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Product Information

Helicobacter pylori IgM ELISA kit

Catalog Number: EA100945
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The *Helicobacter pylori (H. pylori)* IgM ELISA Kit is intended for the detection of IgM antibody to *H. pylori* in human serum or plasma.

Background

H. pylori is detectable in nearly 100% of adult patients with duodenal ulcer and about 80% of patients with gastric ulcer. An association between *H. pylori* and gastric cancer is confirmed. In developing countries, where most children become infected by the age of 10, gastric cancer rates are very high. In the USA and other developed countries, standards of hygiene and the increasing socioeconomic status of the population have reduced the incidence of infection, and in parallel, the rates of peptic ulcers and gastric cancer have declined. There is excellent correlation between the clinical presentation of gastritis, the presence of *H. pylori* in the stomach and elevated serum *H. pylori* IgG and IgA antibodies. ELISA sensitivity and specificity are 90%, and the predictive value of a negative result for is very high. *H. pylori* IgG and/or IgA antibodies falls significantly after successful antibacterial therapy. Eradication of *H. pylori* is associated with a significant reduction in duodenal ulcer recurrence. pylori strains are classified into two broad groups - those that express both VacA and CagA (type I) and those that produce neither (type II). Type I strains are predominate in patients with ulcers and cancer. Up to 50% of adults is infected with *H. pylori*, but most of them are asymptotic and will not develop ulcer. The reason is they are infected with type II. 80-100% of patients with duodenal ulcer disease produce CagA antibodies against a 128 kd antigen compared with 60- 63% of *H. pylori*-infected persons with gastritis only, indicating that serologic responses to the 128 kd protein are more prevalent among *H. pylori*-infected persons with duodenal ulcers than infected persons without peptic ulceration. In *H. pylori*-infected patients who develop gastric cancer, serum IgG against CagA 94% sensitive and 93% specific, indicating that detection of antibodies to CagA is useful marker for diagnosis of duodenal ulcer and gastric cancer.

Principle of the Test

Diluted patient serum (serum diluent contains sorbent to remove rheumatoid factor and human IgG interference) is added to wells coated with purified antigen. IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgM specific antibody in the sample.

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with *H. pylori* antigen	12x8x1
2. Sample Diluent: 1 bottle (ready to use)	22 ml
3. Calibrator: 1 Vial (ready to use)	1ml
4. Positive Control: 1 vial (ready to use)	1ml
5. Negative Control: 1 vial (ready to use)	1ml
6. Enzyme conjugate: 1 bottle (ready to use)	12ml
7. TMB Substrate: 1 bottle (ready to use)	12ml
8. Stop Solution: 1 bottle (ready to use)	12ml
9. Wash concentrate 20X: 1 bottle	25ml

Materials and Equipment Required but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection and Preparation

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8°C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

Reagent Preparation

1. Prepare 1X Wash buffer by adding Wash Concentrate (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
- It is recommended that standards, control and serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 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. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100 µl l of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature. Add 100 µl of stop solution.
8. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm

Calculation of Results

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Example of a Standard Curve

Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5= 0.400
Positive control O.D. = 1.2
Ab Index = 1.2 / 0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6 / 0.4 = 4.0

Quality Control

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

1. If the O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab index for Positive control should be greater than 1.2.

Interpretation

The following is intended as a guide to interpretation of *H. pylori* IgM test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

- **Antibody Index Interpretation**
  - <0.9 No detectable antibody to *H. pylori* IgM by ELISA
  - 0.9-1.1 Borderline positive. Follow-up testing is recommended if clinically indicated.
  - >1.1 Detectable antibody to *H. pylori* IgM by ELISA

References

1. Cutler AF; Prasad VM; Santogade P. Four-year trends in Helicobacter pylori IgG serology following successful eradication. Am J Med 1998;105(1):18-20
2. Holtmann G; Talley NJ; Mitchell H; Hazell S. Antibody response to specific H. pylori antigens in functional dyspepsia, duodenal ulcer disease, and health. Am J Gastroenterol 1998; 93(8):1222-7.
3. Parsonnet J; Replogle M; Yang S; Hiatt R. Seroprevalence of CagA-positive strains among Helicobacter pylori- infected, healthy young adults. J Infect Dis 1997;175(5):1240-2.
4. Klaamas K; Held M; Wadstr¨om T; Lipping A; Kurtenkov O. IgG immune response to Helicobacter pylori antigens in patients with gastric cancer as defined by ELISA and immunoblotting. Int J Cancer 1996; 67(1):1-5.
5. Matsukura N; Onda M; Tokunaga A; Kato S; Yoshiyuki T; Hasegawa H; Yamashita K; Tomtitchong P; Hayashi A. Role of Helicobacter pylori infection in perforation of peptic ulcer: an age- and gender-matched case-control study. J Clin Gastroenterol 1997;10:S235-9.

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