

CALDON BIOTECH INC.

Helicobacter pylori IgM ELISA

Catalog No. HP015M
(96 tests)

NAME AND INTENDED USE

The CALDON BIOTECH INC. (CBI), *Helicobacter pylori* (*H. Pylori*) IgM ELISA is intended for use in the evaluation of patients with suspected *H. pylori* infection.

SUMMARY AND EXPLANATION OF THE TEST

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* antibodies. ELISA sensitivity and specificity are 90%, and the predictive value of a negative result for is very high. *H. pylori*-specific antibodies falls significantly after successful antibacterial therapy. Eradication of *H. pylori* is associated with a significant reduction in duodenal ulcer recurrence.

H. 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 asymptomatic 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, antibodies against CagA are 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.

MATERIALS PROVIDED

1. Microwell Strips: *H. pylori* coated wells (12 x 8 x 1 wells).
2. Sample Diluent: 1 bottle (22 mL). Ready to use.
3. Calibrator: Yellow Cap. (1.50 mL/vial). Ready to use.
4. Positive Control: Red Cap. (1.50 mL/vial). Ready to use.
5. Negative Control: Blue Cap. (1.50 mL/vial). Ready to use.
6. Enzyme Conjugate: 1 bottle (12 mL). Ready to use.
7. TMB Substrate: 1 bottle (12 mL). Ready to use.
8. Stop Solution: 1N H₂SO₄; 1 bottle (12 mL). Ready to use.
9. Wash Concentrate: 1 bottle (50 mL), 20X concentrate.

STORAGE AND STABILITY

1. Store the kit at 2-8 ° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage or usage.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials: The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen

as well as HIV antibody with FDA licensed reagents. However, 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 in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
- Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
 - Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
 - The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
 - Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION AND HANDLING

- Collect blood specimens and separate the serum.
- 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 of serum sample.

PREPARATION FOR ASSAY

- Bring all specimens and kit reagents to room temperature (20-25 °C) and gently mix.
- Prepare washing buffer by adding the contents of the bottle (50 mL, 20X Wash concentrate) to 950 mL of distilled or deionized water in one-liter container. Store at room temperature.

ASSAY PROCEDURE

- Place the desired number of coated strips into the holder.
- 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.
- 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.
- Remove liquid from all wells. Repeat washing three times with wash buffer.
- Dispense 100 µL of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
- Remove enzyme conjugate from all wells. Repeat washing three times with wash buffer.
- Dispense 100 µL of TMB substrate solution and incubate for 10 minutes at room temperature.
- Add 100 µL of 1N H₂SO₄ to stop reaction.
- Read O.D. within 30 min at 450 nm using microwell reader.