FAQ: Frequently Asked Questions

Device: GAP IgA/GAP IgG/GAP IgM ELISA (REF 7004/7008/7006)

Sample Type

What type of samples can I collect and test?
The determination of GAP should be performed on human serum. Collect venous blood in a glass "vacutainer" (red Top) tube. Serum separators may be used but do not use anticoagulants or preservatives. The serum should be promptly separated by centrifugation. Serum can be stored at 2-8°C for up to 10 days; for longer periods, serum should be stored at ≤-20°C.

What should I do if the test sample is hemolyzed or lipemic?
Grossly hemolyzed, lipemic, and icteric samples as well as samples containing particulate matter or showing obvious microbial contamination should not be tested.

How long can samples be stored before testing?
Serum samples may be stored up to 10 days at 2-8°C. For longer periods, serum should be stored at ≤-20°C, with one freeze thaw cycle.

What is the minimum volume of sample required for testing?
For duplicate testing, 200µL of diluted sample is required.

Can I test non-Human samples?
At this time, Biomerica has not validated its GAP ELISA kit with the use of non-Human plasma or serum.

Reagents

Can I interchange lot numbers?
ELISA kit components are lot specific. Do not interchange lot numbers from previous kits.

Can I interchange Reagent Components from GAP IgG, GAP IgA, or GAP IgM?
The GAP ELISA kit components are specific for each test format (IgG, IgA, and IgM). Do not interchange kit components.

What are the storage conditions for the kit components?
All reagents except the Sample Diluent, Working Substrate, and the Wash Concentrate are ready-to-use.
Store all reagents at 2-8 °C.

Can I freeze the kit reagents?
No, the kit reagents should be stored between 2°C and 8°C.

Can I reuse microwell strips?
No. Unused microwell strips must be placed back inside the resealed foil pouch. It is important to protect the strips from moisture.

How quickly should I pipette calibrators, controls and samples into the wells?
All reagents and samples should be brought to room temperature (18-25°C) before starting the assay.

What is the calibrator and control source in the GAP ELISA?
The calibrator and control source in the GAP ELISA is human serum. This kit does not have any recombinant reagents.

Is precipitate present in the wash concentrate normal?
Precipitate present in the Wash Concentrate is normal. This is due to storage at lower temperature such as 4°C. Dissolve by placing the vial in a 37° C water bath. If incubating the wash concentrate in a water bath does not dissolve the precipitate, the precipitate will dissolve once the wash concentrate is diluted to a 1X solution. To eliminate precipitate formation, the wash concentrate can be stored between 2-30°C according to the label.

What is the stability of the working wash solution?
The diluted working wash solution is stable at 2-8 °C for 60 days.

What is the stability of the working sample diluent solution?
The diluted sample diluent solution is stable at 2-8 °C for 60 days.

When should I mix Substrate Solution "A," and "B," in equal proportions?
Substrate Solution "A," and "B," should be mixed in equal proportions 1 hour prior to use. Mixed substrate solution is stable for 60 minutes at room temperature and should be kept away from light until use.

What should I do if a reagent has no label, lot or expiration date?
Do not use any reagents that lack a label, lot number or expiration date. Please contact Biomerica.

What should I do if I receive a reagent that is leaking or broken?
Do not use any reagents that appear to be leaking or broken. Please contact Biomerica.

Sample Preparation

How do I prepare the sample for testing?
Pipette 25µl of the serum sample to 5 ml of Working Sample Diluent Solution. Mix thoroughly before plating.

Calibrator and Control Preparation

Do the calibrators and controls need to be diluted?
No, the calibrators and controls are supplied prediluted and ready to use.

ELISA Testing Procedure

How long does it take to perform the GAP ELISA (IgG, IgA, IgM)?
Assay time is 1 hour 40 minutes.

What is the maximum number of samples that can be tested on one kit?
If calibrators, controls, and samples are run in duplicate, a total number of 41 samples can be run on one ELISA kit.

How long do I have to let the reagents come to room temperature before starting the assay?
Bring all reagents to room temperature before use to ensure proper kit reactivity. You may remove reagents from the foam inset to reduce the time need to come to room temperature. Typical time to come to room temperature is 1 hour.

Can I use an automated microplate washer when running ELISA?
Yes, an automated plate washer may be used. If a microplate washer is unavailable, manual washing is also acceptable.

Can I run assay via automated methods?
Biomerica has only validated its GAP ELISA kit via manual testing as outlined in the approved IFU. You would need to perform your own validation to ensure equivalent performance between the manual method and the chosen automated method.

Can I use incubation times and/or temperatures that are different than what are listed in the protocol?
Using incubation times and/or temperatures that differ from what is listed in the package insert may give erroneous results, and is therefore discouraged.

Can I read the assay at 405 nm instead of 450 nm?
No, this assay must be read at 450 nm, not at 405 nm.

Can I run calibrators, controls, and samples in singlet?
It is recommended that all calibrators, controls, and patient samples are assayed in duplicate. The average absorbance units of duplicate sets should then be used for reduction of data and the calculation of results.

How long do I have to read the plate after adding the Stop Solution?
The plate should be read within 30 minutes of adding Stop Solution

What precautions should I take when running this kit?
The source of the Calibrators and Controls is human serum, in which the human serum used has been found non-reactive to HbsAg, anti-HIV ½, and anti-HCV when tested with FDA licensed reagents. Although 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.

All specimens, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. Common precautions in handling should be exercised, as applied to any untested patient sample.

Stopping Solution consists of 1 N Sulfuric Acid. This is a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves and eye protection, with appropriate protective clothing. Any spill should be wiped immediately with copious quantities of water. Do not breathe vapor and avoid inhalation.

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.

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.

Interpretation of Results

What concentration units are expressed in the test result?
The Biomerica GAP ELISA expresses concentration results in units/mL.

Curve Fit, QC, and Data Analysis

What type of curve fitting method is acceptable to interpret data results?
Computer programs using quadratic curve fit generally give a good fit.

What is considered an invalid Test?
In assays in which one or more of the quality control sample values lay outside the acceptable limits, the results for the patient sample should be considered invalid and retested.

	GAP IgM	GAP IgG	GAP IgA
Negative	< 10 U/mL	< 10 U/mL	< 10 U/mL
Positive	40-70 U/mL	25-50 U/ml	25-50 U/mL
Zero	< 0.2 OD	< 0.2 OD	< 0.2 OD

What is recommended for internal QC of each kit run?
It is recommended that control serum or serum pools should be evaluated with each run of calibrators and patient samples. Results generated from the analysis of the control samples should be evaluated for acceptability using appropriate statistical methods.

What should I do if the sample result is equivocal?
Equivocal samples have concentrations between 18-20 U/mL for GAP IgG and IgA and between 36-40 U/mL for GAP IgM. Equivocal patients should be redrawn after 2 weeks and the second sample assayed together with the first sample.

Performance Characteristics

Does the GAP ELISA have cross-reaction with Rat or other non-human species of GAP?
Unfortunately, we don't have data to support cross-reactivity with rat or other non-human species. This assay has only been validated for use in human serum.

Limitation of Procedure

What are some of the limitations of the Biomerica GAP ELISA?
Like any analyte used as a diagnostic adjunct, GAP results must be interpreted carefully with the overall clinical presentations and other supportive diagnostic tests.

The GAP IgG, IgA and IgM assay is a test 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.

Supplemental Information

Can I purchase reagents individually?
The GAP assay is intended to be sold as a complete kit. Please contact Biomerica for reagent availability.

Troubleshooting Tips

Observation	Probable Cause
Low Absorbance: Standard Curve	Improper handling of calibrators and controls
	Omission of key reagents
	Enzyme or substrate reagent degradation
	Incorrect assay temperature
	Improper buffer/diluent used
	Reagents were cold when added to wells
	Incorrect incubation time
	Inadequate volume of substrate solution added
	Incorrect mixing of substrate A and B solution
	Wells dried out
	Excessive wash/aspiration pressure from automated plate-washer
	Plate reader set at incorrect wavelength
No Signal in Sample Well	Sample dilution error
	Sample handling error
High Background	Incomplete washing/aspiration of wells
	Substrate contamination
	Inaccurate pipetting
Poor Precision	Inadequate mixing of reagents
	Inadequate washing
	Incomplete resuspension of reagents
	Samples contain precipitates
	Wells dried out
	Presence of bubbles in wells
	Contaminated well bottom

GAP ELISA Kit – Procedural Flow Chart