HELICOBACTER PYLORI IgM ENZYME IMMUNOASSAY TEST KIT
Catalog Number: BC-1053

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Enzyme Immunoassay for the Detection of IgM Antibodies to *Helicobacter pylori* in Human Serum

FOR INVESTIGATIONAL USE ONLY

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

H. pylori IgM Enzyme Immunoassay

SUMMARY OF ASSAY PROCEDURE

1. Sample dilution 1:40

5 µl / 200 µl

2. Three incubations at room temperature

Diluted Sample 100 µl	Enzyme Conjugate 100 µl	TMB Reagent (One-Step) 100 µl
30 min.	30 min.	20 min.

3. Stop with 100 µl of acid. Read O.D. at 450 nm

INTENDED USE

The *Helicobacter pylori* IgM Test Kit is intended for use in evaluating the serologic status to *H. pylori* infection in patients with gastrointestinal symptoms.

INTRODUCTION

Helicobacter pylori is a spiral bacterium cultured from human gastric mucosa discovered by B.J. Marshall in 1982. Studies have indicated that the presence of *H. pylori* is associated with a variety of gastrointestinal diseases including gastritis, duodenal and gastric ulcers, non-ulcer dyspepsia, and gastric adenocarcinoma and lymphoma. The organism is present in 95-98% of patients with duodenal ulcers and 60-90% of patients with gastric ulcers. The studies have also demonstrated that removal of the organism by anti-microbial therapy is correlated with the resolution of symptoms and cure of diseases.

Patients who present clinical symptoms relating to the gastrointestinal tract can be diagnosed for *H. pylori* infection by two methods:

(1) Invasive techniques – include biopsy followed by culture or histologic examination of biopsy specimen or direct detection of urease activity.

(2) Non-invasive techniques – include urea breath tests and serological methods.

All of the testing performed on biopsy samples is subject to errors related to sampling and interference of contaminated bacteria. An ELISA test for the presence of *H. pylori* specific IgM antibody is the technique of choice for serologic tests because of its accuracy and simplicity.

PRINCIPLE OF THE TEST

Purified *H. pylori* antigen is coated on the surface of microwells. Diluted patients serum is added to the wells, and the *H. pylori* IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away. Enzyme conjugate is added, which binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and a solution of TMB Reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgM-specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

REAGENTS

Materials provided with the kit:

- Purified *H. pylori* antigen coated microtiter plate, 96 wells.
- Enzyme Conjugate Reagent (red color), 13 ml.
- Sample Diluent (blue color), 22 ml.
- Negative Control, 150 µl.
- Cut-off Calibrator, *H. pylori* IgM EIA Index = 1, 150 µl.
- Positive Control, 150 µl.
- Wash Buffer (20×), 50 ml.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

Materials required but not provided:

- Distilled water.
- Precision pipettes: 5 µl, 100 µl and 200 µl.
- Disposable pipette tips.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.

SPECIMEN COLLECTION AND PREPARATION

1. Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.
2. Specimens may be refrigerated at 2-8°C for up to 7 days or frozen for up to 6 months. Avoid repetitive freezing and thawing of serum sample.

STORAGE OF TEST KITS AND INSTRUMENTATION

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Dilute 1 volume of Wash Buffer (20×) with 19 volumes of distilled water. For example, dilute 50 ml of Wash Buffer (20×) into distilled water to prepare 1000 ml of Wash Buffer (1×). Wash buffer is stable for 1 month at 2-8°C. Mix well before use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Prepare 1:40 dilution of test samples, negative control, positive control, and calibrator by adding 5 µl of the sample to 200 µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrator, and controls into the appropriate wells. For the reagent blank, dispense 100 µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well for 10 seconds.
4. Incubate at room temperature for 30 minutes.
5. At the end of the incubation period, remove liquid from all wells. Rinse and flick the microtiter wells 4 times with diluted wash buffer (1×) and then one time with distilled water. (Please do not use tap water.)
6. Dispense 100 µl of enzyme conjugate to each well. Mix gently for 10 second.
7. Incubate at room temperature for 30 minutes.
8. Remove enzyme conjugate from all wells. Rinse and flick the microtiter wells 4 times with diluted wash buffer (1×) and then one time with distilled water.
9. Add 100 µl of TMB Reagent to each well. Mix gently for 10 seconds.
10. Incubate at room temperature for 20 minutes.
11. Add 100 µl of Stop Solution to each well including the 2 blanks.
12. Mix gently for 30 seconds. *It is important to make sure that all the blue color changes to yellow color completely.*
13. Read the optical density at 450 nm ***within 15 minutes*** with a microtiter plate reader.

Important Note:

The wash procedure is critical. Insufficient washing will result in improper color development.

CALCULATION OF RESULTS

1. Calculate the mean of duplicate calibrator value x_c.
2. Calculate the mean of duplicate positive control, negative control and patient samples.
3. Calculate the *H. pylori* IgM EIA Index of each determination by dividing the mean values of each sample by calibrator mean value, x_c.

Example of typical results:

Cut-off Calibrator *H. pylori* IgM EIA Index = 1

1. Cut-off Calibrator O.D. = 0.650, 0.630 $x_c = 0.640$
2. Negative Control O.D. = 0.210, 0.230 $x_n = 0.220$
   H. pylori IgM EIA Index = $x_n / x_c = 0.220 / 0.640 = 0.340$
3. Positive Control O.D. = 1.105, 1.210 $x_p = 1.200$
   H. pylori IgM EIA Index = $x_p / x_c = 1.200 / 0.640 = 1.80$
4. Patient sample O.D. = 1.501, 1.670 $x_s = 1.600$
   H. pylori IgM EIA Index = $x_s / x_c = 1.600 / 0.640 = 2.50$

INTERPRETATION

Negative: *H. pylori* IgM EIA Index less than 0.90 is seronegative for IgM antibody to *H. pylori*. The serum sample may have been taken too early.

Equivocal: *H. pylori* IgM EIA Index between 0.91-0.99 is equivocal. Retest In a parallel fashion with a new serum Sample drawn 3 weeks later.

Positive: *H. pylori* IgM EIA Index of 1.00 or greater is seropositive.

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.250.
2. If the O.D. value of the Cut-off Calibrator is lower than 0.250, the test is not valid and must be repeated.

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

REFERENCES

1. Marshall, B.J. and J.R. Warren. Unidentified curved bacilli in the stomach of patients with gastritis and Peptic ulceration. Lancet 1: 1311-1314, 1984.
2. Ruaws, E.A.J. and G.N.J. Tytgat. Cure of duodenal ulcer associated with eradication of *Helicobacter pylori*, Lancet 335: 1233-35, 1990.
3. Perez-Perez, G.I., S.S. Wilkin, M.D. Decker and M.J. Blaswer. Seroprevalence of *Helicobacter pylori* infection in couples. J. Clin. Microbiol. 29:642-644, 1991.

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