AccuDiag™ H. pylori Antigen ELISA Kit

REF 1506-12

IVD See External Label 2°C 8°C 96 Tests

H. pylori Antigen ELISA	
Principle	Indirect ELISA
Detection	Quantitative
Sample	Human Stool Specimen
Incubation Time	70 minutes
Sensitivity	96%
Specificity	100%
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The Diagnostic Automation Inc. ELISA, *Helicobacter pylori* Antigen (Ag) is a quantitative assay for the detection of *H. pylori* Ag in human stool specimen. The test results are intended to aid in the diagnosis of *H. pylori* infection, to monitor the effectiveness of therapeutic treatment and to confirm the eradication of *H. pylori* in peptic ulcer patients.

SIGNIFICANCE AND SUMMARY

Helicobacter pylori is a spiral bacterium cultured from human gastric mucosa by Marshall in 1982[1]. Studies have indicated that the presence of *H. pylori* is associated with a variety of gastrointestinal diseases including gastritis, duodenal and gastric ulcer, non-ulcer dyspepsia, gastric adenocarcinoma and lymphoma. The organism is present in 95-98% of patients with duodenal ulcer and 60-90% of patients with gastric ulcers. The studies have also demonstrated that removal of the organism by antimicrobial therapy is correlated with the resolution of symptoms and cure of diseases[2]. Patients who present with 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 histological examination of biopsy specimen or direct detection of urease activity. The cost and discomfort to the patients are very high and biopsy samples are subject to errors related to sampling and interference of contaminated bacteria. 2) Non-invasive techniques include urea breath tests (UBT)[3] and serological methods[4]. The UBT requires a high density and active bacteria and should not be performed until 4 weeks after therapy to allow resisdual bacteria to increase to the detection level. The main limitation of serology test is the inability to distinguish current and past infections. ELISA, H. pylori Antigen, tests the presence of H. pylori antigens in stool specimens for an active infection.

ASSAY PRINCIPLE

Purified *H. pylori* antibody is coated on the surface of microwells. An aliquot of diluted stool sample is added to wells, and the *H. pylori* antigens, if present, bind to the antibody. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and TMB Chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportionated to the amount of *H. pylori* Ag in stool sample.

SPECIMEN COLLECTION & PREPARATION

1. Transfer a small piece of stool (~5mm in diameter; ~150mg) into 1 ml of diluted extraction sample diluent in a test tube, mix thoroughly.
2. If liquid samples such as from culture medium or others are available for test, dilute it 1:1 with Sample Treatment Solution.

REAGENTS

Materials provided with the kit

1. Twelve 1x 8-well strips coated with purified anti *H. pylori* Ag antibody. The strips are packaged in a strip holder.
2. Concentrated Extraction sample diluent (10 X, 11 ml in a blue cap vial), needs to be diluted before use.
3. Prediluted Calibrator 1,2,3,4,5,6 (0.6 ml for each).
4. HRP-conjugate (6 ml in a yellow cap vial).
5. Wash buffer (25 ml, in a clean cap vial) 30 x concentrate.
6. TMB substrate solution (11 ml, in a black cap vial)
7. Stop solution (11 ml, in a white cap vial).

REAGENT PREPARATION

1. Prepare 1x washing buffer. Prepare washing buffer by adding 29-30 portions of distilled or deionized water to 1 portion of 30x wash concentrate.
2. Prepare 1x extraction sample solution by diluting 1 portion of concentrated extraction solution with 9 portions of DI water. Mix well.
3. Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.

ASSAY PROCEDURE

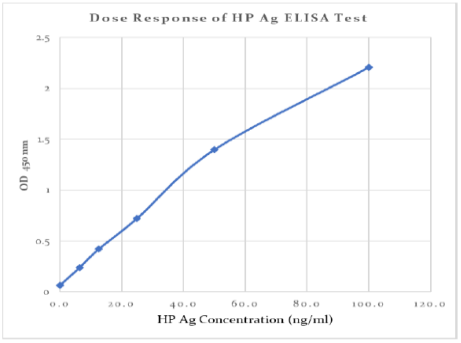
1. Place the desired number of coated strips into the holder.
2. Dispense 100µl of treated sample, standards, and calibrators into the appropriate wells. Tap the holder to remove air bubbles from the liquid and mix well. Incubate at 37°C for 30 minutes.
3. Remove liquid from all wells and repeat washing four times with washing buffer.
4. Dispense 50µl of enzyme conjugate to each well and incubate at 37°C for 30 minutes.
5. Remove enzyme conjugate from all wells. Repeat washing four times with washing buffer.
6. Dispense 100µl of TMB Chromogenic Substrate to each well and incubate at 37°C for 10 minutes.
7. Add 100µl of stop solution to stop reaction. Make sure there are no air bubbles in each well before reading *H. pylori* Ag.
8. Read O.D. at 450/620 nm with a microwell reader.

RESULTS

1. Construct a standard curve by plotting O.D. 450 nm on the y-axis against the concentration of calibrator ng/ml values on the x-axis.
2. Using the O.D. value of each specimen, determine the concentration from the standard curve. If sample results are greater than 100 ng/ml (over the range of standard curve), they can be reported as "high positive". To assess accurate results, samples can be further diluted and retested again.
3. A typical example (for demonstrations only)

Calibrator set	H. pylori Ag (ng/ml)	O.D. (450 nm)
1	0.0	0.067
2	6.3	0.240
3	12.5	0.421
4	25	0.724
5	50	1.397
6	100	2.204

4. A typical illustration of standard dose response.



PERFORMANCE CHARACTERISTICS

Comparative Study

The Diagnostic Automation Inc. H. pylori Antigen ELISA test was compared to another commercially available ELISA assay for detection of *H. pylori* antigen.

A total of 183 specimens were tested by two procedures. These results are summarized in table 1 below:

Table 1

		DAI H. pylori Antigen ELISA		
		Pos.	Neg.	Total
Reference	Pos.	72	3	75
H. pylori	Neg.	108	108	108
Antigen ELISA	Total			183

Sensitivity = 72/75 = 96.0% Specificity = 108/108 = 100%

Precision

Assay reproducibility was determined by assaying 3 positive specimens in replicates of 10 on 2 consecutive runs using the same production lot. The coefficient of variation (%CV) of Intra-assay and Inter-assay were calculated. Table 2 shows reproducibility of assay results:

Table 2

Sample	Number of Tests	Intra-assay Precision %CV	Inter-assay Precision %CV
1	10	6.8	9.2
2	10	7.2	8.6
3	10	8.1	9.4

STORAGE CONDITIONS

1. Store the kit at 2 – 8°C.
2. Always keep microwells tightly sealed in pouch with desiccants. We recommend you use up all wells within 4 weeks after initial opening of the pouch.
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 & PRECAUTIONS

1. Potential biohazardous materials: The calibrator and controls contain human source components which have been tested and found nonreactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as 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. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. This product contains components 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.

INTERPRETATION

Minimum detectable concentration: 0.5 ng/ml

Negative: **< 15 ng/ml**
Positive: **> 20 ng/ml**
Medium Positive: 20-100 ng/ml

REFERENCES

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

bsi ISO 13485 Quality Management for Medical Devices CERTIFIED

Diagnostic Automation/Cortez Diagnostics, Inc.
21250 Califa Street, Suite 102 and 116,
Woodland Hills, California 91367 USA

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