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

A. 510(k) Number:

K110745

B. Purpose for Submission:

To obtain substantial equivalence determination for a new device which detect IgG antibodies to *Helicobacter pylori* (*H. pylori*) in human serum.

C. Measurand:

Detection of IgG Antibodies to *H. pylori*

D. Type of Test:

Qualitative detection of *H. pylori IgG* Enzyme-Linked Immunosorbent Assay (ELISA) Test Kit

E. Applicant:

Gold Standard Diagnostics

F. Proprietary and Established Names:

Helicobacter pylori ELISA IgG Test Kit

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
LYR	I	21 CFR 866.3110	83 - Microbiology

H. Intended Use:

1. Intended use:

The *Helicobacter pylori (H. pylori*) ELISA IgG test kit is intended for the qualitative detection of IgG antibodies to *H. pylori* in human serum in the adult population. This test is intended to aid in the diagnosis of *H. pylori* in patients

suspected of having *H. pylori* infection, and in patients with gastrointestinal symptoms, and is to be used in conjunction with clinical findings.

2. Indication for use:

   The *Helicobacter pylori (H. pylori*) ELISA IgG test kit is intended for the qualitative detection of IgG antibodies to *H. pylori* in human serum in the adult population. This test is intended to aid in the diagnosis of *H. pylori* in patients suspected of having *H. pylori* infection, and in patients with gastrointestinal symptoms, and is to be used in conjunction with clinical findings.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Spectrophotometer - 450nm

I. Device Description:

The Gold Standard Diagnostics *H. pylori* ELISA IgG Test is an enzyme linked immunosorbent assay where antigen is bound to microwells in polystyrene microtiter plates. A detection antibody (Conjugate) labeled with horseradish peroxidase (HRP) to detect antibodies to *H. pyori* in serum is added. The Substrate contains tetramethylbenzidine (TMB) which is oxygenated by the Conjugate to produce a blue end product. The Stop Solution is an acid that stops the reaction and turns the substrate yellow if antibody is present in the patient serum. The kit also includes a Wash Buffer, Diluent, a Negative Control, Positive Control, and a Cutoff Control. The controls are provided to determine if the assay is functioning properly and to determine the antibody level. The kit contains 12 x 8well antigen coated microtiter strips in a frame. The reagents are sufficient for 96 determinations.

J. Substantial Equivalence Information:

1. Predicate Device names:

   Micro Detect, Inc. Pylori Detect IgG

2. Predicate K number:

   K973508

3. Comparison with predicate:

Similarities		
Item	**Gold Standard Diagnostics Device**	Predicate
Intended Use	Detection of H. pylori IgG antibodies as aid in the diagnosis of H. pylori infection	Same
Type of test	Qualitative	Same
Analyte	IgG antibodies to H. pylori	Same
Methodology	Enzyme Linked Immuno Sorbent Assay	Same
Matrix	Serum	Same
Reader/Wavelength	Spectrophotometer /450 nm	Same

Differences		
Item	Gold Standard Diagnostic Device	Predicate
Incubation time	Incubate for 30 minutes at 37°C	Incubate for 20 minutes at Room Temperature
Reagents	Stop Solution – Acid mixture Wash four times with reconstituted Wash Solution Add 50ul of Stop Solution	Stop Solution – Sulfuric Acid Wash three times with reconstituted Wash Solution Add 100ul of Stop Solution

K. Standard/Guidance Document Referenced (if applicable):

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

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

L. Test Principle:

The antibody searched for in the human serum forms an immune complex with the antigen coated on the microtiter-plate. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is again removed by washing processes. After adding the substrate solution (TMB), a blue dye is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision

The intra and inter assay precision was calculated by running six patient sera (four positives and two negatives) at three different sites. The results are summarized in the table below:

		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Site 1 Intra-Assay	**Ave:**	0.884	1.022	0.965	1.302	0.185	0.168
	SD:	0.063	0.043	0.050	0.006	0.011	0.016
	CV:	7.2%	4.3%	5.2%	0.4%	6.0%	9.5%
Site 2 Intra-Assay	**Ave:**	0.935	0.958	0.817	1.329	0.147	0.153
	SD:	0.028	0.034	0.042	0.015	0.005	0.007
	CV:	3.0%	3.6%	5.1%	1.1%	3.4%	4.3%
Site 3 Intra-Assay	**Ave:**	1.013	1.093	0.933	1.476	0.181	0.177
	SD:	0.016	0.103	0.068	0.106	0.013	0.011
	CV:	4.5%	9.4%	7.3%	7.2%	7.0%	6.4%
Inter-Assay	**Ave:**	0.974	1.057	0.925	1.414	0.177	0.172
	SD:	0.070	0.098	0.078	0.114	0.018	0.014
	CV:	7.2%	9.3%	8.4%	8.0%	10.1%	8.4%

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 table below:

		5 day average:	Sample 1	Sample 2	Sample 3
Site 1	Tech 1	OD: SD: CV:	0.338 0.047 13.9%	0.708 0.074 10.4%	1.030 0.095 9.2%
	Tech 2	OD: SD: CV:	0.354 0.043 12.2%	0.692 0.060 8.7%	1.055 0.107 10.1 %
Site 2	Tech 1	OD: SD: CV:	0.329 0.044 13.4%	0.693 0.037 5.4%	1.034 0.050 4.9%
	Tech 2	OD: SD: CV:	0.360 0.052 14.4%	0.693 0.032 4.7%	1.022 0.049 4.8%
Site 3	Tech 1	OD: SD: CV:	0.300 0.048 16.1%	0.516 0.054 10.5%	0.888 0.032 3.6%
	Tech 2	OD: SD: CV:	0.374 0.041 11.0%	0.642 0.094 14.6%	0.928 0.128 13.7 %

The results obtained for both precision and reproducibility studies were acceptable.

b. ***Linearity/assay reportable range:***

Not applicable

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

Controls

The ready to use controls serve for a qualitative determination of antibodies. Their concentration can be expressed in units. Fluctuations resulting from the test procedure can be balanced with this calculation method to produce a high reproducibility. Use the means of the OD values for calculation of the units (see calculation of units below).

Test function control:

a) The OD of the blank should be < 0.150
The OD-values of the negative controls should be lower than the OD-values mentioned in the Quality Control Certificate. The OD-values of the positive

controls as well as of the cut-off controls should be above the OD-values mentioned in the Quality Control Certificate.
b) 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.

Calculation of Units:
The OD of the blank value (450/620nm) has to be subtracted from all other OD's.

Units* = (OD controls or samples / OD of cutoff) x 10

Interpretation of the Test:

Units	**Interpretation**
< 9.0	negative
9.0 – 11.0	equivocal
> 11.0	positive

*Units are defined by the manufacturer.

i. If the measured values are above the defined equivocal range, they are considered to be positive.

ii. If the measured Unit is within the equivocal range, no significant high antibody concentration is present, the samples are considered to be equivocal. Retest the sample and if the results are again equivocal, no significant antibodies are present. For the definitive detection of the presence of *H. pylori* it is necessary to determine the antibody concentration of two serum samples, one IgG and one IgM and in addition histology, culture and / or a urease test. A correct diagnosis based on the evaluation of a single serum sample is not possible.

iii. If the measured values are below the defined equivocal range, no measurable antigen specific antibodies are present in the samples. The samples are considered to be negative.

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 30 fold dilution of the pooled sample giving an OD value of 0.379 with a corresponding unit value of 11.9.

Analytical specificity:

Cross Reactivity

An adsorption study was performed to evaluate cross reactivity. Sera with different levels of antibodies to *H. pylori* were adsorbed with *H. pylori*, and *Candida albicans, E. coli, Borrelia burgdorferi, Clostridium* spp., *Campylobacter, Bacillus, Enterobacter, Pseudomonas, Proteus* and two different strains of *H. influenza.* The identity of the bacteria used were identified by the ATCC and confirmed with the MALDI-TOF method. The bacteria were evaluated at a concentration of 10^7cfu/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	$2.40x10^7$	1.8%
Escherichia coli	$6.90x10^7$	9.5%
Borrelia burgdorferi	$1.00x10^8$	5.5%
Clostridium spp.	$1.20x10^7$	6.0%
Campylobacter	$1.50x10^9$	4.7%
Bacillus Cereus	$4.40x10^7$	18.2%
Enterobacter	$1.80x10^8$	2.1%
Pseudomonas	$1.45x10^8$	5.1%
Haemophilus Influenza	$7.90x10^7$	3.8%
Proteus	$1.40x10^8$	4.6%

The mean percent inhibition for *H. pylori* was 96%, and 1.8% to 9.5% with the other organisms. There seems to be a weak cross reactivity with *Bacillus.* Taking into account the normal test variation, the remaining cross reactivity with *Bacillus* may affect high negative samples close to the equivocal range. With the exception of the cross reactivity, no overall effects on the analytical specificity were seen on the Gold Standard Diagnostics *H. pylori* ELISA IgG assay. A limitation statement was included in the package insert.

Interfering Substances

The effect of potential interfering substances on samples using the Gold Standard Diagnostics *H. pylori* ELISA IgG assay was evaluated. High levels of hemoglobin, bilirubin, cholesterol and triglycerides in serum samples were tested at the assay cutoff (9-11 units) in triplicate. The recommended concentrations from the guideline "Interference Testing In Clinical Chemistry" from the Clinical and Laboratory Standards Institute (18) were used (see table below). The tested substances did not affect the performance of the Gold Standard Diagnostics *H. pylori* ELISA IgG assay.

Substance	Concentration	*H. pylori* concentration	Mean Percent Inhibition
Hemoglobin	2 g/L	9-11 units	3%
Bilirubin	342 µmol/L	9-11 units	11%
Cholesterol	13 mmol/L	9-11 units	1%
Triglyceride	37 mmol/L	9-11 units	-19%

Leukocytes, intestinal secretions or mucus, fat, and medications used to relieve diarrhea or other gastric symptoms were not tested, therefore, it is not known if these substances will interfere with the assay as they were not evaluated

f. Assay cut-off:

Determination of Cutoff

The cutoff was determined by testing normal sera from blood donors, 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 278 samples. The cutoff was determined by taking the negative average plus three standard deviations of the blood donors. 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:

Performance Data

Method Comparison

The performance of the Gold Standard Diagnostics *H. pylori* ELISA IgG assay was determined by conducting a correlation study using 625 samples being

routinely tested for *H. pylori*. No information as to the age, gender or ethnic makeup was provided, therefore, no conclusions can be made on these factors from these samples. The samples were tested on both the Gold Standard Diagnostics *H. pylori* ELISA IgG assay and a commercially available ELISA assay as the predicate device. The results are summarized in the following table:

		Predicate Device IgG ELISA		
		Positive	Equivocal	Negative
Gold Standard Diagnostics IgG ELISA	Positive	203	3	13
	Equivocal	5	2	8
	Negative	11	5	375
	Total	**219**	**10**	**396**

% Positive Agreement = 92.7% (203/219) with 95% CI: 88.5% to 95.5%;
% Negative Agreement = 94.7% (375/396) with 95% CI: 92.0% to 96.5%
% Equivocal Agreement = 20% (2/10) with 95% CI: 5.7% to 51.0%
Overall Agreement = 92.8% (580/625)

The discrepant samples were further tested with a third commercially available assay. Of the eleven Gold Standard Diagnostics negative samples, and predicate device positive samples, nine samples were positive by the third assay. Of the 13 Gold Standard Diagnostics positive samples, and predicate device negative samples, one sample was equivocal, two negative, and ten samples were positive by the third assay.

b. Matrix comparison:

3. Clinical studies:

   a. Clinical Sensitivity:

   No claim can be made for clinical sensitivity since comparison was not done to the gold standard e.g. biopsy (culture and/or histological diagnosis) or the urea breath test.

   *b. Clinical specificity**:***

   Same as above

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

4. Clinical cut-off**:**

   Not applicable

5. Expected values/Reference range:

H. pylori is universally distributed and affect 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 (13-15). An expected values study was performed with the *H. pylori* IgG ELISA testing 625 patients from various populations. Results showed that a total of 203 patients were positive with the assay. The expected values result for the *H. pylori* IgG ELISA is 32%.

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.