Export Only Version

GAP®-IgM

REF **7006 GAP IgM**

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BIOMERICA

I. INTENDED USE

This immunoassay is for the quantitative detection of IgM antibodies to *Helicobacter pylori* in human serum. These reagents are for *in vitro* diagnostic use only.

II. SUMMARY OF THE TEST

Helicobacter pylori has been found in biopsy samples from gastric epithelium from patients showing active type B gastritis. [1,2,3,4,5] Although the route of infection is unknown, reports have shown that *H. pylori* infection is associated with chronic gastritis. [6,7] A correlation has been found between the presence of *H. pylori* and gastric lesions in some cases of duodenal ulcers. [8] Complete resolution of gastritis after eradication of the organism has also been reported. [9]

H. pylori is a gram negative, curved, spiral-shaped rod, 0.2 – 0.8 µ in width by 0.5 – 5.0 µ in length. It colonizes the deep portion of the mucous gel layer and the apical surface of the gastric mucosal epithelial cells. It may also be located in the junction between adjacent mucosal epithelial cells.

The presence of *H. pylori* can be detected by both invasive and non-invasive methods. Invasive methods include testing biopsy specimens by culture, histology and rapid urease. Due to the patchy distribution of *H. pylori* in tissue, false negative results are common. Non-invasive procedures include serum tests for antibodies against *H. pylori* and the urea breath test which utilizes radiolabelled urea.

The mechanisms by which *H. pylori* causes disease is not well understood. It can survive in the gastric mucosa, possibly for several years. *H. pylori* is also common in individuals who are considered healthy, with no clinical symptoms of disease.

III. PRINCIPLE OF THE PROCEDURE

The GAP IgM kit is quantitative ELISA assays for the detection of antibodies specific for H. *pylori* in human serum. Partially purified *H. pylori* antigens are immobilized on the wells of a microtiter plate and diluted patient serum is added to the wells. The antibodies in the serum, specific to *H. pylori*, will bind to the antigen on the wells. Non-specific antibodies are washed away. Anti-human IgM enzyme conjugate is added to the wells and binds to the antibody-antigen complex. Excess enzyme conjugate is washed way. The color is developed by the addition of an enzyme substrate. The intensity of the color is directly proportional to the amount of antibody present in the serum sample.

IV. KIT COMPONENTS

1. **PLA H. pylori = Antigen-Coated Wells** – 96 microtiter wells coated with inactivated *H. pylori* antigens.
2. **CAL 0-4 = Calibrator Set** – 5 vials, 1 mL each of zero, 12.5, 25, 50 and 100 units/mL of IgM antibodies against *H. pylori* in buffered human serum.
3. **CTRL + - = Control Set** – 1 mL each positive and negative for IgM antibodies against *H. pylori* in buffered human serum.
4. **DIL SPE 10X = Sample Diluent Concentrate** – 50 mL of phosphate buffered saline with Tween.
5. **CONJ ENZ = Enzyme Conjugate** – 10 mL of goat anti-human IgM horseradish peroxidase conjugate in a TRIS/BSA buffer.
6. **SUBS A TMB = Substrate Solution A** – 12 mL TMB in an acetate buffer with DMSO.
7. **SUBS B H_2O_2 = Substrate Solution B** – 12 mL hydrogen peroxide in an acetate buffer.
8. **BUF WASH 50X = Wash Concentrate (50x)** – 20 mL phosphate buffered saline with Tween.
9. **SOLN STOPPING = Stop Solution** – 6 mL of 1 N H_2SO_4.

Store all kit components at 2 – 8°C

Calibrators, controls and serum diluent contain 0.09% sodium azide as a preservative.

V. ADDITIONAL EQUIPMENT NEEDED

1. Microplate reader
2. Microplate washer
3. Pipettes capable of dispensing 25, 50 and 100 µL
4. Volumetric pipettes: 5 and 10 mL
5. Graduated cylinders: 500 and 1000 mL
6. Test tubes: 13 x 100 mm
7. Plastic film
8. Deionized water

VI. PRECAUTIONS

The components of this product contain material of human origin which has been tested by FDA approved methods and found to be non-reactive for Hepatitis B Surface Antigen (HbsAg), HIV-1/2 and HCV antibodies. However, no test method can offer complete assurance of safety. These components have NOT been tested for antibodies to HTLV-1 or HTLV-II. For these reasons it is recommended that the components of this product be considered potentially infectious and handled with the same precautions used with patient samples.

Some reagents in this kit contain sodium azide as a preservative. Sodium azide may react with lead and copper plumbing to form explosive metal azides. When disposing of these reagents, always flush with large volumes of water to prevent azide build-up.
The Substrate Solution A contains Dimethyl Sulfoxide (DMSO). DMSO is a skin irritant and can also cause irritation to the mucosal

membranes and upper respiratory tract if inhaled, or ingested. Avoid exposure by wearing personal protective equipment such as gloves and safety glasses. If skin or eye contact occurs, flush with water for a minimum of 15 minutes. If inhaled, move to fresh air. If ingested, obtain medical attention.

A sulphurous odor may be present in the Substrate Solution A and will not affect ELISA results. The handling of Substrate Solution A and preparation of the Working Substrate Solution should be done in a fume hood or a well-ventilated area to minimize exposure.

The Stop Solution contains Sulfuric Acid (H_2SO_4), which is a skin and eye irritant. Avoid skin and eye contact. Wear gloves and safety glasses. If skin or eye contact occurs, flush with water for a minimum of 5 minutes..

VII. REAGENT PREPARATION AND STORAGE

Sample Diluent Solution: Add the entire contents of the vial of Sample Diluent concentrate into a 500 mL container. Rinsing the vial with water to remove any precipitated crystals, add 450 mL of deionized water. Mix thoroughly.

Wash Solution: If crystals are present in the Wash Buffer concentrate due to storage at a lower temperature such as 2-8°C, dissolve by placing the vial in a 37°C water bath or incubator for 30 minutes. Add the entire contents of the Wash Concentrate into a 1 liter container. Rinsing the vial with water to remove any precipitated crystals, add 980 mL of deionized water. Mix thoroughly.

Working Substrate Solution: Within 1 hour of use, mix equal volumes of Substrate Solution A and Substrate Solution B. Prepare only the required amount. Each microwell strip will require a minimum of 800 µL of Working Substrate Solution. As an example, for 6 microwell strips, mix 3.0 mL of Substrate Solution A with 3.0 mL of Substrate Solution B. Store in the dark or cover with aluminum foil. Use within 1 hour.
The wash solution, sample diluent solution and open kit reagents are stable for 60 days, when stored at 2 – 8°C.

VIII. SPECIMEN COLLECTION AND STORAGE

The specimen should be serum. The usual precautions for venipuncture should be observed. Do not use anticoagulants or preservatives. Do not use grossly hemolyzed or grossly lipemic specimens. Serum may be stored at 2-8°C for up to 10 days; for longer periods, serum should be stored at –20°C.

IX. SPECIMEN PREPARATION

Each serum sample should be diluted with the Sample Diluent Solution. Add 25 µL of the serum sample to 5 mL of Sample Diluent Solution. Mix thoroughly. Controls and calibrators are supplied prediluted and are ready to use.

X. PROCEDURE

1. All reagents and samples should be brought to room temperature (18-25°C) before the start of the assay. Assemble the desired number of microwell strips into the strip holder.
2. Pipet 100 µL of each calibrator, control and diluted patient sample into each well. The calibrators and controls are prediluted and ready to use. Cover the plate with plastic film and incubate for 1 hour at 22-26°C.
3. Decant the wells and wash 3 times with 300 µL of Wash Solution. Blot thoroughly after each wash step.
4. Add 100 µL of Enzyme Conjugate to each well. Cover the plate with plastic film and incubate for 30 minutes at 22-26°C.
5. Prepare the Working Substrate Solution. See REAGENT PREPARATION for more information.
6. Decant the wells and wash 3 times with 300 µL of Wash Solution. Blot thoroughly after each wash step.
7. Add 100 µL of the Working Substrate Solution to each well. Cover the plate with plastic film and incubate for 10 minutes **in the dark** at 22-26°C.
8. Immediately after incubate, add 50 µL of Stop Solution to each well. Mix the reagents by gently tapping the plate.
9. Using a wavelength of 450 nm, measure the optical density of each well. The plate should be read within 30 minutes of adding the Stop Solution. Use quadratic curve fitting for the calibration curve and for calculating results.

XI. QUALITY CONTROL

In keeping with good laboratory practice, the Positive and Negative Controls should be run in parallel with patient specimens. Failure to obtain the appropriate values for controls may indicate imprecise manipulations, improper sample handling or deterioration of reagents.

	GAP IgM
Negative	< 10 U/mL
Positive	40-70 U/mL
Zero	< 0.2 mOD

XII. INTERPRETATION OF RESULTS

	GAP IgM
Positive	> 40
Equivocal	36 – 40
Negative	< 36

Patients with equivocal results should be redrawn after 2 weeks and the second sample assayed together with the first sample.

XIII. SPECIFICITY AND SENSITIVITY

Clinical trials with the GAP IgM assay was performed at four clinics on patients presenting with clinical symptoms typical of gastritis. Biopsy samples taken from these patients were tested by culture, urease and/or histological stains. The GAP results were compared to the biopsy results.

	GAP IgM
Sensitivity (%)	93.8
Specificity (%)	82.0
Accuracy (%)	89.3
Patients	109

WITHIN-RUN PRECISION
Within-run precision was determined by testing 12 replicates of three serum samples.

GAP IgM

Mean (U/mL)	Standard Deviation (U/mL	Coefficient of Variation (%)
10.2	0.5	4.9
25.2	1.4	5.7
44.4	2.5	5.7

BETWEEN-RUN PRECISION
Between-run precision was determined by measuring three serum samples on ten separate runs, using four different operators.

GAP IgM

Mean (U/mL)	Standard Deviation (U/mL	Coefficient of Variation (%)
10.1	1.1	11.2
21.2	1.9	8.9
39.7	4.0	10.0

XIV. LIMITATIONS OF THE PROCEDURE

The GAP IgM assay is quantitative tests to detect the presence of antibodies specific for *H. pylori* and does not indicate the titer of the antibody.

A positive test result does not distinguish between colonization or infection by *H. pylori* and does not indicate the presence of gastrointestinal disease.

A negative test result does not preclude the presence of *H. pylori*. Colonization may be present but in its very early stages or the antibody titer may be too low for the test to detect.

Persons over 50 years of age have a higher likelihood of past encounters with *H. pylori* and may have low but detectable levels of antibodies specific for *H. pylori* without clinical symptoms. 10,11,12 Additionally, the incidence of seropositivity can vary by population. 13

The GAP IgM *H. pylori* assay should be used only for patients reporting clinical signs and symptoms related to gastritis. These tests should not be used on patients who are asymptomatic.

XV. SYMBOLS

	Storage Temperature
	Lot Code
	Expiration
	Manufacturer
	Authorized Representative
	Caution, see instructions
	For in vitro diagnostic use
	Catalog No.

XVI. REFERENCES

1. Marshall, B.J. and Warren, J.R.: "Unidentified Curved Bacillus on Gastric Epithelium in Active Chronic Gastritis" (letter), *Lancet,* **1**:1273, 1983.
2. Rollason, T.P. et al: "Spiral Organisms in Endoscopic Biopsies of the Human Stomach," *J. Clin. Pathol.,* **37**:23, 1984.
3. Steer, H.W.: "The Gastro-duodenal Epithelium in Peptic Ulceration," *J. Pathol.,* **146**:355, 1985.
4. Buck, G.E. et al: "Relation of *Campylobacter pyloridis* to Gastritis and Peptic Ulcer," *J. Infect. Dis.,* **153**:664, 1986.
5. Jones, D.M. et al: "*Campylobacter*-like Organisms on the Gastric Mucosa Culture, Histological and Serological Studies," *J. Clin. Pathol.,* **37**:1002, 1984.
6. Strickland, R.G. and Mackay, I.R.: "A Reappraisal of the Nature and Significance of Chronic Atrophic Gastritis," *Amer. J. Diag. Dis.,* **18**:426, 1973.
7. Pettross, C.W. et al: "*Campylobacter pylori* Relationship to Peptic Disease, Gastric Inflammation and Other Conditions," (Abstract) *Gastroenterology,* **90**:1585, 1986.
8. McKenna, D. et al: "*Campylobacter pylori* and Histological Gastritis in Duodenal Ulcer: A Controlled Prospective Randomized Trial," (Abstract) *Gastroenterology,* **912**:1528, 1987.
9. McNulty, C.A., et al: "*Campylobacter pylori* and Associated Gastritis: Investigator Blind, Placebo Controlled Trial of Bismuth Salicylate and Erythromycin Perthylsuccinate,": *Br. Med. J.*, **293**:645, 1986.
10. Jones, D.M. et al: "Antibody to the Gastric *Campylobacter*-like Organism ("*Campylobacter pyloridis*") – Clinical Correlation and Distribution in the Normal Population," *J. Med. Microbiol.*, **22**:57, 1986.
11. Rathbone, B.J. et al: "Systemic and Local Antibody Response to Gastric *Campylobacter pylori* in Nonulcerous Dyspepsia," *Gut,* **27**:642, 1986.
12. Morris, A.G. et al: "Seroepidemiology of *Campylobacter pyloridis*," *N. Z. Med. J.*, **99**:657, 1986.
13. Forman, D. et al: "An International Association Between *Helicobacter pylori* Infection and Gastric Cancer," *Lancet*, **341**:1359, 1993.

XVII. ORDERING INFORMATION

Contact: BIOMERICA, INC.
17571 Von Karman Avenue
Irvine, California 92614

Phone: (949) 645-2111
FAX: (949) 553-1231
E-Mail: bmra@biomerica.com

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MDSS GmbH
Schiffgraben 41
D-30175 Hannover
Germany