DIAGNOSTIC AUTOMATION, INC.

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IVD | See external label | 2°C–8°C | 96 tests | REF 1503Z

H.Pylori IgG

Cat # 1503Z

Test	**H.pylori IgG ELISA**
Method	**ELISA: Enzyme Linked Immunosorbent Assay**
Principle	**ELISA - Indirect; Antigen Coated Plate**
Detection Range	**Qualitative Positive; Negative control & Cut off**
Sample	**5ul Serum**
Specificity	**97%**
Sensitivity	**99 %**
Total Time	**~ 75 min**
Shelf Life	**12 -18 Months from the manufacturing date**

** Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account*

NAME AND INTENDED USE

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

SUMMARY AND EXPLANATION OF THE TEST

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. DIAGNOSTIC AUTOMATION INC. H. pylori IgG, 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 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 IgG specific antibody 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* antigen coated wells.	(12 X 8)
2. Sample Diluent: Blue color solution	1 Bottle (22 mL)
3. Calibrator: Factor value (f) stated on Label. Red Cap	1 Vial (150µL)
4. Negative Control: Range Stated on Label. Natural Cap	1 Vial (150 µL)
5. Positive Control: Range Stated on label. Green Cap	1 Vial (150 µL)
6. Washing Concentrate 10X	1 bottle (100mL)
7. Enzyme Conjugate. Red Color Solution	1 Vial (12mL)
8. TMB Chromogenic Substrate. Amber Bottle	1 Vial (12 mL)
9. Stop Solution	1 Vial (12 mL)

STORAGE AND STABILITY

1. Store the kit at 2 - 8° 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. This test kit is designed for in vitro diagnostic use.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as a integral unit. The components of different lots should not be mixed.
5. 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. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2 - 8° C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing of serum sample.

PREPARATION FOR ASSAY

1. Prepare 1x washing buffer.
   Prepare washing buffer by adding distilled or deionized water to 10x wash concentrate to a final volume of 1 liter.
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. Prepare 1:40 dilutions by adding 5 µl of the samples, negative calibrator, positive calibrator, and cut-off calibrator to 200 µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera and calibrators into the appropriate wells. For the reagent blank, dispense 100 µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.
4. Remove liquid from all wells. Repeat washing three times with washing buffer.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 30 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.
7. Dispense 100 µl of TMB Chromogenic Substrate to each well and incubate for 15 minutes at room temperature.
8. Add 100 µl of Stop Solution to stop reaction.

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

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

CALCULATION OF RESULTS

1. To obtain the Cut off value: Multiply the OD_{450} of Calibrator by Factor (f) printed on label of calibrator.
2. Calculate the IgG Index of each determination by dividing the OD values of each sample by obtained OD value of cut off.

Note: This factor (f) is a variable value of each kit.

For example
If factor (f) value on label = 0.30

Sample	OD 450	Mean OD 450 (A)	Calculated Cut off value (B)	INDEX A/B	Interpretation
Calibrator f = 0.3	1.836 1.791	1.814	0.544		
Positive Control	1.425 1.396	1.411		2.59	Positive
Negative Control	0.254 0.191	0.223		0.409	Negative
Patient Sample 1	1.126 1.209	1.168		2.146	Positive
Patient Sample 2	0.374 0.355	0.365		0.670	Negative

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.150.
2. If the O.D. value of the Calibrator is lower than 0.250, the test is not valid and must be repeated.
3. The IgG Index for Negative and Positive control should be in the range stated on the labels

INTERPRETATION

Negative: H. pylori G Index of 0.90 or less are seronegative for IgG antibody to *H. pylori*. The serum sample may have been taken too early.

Equivocal: H. pylori G Index of 0.91 - 0.99 is equivocal. Retest in a parallel fashion with a new serum sample drawn 3 weeks later.

Positive: H. pylori G Index of 1.00 or greater are seropositive.

PERFORMANCE CHARACTERISTICS

A total of 347 patient samples were used to evaluate specificity and sensitivity of the test. The DIAGNOSTIC AUTOMATION INC. H . pylori IgG test results were compared to the endoscopic biopsy findings.

		Endoscopic Biopsy			
		N	E	P	Total
DIAGNOSTIC	N	134(D)	0	2 (B)	136
AUTOMATION	E	6	0	5	11

INC.	P	4(C)	0	196(A)	200
	Total	144	0	203	347

Sensitivity = A / (A + B) = 196 / 198 = 99%
Specificity = D / (C + D) = 134 / 138 = 97%
Accuracy = (A + D) / (A + B + C + D) = 330 / 336 = 98%

The comparison of DIAGNOSTIC AUTOMATION INC. H. pylori IgG test to a commercial ELISA kit results are summarized.

		Reference ELISA			
		N	E	P	Total
DIAGNOSTIC	N	96(D)	1	4 (B)	101
AUTOMATION	E	2	2	1	5
INC.	P	3(C)	0	105(A)	108
	Total	101	3	110	214

Sensitivity = A / (A + B) = 107 / 109 = 96%
Specificity = D / (C + D) = 96 / 99 = 97%
Accuracy = (A + D) / (A + B + C + D) = 201 / 208 = 97%

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

	Negative	Low positive	Positive
Intra-assay	9.1%	8.5%	6.4%
Inter-assay	10.5%	8.9%	7.5%

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 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.

SUMMARY OF ASSAY PROCEDURE

Step	(20-25°C Room temp.)	Volume	Incubation time
1	Sample dilution 1:40 = 5 µl / 200 µl		
2	Diluted sample & 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		

Date Adopted	Reference No.
2012-09-04	DA-H.Pylori IgG-2009

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