

CALDON BIOTECH INC. Helicobacter pylori IgA ELISA

Catalog No. HP014A
(96 tests)

NAME AND INTENDED USE

The CALDON BIOTECH, INC. (CBI), *Helicobacter pylori* (*H. pylori*) IgA ELISA is intended for use in evaluating 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* 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. *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, 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 is added to wells coated with purified antigen. IgA 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 IgA specific antibody in the sample.

MATERIALS PROVIDED

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

STORAGE AND STABILITY

- Store the kit at 2-8°C.
- Keep microwells sealed in a dry bag with desiccants.
- The reagents are stable until expiration of the kit.
- 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.

2. 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.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. 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

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 of serum sample.

PREPARATION FOR ASSAY

1. Bring all specimens and kit reagents to room temperature (20–25°C) and gently mix.
2. 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

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. Repeat washing three times with wash buffer.
5. Dispense 100 µL of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Repeat washing three times with wash buffer.
7. Dispense 100 µL of TMB substrate solution and incubate for 10 minutes at room temperature.
8. Add 100 µL of 1N H₂SO₄ to stop reaction.

9. Read O.D. within 30 min at 450 nm using microwell reader.

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 typical results:

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:

- The O.D. of the Calibrator should be greater than 0.250.
- The Ab index for Negative control should be less than 0.9.
- The Ab Index for Positive control should be greater than 1.2.

INTERPRETATION

The following is intended as a guide to interpretation of CBI *H. pylori* IgA 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* IgA by ELISA.

0.9-1.1 Borderline positive. Follow-up testing is recommend if clinically indicated.

>1.1 Indicative of *H. pylori* infection.

LIMITATIONS OF THE TEST

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Lipemic or hemolyzed samples may cause erroneous results.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity

94 sera from patients with suspected *H. pylori* infections were tested by both CBI *H. pylori* IgA ELISA and a reference ELISA method. 14 sera were positive and 73 were negative by both methods (95% agreement). The results are summarized below:

	CBI <i>H. pylori</i> IgA ELISA		
	+	-	Total
Reference ELISA	14	3	
+ Kit	17		
-	4	73	
	77		
Total	18	76	
	94		

**2. Precision
Intra-Assay Study**

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation%
1	16	1.43	0.096	6.71
2	16	0.98	0.067	6.83
3	16	0.26	0.019	7.30

Inter-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation%
1	10	1.29	0.121	9.37
2	10	0.91	0.089	9.78
3	10	0.23	0.025	10.86

REFERENCES

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