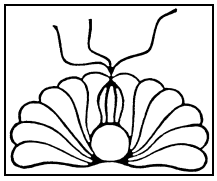
GAP®- IgM

REF **7006R**



ELISA Test for the Detection of *Helicobacter pylori* Specific IgM Antibodies

For Research Use Only
Not for Use in Diagnostic Procedures
This product must be used with
Biomerica's Certification Program

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BIOMERICA

I. INTENDED USE

The GAP IgM test is intended for in vitro research use only. It measures IgM antibodies specific to *Helicobacter pylori (H. pylori)* in human serum.

II. INTRODUCTION

H. pylori is a gram negative, curved, spiral shaped rod (0.2 - 0.8 um in width by 0.5 - 5.0 um 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*. *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. Urease splits urea to produce ammonia, which provides conditions needed for the multiplication and sustenance of the organism in the gastric environment.

III. PRINCIPLE OF THE TEST

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

IV. WARNINGS AND PRECAUTIONS

1. Potential Biohazardous Material

The matrix of the Calibrators and controls is human serum. The human serum used has been found negative for HIV antibodies as well as for Hepatitis B surface antigen when tested with FDA licensed reagents. However, as 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 at the Biosafety Level 2, as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Micro-biological and Biomedical Laboratories," 1984.

The microwell strips do not contain viable *H. pylori* organisms. However, the strips should be considered potentially infectious and handled at the Biosafety Level 2.

2. Sodium Azide

The 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.

V. PROCEDURAL NOTES

1. Strict adherence to the specified time and temperature 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.

VI. ADDITIONAL MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipet with disposable tips to deliver 25 ul and 100 ul.
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 pipet for sample dilution and 10 ml pipet for substrate buffer delivery.
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.

VII. 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 ziplock bag. Reseal carefully, any unused strips.
2. GAP *H. pylori* IgM Calibrators, human serum base, 5 x 1.0 ml vials:

   GAP IgM Calibrator 0

   GAP IgM Calibrator 1 (12.5 Units/ml)* (Dil. Human Serum)

   GAP IgM Calibrator 2 (25 Units/ml)* (Dil. Human Serum)

   GAP IgM Calibrator 3 (50 Units/ml)* (Dil. Human Serum)

   GAP IgM Calibrator 4 (100 Units/ml)* (Dil. Human Serum)
3. GAP IgM Negative Control (1 x 1.0 ml).
4. GAP IgM Positive Control (1 x 1.0 ml).
5. Anti Human IgM-HRP Conjugate (1 x 10.0 ml).
6. Substrate Solution A (1 x 12 ml).
7. Substrate Solution B (1 x 12 ml).
8. Serum Sample Diluent (10x concentrate) (1 x 50 ml).
9. Washing Buffer (concentrate). Phosphate buffered saline with Tween (1 x 20 ml vial).
10. Stopping Solution (1N H_2SO_4) (1 x 6 ml bottle).

    ** Biomerica arbitrary reference units*

Serum diluent, calibrators and controls contain .01% sodium azide as a preservative.

Store kit at 2-8°C.

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) up to 10 days. If the sample cannot be tested in this period, store 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 (1:10 Dilution):**
   Add the entire contents of the vial into a 500 ml container with 450 ml of distilled water. 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 serum sample.

2. **Washing Buffer (1:50 Dilution):**
   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 vial into 1000 ml of distilled or deionized water in a 1000 ml container. 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 solution 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.

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. The open test kit reagents are stable for 60 days when stored at 2-8°C.

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 ul of each serum sample 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.

Well Positions:

A1, 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, shown in the above diagram, dispense 100 ul of Calibrators 0 through 4, Negative Control, Positive Control, and diluted serum samples.
b. Cover the plate with parafilm and let it stand for 1 hour at room temperature (22-26°C).

2. Wash Procedure

After a one hour incubation, discard the 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 ul 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 ul of Anti-Human IgM-HRP Enzyme Conjugate reagent to all microwells.
b. Cover the plate (as in step #1b) and let it stand for 30 minutes at room temperature (22-26°C).

4. **Wash (same step #2)**.

5. **Substrate Incubation**

a. Add 100 ul of the "Working Substrate" Reagent (see "Reagent Preparation" Section, #3), into all wells.
b. Cover the plate (as in step #1b) 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 ul of Stopping Solution to all of the wells.
b. Set the microplate reader to read at a wavelength of 450 nm and measure the optical density (O.D.) of each well. The plate should be read within one hour after the 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 IgM 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. Duplicates		Mean O.D.	Value U/ml
Calib. 0	0.096	0.101	0.099	0.0
Calib. 1	0.453	0.468	0.461	12.5
Calib. 2	0.802	0.816	0.809	25.0
Calib. 3	1.377	1.389	1.383	50.0
Calib. 4	2.103	2.111	2.107	100.0
Negative Control	0.199	0.152	0.176	2.4
Positive Control	1.131	1.156	1.144	38.6
Patient Sample 1	0.405	0.412	0.409	10.1
Patient Sample 2	1.696	1.705	1.701	62.3

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 40 - 70 units/ml*.

** Biomerica Reference Values*

XIII. RESULTS

Positive Results: (>40 units/ml) Samples with values greater than 40 units/ml are considered to be positive for the presence of IgM specific antibodies to *H. pylori*.

Negative Results: (<36 units/ml) Samples with values less than 36 units/ml should be considered negative for the presence of IgM specific antibodies to *H. pylori*.

Equivocal Results: (Between 36 and 40 units/ml) Samples with values greater than or equal to 36 and less than or equal to 40 units/ml are equivocal.

XIV. LIMITATIONS

1. The GAP IgM ELISA is a qualitative test to detect the presence of IgM specific antibodies to *H. pylori* and does not indicate the titer of the antibody.

2. The GAP IgM ELISA test should be used for research purposes only and not for use in diagnostic procedures. Contact Biomerica for compliance program requirements.

XV. 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* serum samples.

	Low	Medium	High
Number of determinations:	12	12	12
Mean value:	10.24	25.16	44.42
S.D.0.50	1.44	2.52	
C.V.	4.9%	5.7%	5.7%

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 serum samples over a period of five months, using four different operators.

	Low	Medium	High
Number of determinations:	10	10	10
Mean value:	10.12	21.15	39.68
S.D.	1.13	1.89	3.96
C.V.	11.2%	8.94%	10.0%

XVI. REFERENCES

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XVII. INQUIRIES AND TECHNICAL ASSISTANCE

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