MONOCENT
Innovation & Excellence

ENGLISH

H. pylori Ag
ELISA TEST SYSTEM

CE

REF EL45-1138 Σ 96 TESTS IVD

INTENDED USE

The Monocent, Inc.'s 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.

SUMMARY AND EXPLANATION

Helicobacter pylori is a spiral bacterium cultured from human gastric mucosa by Marshall in 1983[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. 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 residual bacteria to increase to the detection level. The main limitation of serology test is the inability to distinguish current and past infections. The H. pylori Antigen ELISA tests for the presence of H. pylori antigens in stool specimens for an active infection.

PRINCIPLE OF THE TEST

The Monocent, Inc.'s 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 intensity of the blue color generated is proportionated to the amount of H. pylori Ag in stool sample

MATERIALS AND COMPONENTS

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

STORAGE CONDITIONS

- Store the kit at 2 - 8 °C.
- 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.
- The reagents are stable until expiration of the kit.
- Do not expose test reagents to heat, sun or strong light during storage or usage

SPECIMEN COLLECTION

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

ASSAY 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 diluent by diluting 1 portion of 10 X concentrated extraction diluent with 9 portions of DI water. Mix well.
3. Bring all specimens and kit reagents to room temperature (20-25 °C) and gently mix.

PRECAUTIONS

1. Potential biohazardous materials: The calibrators 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 respective biosafety level.
2. As recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories"[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.

TEST PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Dispense 100 µl of treated sample and/or standards 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 50 µl of HRP 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 100 µl of TMB Chromogenic Substrate to each well and incubate 37 °C for 10 minutes.
7. Add 100 µl 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

INTERPRETATION OF RESULTS

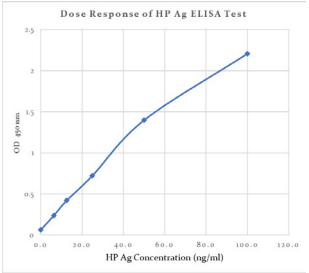
Minimum detectable concentration: 0.5 ng/ml
Negative: < 15 ng/ml
Positive: > 20 ng/ml
Medium positive 20-100 ng/ml

CALCULATION OF 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 demonstration 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

1. Comparative Study

The Monocent 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:

		Monocent H. Pylori Antigen Elisa		
		Pos.	Neg.	Total
Reference	Pos.			75
H. Pylori	Neg.	72	3	108
Antigen Elisa	Total	108	108	183

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

2. 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 the were calculated.

Table shows reproducibility of assay results:

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

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