GRUPO MEXLAB®
ALTA CALIDAD EN PRUEBAS PARA SU LABORATORIO

Helicobacter pylori Antigen
ENZYME IMMUNOASSAY TEST KIT

(96 tests)

SUMMARY OF ASSAY PROCEDURE

Step	(20-25°C Room temp.)	Volume	Incubation time
1	Sample treatment	1 ml	
2	Treated samples, calibrators & controls	100 µl	30 minutes
3	Washing buffer (3 times)	350 µl	
4	Enzyme conjugate	100 µl	30 minutes
5	Washing buffer (3 times)	350 µl	
6	TMB Chromogenic Substrate	100 µl	15 minutes
7	Stop solution	100 µl	
8	Reading OD 450 nm		

NAME AND INTENDED USE

The Helicobacter pylori Antigen Enzyme Immunoassay (ELISA) Test Kit is a quantitative assay for the detection of *H. pylori* antigens 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 OF THE TEST

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 ulcers 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.

H. pylori Antigen ELISA tests the presence of *H. pylori* antigens in stool specimens for an active infection.

PRINCIPLE OF THE TEST

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 proportional to the amount of antigen in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

MATERIALS PROVIDED

1. Microwell Strips: Purified *H. pylori* antibody coated wells.(12 x 8 wells)
2. Sample Treatment Solution — 1 Bottle (100 ml)
3. Washing Concentrate 50x — 1 Bottle (15 ml)
4. TMB Chromogenic Substrate: Amber bottle. — 1 vial (12 ml)
5. Enzyme Conjugate: Red color solution. — 1 vial (12 ml)
6. Calibrator Set: 0, 6.3, 12.5, 25, 50, 100 ng/ml — 1 ml/ vial
7. Control Set: Negative and Positive Controls
   Ranges are indicated on labels — 1 ml/vial
8. Stop Solution — 1 vial (12 ml)

STORAGE AND STABILITY

1. Store the kit at 2 - 8 ^{O}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 AND 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.

SPECIMEN COLLECTION AND HANDLING

1. Transfer small piece of stool (~5mm in diameter; ~150mg) into 1ml of Sample Treatment Solution 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.

PREPARATION FOR ASSAY

1. Prepare 1x washing buffer.
   Prepare washing buffer by adding distilled or deionized water to 50x wash concentrate to a final volume of 750 ml.
2. Bring all specimens and kit reagents to room temperature (20-25 ^{O}C) and gently mix.

ASSAY PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Dispense 100µl of treated sample, calibrators, and controls into the appropriate wells. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.
3. Remove liquid from all wells and repeat washing three times with washing buffer.
4. Dispense 100µl of enzyme conjugate to each well and incubate for 30 minutes at room temperature.
5. Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.
6. Dispense 100µl of TMB Chromogenic Substrate to each well and incubate for 15 minutes at room temperature.
7. Add 100µl of stop solution to stop reaction.

Make sure there are no air bubbles in each well before reading

8. Read O.D. at 450 nm with a microwell reader.

CALCULATION OF RESULTS

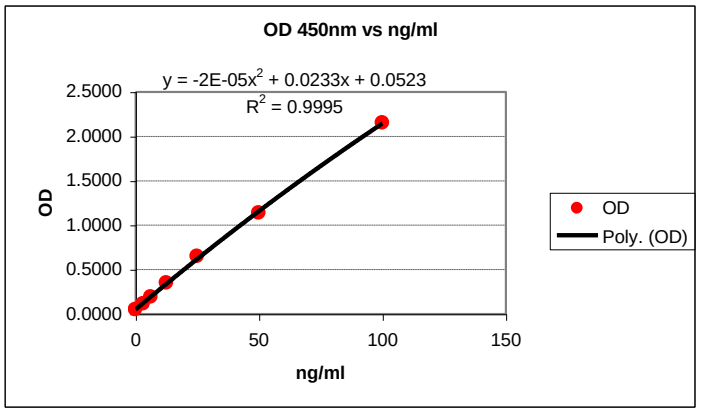
1. Construct a standard curve by plotting O.D. 450 nm on the y-axis against the concentration of calibrator ng/ml values on the x-axis with an order 2 Polynomial trendlines.

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 and greater than 100 ng/ml". To assess accurate results, samples can be further diluted and retested again.

3. A typical example (for demonstration only):

Calibrator Set	H. Pylori Antigen (ng/ml)	O.D. 450 nm		O.D. 450 nm Mean	SD	CV %
Calibrator 1	0.0	0.019	0.022	0.021	0.0021	10.3%
Calibrator 2	6.3	0.153	0.161	0.157	0.0057	3.6%
Calibrator 3	12.5	0.300	0.299	0.300	0.0007	0.2%
Calibrator 4	25	0.555	0.536	0.546	0.0134	2.5%
Calibrator 5	50	1.096	1.042	1.069	0.0382	3.6%
Calibrator 6	100	1.942	1.891	1.917	0.0361	1.9%

4. A typical illustration of standard curve:



QUALITY CONTROL

1. The negative control and positive control should be run with every batch of samples tested, and the concentration must be within the range stated on its label.
2. The O.D. value of calibrator 0 ng/ml must be lower than 0.15 and the O.D. value of calibrator 100 ng/ml must be greater than 1.0.

INTERPRETATION

Minimum detectable concentration: 0.5 ng/ml

Negative: < 15 ng/ml

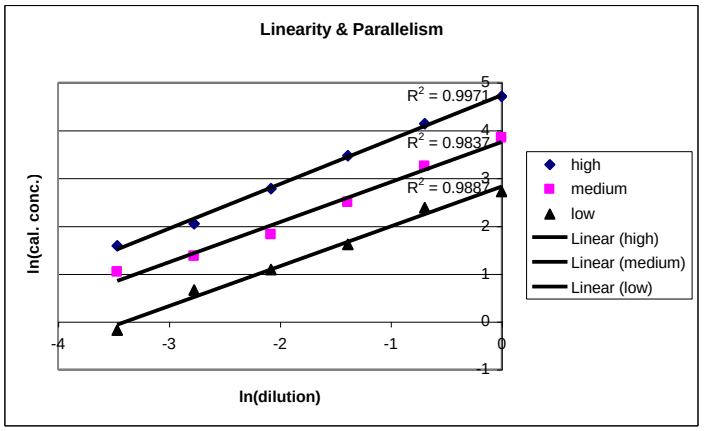
Positive: > 20 ng/ml

LINEARITY and PARALLELISM:

A study was conducted to demonstrate linearity of the assay. Three positive patient samples were serially diluted. Ng/ml values were calculated for individual OD readings of the diluted samples. The linearity of R squared values are listed in the following table:

Sample #	Neat	1:2	1:4	1:8	1:16	1:32	R^2
1	110.9	62.8	32.1	16.1	7.8	4.9	0.9971
2	46.6	25.7	12.1	6.2	3.9	2.8	0.9837
3	15.2	10.8	5.0	3.0	1.9	0.8	0.9887

The linear regression graph of above three positive samples:



PRECISION:

The precision of the assay was evaluated by testing three different sera and eight replicate readings in 3 days. The intra-assay and inter-assay %CV are summarized below:

N = 8	Low Positive	Middle positive	High Positive
Intra-assay	6.7%	4.7%	3.3%
Inter-assay	7.4%	2.7%	16.7%

CROSS-REACTIVITY:

A study was performed to determine the cross-reactivity with the following bacterial and viral strains: Camplylobactor coli, Camplylobactor fetus, Camplylobactor jejuni, Camplylobactor lari, Candida albicans, Enterobacter cloacae, Helicobacter cinaedi.
All above positive samples were tested negative for Helicobacter pylori Antigen test.

LIMITATIONS OF THE PROCEDURE

1. The assay should be used only to evaluate patients with clinical signs and symptoms suggestive of gastrointestinal disease.
2. A positive test result indicates an active infection and colonization by *H. pylori*. It does not necessarily indicate that gastrointestinal disease is present.

REFERENCES

1. Marshall, B.J. and J. R. Warren. Unidentified curved bacilli in the stomach of patients with gastritis and Peptic ulceration, *Lancet 1*:1311-1314, 1984.
2. Ruaws, E.A.J. and G.N.J. Tytgat. Cure of duodenal ulcer associated with eradication of *Helicobacter pylori*, Lancet 335:1233-35, 1990.
3. Klein PD, Malaty HM, Martin RF, et al. Noninvasive detection of *Helicobacter pylori* infection in clinical practice: the ^{13}C urea breath test. *Am J. Gastroenterol.* 1996;91:690-694
4. Cutler AF. Testing for *Helicobacter pylori* in clinical practice. Am J. Med.1996;100:35S-41S.