

ReQuest® H. Pylori IgG

REF 01-550H 96 Test Set

IVD

Enzyme Immunoassay for the Detection of Anti- *Helicobacter pylori* IgG Antibodies in Human Serum

Intended use

The SeraQuest® H. Pylori IgG is a qualitative enzyme immunoassay (EIA) kit for the detection of IgG antibodies against *H. pylori* in human serum. The test is intended as an aid in the diagnosis of infection by *H. pylori* in patients with gastrointestinal symptoms. FOR *IN VITRO* DIAGNOSTIC USE ONLY.

Summary

Gastritis and peptic ulcers are major illnesses that affect a significant number of individuals each year. Since the discovery of *H. pylori* (Warren and Marshall, 1983) (1), many reports suggested a relationship between the presence of *H. pylori* and gastrointestinal disorders (2,3). Evidence of such correlation between the presence of *H. pylori* on the gastric mucosa of patients and histologically confirmed gastritis, and peptic ulcer disease stimulated the development of serological techniques for the detection of antibodies to *H. pylori* (4-8). Many reports have shown that such serological techniques are simple and convenient for the detection of *H. pylori* in infected individuals. Detection of IgG antibodies against *H. pylori* in patient serum has been shown to be an aid for detection of *H. pylori* infection in symptomatic patients.

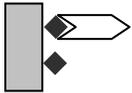
There are significant differences among antigens used for detection of antibodies to *H. pylori*. For detection of *H. pylori* infection by serological methods, in addition to major antigenic components such as urease, the presence of proteins associated with CagA and VacA genes in the antigenic mixture are essential. Antibodies to CagA and VacA proteins are found in patients with peptic ulcer.

Principles of the test

1. Purified specific antigen of *H. pylori* prepared from ATCC strains 43504 and 43629 has been attached to the surface of each micro well.

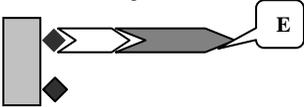


2. Antibodies to *H. pylori* present in patient serum will bind to antigen.



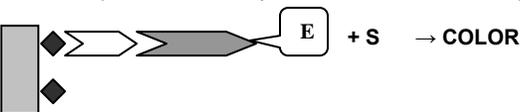
3. After 20 min incubation, washing removes unbound non-specific components present in patient serum.

4. Anti-human IgG conjugated with enzyme (peroxidase) is added. During the incubation the conjugate binds to IgG antibody specific to *H. pylori* that is bound to the antigen.



5. After 20 min incubation, excess unbound conjugate is removed by washing.

6. Substrate specific to the enzyme is added. Color development begins.



7. The enzyme reaction is stopped after 15 min.
8. The color developed after stopping the reaction is measured using a spectrophotometer at 450nm.

Reagents and materials supplied

Each PYLORI IgG kit contains:

- | | |
|---|-----------|
| 1. 12 x 8-well microtiter strips, coated with <i>H. pylori</i> specific antigen | 1 Plate |
| 2. Diluent/Wash (concentrate), containing PBS, Tween | 1 x 30 mL |
| 3. Conjugate (goat anti-human IgG peroxidase labeled), containing | |
| 4. Protein, Buffer, and Proclin (0.001%). Ready to use. | 1 x 11 mL |
| 5. Substrate (TMB). Ready to use. | 1 x 12 mL |

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| 6. Stop Solution (0.5 N H ₂ SO ₄) Ready to use. | 1 x 12 m |
| 7. <i>H. pylori</i> IgG Positive Control, Human Serum. Ready to use. | 1 x 1 mL |
| 8. <i>H. pylori</i> IgG Calibrator, Human Serum. Ready to use. | 1 x 1 mL |
| 9. <i>H. pylori</i> IgG Negative Control, Human Serum. Ready to use. | 1 x 1 mL |
| 10. Package Insert. | |

Materials required (not provided)

- Distilled or deionized water
- Micropipettors (10 µL, 100 µL, and 1 mL)
- Microplate reader with 450nm wavelength capability
- Squeeze bottle for "Working Diluent/Wash" (500 mL or 1000 mL capacity) or microplate washer (required wash volume: at least 300 µL per well)
- Timer
- Sample test tube, 5 mL
- Multi-channel pipette (optional)

Specimen collection and handling

Serum collected aseptically kept at 2-8°C for up to 72 hours can be used in this assay. Freeze specimens at -20°C if not used within 72 hours; avoid repeated freezing and thawing. Avoid use of lipemic, hemolyzed, or contaminated specimens. Do not use heat-inactivated specimens.

Warnings and precautions

- For *in vitro* diagnostic use only
- Do not pipette by mouth
- Stop solution is 0.5 N H₂SO₄, avoid contact with skin
- Controls contain human serum. Sera have been tested and found non-reactive for HbsAg and antibodies to HCV and HIV. Since no known method can offer complete assurance that infectious agents are absent, controls must be handled as recommended in the CDC/NIH health manual.
- Controls and calibrator contain 0.2% sodium azide.
- Sodium azide is a toxic substance. In case of contact, flush immediately with copious amounts of water. Sodium azides may react with lead and copper plumbing to form explosive metal azides. Dispose of reagents with large volumes of water according to state and local regulations.
- The only reagents that may be used with different lots of PYLORI DETECT IgG kits are the Negative Control, Substrate, Stop Solution and Diluent/Wash.

Reagent preparation

Before running the test, prepare the following:

1. Diluent/Wash preparation: Add the content of the Diluent/Wash bottle to 720 mL of distilled or deionized water. Make sure all material in the Diluent/Wash bottle is transferred before bringing it to the final volume (750-mL). Label the resulting solution "Working Diluent/Wash". Store at 2-8°C.
2. Sample dilution: Dilute each patient specimen with "Working Diluent/Wash" by adding 10 µL of specimen to 1 mL of "Working Diluent/Wash". Mix thoroughly and use this 1:101 diluted specimen in the assay.
3. Antigen plate Preparation: Each plate contains 12 strips each of 8 wells. Each patient specimen and Control occupies one well. Calculate the number of wells needed. Leave the required number of strips in the strip holder and keep unused strips in a resealable bag. Store at 2-8°C.

Reagent stability

All kit components should be stored at 2-8°C. **Do not freeze kit components.** All components are stable up to the expiration date printed on the label when stored properly.

Procedure

1. All kit components must be at room temperature (18-27°C) for 20-30 minutes before use.
2. Place the required number of strips in the strip holder.
3. Set the Controls and Calibrator (Ready to use) and diluted specimens in strip # 1 as suggested below. Use additional strips for more specimens:

	1	2
A	Blank	Specimen # 5
B	Calibrator	Specimen # 6
C	Positive Control	Specimen # 7
D	Negative Control	Specimen # 8
E	Specimen # 1	Specimen # 9
F	Specimen # 2	Specimen # 10
G	Specimen # 3	Specimen # 1
H	Specimen # 4	Specimen # 12

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4. Add 100 µL of Controls, Calibrator (Ready to use) and diluted specimens to corresponding wells. A new pipette tip **must** be used for each patient specimen, Calibrator and Controls. Add 100 µL of the "Working Diluent/Wash" to blank well.
5. Allow the strips to incubate at room temperature (18-27°C) for 20 minutes.
6. Discard the contents of all wells and gently wash each well by filling with "Working Diluent/Wash" and discarding. Repeat washing two additional times. Invert the strips over a paper towel and blot. Avoid getting air bubbles in the wells.
7. Add 100 µL of Conjugate to all wells and incubate the strips at room temperature for 30 minutes.
8. Repeat step 6.
9. Add 100 µL of the Substrate to all wells. Incubate for 20 minutes at room temperature (18-27°C).
10. Add 100 µL of Stop Solution to all wells in the same order and rhythm used for addition of Substrate. Gently tap the strips. A color change takes place immediately after addition of Stop Solution.
11. Read the absorbance of each well at 450nm using a suitable spectrophotometer within 15 minutes.

NOTE: When running more than 2 strips, it is recommended that you use a multi-channel pipette for dispensing Conjugate, Substrate, and Stop Solution.

Results

Compare the optical density (OD) of specimens with the OD of Calibrator. The results can be calculated by the following method:

Determine the titer of antibody to *H. pylori* for each patient specimen by converting OD to ELISA VALUE using the following formula:

$$\frac{\text{OD of Test Specimen}}{\text{OD of Calibrator}} = \frac{\text{ELISA VALUE of the specimen}}{1.0}$$

Report the results as ELISA VALUE.

EXAMPLE:

Specimen	OD 450nm*	ELISA VALUE
Calibrator	1.1	1.0
Specimen #1	1.6	1.45
Specimen #2	1.0	0.91
Specimen #3	0.5	0.45

* The OD values showed in the above table are **Examples only**.

Interpretation of results using ELISA VALUE:

ELISA VALUE	Interpretation
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≥ 1.0	Positive
0.89- 0.99	Equivocal (Retest the specimen)
< 0.89	Negative

Report for the above Example:

Specimen # 1	Positive
Specimen # 2	Equivocal (Retest the specimen)
Specimen # 3	Negative

A positive result indicates the presence of IgG antibodies to *H. pylori* antigen. A negative result does not necessarily rule out the presence of the antibody because the level of IgG antibody to *H. pylori* may be undetectable. The equivocal samples must be re-tested and results of the second assay should be reported.

Quality Control

Ensure the blank value has been subtracted from all control, calibrator and specimen wells prior to applying the Quality Control criteria below:

1. Positive and Negative Controls provided in the kit verify test performance. If the OD of the Positive Control is less than 0.4 there may be a reagent problem (contaminated reagent for example).
2. If the OD of the Negative Control is more than 0.2, or if the OD of the blank is more than 0.1, there may be a procedural problem (inadequate washing for example).
3. If a repeat assay is performed, always use a fresh dilution of the patient specimen.
4. Controls and Calibrator should be included in each assay.

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- Bring the kit components to room temperature (18- 27° C) before use.

Limitations

- The result of this assay should only be used as an aid for diagnosis of *H. pylori* infection.
- A positive result indicates the presence of IgG antibodies to *H. pylori*. Diagnosis of gastritis and peptic ulcer should be confirmed with other clinical findings.
- A negative result does not rule out infection by *H. pylori* because the level of antibody may be too low for detection.
- A non-significant level of cross-reactivity has been reported between *Campylobacter jejuni* and *H. pylori*. Serum specimens from patients positive for *C. jejuni* may produce low level of cross-reactivity in the assay.

Expected values

H. pylori is universally distributed and affects both genders, all ages and races. The prevalence of infection with *H. pylori* is higher in underdeveloped countries and in the communities with low standard of living and poor hygiene. Studies in asymptomatic Caucasians in the United States show that there is an increase in the prevalence of *H. pylori* infection with age (6, 9, 10). Testing of asymptomatic volunteers by ELISA showed that seropositivity of IgG to *H. pylori* went from 10 % in 20 to 39-year old subjects to 50 % in 40 to 59- year old subjects (11). 91 random specimens were selected and tested for IgG to *H. pylori* using Pylori Detect IgG kit. Of the 91 sera tested, 37.3 % were positive for IgG to *H. pylori*.

Performance characteristics

The performance of SeraQuest® H. Pylori IgG was determined using clinically confirmed patient specimens. The assay results were compared with endoscopy results (Culture, Histology and Urease CLO test). A correlation study was conducted on 368 patients with diagnosed gastro-intestinal disorders. Of the 368 patients participating in this study 126 were negative and 242 were positive for the presence of *H. pylori* by endoscopic evaluation. The performance of SeraQuest® H. Pylori IgG assay for detection of IgG antibodies to *H. pylori* on tested sera gave the following results:

Endoscopy		SeraQuest® H. Pylori Positive	SeraQuest® H. Pylori Negative	SeraQuest® H. Pylori Borderline **	Total
+	242	233(A)	4(B)	5	242
-	126	7(D)	119(C)	0	126
Total	368	240	123	5	368

* *H. pylori* Serology assay results

** Sera falling in the borderline category were omitted from the following calculations.

Sensitivity = $A/(A+B) = 233/(233+4) \times 100 = 98.3\%$

Specificity = $C/(C+D) = 119/(119+7) \times 100 = 94.4\%$

Assay precision

The *intra and inter* assay precision was calculated by running three patient sera (Negative, Low positive, and High positive) in 24 replicates using two independent operators. The following results were obtained:

Intra and Inter assay precision

		Serum 1	Serum 2	Serum 3
Operator 1	N		24	
	X	24	0.956	24
	S.D	0.255	0.046	1.701
	% C.V.	0.01	4.79	0.024
	Intra-assay	3.85		1.39
Operator 2	N	24	24	24
	X	0.268	1.04	1.89
	S.D.	0.009	0.062	0.029
	% C.V.	3.22	5.91	1.56
	Intra-assay			

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	N	48	48	48
	X	0.261	0.998	1.795
	S.D.	0.011	0.068	0.099
Inter-assay	% C.V.	4.35	6.86	5.53

Determination of cutoff

The absorbance values of all positive and negative sera tested on SeraQuest® H. Pylori IgG were compared with the absorbance values of a serially diluted serum specimen used as a positive reference for antibodies to *H. pylori*. Using the results of endoscopy as the gold standard and measuring the serum IgG to *H. pylori* in all positive and negative specimens, the best dilution of the reference positive specimen, which could distinguish between confirmed positives and confirmed negatives was selected as the cutoff point. The absorbance values of a small number of specimens that were close to cutoff but did not exactly match the absorbance of the cutoff were considered "Equivocal".

References

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Symbols Glossary

Symbol	Standard Title and Number	Title of Symbol	Symbol reference #	Explanatory Text
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Manufacturer	5.1.1	Indicates the medical device manufacturer, as defined in EU Directives 90/385/EEC, 93/42/EEC and 98/79/EC.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Authorized representative in the European Community	5.1.2	Indicates the Authorized representative in the European Community.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Use-by-date	5.1.4	Indicates the date after which the medical device is not to be used.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Batch code	5.1.5	Indicates the manufacturer's batch code so that the batch or lot can be identified.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Catalog number	5.1.6	Indicates the manufacturer's catalogue number so that the medical device can be identified.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Temperature limit	5.3.7	Indicates the temperature limits to which the medical device can be safely exposed.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Consult instruction for use	5.4.3	Indicates the need for the user to consult the instructions for use.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	<i>In vitro</i> diagnostic medical device	5.5.1	Indicates a medical device that is intended to be used as an <i>in vitro</i> diagnostic medical device.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Contains sufficient for 96 tests	5.5.5	Indicates the total number of IVD tests that can be performed with the IVD kit reagents.
Rx Only	Guidance for Industry and FDA on Alternative to Certain Prescription Device Labeling Requirements	Rx Only	N/A	Caution: Federal law prohibits dispensing without prescription.