510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

K110899

B. Purpose for Submission:

To obtain a substantial equivalence determination for *Helicobacter pylori (H. pylori)* ELISA IgA test Kit

C. Measurand:

IgA antibodies to *H. pylori*

D. Type of Test:

Enzyme linked immunosorbent assay

E. Applicant

Gold Standard Diagnostics

F. Proprietary and Established Names:

Helicobacter pylori IgA test kit

G. Regulatory Information:

1. Regulation section:

   26 CFR 866.3110 – Campylobacter fetus serological reagents

2. Classification:

   Class I

3. Product code:

   LYR – Campylobacter pylori

4. Panel:

   83 - Microbiology

H. Intended Use:

1. Intended use:

   The *Helicobacter pylori (H. pylori*) ELISA IgA test kit is intended for the qualitative detection of IgA antibodies to *H. pylori* in human serum in the adult population. This test is intended as a second test to aid in the diagnosis of *H. pylori* in patients with gastrointestinal symptoms, in conjunction with clinical findings. It should be performed and interpreted with another assay for detection of IgG antibodies to *H. pylori.*

2. Indications for use:

   The *Helicobacter pylori (H. pylori*) ELISA IgA test kit is intended for the qualitative detection of IgA antibodies to *H. pylori* in human serum in the adult population. This test is intended as a second test to aid in the diagnosis of *H. pylori* in patients with gastrointestinal symptoms, in conjunction with clinical findings. It should be performed and interpreted with another assay for detection of IgG antibodies to *H. pylori.*

3. Special conditions for use statement:

   For Prescription Use

4. Special instrument requirements:

   N/A

I. Device Description:

The *Helicobacter pylori (H. pylori*) ELISA IgA test is an enzyme linked immunosorbent assay. The device consists of a kit containing wash buffer, diluent, a negative control, positive control, and a cut-off control, IgA conjugate, TMB solution and Citrate - Stopping solution. The kit contains a microtiter plate consisting of 96 antigen coated, breakable single wells. The reagents are sufficient for 96 determinations.

J. Substantial Equivalence Information:

1. Predicate device name:

   Micro Detect Inc. Pylori Detect IgA

2. Predicate K number(s):

K003794

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	For the detection of IgA antibodies to H. pylori in human serum	Same
Methodology	Enzyme linked immunosorbent assay	Same
Matrix	Serum	Same
Reader/Wavelength	Spectrophotometer /450 nm	Same
Reagents	Substrate (TMB), positive and negative controls	Substrate (TMB), positive and negative controls
Indications for Use	Use as a second test. To be performed in conjunction with IgG	Use as a second test. to be performed in conjunction with IgG

Differences		
Item	Device	Predicate
Incubation time	90 minutes	55 minutes
Reagents	Wash solution-20x Diluent – ready to use Kit contains *H. pylori* cut off control	Diluent wash concentrate – 25x Kit contains *H. pylori* calibrator

K. Standard/Guidance Documents Referenced :

EP 17-A Protocols for determination of Limits of Detection and Limits of Quantitation; Approved Guideline, CLSI Vol 24, No.34, 2004

EP 7-A2 Interference Testing in Clinical Chemistry, Approved Guideline, 2nd ed., CLSI Vol 25, No 27, 2005

L. Test Principle:

The *Helicobacter pylori (H. pylori*) ELISA IgA test is an enzyme-linked immunosorbent assay. Purified antigen is bound to microwells in a polystyrene

microtiter plate. Serum is added to each well and incubated for 30 minutes at 37° C. If *H. pylori* IgA antibodies are present they will bind to the antigen in the well. Unbound serum is removed by washing the wells three times. An HRP-conjugated anti-human IgA is then added to each well and incubated for 30 minutes at 37 °C. The wells are washed three times to remove any unbound conjugate. A TMB substrate is added to each well and the plate is incubated for 30 minutes at 37 °C. This reaction generates a blue color produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added and the color intensity is measured spectrophotometrically

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision

Intra and inter assay precision were calculated by running six patient sera (four positives and two negatives at three different sites. Results are summarized in the table below:

		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Site 1	**Ave:**	0.945	0.803	0.860	0.498	0.091	0.115
	SD:	0.024	0.034	0.039	0.025	0.006	0.008
Intra-Assay	**CV:**	2.5%	4.2%	4.5%	5.0%	6.3%	7.1%
Site 2	**Ave:**	1.030	0.737	0.716	0.579	0.091	0.137
	SD:	0.060	0.057	0.041	0.046	0.004	0.009
Intra-Assay	**CV:**	5.9%	7.7%	5.7%	7.9%	4.8%	6.9%
Site 3	**Ave:**	0.996	0.667	0.768	0.579	0.091	0.151
	SD:	0.108	0.035	0.057	0.042	0.010	0.009
Intra-Assay	**CV:**	10.8%	5.3%	7.4%	7.3%	12.2%	6.1%
	Ave:	0.964	0.770	0.812	0.527	0.090	0.126
Inter-Assay	**SD:**	0.062	0.063	0.074	0.049	0.007	0.017
	CV:	6.4%	8.1%	9.1%	9.4%	7.4%	13.7%

Reproducibility

The reproducibility of the assay was done by testing three samples in triplicate (a high negative, low positive and a moderate positive) for five days, twice a day, at three sites with two technicians per site. The results are summarized in the following table:

		5 day average:	Sample 1	Sample 2	Sample 3
Site 1	Tech 1	OD:	0.614	0.724	1.545
		SD:	0.045	0.066	0.091
		CV:	7.3%	9.1%	5.9%
	Tech 2	OD:	0.588	0.717	1.494
		SD:	0.059	0.073	0.146
		CV:	10.1%	10.2%	9.8%
Site 2	Tech 1	OD:	0.594	0.704	1.546
		SD:	0.034	0.045	0.082
		CV:	5.7%	6.4%	5.3%
	Tech 2	OD:	0.618	0.845	1.741
		SD:	0.036	0.044	0.071
		CV:	5.8%	5.2%	4.1%
Site 3	Tech 1	OD:	0.356	0.480	1.046
		SD:	0.048	0.087	0.128
		CV:	13.4%	18.0%	12.2%
	Tech 2	OD:	0.478	0.636	1.323
		SD:	0.086	0.110	0.148
		CV:	18.0%	17.3%	11.2%

b. Linearity/assay reportable range:

N/A

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Controls provided are ready to use. Units of the cut-off controls are defined as 10 units. The calculated units of the positive controls should be within the ranges mentioned in the quality control certificate. If those requirements (OD values, units) are not fulfilled, the test has to be repeated.

d. Detection limit:

The limit of detection was determined by testing the kit controls along with a pooled positive sample and several dilutions of the pooled sample. The limit of detection was determined at a 3.3 fold dilution of the pooled sample giving an OD value of 0.558 with a corresponding unit value of 11.3

e. Analytical specificity:

Cross reactivity

An adsorption study was performed to evaluate cross reactivity. Briefly, sera with different levels of antibodies to *H. pylori* were adsorbed with either of the following organisms; *H. pylori, Candida albicans, E. coli, Borrelia burgdorferi, Clostridium* spp., *Campylobacter, Bacillus, Enterobacter, Pseudomonas, Proteus* , or two different strains of *H. influenza.* The identity of the bacteria used were identified by the ATCC and confirmed by mass

spectrometry. The bacteria were evaluated at a concentration of 10^7 CFU/ml or higher. The adsorbed samples were compared to the untreated samples and the mean percent inhibition was calculated. The results are summarized in the following table:

Organism	**Concentration (CFU/ml)**	**Mean percent inhibition**
Helicobacter pylori		96%
Candida albicans	2.40×10^7	0.8%
Escherichia coli	6.90×10^7	2.4%
Borrelia burgdorferi	1.00×10^8	4.3%
Clostridium spp.	1.20×10^7	1.7%
Campylobacter	1.50×10^9	3.3%
Bacillus	4.40×10^7	10.9%
Enterobacter	1.80×10^8	4.1%
Pseudomonas	1.45×10^8	3.8%
Haemophilus Influenza	7.90×10^7	5.8%
Proteus	1.40×10^8	3.1%

The mean percent inhibition for *H. pylori* was 96%, and 0.8% to 10.9% with the other organisms. Overall no effects on the analytical specificity were seen with the Gold Standard Diagnostics *H. pylori* ELISA IgA assay.

Interfering Substances

Interference

The effect of potential interfering substances on samples using the Gold Standard Diagnostics *H. pylori* ELISA IgA assay was evaluated. High levels of hemoglobin, bilirubin, cholesterol and triglycerides in serum samples were tested. The concentrations, recommended in the guideline "Interference Testing in Clinical Chemistry" from the Clinical and Laboratory Standards Institute, were used. The tested substances did not affect the performance of the Gold Standard Diagnostics *H. pylori* ELISA IgA assay.

Substance	Concentration	*H. pylori* concentration	Mean Percent Inhibition
Hemoglobin	2 g/L	9-11 units	9%
Bilirubin	342 µmol/L	9-11 units	7%
Cholesterol	13 mmol/L	9-11 units	0%
Triglyceride	37 mmol/L	9-11 units	-32%

f. Assay cut-off:

The cut-off was determined by testing normal sera, clinical defined samples, proficiency samples, along with sera positive for *H. pylori*, borderline positive for *H. pylori*, and high negative for *H. pylori* for a total of 254 samples. The cutoff was determined by taking the negative average plus three standard deviations from normal sera samples. The cutoff was further adjusted so that the clinically defined samples were positive, the proficiency samples met their criteria, the positive *H. pylori* sera were positive, and the *H. pylori* negative sera were negative.

2. Comparison studies:

a. Method comparison with predicate device:

The performance of the Gold Standard Diagnostics *H. pylori* ELISA IgA assay was determined by conducting a correlation study using 628 samples being routinely tested for *H. pylori.* Testing was conducted at three geographically diverse clinical sites. The samples were tested with both the Gold Standard Diagnostics *H. pylori* ELISA IgA assay and the predicate device. The results are summarized in the following table:

		Predicate Device IgA ELISA		
		Positive	Borderline	Negative
Gold Standard Diagnostics IgA ELISA	Positive	121	31	26
	Borderline	13	8	23
	Negative	7	5	394

%Positive Agreement = 94.5% (C.I. 76.0 - 100%)
%Negative Agreement = 93.8% (C.I. 88.8% - 99.5%)
Overall Agreement = 94.0% (C.I. 85.9% - 100%)

The discrepant samples were further tested with a second commercially available assay. Of the seven samples, that were negative by the Gold Standard assay and positive by the predicate device, five samples were positive by the second assay. Of the 26 Gold Standard Diagnostics positive samples, which were negative by

the predicate device, two samples were borderline, one sample was negative and 23 samples were positive by the second assay.

b. Matrix comparison:

N/A

3. Clinical studies:

a. Clinical Sensitivity:

N/A (comparison was not done to the gold standard e.g. biopsy, culture and/or histological examination, or the urea breath test.

b. Clinical specificity:

N/A (comparison was not done to the gold standard.)

c. Other clinical supportive data (when a. and b. are not applicable):

N/A

4. Clinical cut-off:

N/A

5. Expected values/Reference range:

H. pylori is universally distributed and affects all genders, ages and races. The prevalence of infection with *H. pylori* is higher in underdeveloped countries and in the communities with a 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. An expected values study was performed with the *H. pylori* ELISA Ig A assay testing 628 patients from various populations. Results showed that a total of 121 patients were positive with the assay. The expected values result seen in this study for the *H. pylori* IgA assay is 19%.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.