

H.pylori IgA ELISA Kit

48 Tests Kit
Enzyme Immunoassay for the
Determination of *H.pylori* IgA level
in Human Serum/Plasma
(For In Vitro Diagnostic Use Only)
Catalogue No. MA_H.P.IgA_48_01

PISHTAZ TEB DIAGNOSTICS

Introduction

Helicobacter pylori (*H.pylori*) is a gram negative bacterium which is located at gastric mucosa. The organism is present in 95-98% of patients with duodenal ulcer and 60-90% of patients with gastric ulcers. Estimation of infection rate by various diagnostic tests including bacteriological, histological and serological tests have revealed that 90% of symptomatic patients are affected and 50% of old age adults (> 50 years) is only colonized by bacteria lifelong without any clinical symptoms. Studies also have been demonstrated that removal of the organism by antimicrobial therapy is correlated with the resolution of symptoms and cure of diseases. Patients who present 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.
- 2) Non-invasive techniques include urea breath tests and serological methods.

All of the tests performed on biopsy samples are subject to errors related to sampling and interference of bacterial contamination. *Helicobacter pylori* infection stimulates humoral immune response and provokes specific antibodies like IgG, IgM, and IgA. *H. pylori* IgA ELISA test is an accurate and simple technique to determine colonization of bacteria and ELISA test is technique of choice for detection of IgA response.

Test Principle

The test principle is based on indirect ELISA technique. In this technique wells are coated with certain amount of specific *H.pylori* antigens. Then diluted serum sample is allowed to react with solid phase antigens. If specific antibodies are presented in the sera they will bind to *H.pylori* antigens. After incubation at room temperature, the wells are washed with washing solution to remove unbound antibodies. After washing the wells, the secondary conjugated antibody which is anti-human IgA-horseradish peroxidase (HRP) conjugate is added into the wells. Following another incubation and wash step. A solution of TMB is added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of specific anti-*H.pylori* IgA is directly proportional to the color intensity of the test sample.

Materials Provided with Test Kit

1. *H.pylori* antigen coated wells (1 plate, 48 wells): Microtiter wells coated with *H.pylori* antigen
2. Sample diluent (1 vial, 50 ml): Contains phosphate buffer solution with protein as stabilizer and kathon CG as preservative.
3. Enzyme conjugate (1 vial, 6 ml): Contains polyclonal anti human IgA labeled with HRP, ready to use.
4. Standards set (1ml / vial): Including 0.0, 10, 20, 50, 100 U/ml of anti- *H.pylori* IgA in buffer containing protein as stabilizer and kathon CG as preservative , ready to use.
5. Wash solution (1 vial, 25ml): Contains phosphate buffer solution with 0.05 % tween 20 as detergent, pH=6 concentrated (20X).
6. Chromogen substrate reagent (1 vial, 6 ml): Contains tetra-methyl-benzidine and hydrogen peroxide, ready to use.
7. Stop solution (1 vial / 6 ml): Contains 1 molar hydrochloric acid, pH< 1.
8. Cardboard sealer.



Materials/Equipments required but not provided with Test Kit

1. ELISA reader.
2. Precision pipettes
3. Distilled water.
4. Disposable pipette tips.
5. Vortex mixer or equivalent.
6. Absorbent paper.
7. Graph paper.

General Information

1. Do not mix kit reagents from different lot numbers. All kit components must be used only in original kit.
2. All reagents obtained from human sources are negative for HBs Ag, HCV and HIV antibodies. To prevent risk of contamination, use personal protective equipments like gloves, lab coats, etc. and avoid direct contact with reagents.

Storage Conditions

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.
2. Keep un-used wells in their sealed bag with desiccants.
3. Do not use expired date reagents.

Specimen Collection and Preparation

The kit is for use with serum or plasma. Serum or plasma should be prepared from a whole blood specimen obtained by approved aseptic technique. If testing cannot be done within an hour after sample collection, refrigerate (maximum 48 hours) the specimen immediately and let it return to room temperature before testing. If prolong storage is required, samples should be stored at -20°C. Avoid freeze-thaw of specimen during storage.

Reagents & Specimens Preparation

1. All reagents should be allowed to reach room temperature (22-28°C) before use.
2. Working wash solution: dilute concentrated wash solution 1:20 with

distilled water before use.

3. Dilute specimens 1:101 with sample diluents (i.e. 10 µl of specimen with 1 ml of sample diluents)

Note: Kit standards are ready to use and do not need to be diluted.

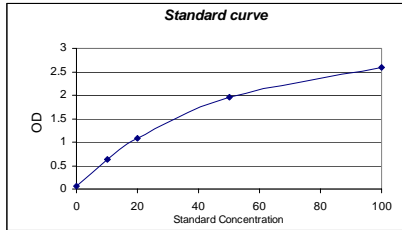
Assay Procedure

1. Use required number of wells and keep the remaining with desiccants in tightly closed sealed bag.
2. Add 100 µl of each standard, serum control and diluted specimen into appropriate wells.
3. Seal the plate with Cardboard sealer tightly. Leave wells for 30 minutes at room temperature.
4. Remove the wells content by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times (each with 300 µl of Working wash solution).
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
6. Add 100 µl of ready to use anti-human IgA -HRP conjugate into the wells.
7. Cover the plate with cardboard sealer tightly. Leave wells for 30 minutes at room temperature.
8. Repeat step 4 and 5
9. Dispense 100 µl of chromogen/ substrate to each well.
10. Incubate the microplate wells at room temperature and dark for 15 minutes to develop color.
11. Add 100 µl of stop solution to the wells to stop reaction.
12. Read absorbance at 450 nm by ELISA reader (Use 630 nm filter as reference filter if it's available). Reference filter are highly recommended.



Example of Standard curve

Standards (U/ml)	Absorbance
0	0.05
10	0.57
20	1.00
50	1.97
100	2.60



Note: All absorbances shown in above curve are for the purpose of illustration only, and should not be used to calculate unknowns. Users should obtain their own data and standard curves.

Validity of the Assay

The assay is to be considered valid if:

1. The OD (450 nm) of 0.0 U/ml standard is lower than 0.1. Higher values indicate chromoge/substrate contamination. In such a case, repeat the assay carefully checking the reagent.
2. The OD (450 nm) of 10 U/ml Standard is higher than the OD 450nm of 0 U/ml standard. Lower values indicate kit reagents decay. In such a case, check expiry date of the kit before repeating the assay.
3. The OD (450 nm) of 100 U/ml standard is higher than 1.0. Lower values indicate kit reagents decay. In such a case, check expiry date of the kit before repeating the assay.

Result Calculation

1. Calculate mean absorbance value of standards and samples at 450 nm (Use 630 nm filter as reference filter if it's available).
2. Construct a point to point standard curve by plotting the mean absorbance obtained

for each reference standard against its concentration in U/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.

3. Use the mean absorbance value for each sample; determine the corresponding concentration of Specific Anti *H.pylori* IgA in U/ml from the standard curve.

Expected Values

Based on study on normal healthy population:

1. Sera which contain anti-H.pylori IgA antibody greater than 20 U/ml should be considered as positive.
2. Sera which contain anti-H.pylori IgA antibody less than 15 U/ml should be considered as negative.
3. Sera which contain anti-H.pylori IgA antibody between 15-20 U/ml should be considered as equivocal or suspicious and should be re-tested with fresh specimen after a few days or checked by other valid diagnostic tests.

Test Interpretation

Results should be interpreted on the context of clinical manifestation, medical history and other diagnostic test. Positive results are only indicating presence of specific anti-H. pylori antibody but not representative of H. pylori infection or colonization. The ELISA test also could not differentiate previous infection from active current infection.

Performance Characteristics
1. Sensitivity and Specificity

A total of 134 patients with clinical signs and symptoms of gastric disease who had been referred to physicians, were evaluated. Of these, 75 were confirmed *H.pylori* positive and 69 were confirmed *H.pylori* negative by biopsy finding (using histological techniques, culture and CLO test). The ELISA test results were compared to the endoscopic biopsy sample findings.



**Pishtaz teb diagnostics *H.pylori* IgA
ELISA kit**
Biopsy

	Pos	Equivocal	Neg	Total
+	60	2	3	65
-	2	2	65	69
Total Samples				134

Sensitivity = $60 / 65 = 92\%$
 Specificity = $65 / 69 = 94\%$
 Accuracy = $125 / 134 = 93\%$

Correlation Test

161 random sera were tested by Pishtazteb *H.pylori* IgA ELISA Kit and a CE marked commercial kit. 21 sera were positive and 116 were negative by both methods (92% agreement). Comparative study with FDA and CE approved ELISA kits revealed relative sensitivity of 85% and relative specificity of 97%. The relative correlation of kit was 92%.

		Pishtaz teb <i>H.pylori</i> IgA ELISA kit			
		+	-	SUSP	Total
Reference ELISA kit	+	21	2	2	27
	-	3	116	1	120
	SUSP	2	1	11	14
	Total	28	119	14	161

2. Reproducibility

Three serum samples with different concentrations of Anti *H.pylori* IgG were repeatedly tested. Results are shown in table 1 and 2:

Table 1: Intra-assay

No.	No. of Tests Performed	Means U/ml	SD U/ml	CV%
1	24	14	0.7	5.0
2	24	33	1.5	4.5
3	24	46	2.7	5.8

Table 2: Inter-assay

No.	No. of Tests Performed	Means U/ml	SD U/ml	CV%
1	10	13.9	1.23	8.9
2	10	32.6	2.73	8.4
3	10	48.2	4.1	8.5

*Each test has been run in duplicate



References

1. Peterson W.L. (1991). *H.pylori* and peptic ulcer disease. N. Engl. J. Med. 324: 1024-1047
2. M C Guigan J.E. (1988) Peptic ulcer and gastritis. Harrison's principles of internal medicine. 12th edition, chapter 238, 1229-1248.
3. Podolsky I. (1989). Prevalence of *H.pylori* in healthy subject and patients with peptic disease. Gastroenterology 96 suppl. A394.
4. C.I. Perez and M.O. Blaser (1991) Serodiagnosis of *H.pylori*: comparison of enzyme linked immunosorbent assay. H. Clin. Microbiol 29: 1635-1639.



<i>H.pylori</i> IgA ELISA Test Procedure

Step 1



Reagent	Standard	Diluted Sample
		
Standard	100 µl	None
Diluted Sample	None	100 µl

Cover the microplate wells with cardboard sealer tightly and incubate them for 30 minutes at room temperature.


Step 2

Remove plate cover and discard contents of the wells. Wash the microplate wells for 5 times according to test manual.




Step 3

Ready to use-HRP conjugate	100 µl	100 µl
		

Cover the microplate wells with cardboard sealer and incubate for 30 minutes at RT.




Step 4

Remove plate cover and discard contents of the wells. Wash the microplate wells for 5 times according to test manual.

Chromogen-substrate solution	100 µl	100 µl
		

Incubate wells for 15 minutes at room temperature and dark.


Step 5

Stop Solution	100 µl	100 µl
		

Read absorbance at 450 nm (Use 630 nm filter as reference filter if it's available).