

# INSTRUCTIONS FOR USE



EN

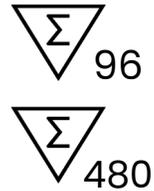
## Anti-*H. pylori* IgG

REF 3Z51021G  
SM3Z51021G  
3Z51021GB

IVD



Rx Only



### INTENDED USE

Anti-*H. pylori* IgG is an ELISA-based test system for the qualitative detection of IgG antibodies to *H. pylori* in sera from symptomatic adults. This device is for *In Vitro* diagnostic use.

### SIGNIFICANCE AND BACKGROUND

Scientists do not completely understand the epidemiology and transmission of *H. pylori*. Studies show that the organism is usually acquired in childhood and early adulthood, and up to 50% of the world's population is infected, many without symptoms (1). Developing countries display the highest infection rates. Lower socioeconomic class and crowded living conditions are associated with an increased prevalence, although recent epidemiological data suggest that the prevalence of *H. pylori* infection is decreasing (2).

Due to protection they receive from gastric acid and the mucous layer, the organisms prefer to reside in the gastric mucosa and in the gastric crypts throughout the stomach. Individuals with *H. pylori* present in the stomach may also have the organism in the metaplastic gastric epithelium cells of the esophagus or duodenum (3). Although the precise mechanism by which *H. pylori* causes mucosal damage remains unknown, the commonly accepted factors that are likely to contribute to the organism's virulence are listed below.

1. The presence of flagella facilitates movement of the organism through the viscous gastric mucus layer, thereby protecting it from the acidic milieu of the stomach (4).
2. The organism attaches to epithelial membranes by adherence pedestals and proteins similar to those found in enteropathogenic *Escherichia coli* (5).
3. The organism produces a variety of catalytic enzymes, such as catalase, lipase, phospholipase, protease, and cytotoxins harmful to both the integrity of the mucus layer and the underlying cells (6 - 9).
4. The organism also produces the enzyme urease, which catalyzes the transformation of urea to ammonium and bicarbonate, thereby forming a microenvironment that protects the organism from gastric acid (10 - 13).

The organism does not appear to invade the bloodstream since no isolates yet have been detected using commercial blood culture method. Varieties of invasive and noninvasive tests are currently available for the diagnosis of *H. pylori* infection. The invasive diagnosis of *H. pylori* infection includes biopsy samples, histologic examination of stained biopsy specimens, or direct detection of the urease activity in the biopsy. Non-invasive techniques included urea breath tests and serological methods. The desirability for cost-effective and noninvasive tests to detect *H. pylori* infection has led to the development of methods that do not require endoscopy.

### PRINCIPLE OF THE ASSAY

The Anti-*H. pylori* IgG is designed to detect IgG class antibodies to *H. pylori* in human sera. Creation of the sensitized wells of the plastic microwell strips occurred using passive adsorption with *H. pylori* antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgG is added to the wells and the plate is incubated. The Conjugate will react with the specific antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted Conjugate.
3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

### TEST SYSTEM COMPONENTS

#### Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE:** The following components contain Sodium Azide as a preservative at a concentration of <0.1% (w/v): Controls, Calibrator, and SAVe Diluent®.

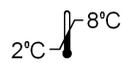
Kit Component	Quantity 	Quantity 	Description
<b>PLATE</b>	1	5	Plate: 96 wells configured in twelve, 1x8-well, strips coated with inactivated <i>H. pylori</i> (ATCC No. 49503) antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
<b>CONJ</b>	1	5	Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgG (Fc chain specific) in 15mL, white-capped bottle(s). Ready to use.
<b>CTRL +</b>	1	2	Positive Control (Human Serum): 0.35mL, red-capped vial(s). 21X concentrate.
<b>CAL</b>	1	4	Calibrator (Human Serum): 0.5mL, blue-capped vial(s). 21X concentrate.
<b>CTRL -</b>	1	2	Negative Control (Human Serum): 0.35mL, green-capped vial(s). 21X concentrate.
<b>DIL SPE</b>	1	4	SAVe Diluent®: 30mL, green-capped, bottle(s) containing Tween-20, bovine serum albumin and phosphate-buffered-saline. Ready to use. <b>NOTE: The SAvE Diluent® will change color when combined with serum.</b>
<b>SOLN TMB</b>	1	5	TMB: 15mL, amber-capped, amber bottle(s) containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use.
<b>SOLN STOP</b>	1	3	Stop Solution: 15mL, red-capped, bottle(s) containing 1M H2SO4, 0.7M HCl. Ready to use.
<b>WASH 10X</b>	1	5	Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. 100mL, clear-capped, bottle(s) containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). <b>NOTE: 1X solution will have a pH of 7.2 ± 0.2.</b>

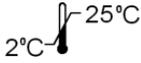
**NOTE:** The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS ELISA Test Systems: TMB, Stop Solution, and Wash Buffer. SAvE Diluent® may be used interchangeably with any ZEUS ELISA Test System utilizing Product No. 005CC.

## MATERIALS REQUIRED BUT NOT PROVIDED

- ELISA microwell reader capable of reading at a wavelength of 450nm. **NOTE: Use of a single (450nm), or dual (450/620 - 650nm), wavelength reader is acceptable. Dual wavelength is preferred, as the additional reference filter has been determined to reduce potential interference from anomalies that may absorb light.**
- Pipettes capable of accurately delivering 10 - 200µL.
- Multichannel pipette capable of accurately delivering 50 - 200µL.
- Reagent reservoirs for multichannel pipettes.
- Wash bottle or microwell washing system.
- Distilled or deionized water.
- One-liter graduated cylinder.
- Serological pipettes.
- Disposable pipette tips.
- Paper towels.
- Laboratory timer to monitor incubation steps.
- Disposal basin and disinfectant (i.e., 10% household bleach - 0.5% sodium hypochlorite).

## STORAGE CONDITIONS

	Coated Microwell Strips: Immediately reseal extra strips with desiccant and return to proper storage. After opening, strips are stable for 60 days, as long as the indicator strips on the desiccant pouch remain blue.
---	---

	Conjugate – DO NOT FREEZE.
	Unopened Kit, Calibrator, Positive Control, Negative Control, TMB, Sample Diluent.
 25°C	Stop Solution: 2 – 25 °C Wash Buffer (1X): 20 – 25°C for up to 7 days, 2 – 8°C for 30 days Wash Buffer (10X): 2 – 25°C

## PRECAUTIONS

1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, consider the strips **potentially biohazardous materials** and handle accordingly.
4. The Controls are **potentially biohazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, handle these products at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": Current Edition; and OSHA's Standard for Bloodborne Pathogens (17).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 – 25°C) before starting the assay.** Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The SAVE Diluent®, Controls, and Calibrator contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions upon hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
8. The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
9. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
11. Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Do not use reagents from other sources or manufacturers.
14. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with Conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach – 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. Do not allow the Conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing Sodium Azide as a preservative. Residual amounts of Sodium Azide may destroy the Conjugate's enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.

## SPECIMEN COLLECTION

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Infectious Disease (Current Edition).
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, consider all blood derivatives potentially infectious.
3. Use only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures in this assay (15, 16). Do not use if there are any added anticoagulants or preservatives. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2 – 8°C, for no longer than 48 hours. If a delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (18).

## ASSAY PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20 – 25°C).
2. Determine the number of microwells needed. Allow for six Control/Calibrator determinations (one Reagent Blank, one Negative Control, three Calibrators and one Positive Control) per run. Run a Reagent Blank on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2 – 8°C.

EXAMPLE PLATE SET-UP		
	1	2
A	Blank	Patient 3
B	Negative Control	Patient 4
C	Calibrator	Etc.
D	Calibrator	
E	Calibrator	
F	Positive Control	
G	Patient 1	
H	Patient 2	

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of SAve Diluent\*) of the Negative Control, Calibrator, Positive Control, and each patient serum. **NOTE: The SAve Diluent\* will undergo a color change confirming that the specimen has been combined with the diluent.**
4. To individual wells, add 100µL of each diluted Control, Calibrator and patient specimen. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100µL of SAve Diluent\* to well A1 as a Reagent Blank. Check software and reader requirements for the correct Reagent Blank well configuration.
6. Incubate the plate at room temperature (20 – 25°C) for 25 ± 5 minutes.
7. Wash the microwell strips 5 times.
  - a. **Manual Wash Procedure:**
    1. Vigorously shake out the liquid from the wells.
    2. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
    3. Repeat steps 1. and 2. for a total of 5 washes.
    4. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with disinfectant at the end of the day's run.
  - b. **Automated Wash Procedure:**

If using an automated microwell wash system, set the dispensing volume to 300 – 350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.
8. Add 100µL of the Conjugate to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
9. Incubate the plate at room temperature (20 – 25°C) for 25 ± 5 minutes.
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100µL of TMB to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.

12. Incubate the plate at room temperature (20 – 25°C) for 10 – 15 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including the Blank well, at the same rate and in the same order as the TMB. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the Reagent Blank. Read the plate within 30 minutes of the addition of the Stop Solution.

**ABBREVIATED TEST PROCEDURE**

1. Dilute Serum 1:21.
2. Add diluted sample to microwell – 100µL/well.
3.  $\longrightarrow$  *Incubate 25 ± 5 minutes.*
4. Wash.
5. Add Conjugate – 100µL/well.
6.  $\longrightarrow$  *Incubate 25 ± 5 minutes.*
7. Wash.
8. Add TMB – 100µL/well.
9.  $\longrightarrow$  *Incubate 10 – 15 minutes.*
10. Add Stop Solution – 50µL/well – Mix.
11. READ within 30 minutes.

**QUALITY CONTROL**

1. Each time the assay is performed, the Calibrator must be run in triplicate. A Reagent Blank, Negative Control, and Positive Control must also be included.
2. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.
3. The mean OD value for the Calibrator, Positive Control, and Negative Control should fall within the following ranges:

	<u>OD Range</u>
Negative Control	≤0.250
Calibrator	≥0.300
Positive Control	≥0.500

- a. The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤0.9.
- b. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥1.25.
- c. If the above conditions are not met the test should be considered invalid and should be repeated.
4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure, but will not ensure precision at the assay Cutoff.
5. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. Refer to CLSI document C24: Statistical Quality Control for Quantitative Measurement Procedures for guidance on appropriate QC practices.

**INTERPRETATION OF RESULTS**

1. **Calculations:**
  - a. *Correction Factor:* The manufacturer determined a Cutoff OD Value for positive samples and correlated it to the Calibrator. The Correction Factor (CF) allows for the determination of the Cutoff Value for positive samples. It will also correct for slight day-to-day variations in test results. The Correction Factor is determined for each lot of components and is printed on the Component Label located in the Test System box.
  - b. *Cutoff OD Value:* To obtain the Cutoff OD Value, multiply the CF by the mean OD of the Calibrator determined above.  
( $CF \times \text{Mean OD of Calibrator} = \text{Cutoff OD Value}$ )

- c. *Index Values/OD Ratios*: Calculate the Index Value/OD Ratio for each specimen by dividing its OD Value by the Cutoff OD from step b.

Example: Mean OD of Calibrator	=	0.793
Correction Factor (CF)	=	0.25
Cutoff OD	=	0.793 x 0.25 = 0.198
Unknown Specimen OD	=	0.432
Specimen Index Value/OD Ratio	=	0.432/0.198 = 2.18

2. **Interpretations:** Index Values/OD Ratios are interpreted as follows.

	Index Value/OD Ratio
Negative Specimens	≤0.90
Equivocal Specimens	0.91 to 1.09
Positive Specimens	≥1.10

- An OD ratio ≤0.90 indicates no significant amount of IgG antibodies to *H. pylori* detected. A negative test result indicates that IgG antibody to *H. pylori* is not present or is at a level that the assay cannot detect.
- An OD ratio ≥1.10 indicates that IgG antibodies specific to *H. pylori* were detected. A positive test result only indicates the presence of IgG antibody to *H. pylori* and does not necessarily indicate that gastrointestinal disease is present.
- Specimens with OD ratio values in the equivocal range (0.91 – 1.09) should be retested in duplicate. Report any two of the three results which agree. Evaluate repeatedly equivocal specimens using an alternate serological method and/or re-evaluate by drawing another sample one to three weeks later.

## LIMITATIONS OF THE ASSAY

- The Anti-*H. pylori* IgG is a laboratory diagnostic aid and by itself is not diagnostic.
- The Anti-*H. pylori* IgG is a qualitative test. Make no quantitative interpretation with respect to the values.
- Only use the test to evaluate patients with clinical signs and symptoms suggestive of gastrointestinal disease. Do not use with asymptomatic patients.
- Literature references have suggested cross reactivity of ELISA IgG antibody with other closely related organisms such as *Borrelia burgdorferi*, *Campylobacter fetus*, *Campylobacter jejuni*, and *Escherichia coli*. However, ZEUS Scientific conducted no evaluation of the performance of this assay with *C. fetus*, *C. jejuni*, and *E. coli*. Therefore, the specificity of this device is unknown if the host is exposed to these organisms.
- ZEUS Scientific conducted no evaluation of the use of specimens containing high levels of potentially cross-reacting endogenous substances such as lipids, hemoglobin, or bilirubin.
- Do not use this assay with pediatric patients.
- A positive result does not allow one to distinguish between active infection and colonization by *H. pylori*.
- The comparative studies performed were based on specimens from adults 18 years or older.

## EXPECTED RESULTS

*H. pylori* infection is usually acquired in childhood and early adulthood, and up to 50% of the world's population is infected, many without symptoms (5). Reports show a low prevalence of the organism in the pediatric population of industrialized nations. In developed countries, a slow increase in acquiring the organism occurs after age 20. The highest prevalence occurs in the over 60 age group, with 40 – 50% of individuals harboring the bacterium (14). Developing countries show the highest infection rates, with approximately 75% of children demonstrating infection. This is most likely due to lower socioeconomic class and crowded living conditions that exist. A study was conducted to establish/estimate expected reactivity rate. Two clinical sites tested a total of 277 routine, random specimens for analysis. Using the Anti-*H. pylori* IgG and with respect to this population, 159/277 (57.4%) specimens were negative, 8/277 (2.9%) specimens were equivocal, and 110/277 (39.7%) were reactive.

## PERFORMANCE CHARACTERISTICS

### 1. Comparative Study

There were two separate clinical investigations conducted to assess the performance of the Anti-*H. pylori* IgG. The first study evaluated the performance of the Anti-*H. pylori* IgG against a commercially available ELISA test assay in a two-site clinical investigation. Briefly, there were 411 specimens tested: 211 at Site One, and 200 at Site Two. Specimens tested from Site One included 84 normal specimens and 127 clinical specimens. Specimens tested from Site Two included 50 normal specimens and 150 clinical specimens. A summary of the combined results follows in Table 1. The second clinical study evaluated 237 specimens (142 clinically characterized) in-house using the Anti-*H. pylori* IgG and a commercially available ELISA test system. The study was designed to assess the performance of Anti-*H. pylori* IgG against the results of a commercially available ELISA test system, and against clinical

results. Table 2 shows the results of Anti-H. pylori IgG versus a commercially available test system using all 237 specimens. Table 3 shows both test systems against clinical results (142 specimens). Of the 142 clinically characterized specimens, 98 were culture positive, 104 were urease (CLO) positive, and 72 were Giemsa stain (histology) positive. Tables 4 - 6 summarize the results of this breakdown.

**Table 1: Combined Clinical Sites**

	Predicate <i>H. pylori</i> Result	Anti-H. pylori IgG Result	Total Number	Percent Total
Concordance	+	+	138	93.1%
	-	-	228	
Discordance	+	-	17	6.9%
	-	+	10	
*Total Specimens Tested			393	

**Table 2: In-House Study**

	Predicate <i>H. pylori</i> Result	Anti-H. pylori IgG Result	Total Number	Percent Total
Concordance	+	+	156	97.8%
	-	-	67	
Discordance	+	-	0	2.2%
	-	+	5	
*Total Specimens Tested			228	

**Table 3: Clinically Characterized Specimens**

(n = 142)

	Anti-H. pylori IgG			
	-	± *	+	Total
Clinical Results				
-	23	2	7	32
+	5	1	104	110
Total	28	3	111	142

**Clinical Sensitivity = 104/109 = 95.4%**

95% Confidence Interval = 91% to 99%

**Clinical Specificity = 23/30 = 76.7%**

95% Confidence Interval = 62% to 92%

**Agreement = 127/139 = 91.4%**

95% Confidence Interval = 87% to 96%

	Commercial ELISA Test System			
	-	± *	+	Total
Clinical Results				
-	28	1	3	32
+	6	0	104	110
Total	34	1	107	142

**Clinical Sensitivity = 104/110 = 94.5%**

95% Confidence Interval = 90% to 99%

**Clinical Specificity = 28/31 = 90.3%**

95% Confidence Interval = 80% to 100%

**Agreement = 132/141 = 93.6%**

95% Confidence Interval = 90% to 98%

**Table 4: Culture Methodology Specimens**

(n = 98)

	Anti-H. pylori IgG			
	-	± *	+	Total
Culture				
+	5	1	92	98
Total	5	1	92	98

**Clinical Sensitivity = 92/97 = 94.8%**

95% Confidence Interval = 90% to 99%

	Commercial ELISA Test System			
	-	± *	+	Total
Culture				
+	6	0	92	98
Total	6	0	92	98

**Clinical Sensitivity = 92/98 = 93.9%**

95% Confidence Interval = 89% to 99%

**Table 5: Urease (CLO) Test Specimens**

(n = 104)

	Anti-H. pylori IgG			
	-	± *	+	Total
Urease				
+	5	1	98	104
Total	5	1	98	104

**Clinical Sensitivity = 98/103 = 95.1%**

95% Confidence Interval = 91% to 99%

	Commercial ELISA Test System			
	-	± *	+	Total
Urease				
+	6	0	98	104
Total	6	0	98	104

**Clinical Sensitivity = 98/104 = 94.2%**

95% Confidence Interval = 90% to 99%

Table 6: Giemsa Stain Specimens					Commercial ELISA Test System						
(n = 73)											
		Anti-H. pylori IgG					Commercial ELISA Test System				
		-	± *	+	Total			-	± *	+	Total
Giemsa	+	0	0	72	72	Giemsa	+	0	0	72	72
	Total	0	0	72	72		Total	0	0	72	72
<b>Clinical Sensitivity = 72/72 = 100.0%</b>					<b>Clinical Sensitivity = 72/72 = 100.0%</b>						
95% Confidence Interval = 100% to 100%					95% Confidence Interval = 100% to 100%						

\* Equivocal specimens were excluded from any calculations.

## 2. Reproducibility

Reproducibility was determined at both clinical sites. Briefly, six specimens were tested: two strong positive specimens, two specimens near the cutoff zone, and two low negative specimens. In addition to these six panel members, the Test System's Positive and Negative Controls were included as two additional precision members. On each of three days, a technician tested each specimen once a day, eight times each, resulting in 24 test points. A responsible party then calculated the intra-assay and inter-assay precision from the resulting data. The results of the experiment are in Tables 7 and 8.

**Table 7: Site One**

Sample Number	Intra-Assay (8)						Inter-Assay (n=24)	
	Day 1		Day 2		Day 3			
	Mean Ratio	% CV	Mean Ratio	% CV	Mean Ratio	% CV	Mean Ratio	% CV
1	7.51	1.1	7.46	11.0	7.48	2.7	7.48	6.3
2	4.05	3.0	3.76	22.3	3.63	5.8	3.81	13.5
3	7.19	5.5	8.30	2.7	7.43	1.3	7.64	7.2
4	0.94	3.2	0.92	13.5	0.78	8.6	0.88	12.4
5	0.58	13.0	0.50	10.6	0.44	2.4	0.51	15.3
6	0.59	5.5	0.53	5.1	0.48	6.5	0.53	9.9
NC	0.17	14.0	0.22	17.4	0.13	10.1	0.17	26.3
PC	10.54	0.9	13.19	1.3	11.10	1.7	11.61	10.1

**Table 8: Site Two**

Sample Number	Intra-Assay (8)						Inter-Assay (n=24)	
	Day 1		Day 2		Day 3			
	Mean Ratio	% CV	Mean Ratio	% CV	Mean Ratio	% CV	Mean Ratio	% CV
1	7.25	6.4	7.96	5.5	7.21	6.3	7.47	7.4
2	3.79	4.7	4.01	5.9	3.57	3.0	3.79	6.7
3	7.17	5.4	7.98	4.9	7.21	1.4	7.45	6.6
4	0.89	25.1	1.21	15.1	1.06	17.9	1.06	22.1
5	0.36	50.1	0.65	36.3	0.49	45.8	0.50	48.1
6	0.89	16.2	0.86	22.5	0.67	25.2	0.81	23.7
NC	0.32	45.0	0.25	44.8	0.14	132.4	0.24	68.6
PC	9.57	5.0	11.19	6.3	9.86	8.0	10.21	9.4

## 3. Cross Reactivity

Two sets of studies were conducted to test for cross reactivity on the Anti-H. pylori IgG. The first was to evaluate for potential cross reactivity with *Borrelia burgdorferi*. Testing of ten samples positive for *B. burgdorferi* side by side on both the Anti-H. pylori IgG and the Anti-B. burgdorferi IgG, showed results that the Anti-H. pylori IgG did not produce any cross reactivity toward samples that are IgG positive for *B. burgdorferi*. The second was an "alternate study" conducted to investigate for potential cross reactivity with *C. jejunii*. Results of this study would show if samples, reactive to *H. pylori* antigens, would react with *C. jejunii* antigens. This involved the development of qualitative IgG ELISA test system for *C. jejunii* and a test procedure similar to the *H. pylori* test system. To ensure that the microwells coated with *C. jejunii* antigens were fully functional, developers verified reactivity using rabbit anti *C. jejunii* polyclonal antibodies and goat anti-rabbit IgG labeled with HRP. A total of 20 *H. pylori* positive specimens were tested on both the *H. pylori* IgG and the *C. jejunii* IgG test systems. The results showed that the possibility of cross-reactivity between *H. pylori* and *C. jejunii* is unlikely.

## REFERENCES

1. Goodwin, C.S, Mendall. M.M., Northfield T.C., 1997. *Helicobacter pylori* infection. *Lancet* Vol.349: 265-269.
2. Parsonnet J., Blaster M., Perez-Perez F., et al. 1992. Symptoms and risk factors of *Helicobacter pylori* in a cohort of epidemiologists. *Gastroenterology* 102:41-46.
3. Review Criteria for Assessment of Laboratory Tests for the Detection of Antibodies to *Helicobacter pylori*. 1992. FDA
4. Hazell S., Lee A., Brady L., et al. 1986. *Campylobacter pyloridis* and gastritis: association with intercellular spaces and adaptation to an environment of mucus as important factors in colonization of gastric epithelium. *J. Clin Pathol* 153:685-663.
5. Goodwin, C., Armstrong J., Marshall B., 1986. *Campylobacter pyloridis* gastritis and peptic ulceration. *J. Clin Pathol* 39:353-365
6. Sarosiek J., Slomiany A., Slomiany B. 1988. Evidence for weakening of gastric mucus integrity by *Campylobacter pyloridis*. *Scand J Gastroenterol* 23:585-590
7. Slomiany B., Bilsky J., Sarosiek J., 1987. *Campylobacter pyloridis* degrades mucin and undermines mucosal integrity. *Biochem Biophys Bio Commun* 144:307-312.
8. Figura N., Guglielmetti P., Rossolini A., et al. 1989. Cytotoxin production by *Campylobacter* strains isolated from patients with peptic ulcers and from patients with chronic gastritis only. *J Clin Microbio* 27:225-226.
9. Covacci A., Censini S., Bugnoli M., 1993. Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. *Proc Natl Sci USA* 90:5791-5795.
10. Marshall B., Barret L., Prakash C., et al. 1990. Urea protects *Helicobacter (Campylobacter) pylori* from the bacterial effect of acid. *Gastroenterology* 99:697-702.
11. Mobley H., Cortesia M., Rosenthal L., et all. 1988. Characterization of urease from *Campylobacter pylori*. *J Clin Microbio*. 26:831-836.
12. Nujami E., Rowe P., Dahill D., et al 1992. Role of ammonia in the pathogenesis of the gastritis, hypergastrinemia, and hyperpepsinogemia caused by *Helicobacter pylori* infection. *Gut* 33:1612-1616.
13. Tsuji S., Kawano S., Tsuji S., et al. 1992. Mechanism of gastric mucosal damage by ammonia. *Gastroenterology* 102: 881-888.
14. Vellozzi E.M., 1993. The evolving role of *Helicobacter pylori* in gastric disease. *Clin Micro Update*. Hoechst-Roussel Pharm., Inc. Vol 6, No. 4.
15. Procedures for the collection of diagnostic blood specimens by venipuncture – Second Edition: Approved Standard (1984). Published by National Committee for Clinical Laboratory Standards.
16. Procedures for the Handling and Processing of Blood Specimens. NCCLS Document H18-A, Vol. 10, No. 12, Approved Guideline, 1990.
17. U.S. Department of Labor, Occupational Safety and Health Administration: Occupational Exposure to Bloodborne Pathogens, Final Rule. Fed. Register 56:64175-64182, 1991.
18. Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guidelines – 4<sup>th</sup> Edition (2010). CLSI Document GP44-A4 (ISBN 1-56238-724-3). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, PA 19087.

## GLOSSARY OF SYMBOLS

The following symbols **may** have been used in the labelling of this product or products associated with this product.

Symbol	Description	Symbol	Description
	Manufacturer		Keep away from sunlight
<b>IVD</b>	<i>In vitro</i> diagnostic medical device	<b>PLATE</b>	Plate
<b>REF</b>	Catalogue number	<b>CONJ</b>	Conjugate
$\Sigma_n$	Sufficient for <i>n</i> tests	<b>CTRL +</b>	Positive Control
<b>LOT</b>	Batch code	<b>CTRL -</b>	Negative Control
	Use by	<b>CAL</b>	Calibrator
	Temperature limitation	<b>DIL</b> <b>SPE</b>	Sample Diluent
<b>CONT</b>	Contents	<b>SOLN</b> <b>TMB</b>	TMB
<b>UDI</b>	Unique Device Identifier	<b>SOLN</b> <b>STOP</b>	Stop Solution
	Consult the warnings and precautions	<b>WASH</b> <b>10X</b>	Wash Buffer Concentrate (10X)
	Consult electronic instructions for use	<b>EN</b>	English
	Store in the upright position	<b>Made in the USA</b>	Made in the USA
<b>RX Only</b>	Applicable for U.S.A: Prescription <i>in vitro</i> diagnostic product		Corrosive
	Hazardous Communication	<b>EC</b> <b>REP</b>	European Commission Authorized Representative
<b>CE</b>	Conformity with Directive 98/79		

Australian Sponsor:  
Southern Cross Diagnostics Pty Ltd  
Unit 7, 17 Green Street  
Banksmeadow, NSW 2019, Australia  
Website: [www.scdiagnostics.com.au](http://www.scdiagnostics.com.au)

**ZEUS Scientific**  
200 Evans Way, Branchburg, New Jersey, 08876, USA  
Toll Free (U.S.): 1-800-286-2111, Option 2  
International: +1 908-526-3744  
Fax: +1 908-526-2058  
Website: [www.zeusscientific.com](http://www.zeusscientific.com)

**EC** **REP**

**EMERGO EUROPE**  
Westervoortsedijk 60  
6827 AT Arnhem  
The Netherlands

For US Customer Service contact your local distributor.  
For US Technical Support contact ZEUS Scientific, call toll free or e-mail [support@zeusscientific.com](mailto:support@zeusscientific.com).  
For Customer Service and Technical Support inquiries outside the US, please contact your local Sebia subsidiary or authorized distributor.

©2025 ZEUS Scientific All Rights Reserved.