrators and controls contain .01% sodium azide as a preservative. Store kit at 2-8°C.

V. WARNINGS AND PRECAUTIONS

1. **Potential Biohazardous Material**

The matrix of the Calibrators and Controls is human serum. The human serum used has been found non-reactive to HbsAg, anti-HIV 1/2 and anti-HCV when tested with FDA licensed reagents. Because there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled as if potentially infectious

2. **Sodium Azide**

Some reagents contain sodium azide as a preservative. Sodium azide may react with lead, copper or brass to form explosive metal azides. When disposing of these materials, always flush with large volumes of water to prevent azide buildup.

3. **1N H_2SO_4**

The stopping solution, provided in the test kit, contains $1NH_2SO_4$.Contact with eyes and skin should be avoided. Wear gloves and eye cover while handling the material. Wash with large volumes of water if the solution gets in contact with the skin.

VI. PROCEDURAL NOTES

1. Strict adherence to the specified times and temperatures of incubations is essential for accurate results.
2. Do not freeze reagents.
3. Store all components of the test kit at 2-8°C at all times. Do not allow reagents to stand at room temperature for extended periods of time.
4. Positive and Negative Controls must be run each time the test is performed.
5. Do not mix reagents from different lots.
6. Do not use expired reagents.
7. Use only clear sera as test specimens. The samples should not have gross turbidity, hemolysis or any microbial contamination.

VII. ADDITIONAL MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipet with disposable tips capable of delivering 25µl and 100µl.
2. For washing, use either a microtiter plate washer (automated or manual), or a squeeze wash bottle.
3. Distilled or deionized water.
4. 5 ml and 10 ml disposable serological pipettes.
5. A 500 ml and 1000 ml graduated container.
6. Microtiter plate reader with 450 nm wavelength absorbance capability.
7. Absorbent paper towels.
8. Test tubes (13 x 100 mm) for serum dilution.
9. Parafilm or plastic wrap.

VIII. SPECIMEN COLLECTION

Collect about 5 ml of venous blood by venipuncture into a glass "vacutainer" (red top) tube. The use of serum separators is allowed, but do not use anticoagulants or preservatives. Separate serum by centrifugation. Serum samples may be stored refrigerated (2-8°C) for up to 10 days. If the sample cannot be tested in this period, store it frozen at -20°C. Excessive hemolysis and lipemia, the presence of large clots or bacterial growth in the specimen, may interfere with the performance and accuracy of the test.

IX. REAGENT PREPARATION AND STORAGE

1. **Serum Sample Diluent Buffer (1:25 Dilution):**
   Add the entire contents of the vial into a 500 ml container with 480 ml of distilled water. Rinse out any crystals that may be present. Mix thoroughly. Label the container as "**Working Sample Diluent Buffer**", and store at 2-8°C until use. The reconstituted reagent is stable for the shelf-life of the kit when stored at 2-8°C. Use 5 ml for each patient sample.
2. **Washing Buffer (1:50 Dilution):**
   Add the entire contents of the vial into 980 ml of distilled or deionized water in a 1000 ml container. Rinse out any crystals that may be present. Mix thoroughly. Label the container as "**Working Washing Buffer**" and store at 2-8°C until use. The "Working Washing Buffer" is stable for the shelf-life of the kit when stored at 2-8°C.
3. **Substrate Reagent:**
   Mix Substrate Solution A and Substrate Solution B in the ratio of 1:1 and label as **"Working Substrate".** Prepare the Working Substrate within one hour prior to use. Prepare only the required volume. For example: For six microwell strips mix 2.5 ml of Substrate Solution A with 2.5 ml of Substrate Solution B. The Working Substrate is stable for 60 minutes at room temperature.
4. **Storage of Remaining Kit Components**
   H. pylori antigen coated strips, calibrators and controls, substrate solutions and stopping solution should be stored at 2-8°C and are stable until the kit expiration date.

X. ASSAY PROCEDURE

The test kit contains 12 microwell strips. Assemble the microwell strips (the number depends on the number of specimen samples to be tested) into the left side of the strip holder. Return the unused strips to the original ziplock bag and store at 2 - 8°C.

NOTES:

Sample Dilution: Accurately pipet 25 µl of each patient serum to 5 ml of Working Sample Diluent Buffer (see #1 of the above) in a 13 x 100 mm test tube (1:200 dilution). Mix thoroughly by inversion or vortexing. Appropriately label all tubes containing diluted test samples. The diluted samples are stable for 2 weeks at 2-8°C.

Well Positions Chart:

Al,B1	Calibrator 0	C2,D2	Negative Control
C1,D1	Calibrator 1	E2,F2	Positive Control
E1,F1	Calibrator 2	G2,H2	Patient Sample #1
G1,H1	Calibrator 3	A3,B3	Patient Sample #2
A2,B2	Calibrator 4		

1. **Serum Incubation:**
   a. Into the appropriate wells, as shown in the "well positions chart", dispense 100 µl of Calibrators 0 through 4, Negative Control, Positive Control, and diluted patient serum samples.
   b. There should be 100 µl of solution in each microwell.
   c. Cover the plate with a plate cover and let it stand for 1 hour at room temperature (22-26°C).
2. **Wash Procedure:**
   After a one hour incubation, discard contents of all wells into the sink by quick decantation. Blot the plate dry with a paper towel. If you use an automatic or manual plate washer, wash each well three times with about 300 µl of washing buffer according to the instrument manufacturer's instructions. If you use a squeeze bottle, fill it with washing buffer. Carefully fill the wells up one by one with the washing buffer by squeezing the bottle (avoid air bubbles in the well during washing), discard the washing buffer, blot dry with a paper towel, and then repeat the procedure two more times, making sure that the plate is blotted dry each time between washes.
3. **Conjugate Incubation:**
   a. Add 100 µl of Anti-Human IgG-HRP Enzyme Conjugate reagent into all wells.
   b. Cover the plate (as in step # 1c) and let it stand for 30 minutes at room temperature (22-26°C).
4. **Wash (same as step #2)**.
5. **Substrate Incubation:**
   a. Add 100 µl of the "Working Substrate" Reagent (see "Reagent Preparation" Section, #3), into all wells.
   b. Cover the plate (as in step #1c) and let it stand **in the dark** at room temperature (22-26°C) for 10 minutes.
6. **Stop Reaction/Read Absorbance:**

a. After exactly 10 minutes, add 50 µl of Stopping Solution to all of the wells.
b. Set the microplate reader to read at a single wavelength of 450 nm and measure the optical density (O.D.) of each well. The plate should be read within 30 minutes after addition of the stop solution.

XI. CALCULATION OF DATA

1. Read the O.D. of the Calibrators, Controls and Patient Samples and record the data as shown in Table #1.
2. Calculate the net O.D. of the Calibrators, Controls and Patient Samples by subtracting the O.D. of the Calibrator 0 from the O.D of Calibrators, Controls and Patient Samples as shown in Table #1. Note, calculation of net O.D. is optional.
3. Use an automated regression program to reduce the data, or construct a calibration curve on linear graph paper using the net O.D. on the Y axis and the calibrator value on the X axis. If an automated regression system is used, acceptable results will be obtained by using (1) Quadratic, (2) Cubic Spline or (3) Point to Point. Once your regression type has been validated on the GAP IgG do not change unless a full validation is again performed.
4. Interpolate the patient values from the calibration curve.

TABLE 1

Sample Calculations:

I.D.	O.D.	Net. O.D.	Value* units/ml
Calib. 0	.138	0.0	0.0
Calib. 1	.529	.391	12.5
Calib. 2	.943	.805	25.0
Calib. 3	1.457	1.319	50.0
Calib. 4	2.070	1.794	100.0
Negative Control	.298	.160	5.0
Positive Control	1.078	.940	32.0
Pat. Spl. 1	1.042	.904	29.5
Pat. Spl. 2	.306	.168	5.2

XII. QUALITY CONTROL

1. The O.D. of the Calibrator 0 must be < 0.20
2. Negative Control should read less than 10 units/ml*
3. Positive Control should read 25 - 50 units/ml*

** Biomerica Reference Units*

XIII. INTERPRETATION

Positive Result: (>20 Units/ml) Patient samples with values greater than 20 units/ml are considered to be positive for the presence of IgG specific antibodies to *H. pylori*.

Negative Result: (<18 Units/ml) Patient samples with values less than 18 units/ml should be considered negative for the presence of IgG specific antibodies to *H. pylori*.

Equivocal Result: (Between 18 and 20 Units/ml) Patient samples with values greater than or equal to 18 and less than or equal to 20 Units/ml are equivocal. Patients with equivocal results should be redrawn after two weeks, and the second sample run together with the first sample.

NOTE: Depending on the locale and population, each customer should establish its own cut-off points for negative and positive determination. The above cut-off values should be used as guidelines.

XIV. EXPECTED VALUES

Elderly people (50 years of age or more) have a very high chance of past encounters with *H. pylori* and may have a low but detectable level of *H. pylori* specific IgG antibodies throughout their life. These patients may have a detectable *H. pylori* antibody level but no clinical symptoms and no active infection. This age related phenomenon, with elevated *H. pylori* specific antibodies, has been reported by a number of investigators (19,20,21,22).

DIAGNOSIS	INCIDENCE*
Chronic Atrophic Gastritis	85-100%
Superficial Gastritis	70-95%
Duodenal Ulcer	85-95%
Gastric Ulcer	70-90%

* The data was compiled from 173 patients reporting clinical signs of symptoms related to gastritis in a study performed at four separate clinics using the Biomerica GAP-IgG ELISA procedure.

XV. LIMITATIONS

1. The GAP-IgG ELISA is a qualitative test to detect the presence of IgG specific antibodies to *H. pylori* and does not indicate the titer of the antibody.
2. A positive test result does not distinguish between colonization and infection by *H. pylori*, and does not indicate the presence of gastrointestinal disease. It should be used as an aid to the detection of such a disease with other clinical tests such as Histology, Urease and Culture.
3. A negative test result does not preclude the absence of antibodies to *H. pylori*. The colonization may be there, but may be in its very early stages, or the antibody titer may be too low for the test to detect.
4. If the clinical symptoms and test results are inconclusive, additional testing should be done on a subsequent sample.
5. The GAP IgG ELISA test should be used only for patients reporting clinical signs and symptoms related to gastritis. The test should not be used for patients who are asymptomatic.

XVI. PERFORMANCE CHARACTERISTICS

Specificity and Sensitivity:

A total of 277 patients, reporting to the physicians with clinical signs of symptoms related to gastric disease, were evaluated. Of these, 104 were confirmed *H. pylori* negative and 173 were confirmed *H. pylori* positive by biopsy. Four non-affiliated individual clinics were included in the study. The GAP ELISA test results were compared to the endoscopic biopsy sample findings.

GAP-IgG ELISA

		Positive+	Equiv.±	Negative –	Total
Biopsy(1)	+	172	0	1	173
	–	6	11(2)	87	104
				Total Samples	277

Relative Accuracy = 97.4%
Relative Specificity = 93.5%
Relative Sensitivity = 99.4%

(1) By histological techniques (culture or urease on the biopsy samples).
(2) Equivocal results have not been considered in the calculations for percent accuracy and percent specificity. Equivocal results (± values) are considered negative in the GAP-IgG ELISA procedure.

Precision:

1. Intra-assay precision was determined by measuring the units/ml of 12 replicates of confirmed Low, Medium, and High antibody positive *H. pylori* patient samples.

	Low	Medium	High
Number of determinations:	12	12	12
Mean value:	11.4	21.2	45.9
S.D.	0.82	2.3	3.6
C.V.	7.1%	11.2%	7.9%

2. Inter-assay variability of the test for sample results was calculated from values obtained from confirmed Low, Medium, and High antibody

positive *H. pylori* patient samples over a period of five months, using four different operators.

	Low	Medium	High
Number of determinations:	10	10	10
Mean value:	11.6	20.6	45.3
S.D.	1.6	2.2	3.3
C.V.	13.7%	10.6%	7.2%

Cross-Reactivity: Inhibition assays were performed using extracts from pure strains of *Campylobacter jejuni*, *C. coli*, *C. fetus*, and *Escherichia coli*. Less than 1% cross-reactivity was observed when the human antibodies to *H. pylori* were absorbed with 10 µg per sample of the above bacterial extracts and then run on the GAP-ELISA assay procedure (23).

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XVIII. ORDERING, TERMS, DISCOUNT AND DELIVERY

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