

For Individual Laboratory to Complete:

***H.pylori* IgG
Enzyme Immunoassay**

Laboratory Name		
Adopted		
Reviewed		
Reviewed		
Revised		
Supercedes		

Method: Diamedix Corp., Immunosimplicity®

Manual or in conjunction with one of the Diamedix Automated EIA Systems such as the MAGO Plus, the DSX, or the DS2. For *In Vitro* Diagnostic Use.

Clinical Significance

H. pylori infection has been strongly associated with gastrointestinal diseases including gastritis, duodenal ulcer, and gastric ulcer (1,2). *H. pylori* is detectable in 80-100% of these patients (1,3). There are two categories of testing for the presence of *H. pylori*, invasive and non-invasive. Invasive techniques include culture of biopsy samples, direct detection of urease activity in biopsy sample, and histologic examination of biopsy sample (4). Non-invasive techniques include urea breath tests and serological methods. Due to the risk to the patient and expense, non-invasive techniques are preferred over invasive techniques. In the urea breath test patients are administered labeled urea and the production of labeled carbon dioxide is measured. One method of labeling the urea used exposes the patient to small amounts of radiation while the other method requires the use of a mass spectrophotometer (5,6). The advantage of the serologic test over the breath test is that it is more convenient to the patient, requires only common lab equipment and poses less risk to the patient. There has been correlation between the clinical presentation, presence of *H. pylori*, and elevated serum levels of *H. pylori* antibodies (1,7,8).

The Immunosimplicity® Is-*H. pylori* IgG Test Kit is an EIA procedure intended for the qualitative detection of *H. pylori* IgG antibodies and can be performed either manually or in conjunction with one of the Diamedix Automated EIA Systems.

Principle of the Procedure

Purified *H. pylori* antigen is bound to microwells. Diluted patient sera, Cut-Off Calibrator and controls are placed in the microwells and incubated. Anti-*H. pylori* IgG antibodies, if present, will bind to the antigen forming antigen-antibody complexes. Residual sample is eliminated by aspirating and washing. Conjugate (horseradish peroxidase-labeled anti-human IgG) is added and will bind to these complexes. Unbound conjugate is removed by aspiration and washing. Substrate is then added and incubated. In the presence of bound enzyme the substrate is converted to an end product. The

absorbance of this end product can be read spectrophotometrically at 450 nm (reference 600-630 nm). Color development above a certain level denotes the presence of antibody.

Specimen Collection

Whole blood should be collected by accepted medical techniques. Separated serum should remain at 22°C for no longer than 8 hours. If assays are not completed within 8 hours, serum should be refrigerated (2-8°C). If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -20°C. Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen sera to room temperature slowly and mix gently, avoiding foam formation. Specimens containing visible particulate matter should be clarified by centrifugation before testing. Grossly contaminated, hemolyzed, lipemic, or icteric specimens should not be used.

CAUTION: Serum samples must not be heat-inactivated prior to use.

Reagents

- Antigen Wells** Twelve, 8-well microwell breakapart strips, color-coded dark green, coated with purified *H. pylori* antigen(ATCC# 49503).
- Cut-off Calibrator** One vial with blue cap containing 0.5 ml of human serum or defibrinated plasma weakly reactive for *H. pylori* IgG antibodies, 0.1% sodium azide. The Cut-Off Calibrator is used to determine the cut-off of the assay.
- Positive Control** One vial with white cap containing 0.25 ml of human serum or defibrinated plasma, reactive for *H. pylori*, 0.1% sodium azide. Assigned range printed on label. The positive control is used to control the low range of the assay.
- Negative Control** One vial with black cap containing 0.25 ml of human serum or defibrinated plasma non-reactive for *H. pylori* antibodies, 0.1% sodium azide. Assigned range printed on label. The negative control is used to control the negative range of the assay.

Note: The Cut-Off Calibrator and controls are prepared from different serum lots.

- Sample A Diluent** One bottle with blue cap containing 60 ml Phosphate buffer with protein stabilizers. Contains 0.2% sodium azide, Proclin™ 300, 90 ppm active ingredient. Color-coded blue.
- Wash T Concentrate (20X)** Two bottles with clear caps containing 50 ml of Tris buffer with detergent and Proclin™ 300, 15ppm active ingredient. Each bottle is sufficient to make 1 liter of wash solution.

- Conjugate** One bottle with red cap containing 25 ml goat anti-human immunoglobulin G labeled with horseradish peroxidase. Also includes protein stabilizers and preservatives. Color-coded pink.
- Substrate HRP** One amber bottle with brown cap containing 25 ml buffered TMB solution (3,3',5,5' tetramethylbenzidine).
- Stop N Solution** One bottle with white cap containing 30 ml of 1 N Sulfuric Acid. **CAUTION:** Acids are corrosive. Avoid contact with skin or eyes. If contact is made, flush area with copious amounts of water. See Precautions section.

Store these reagents at 2 to 8° C.

Other Materials Required

Manual Users:

1. Wash bottle or automated microplate washer
2. Pipettors capable of dispensing appropriate volumes
3. Timer
4. One liter graduated cylinder
5. One liter wash solution reservoir
6. Deionized or distilled water
7. Absorbent toweling
8. Tubes or microwell plate for sample dilution
9. Reader capable of reading absorbance at 450nm, reference at 600-630 nm (Performance characteristics have not been established for a single wavelength reader.)

Diamedix Automated EIA System Users:

1. One liter graduated container
2. Deionized or distilled water
3. Dilution containers as appropriate to system
4. Sample and Reagent tips required by system
5. Reagent containers required by system

Warnings:

1. Handle samples, Calibrator, controls and the materials that contact them as potential biohazards. Each donor unit in the Calibrator and controls has been found negative for Hepatitis B surface antigen and HIV-I antibodies by FDA-approved third generation tests. However, because no method can offer complete assurance that HIV-1, Hepatitis B virus, or other infectious agents are absent, these materials should be handled at the Biosafety Level 2 as recommended for any potentially infectious serum or blood specimen in the Centers for Disease Control/National Institutes of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories", 1993.
2. Never pipette by mouth.
3. Avoid contact with open skin and mucous membranes.

4. Certain of the test reagents contain Proclin™ 300 as a preservative. When disposing of reagents containing Proclin™ 300, flush drains with copious amounts of water to dilute the active components below active levels.
5. Serum components contain sodium azide as preservative. Azides are reported to react with lead and copper in plumbing to form compounds that may become explosive. When disposing of solutions containing sodium azide, flush with copious amounts of water to minimize the build up of metal azide compounds.
6. Sodium azide inhibits horseradish peroxidase activity. Care must be taken to ensure that azide is not carried over from other reagents into conjugate and substrate steps.
7. Avoid contamination of the TMB substrate solution with conjugate or other oxidants which will cause the solution to change color prematurely.
8. The substrate contains 3,3', 5,5' Tetramethylbenzidine (TMB) which has shown possible mutagenic effects in laboratory experiments.

Calibration

This test uses an in-house reference standard (or Calibrator). The Calibrator has been derived from weakly positive sera and is titrated to an absorbance value equivalent to the cut-off of the assay. Samples whose absorbances exceed this value are considered positive for *H. pylori* IgG antibodies and samples whose absorbances are less than this value are considered negative for *H. pylori* IgG antibodies. To account for the inherent variations in enzyme immunoassays an equivocal range of +10% has been included at the assay cut-off.

Quality Control

- a) The Positive and Negative Controls must be included in each test run.
- b) The absorbance of the of the Blank must be < 0.25.
- c) The absorbance of the Cut-Off Calibrator must be >0.10.
- d) The Positive and Negative Controls must be within their assigned ranges.

Note: Additional controls may be tested according to guidelines or requirements of local, state, or federal regulations or accrediting organizations.

Procedure

Allow all test components and patient samples to warm to room temperature before use. Invert reagent bottles gently several times before use. Return promptly to the refrigerator after use.

Prepare Wash Solution by adding 50 ml of Wash Concentrate (20X) to one liter with deionized or distilled H₂O.

Manual Users:

1. Prepare 1:21 dilutions of the Cut-Off Calibrator (in triplicate), controls and patient samples in Sample Diluent. (e.g., by addition of 10 µl sample to 200 µl Sample Diluent).
2. Mix sample dilutions gently by withdrawing and expelling in a pipette 2 or 3 times or by vortex mixing for 2 or 3 seconds. Transfer 100 µl of Calibrator, controls and diluted patient samples, to the antigen wells. Avoid formation of bubbles when transferring diluted samples.

NOTE: *Include one well which contains 100 µl of Sample Diluent as a reagent blank. This will ultimately be used to "zero" the photometer before reading test results.*

3. Allow the wells to incubate at room temperature (18-30°C) for 30 ± 5 minutes.
4. Aspirate or discard the contents of the wells. Remove excess moisture in the wells by tapping on paper toweling. Wash the wells by rinsing 3 times with at least 300 µl of Wash Solution. Remove excess moisture from the wells after washing. When using an automated washer, follow the manufacturer's instructions.
5. Place 100 µl of Conjugate into each well, avoiding bubble formation.
6. Allow the wells to incubate uncovered at room temperature (18-30°C) for 30 ± 5 minutes.
7. Wash the wells as described in Step 4 above.
8. Place 100 µl of Substrate into each well, avoiding bubble formation.
9. Allow the wells to incubate uncovered at room temperature (18-30°C) for 30 ± 5 minutes.
10. Place 100 µl of Stop Solution into each well, avoiding bubble formation.
11. Read the absorbance of each well at 450 nm using a reference wavelength of 600-630 nm. The plate should be read within 60 minutes of adding Stop Solution.

Diamedix Automated EIA System Users:

If using one of Diamedix's Automated EIA Systems, please refer to the corresponding Operating Manual for the test setup, procedure, and accessories/consumables needed.

Calculation of Results

Calculate the mean absorbance of the Cut-Off Calibrator. Note : When calculating the mean absorbance value for the Cut-Off Calibrator exclude any absorbance value that deviates by more than 15% from the mean of the three

absorbance values. Use the mean of the remaining two replicates in calculations. Exclusion of more than one of the three absorbance values invalidates the run.

Determine the Index Value for each patient specimen or control using the following formula:

$$\frac{\text{Absorbance of Sample}}{\text{Mean Absorbance of Cut-off Calibrator}} = \text{Index Value}$$

The Diamedix Automated EIA Systems will calculate results using the above formula and print them automatically.

Example: Absorbance values obtained for the Cut-Off Calibrator: 0.276, 0.288, 0.258 (after subtraction of Blank)

Mean Absorbance of Cut-off Calibrator = 0.274

Sample Absorbance = 1.150

Index Value $1.150 / 0.274 = 4.2$

Reference Ranges

The following is only a guide to interpretation. **Each laboratory can establish its own "normal" ranges based on populations encountered.**

Index < 0.90	No detectable antibodies to <i>H.pylori</i> .
Index \geq 1.10	<i>H.pylori</i> IgG antibody detected.
Index 0.90-1.09	Equivocal for IgG antibodies to <i>H. pylori</i> . Sample should be retested. If retest results are equivocal, the sample should be reported as equivocal, tested by another method, or a new sample should be tested.**

**Equivocal samples that give positive results on retest should be reported as positive. Equivocal samples that give negative results on retest should be reported as negative.

Reporting Results

When the Index Value is reported for a single specimen the following statement should be included: "The following results were obtained with the Is-*H. pylori* IgG Test Kit. The magnitude of the measured result, above the cut-off, is not indicative of the total amount of antibody present. The magnitude of the reported IgG level cannot be correlated to an endpoint titer".

Procedure Notes

1. Do not interchange reagents from different reagent lots except for Sample **A** Diluent, Wash **T** Concentrate, Substrate **HRP** and Stop **N** Solution.
2. Do not use reagents beyond their expiration date.
3. Store unused reagents at 2 to 8°C.

4. Incubations above or below the recommended temperatures or times may give erroneous results.
5. The EIA method is a very sensitive technique. Maintain consistent pipetting technique, incubation times and temperature conditions throughout the test procedure. Cross contamination between reagents can invalidate the test.
6. Antigen coated microwells should be stored with the desiccant in the resealable bag provided and returned to the refrigerator immediately after use.
7. (*Manual Procedure Only*) The washing procedure is very important and requires special attention. (Please refer to the Procedure section)
NOTE: *Improperly washed wells may give erroneous results.*
8. The concentration of anti-*H. pylori* IgG in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

Limitations

1. The results obtained with the Is-*H. pylori* IgG Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.
2. Assay performance characteristics have not been established for visual result determination.
3. The test should be performed on serum. The use of whole blood or plasma has not been established.
4. The performance characteristics of the Is-*H. pylori* IgG Test Kit have not been determined for a pediatric population.
5. Screening of the general population should not be performed due to the large percentage of people colonized with *H. pylori*. Testing should only be performed when clinical symptoms are present.
6. Results from immunosuppressed patients should be interpreted with caution.
7. The performance characteristics of the Is-*H. pylori* IgG Test Kit with automated equipment other than Diamedix Automated EIA Systems have not been established.
8. Icteric, lipemic, hemolyzed, or heat inactivated sera may cause erroneous results and should be avoided.
9. A positive result does not differentiate between a current or past infection. It also does not mean the clinical symptoms are due to infection with *H. pylori*. The clinical diagnosis has to be made with the clinical signs and symptoms of the patient.
10. Patients infected with *C. jejuni*, *C. coli*, and *C. fetus* may cause false positives in the Is-*H. pylori* Test Kit.
11. The performance characteristics have not been established on fresh serum samples.

References

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