پادتن علم
PADTAN ELM

THE PADTAN ELM EIA KIT FOR 96 TESTS

Helicobacter Pylori IgM

Intended use:
Semi-quantitative detection of IgM antibadies to Helicobacter Pylori in human serum and plasma

For *in vitro* diagnostic use.

I. Introduction

Helicobacter pylori (H. pylori), a spiral shaped bacterium, was described in 1983 by Marshall and Warren. This gram negative bacterium that causes one of the most common bacterial infections in humans, lives in the stomach and duodenum.
Infection with H. pylori occurs world-wide but the overall prevalence of this infection correlates with socioeconomic conditions . The prevalence among middle-aged adults is over 80% in developing countries, as compared with 20 to 50 percent in industrialized countries[1-3].
The importance of this infection has increased greatly since Marshall and Warren described the presence of H. pylori in the antral mucosa of patients with histological evidence of antrum gastritis and peptic ulcers, especially duodenal ulcers.
Chronic H. pylori infection may lead to gastric and duodenal ulcers, gastric cancers, gastric lymphomas and other gastro-intestinal diseases. Nearly 90% of patients with duodenal ulcer[4-5], 70% of those with gastric ulcer [6-8] and 80% with stomach cancer ,[9-10] have H. pylori infection. Successful eradication of H. pylori leads to disappearance of gastric inflammation[11] and healing of the duodenal ulcer [12-13].
Several techniques (invasive and non invasive) are available for diagnosing H. pylori infection.
Invasive methods require the collection of multiple punch biopsy samples taken during upper gastrointestinal endoscopy. H. pylori is identified by culture, histological examination or urease testing of biopsy specimens.
Non-invasive methods include the urea breath test, stool antigen assays and serological detection of specific antibodies[14].
In most patient infected with HP on initial serum IgM response was observed 2 to 4 weeks after infection . Seroconversion to IgG and IgA was demonstrated between 20 and 30 days after infection in one volunteer study. IgG and IgA antibodies acquire up to 2 months after infection before becoming detectable[15-16].
The PADTAN ELM H.pylori IgM EIA kit is intended for use in evaluating the serologic status to HP infection in patients with gastrointestinal symptoms and it is a useful tool in early diagnosis of HP acute infection.

II. Principle of the test

The H. pylori IgM EIA kit is based on the solid phase enzyme immunoassay (EIA). In this assay system, calibrators, controls and diluted serum samples are incubated with H. pylori bacterial cell lysate immobilised on polystyrene wells.
The IgM antigens to H. pylori present in the wells, are bound to the H. pylori antibody. After washing, away unbound serum components, mouse anti-human IgM conjugated to HRP is added to the wells.
After second incubation, the unbound conjugate is washed away and a solution of TMB (3, 3', 5, 5' tetra-methylbenzidin) is added to each well, resulting in the development of a blue color. The intensity of the color is proportional to antibody activity in the sample.
The color development is arrested by addition of stop solution, causing the blue color to change to yellow. The color intensity is determined in a microtiter plate spectrophotometer at 450 nm. Standard curves are constructed for each assay by plotting absorbance value against the concentration of each standard. The AFP concentrations of patient samples are then read from the standard curve.

III. Kit contents

The reagents provided with the H. pylori Kits (Cat.No.P-HMI) are sufficient for 96 wells. The expiry date of each reagent is shown on the vial label.

Store the Kit at 2-8° C.

1.H. pylori Coated microtiter plate: 96 wells, coated with purified H. pylori antigen.

2.Calibrators (ready to use): 4 vials (1.0 ml) of processed human IgM antibodies to H. pylori, in phosphate buffered saline (PBS) with proteins and thimerosal. The arbitrary units (AU) of the calibrators are given on the vial label.

3.Controls (ready to use): 2 vials (1.0 ml) of human IgM antibodies to H. pylori in phosphate buffered saline (PBS) with proteins and thimerosal. The arbitrary units (AU) of the negative and positive controls are given on the vial label.

4.Enzyme tracer (ready to use): 1 vial (6ml) of mouse anti-human IgM conjugated to horseradish peroxidase (HRP) in phosphate buffered saline (PBS) with proteins and thimerosal.

5.Assay buffer: 1 vial (6 ml) of PBS with proteins and thimerosal and RF Absorbent. In order to avoid interference of Rheumatoid Factor and serum IgM it is necessary that serum samples should be treated with RF absorbent.

6.Serum diluent: 2 vial (25 ml) of PBS with proteins and thimerosal

7.Wash solution (concentrate 20X): 1 vial (25 ml) of PBS - Tween 20 and thimerosal.

8.TMB HRP-Substrate: 1 vial (12 ml) of buffered H_20_2 and 3,3' ,5,5' tetra-methyl benzidine.

9.Stop solution: 1 vial (12 ml) of 2N H_2SO_4.

IV. Materials required (but not supplied with the kit)

1.Distilled or deionized water for preparation of diluted Wash Solution.

2.Microtiter plate spectrophotometer reader with a wavelength of 450nm(with reference wavelength at 630 nm) and an absorbance range of 0 to 3.0.

3.Precision 10- 500 µl micropipettes.

V. Specimen collection and preparation

The assay can be performed on serum or heparinized plasma samples. Keep samples at 2-8 Cfor 1-2 weeks; for longer periods it is recommended to store the sample in aliquoted form at -20°C. Avoid repeated freezing and thawing of samples. Prior to assay, frozen specimens should be slowly brought to room temperature and gently mixed by hand. Do not vortex patient samples.

VI. Reagent preparation

1.Prepare 1:50 dilution of each patient specimen, by mixing 10 µl of specimen with 500µl sample diluent.

2.Concentrated wash solution must be diluted with distilled or deionizer water (Dilute one part concentrated wash solution with 19 parts of water). Diluted wash solution is stable for 7 days at 2 to 8°C. In presence of undissolved crystals, resuspend the solution by placing the vial at 37° C for a few minutes.

VII. Assay procedures

All reagents should be brought to room temperature prior to use.

Use Calibrators and Controls undiluted.

1.Dispense 50 µL standards, control serums and patient diluted samples into appropriate wells. Pipette 50 µl of Assay Buffer to each well .

2.Incubate the strips for 10 min. at room temperature.

3.After the first incubation, aspirate and wash each well 4 times with 300 µl of diluted Wash solution.

4.Add 50 µl of HRP anti-human IgM conjugate to each well. Then incubate for 10 min. at room temperature.

5.After the second incubation, aspirate and wash each well 4 times with 300 µl of diluted Wash solution.

6.Dispense 100 µl of TMB HRP-Substrate into each well.

7.Incubate at room temperature in the dark for 5 min.

8.Add 100µl of Stop Solution and mix for 10 sec. Read absorbance at 450 nm (with reference wavelength at 630 nm) in a microtiter plate reader within 15 min. after addition of Stop solution.

SUMMARY OF ASSAY PROCEDURE

Pipette 50 µl standard, control or diluted sample
Pipette 50 µl Assay buffer
Incubate 10 min. at RT
Wash 4 x (300 µl)
Pipette 50 µl anti-human IgM-HRP
Incubate 10 min. at RT
Wash 4 x (300 µl)
Pipette 100 µl TMB
Incubate 5 min. at RT
Pipette 100 µl stop solution
Read at 450/630 nm

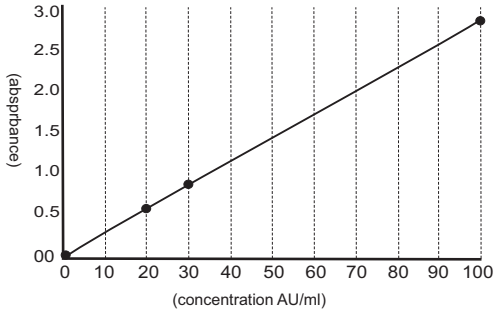
VIII. Calculation of results

The results can be calculated by either microtiter plate, spectrophotometer reader or manual evaluation. If a microtiter plate spectrophotometer reader with data calculation program is used, refer to the plate reader and create a program using the concentration of each of the H. pylori calibrators (in AU/ml).
For manual evaluation, a standard curve is constructed by plotting the absorbance (A) values obtained for each H. pylori calibrator against the corresponding anti- H. pylori IgM.The unknown anti IgM concentration, in AU/ml can then be read from the standard curve using the absorbance value of each patient specimen.

Example of calculation:

The values shown below are examples and must not be used in place of experimental data.

Specimen	Absorbance	anti-HP IgM concentration(AU/ml)
Calibrator A	0.04	0
Calibrator B	0.52	20
Calibrator C	0.83	30
Calibrator D	2.82	100
Sample	1.05	38.1



IX.Validation of test

Test may be considered valid if the following conditions are met:
1.The absorbance value for the calibrator A is higher than 0.1 .
2.The positive and negative control value are in the expected range.
3.The absorbance value for the calibrator D is higher than 1.5.
If above criteria are not met, it may indicate deterioration of the reagents or errors in technique. It should be repeat the test.

X.Interpretation of results

Negative result: If the concentration or absorbance value of the serum sample is lower than that of the calibrator B, the serum sample is considered to be negative. A negative test result indicates that the patient does not have detectable levels of IgM antibodies to H. pylori or that the IgM level is below that which can be detected by this test. This may occur at a too early stage of infection, before an immune response is mounted.
If H. pylori infection is suspected and a negative result is obtained, a second sample should be investigated by bacterial isolation or other diagnostic testing.
Equivocal result: Serum samples with concentration or absorbance values between than those of calibrators B and C, are considered equivocal. In these samples, presence or absence of detectable levels of IgM antibody to H. pylori Samples which remain equivocal upon retesting should be tested by an alternative method.
Positive result: If the concentration or absorbance values of the serum sample is higher than that of calibrator C, the serum sample is considered to be positive and an infection with H. pylori is suspected.
A positive test result does not distinguish between the presence of active or passive disease or colonization in the patient and does not necessarily indicate a gastrointestinal disorder.

result	(AU/ml)	Interpretation
<Calibrator B	<20	Negative
Calibrator B-Calibrator C	20-30	Equivocal
>Calibrator C	>30	Positive

XI. Normal values

In a random local population (Tehran) of 502 individuals (age<20 years and age> 20 years) without clinical symptoms of a gastric disorder (obtained from samples submitted to laboratory for diagnostic testing) 4.3% from age<20 and only 0.3% from age>20 were reactive for H. pylori IgM.
Expected values will vary depending upon age, geographic location and socioeconomic condition of the population being tested. We suggest that each laboratory establishes its own normal ranges based on patient population.

XII. Specific assay characteristics

1.Sensitivity and Specificity: The sensitivity and specificity of Padtan H. pylori IgM Kit was determined by comparing Padtan H. pylori IgM Kit results with IBL kit for 383 sera . The results are summarized in table :

	Padtan H. pylori IgM -	+/-	kit results +
-	320	-	-
Reference Kit results +/-	-	42	1
+	-	1	19

2. Precision: Precision was evaluated for intra- and inter-assay variability. Intra-assay reproducibility was determined by measurement of 10 replicates of 3 samples (positive, equivocal and negative) in a single run. Inter-assay reproducibility was determined by replicate measurement of 3 samples in 10 separate runs.

Reproducibility

Inter-assay			Intra-assay		
$\bar{X}$(AU/ml)	SD	%CV	$\bar{X}$(AU/ml)	SD	%CV
10.37	0.41	3.99	8.43	0.23	2.68
27.0	0.57	2.10	19.19	0.26	1.33
46.19	2.84	6.16	31.2	1.84	5.89

3.Intereference :

- Cross - reactivity of HpM conjugate with HpA and HpG : No cross-reactivity to HpA and HpG (>40 AU/ml) in any samples were found by PADTAN ELM HpM kit.
- Cross - reactivity of HpM conjugate with samples rheumatoid factors: any cross-reactivity with rheumatoid factors of samples (>80 AU/ml) were not found .
- Cross -reactivity of coating helicobacter pylori antigen with non-specific IgM : In study to determine the cross-reactivity of samples with positive IgM of EBV,HSV,CMV,Rubella , all results were negative .

All above results confirm no interferences in PADTAN ELM HpM kit.

XIII. References

1.Malaty HM, Graham DY. Importance of childhood socioeconomic status on the current prevalence of Helicobacter pylori infection. Gut. 35:742,1994.

2.Marshall, B.J. and J.R. Warren. Unidentified curved bacillin in the stomach of patients with gastritis and peptic ulceration. Lanceti:1311 1984.

3.Buck, G.E. Campylobacter pylori and gastroduodenal disease. Clin. Microbiol. Rev. 3:11990.

4.Graham DY. Campylobacter pylori as a Pathogenetic Factor in Duodenal Ulcer: the Case for. Scand. J. Gastroenterol. 24 (supple. 160): 46,1989.

5.Vaira D et al. Four hour rapid urease test (RUT) for detecting Campylobacter pylon: is it reliable enough to start treatment? J. Clin. Pathol. 41:355,1988.

6.Price AB. Histological Aspects of Campyfofiacterpy or Colonisation and Infection of Gastric and Duodenal Mucosa. Scand. J. Gastroenterol. 23 (supple. 142): 21,1988.

7.Dooley CP and Cohen H. Ann. Intern. The Clinical Significance of Campylobactor pylori. Med. 108:70,1988.

8.Parsonnet J etal. Helicobacter pylori\nfac\on and the Risk of Gastric Carcinoma. N. Engl. J. Med. 325:1127,1991.

9.Nomura A et al. Helicobacter pylori Infection and Gastric Carcinoma among Japanese Americans in Hawaii. N. Eng. J. Med. 325:1132,1991.

10.Valle J et al.Disappearance of Gastritis after Eradication of Helicobacter pylori. Scand. J. Gastroenterol. 26:1057,1991.

11.Marshall BJ et al. Prospective Double-Blind Trial of Duodenal Ulcer Relapse after Eradication of Campylobacter pylori. Lancet, ii: 1437,1988.

12.SeppSIS KM etal. Triple Therapy of Helicobacter pylori Infection in Peptic Ulcer. A 12-Month Follow-up Study of 93 Patients.Scand. J. Gastroenterol. 27:973,1992

13.Bazzoli F. Key pointsfromthe revised MaastrichtConsensus Reporttheimpacton general practice. Eur J Gastroenterol Hepatol 2001; 13:Suppl 2:S3-s7. [Medline]

14.Evans, D.J. Jr., D.G. Evants, D.Y. Graham, and P.D. Klein. A sensitive and specific serologic test for detecting of Campylobacter pylori infection. Gastroenterology. 96:1004,1989.

15. Morris A,Nicholson G. Ingestion of Camplyobacter pyloridis causes gastritis and raised fasting gastric pH. AmJ Gastroenterol. 82:192,1987.

16. Sobala GM, Crabtree JE,Dixon MF, et al. Acute Helicobacter pylori infection: clinical features, local and systemic immune response, gastric mucosal histology, and gastric juice ascorbic acid concentrations. Gut. 32:1415,1991.

WARNING!

This kit contains toxic materials, animal and human sourced components. Since no method can completely rule out the presence of blood-borne disease(e.g. HIV,HCV and HBV) therefore,all human sourced material must be considered potentially infectious.
In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary. Never pipette by month. Do not eat, smoke or apply cosmetics in the laboratory.

Rev: Jul.2017

PADTAN ELM Co.
No.26, Shahid Sarparast Ave.West Taleghani Ave.Tehran 14168-IRAN

Tel & Fax: (+98-21) 63481
Email: office@padtan.com