

INTENDED USE

The ZEUS ELISA *Helicobacter pylori* IgG Test System is an enzyme-linked immunosorbent assay 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 ZEUS ELISA *H. pylori* IgG Test System 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®.**

Component				Description
PLATE		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.
CONJ		1	5	Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgG (Fc chain specific) in 15mL, white-capped bottle(s). Ready to use.
CONTROL	+	1	2	Positive Control (Human Serum): 0.35mL, red-capped vial(s).
CAL		1	4	Calibrator (Human Serum): 0.5mL, blue-capped vial(s).
CONTROL	-	1	2	Negative Control (Human Serum): 0.35mL, green-capped vial(s).
DIL	SPE	1	4	SAVE Diluent®: 30mL, green-capped, bottle(s) containing Tween-20, bovine serum albumin and phosphate-buffered-saline. Ready to use. NOTE: The SAVE Diluent® will change color when combined with serum.
SOLN	TMB	1	5	TMB: 15mL, amber-capped, amber bottle(s) containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use.
SOLN	STOP	1	3	Stop Solution: 15mL, red-capped, bottle(s) containing 1M H ₂ SO ₄ , 0.7M HCl. Ready to use.
WASHBUF	10X	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). NOTE: 1X solution will have a pH of 7.2 ± 0.2.

NOTES:

1. 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.
2. Test System also contains a Component Label containing lot specific information inside the Test System box.

PRECAUTIONS

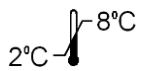
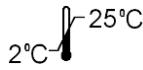
1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.

- The wells of the ELISA plate do not contain viable organisms. However, consider the strips **potentially biohazardous materials** and handle accordingly.
- 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).
- 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.
- 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.
- 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.
- 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.
- The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
- The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
- Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
- Dilution or adulteration of these reagents may generate erroneous results.
- Do not use reagents from other sources or manufacturers.
- 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.
- Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
- Avoid microbial contamination of reagents. Incorrect results may occur.
- Cross contamination of reagents and/or samples could cause erroneous results.
- Reusable glassware must be washed and thoroughly rinsed free of all detergents.
- Avoid splashing or generation of aerosols.
- Do not expose reagents to strong light during storage or incubation.
- Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
- 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.
- Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
- Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
- 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.
- 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.

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 remains blue.
	Conjugate – DO NOT FREEZE.
	Unopened Test System, Calibrator, Positive Control, Negative Control, TMB, SAVE Diluent®
	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

SPECIMEN COLLECTION

- 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).
- No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, consider all blood derivatives potentially infectious.
- 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.
- 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

- Remove the individual components from storage and allow them to warm to room temperature (20 - 25°C).

- 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	

- 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.**
- 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.
- Add 100µL of SAVE Diluent® to well A1 as a Reagent Blank. Check software and reader requirements for the correct Reagent Blank well configuration.
- Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
- Wash the microwell strips 5 times.
 - Manual Wash Procedure:**
 - Vigorously shake out the liquid from the wells.
 - Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
 - Repeat steps 1. and 2. for a total of 5 washes.
 - 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.
 - 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.
- 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.
- Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
- Wash the microwells by following the procedure as described in step 7.
- Add 100µL of TMB to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
- Incubate the plate at room temperature (20 - 25°C) for 10 - 15 minutes.
- 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.
- 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

- Dilute Serum 1:21.
- Add diluted sample to microwell - 100µL/well.
- *Incubate 25 ± 5 minutes.*
- Wash.
- Add Conjugate - 100µL/well.
- *Incubate 25 ± 5 minutes.*
- Wash.
- Add TMB - 100µL/well.
- *Incubate 10 - 15 minutes.*
- Add Stop Solution - 50µL/well - Mix.
- READ within 30 minutes.

QUALITY CONTROL

- 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.
- 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.
- 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

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

INTERPRETATION OF RESULTS

- Calculations:**
 - 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.
 - Cutoff OD Value:** To obtain the Cutoff OD Value, multiply the CF by the mean OD of the Calibrator determined above.

(CF x Mean OD of Calibrator = 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.

	<u>Index Value/OD Ratio</u>
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 ZEUS ELISA *H. pylori* IgG Test System is a laboratory diagnostic aid and by itself is not diagnostic.
- The ZEUS ELISA *H. pylori* IgG Test System 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 ZEUS ELISA *H. pylori* IgG Test System 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 ZEUS ELISA *H. pylori* IgG Test System. The first study evaluated the performance of the ZEUS ELISA *H. pylori* IgG Test System 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 ZEUS ELISA *H. pylori* IgG Test System and a commercially available ELISA test system. The study was designed to assess the performance of ZEUS ELISA *H. pylori* IgG Test System against the results of a commercially available ELISA test system, and against clinical results. Table 2 shows the results of ZEUS ELISA *H. pylori* IgG Test System 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	ZEUS ELISA <i>H. pylori</i> 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	ZEUS ELISA <i>H. pylori</i> 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)					Commercial ELISA Test System				
Clinical Results	ZEUS ELISA <i>H. pylori</i> IgG Test System				Total	-	± *	+	Total
	-	± *	+	Total					
	-	23	2	7					
+	5	1	104	110	6	0	104	110	
Total	28	3	111	142	34	1	107	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%

Table 4: Culture Methodology Specimens (n = 98)					Commercial ELISA Test System				
Culture	ZEUS ELISA <i>H. pylori</i> IgG Test System				Total	-	± *	+	Total
	-	± *	+	Total					
	+	5	1	92					
Total	5	1	92	98	6	0	92	98	

Clinical Sensitivity = 92/97 = 94.8%
 95% Confidence Interval = 90% to 99%

Table 5: Urease (CLO) Test Specimens (n = 104)					Commercial ELISA Test System				
Urease	ZEUS ELISA <i>H. pylori</i> IgG Test System				Total	-	± *	+	Total
	-	± *	+	Total					
	+	5	1	98					
Total	5	1	98	104	6	0	98	104	

Clinical Sensitivity = 98/103 = 95.1%
 95% Confidence Interval = 91% to 99%

Table 6: Giemsa Stain Specimens (n = 73)					Commercial ELISA Test System				
Giemsa	ZEUS ELISA <i>H. pylori</i> IgG Test System				Total	-	± *	+	Total
	-	± *	+	Total					
	+	0	0	72					
Total	0	0	72	72	0	0	72	72	

Clinical Sensitivity = 72/72 = 100.0%
 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 ZEUS ELISA *H. pylori* IgG Test System. 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 ZEUS ELISA *H. pylori* IgG Test System and the ZEUS ELISA *B. burgdorferi* IgG Test System, showed results that the ZEUS ELISA *H. pylori* IgG Test System 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.

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