GRUPO MEXLAB®

ALTA CALIDAD EN PRUEBAS PARA SU LABORATORIO

HELICOBACTOR PYLORI IgG QUANTITATIVE

An Enzyme Immunoassay for the Quantitative Determination of IgG Antibodies to Helicobacter Pylori in Human Serum

Intended use

The Helicobacter pylori IgG Test Kit is intended for use in evaluating the serologic status to *H. pylori* infection in patients with gastrointestinal symptoms.

Introduction

Helicobacter pylori is a spiral bacterium cultured from human gastric mucosa by Marshall in 1982. 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 histologic examination of biopsy specimen or direct detection of urease activity. 2) non-invasive techniques include urea breath tests and serological methods.

All of the testing performed on biopsy samples are subject to errors related to sampling and interference of contaminated bacteria. The Helicobacter pylori IgG Test Kit, testing the presence of *H. pylori* specific IgG antibody, is the technique of choice for serologic tests because of its accuracy and simplicity.

Principle of the test

Purified *H. pylori* antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and the *H. pylori* IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and substrate and chromogen are added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

Materials and components

Materials provided with the test kits:

1. Microwell strips: purified *H. pylori* antigen coated wells, 96 wells per pouch.
2. Sample diluent: 1 bottle,100 ml
3. Wash Buffer Concentrate: 1 bottle (15ml, 50x)
4. Zero Buffer: 1 bottle, 12 ml
5. TMB Substrate, 1 bottle, 12 ml
6. Enzyme Conjugate Reagent: 1 bottle,12 ml
7. Human anti-H. Pylori IgG Standard set, contains 0, 5, 10, 20, and 70 units/ml. 1.0 ml each dose. Prediluted, ready to use.
8. Stop Solution: 1 bottle ,12 ml
9. Control Set: 1.0 ml, 1 set. Prediluted, ready to use.

Materials required but not provided:

- Precision pipettes: 0.01, 0.10, and 1.0 ml.
- Disposable pipette tips.
- Distilled water.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph paper.
- Microtiter plate reader.

Specimen collection and preparation

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

Storage of test kits and instrumentation

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2.5 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

Reagent preparation

1. All reagents should be brought to room temperature (18-22°C) before use.
2. Dilute 1 volume of Wash Buffer Concentrate (50x) with 49 volumes of distilled water. For example, dilute 15 ml of Wash Buffer Concentrate (50x) into distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

Assay procedures

1. Secure the desired number of coated wells in the holder.
2. Prepare 1:100 dilution of test samples, by adding 10 µl of sample to 1.0 ml of sample diluent. Mix well.
   The standards and controls have already been prediluted and are ready for use.
3. Dispense 100 µl of Zero Buffer into each wells. Then dispense 100 µl of H. Pylori IgG standards, controls and diluted specimens into the appropriate wells. For the reagent blank, dispense 100µl of sample diluent in a designed well position. Incubate for 30 minutes at room temperature.
4. Remove the incubation mixture by flicking plate content into a waste container.
5. Rinse and flick the microtiter wells 5 times with washing buffer (1X).
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
7. Dispense 100µl of enzyme conjugate reagent to each well and incubate for 30 minutes at room temperature.
8. Remove enzyme conjugate reagent from all wells. Rinse and flick the microtiter wells 5 times with washing buffer (1X).
9. Dispense 100µl of TMB Substrate and incubate for 30 minutes at room temperature.
10. Add 100µl of Stop Solution to stop reaction.
11. Gently mix for 30 seconds. *Make sure that the blue color changes to yellow color completely and there are no air bubbles in each well.*
12. Read optical density at 450 nm with a microtiter reader within 15 minutes.

Important Note:

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

Calculation of results

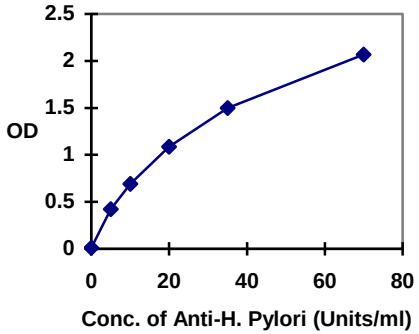
Calculate the mean absorbance value (A_{450}) for each set of reference standards, specimens, controls and patient samples. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in unit/ml on graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of H. Pylori IgG in units/ml from the standard curve.

Example of standard curve

Results of typical standard run with optical density reading at 450nm shown in the Y-axis against H. Pylori IgG concentrations shown in the X-axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

Alternatively, the best-fit line can be determined by regression methods. A second order (Quadratic), third order (cubic spline) or four parameter polynomial of the form will give an excellent fit to the absorbance results for the standards.

H. Pylori IgG (units/ml)	Absorbance (450nm)
0	0.009
5	0.424
10	0.691
20	1.085
70	2.067



Interpretation Validation:

The Standard 1 Readings must give an A_{450} values of less than or equal to 0.100 (After subtracted blank).

Negative:
Specimens yielding an A_{450} value which gives Antibody Unit/mL concentration of less than or equal to ***12*** Antibody Unit/mL are considered negative for antibody to H. Pylori.

Positive:
Specimens yielding an an A_{450} value which gives Antibody Unit/mL concentration of greater than or equal to ***20*** Antibody Unit/mL are considered positive for antibody to H. Pylori.

Equivocal:
Some specimens are neither clearly positive nor clearly negative and the results are considered equivocal. Specimens yielding an A_{450} value which gives Antibody Unit/mL concentrations falling between ***12~20*** Antibody Unit/mL are considered equivocal and may be retested using the same specimen.

Performance characteristics

1. Accuracy:

The comparison of the H. pylori IgG EIA test to a commercial ELISA kit results are summarized.

		Reference ELISA			
		N	E	P	Total
The EIA Kit	N	42 (D)	1	2 (B)	45
	E	0	2 (E)	0	2
	P	1 (C)		52 (A)	53
	Total	43	3	54	100

* N = Nagative; P = Positive; E = Equivocal
Sensitivity = A/(A+B) = 52 / 54 = 98 %
Specificity = D/(C+D) = 42 / 43 = 96 %
Accuracy = (A+D)/(A+B+C+D) = 94 / 97 = 97%

2. Precission:

The precision of the assay was evaluated by testing three different sera 20 replicates on 3 days. The intra-assay and inter-assay C.V. are summarized below:

1]. Intra-Assay

Sample	N	Mean	S.D.	% CV
Level I	20	7.56	0.742	9.81
Level II	20	21.51	1.407	6.54
Level III	20	40.21	2.091	5.20

2]. Inter-Assay

Sample	N	Mean	S.D.	% CV
Level I	20	7.67	0.856	11.20
Level II	20	20.89	1.788	8.56
Level III	20	40.05	3.008	7.51

Limitations of the procedure

There are some limitations of the:

1) The assay should be used only to evaluate patients with clinical signs and symptoms suggestive of gastrointestinal disease. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2) Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee it will eliminate all the effects of that.
3) A positive test result does not allow one to distinguish between 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. Perez-Perez, G.I., S.S. Wilkin, M.D. Decker and M.J. Blaswer. Seroprevalence of *Helicobacter pylori* infection in couples. J. Clin. Microbiol. 29:642-644, 1991.
4. Ansorg, R. et al. Evaluation of techniques for isolation, subcultivation, and preservation of H. Pylori. J. Clin. Microbiology 29: 51-53, 1991.