510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K232892

B Applicant

Biomerica, Inc.

C Proprietary and Established Names

Hp Detect Stool Antigen ELISA

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
LYR	Class I, reserved	21 CFR 866.3110 - Campylobacter Fetus Serological Reagents	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for the detection of *Helicobacter pylori* antigen in human stool.

B Measurand:

Helicobacter pylori antigen

C Type of Test:

Qualitative enzyme immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Hp Detect Stool Antigen ELISA is an in vitro diagnostic qualitative enzyme immunoassay for the detection of *Helicobacter pylori* (*H. pylori*) antigens in human stool or feces. The Hp Detect Stool Antigen ELISA is intended to aid in the initial diagnosis and post-therapy diagnosis of *H. pylori* infection. Additionally, the test may be used to assess *H. pylori* infection status after treatment. Retesting at a minimum of 4 weeks after the completion of treatment may be done to assess *H. pylori* status. Test results should always be taken into consideration by the physician in conjunction with patient's clinical information (history and symptoms).

For Prescription Use Only

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

An ELISA plate reader capable of reading absorbance at one or more of the following settings is required but not provided: 450 nm, 450/620 nm, or 450/630 nm.

IV Device/System Characteristics:

A Device Description:

The Hp Detect Stool Antigen ELISA is an enzyme immunoassay which detects the *H. pylori* antigen in human fecal samples. The Hp Detect Stool Antigen ELISA comes in a kit that contains materials to assay a total of 92 samples. The device consists of a 96-well clear flat bottom polystyrene high bind microplate coated with affinity purified rabbit anti-human *H. pylori* polyclonal antibody. The device is provided with detection antibody which is a purified mouse monoclonal antibody specific for *H. pylori* antigen and has been conjugated to horseradish peroxidase (HRP). The device kit is also provided with sample diluent buffer, wash buffer, substrate solution, stop solution along with negative and positive controls. Negative control is a phosphate buffered protein solution and positive control is composed of purified *H. pylori* antigen (ATCC strain 43504) from cell lysate.

B Principle of Operation:

The Hp Detect Stool Antigen ELISA is an enzyme immunoassay which detects the *H. pylori* antigen in human fecal samples. Polyclonal anti-*H. pylori* capture antibodies are immobilized on microwells. Patient samples prepared in sample diluent are added to the microwells and incubated for one hour at 37 ± 2°C. If the *H. pylori* antigen is present in the sample, it will bind to the immobilized antibody on the plate. Following this incubation, the plate is washed thoroughly. A peroxidase conjugated anti-*H. pylori* monoclonal antibody is then added to the

microwells and incubated for 30 minutes at 37 ± 2°C. If *H. pylori* antigen is bound to the microwells in the first step, the detection antibody would now bind in this step to form a sandwich complex. Following this incubation, a thorough wash step is performed to remove non-specific and non-binding materials. Substrate is then added and incubated for 10 minutes at 37 ± 2°C to generate a color in the presence of the enzyme complex. Stop solution is then added to end the reaction. The results are read spectrophotometrically at the following wavelengths:

1. Single Wavelength Measurement at 450 nm
2. Dual Wavelength Measurement 450/620 nm or 450/630 nm

V Substantial Equivalence Information:

A Predicate Device Name(s):

PREMIER Platinum HpSA PLUS

B Predicate 510(k) Number(s):

K182559

C Comparison with Predicate(s):

Device & Predicate Device(s):	**Device: K232892**	**Predicate: K182559**
Device Trade Name	Hp Detect Stool Antigen ELISA	PREMIER Platinum HpSA PLUS
General Device Characteristic Similarities		
Intended Use/Indications For Use	The Hp Detect Stool Antigen ELISA is an *in vitro* diagnostic qualitative enzyme immunoassay for the detection of *Helicobacter pylori* (*H. pylori*) antigens in human stool or feces. The Hp Detect Stool Antigen ELISA is intended to aid in the initial diagnosis and post-therapy diagnosis of *H. pylori* infection. Additionally, the test may be used to assess *H. pylori* infection status after treatment. Retesting at a minimum of 4 weeks after the completion of treatment may be done to assess *H. pylori* status. Test results should always be taken into	The PREMIER Platinum HpSA PLUS enzyme immunoassay (EIA) is an *in vitro* qualitative procedure for the detection of *Helicobacter pylori* antigens in human stool. Test results are intended to aid in the diagnosis of *H. pylori* infection and to monitor response during and post-therapy in patients. Accepted medical practice recommends that testing by any current method, to confirm eradication, be done at least four weeks following completion of therapy.

Device & Predicate Device(s):	Device: K232892	Predicate: K182559
	consideration by the physician in conjunction with patient's clinical information (history and symptoms). For Prescription Use Only.	
Measured Analyte	Detection of *H. pylori* antigen	Same
Type of Test	Qualitative	Same
Specimen Type	Human fecal specimens	Same
Controls	Positive and negative control included in the kit	Same
Target Population	Persons suspected of having *H. pylori* infection	Same
Storage	Refrigerated (2°C – 8°C)	Same
General Device Characteristic Differences		
Specimen Storage	Specimens may be held up to 120 hours at 2°C – 8°C or should be frozen at ≤ -12°C or ≤ −60°C	Specimens may be held up to 72 hours at 2°C – 8°C or at 20°C – 25°C prior to testing
Time to Result	Approximately 1 hour and 40 minutes	Approximately 1 hour and 10 minutes
Antibody Format	Polyclonal/Monoclonal	Monoclonal
Incubation Temp	37°C ± 2°C	19-27°C
Reading Method	Spectrophotometric	Visual, Spectrophotometric

VI Standards/Guidance Documents Referenced:

Not Applicable

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Reproducibility:
   To demonstrate the reproducibility of the Hp Detect Stool Antigen ELISA, a panel of four (4) specimens was prepared at high negative (0.42 × LoD), low positive (1.60 × LoD), low positive (2.43 × LoD) and moderate positive (3.93 × LoD) by spiking *H. pylori* antigen (ATCC strain 43504) into a negative pooled fecal matrix. The reproducibility panel was blinded, and each run included a positive and negative controls. Testing was performed at three sites, two independent laboratories and one in-house at Biomerica, Inc. The samples were tested in triplicate twice per day over a 5-day period by two technicians at each site

using one kit lot (2 Technicians × 3 sites × 3 replicates × 2 runs × 5 days = 180 results/panel member). The results for the reproducibility study were measured using the dual wavelength (450/620 or 450/630 nm) and are summarized in Table 1 below. Testing at the dual and single wavelength (450 nm) produced equivalent results.

Table 1. Reproducibility of the Hp Detect Stool Antigen ELISA measured in dual wavelength.

Sample ID	*H. pylori* Antigen	Combined (3 sites)	
		Positive/Tested	% Detection
High Negative	0.42 × LoD	7/180	3.9%
Low Positive	1.60 × LoD	180/180	100%
Low Positive	2.43 × LoD	180/180	100%
Moderate Positive	3.93 × LoD	180/180	100%

2. Within-Lab precision/repeatability:
The Within-Lab precision/repeatability of the Hp Detect Stool Antigen ELISA was determined onsite at Biomerica using a panel of four (4) specimens as prepared for the reproducibility study as described above. Two operators performed the test with two validation lots per run over the period of 12 non-consecutive days. Two runs per day with a minimum of 3 hours separation between runs. Three replicates of each sample per run were tested along with positive and negative controls. The results for the Within-Lab precision/repeatability study measured in dual wavelength (450/620 or 450/630 nm) are summarized in Table 2. Testing at the dual and single wavelength (450 nm) produced equivalent results.

Table 2. Within-Lab precision/repeatability of the Hp Detect Stool Antigen ELISA measured in dual wavelength.

Sample ID	*H. pylori* Antigen	Combined (Two kit lots)	
		Positive/ Tested	% Detection
High Negative	0.42 × LoD	13/288	5%
Low Positive	1.60 × LoD	278/288	97%
Low Positive	2.43 × LoD	288/288	100%
Moderate Positive	3.93 × LoD	288/288	100%

The results for reproducibility and precision/repeatability studies are acceptable.

3. Linearity:
Not applicable.

4. Analytical Specificity/Interference:

A. Microbial cross-reactivity and microbial interference:
The Hp Detect Stool Antigen ELISA test was evaluated for cross reactivity and microbial interference with the bacteria, fungi and viruses listed below. A contrived positive matrix was prepared by spiking *H. pylori* purified antigen (ATCC strain 43504) at 2.2 × LoD (5.61 ng/mL) into the negative fecal matrix. Bacteria and fungi were spiked at concentrations of 10^6-10^7 CFU/mL and viruses at a range from 10^4-10^5 $TCID_{50}$ units per mL into either the

contrived positive sample (for microbial interference evaluation) or into the negative fecal matrix (for cross-reactivity evaluation). Positive and negative controls were included with each run. The sample panel were assayed in duplicates. The list of microorganisms and concentrations tested for cross-reactivity and microbial interference studies are shown in Table 3 below. No cross-reactivity or microbial interference was observed with the listed microorganisms when tested at the given concentrations when spiked into the matrix. The study was conducted with both dual (450/620 or 450/630 nm) and single wavelength (450 nm). All three-wavelength measurements produced equivalent results.

Table 3. Microorganisms tested* for cross-reactivity and microbial interference.

Bacteria and Fungi	
Aeromonas hydrophila	*Listeria monocytogenes*
Bacillus subtilis	*Peptostreptococcus anaerobius*
Campylobacter coli	*Plesiomonas shigelloides*
Campylobacter fetus	*Proteus mirabilis*
Campylobacter hyointestinalis	*Pseudomonas aeruginosa*
Campylobacter jejuni	*Pseudomonas fluorescens*
Campylobacter upsaliensis	*Salmonella enterica (Group B)*
Candida albicans	*Salmonella enterica (Group C)*
Citrobacter freundii	*Salmonella enterica (Group D)*
Clostridium difficile	*Salmonella enterica (Group E)*
Clostridium perfringens	*Serratia liquefaciens*
Clostridium sordellii	*Shigella boydii*
Enterobacter cloacae	*Shigella flexneri*
Enterococcus faecalis	*Shigella sonnei*
Escherichia coli	*Staphylococcus aureus*
Escherichia hermannii	*Staphylococcus epidermidis*
Eschericia fergusonii	*Vibrio parahaemolyticus*
Klebsiella pneumonia	*Yersinia enterocolitica*
Lactobacillum lactis	
Viruses	
Adenovirus Type 2	Coxsackievirus Type B5
Adenovirus Type 40	Echovirus Type 9
Adenovirus Type 41	Rotavirus
Coxsackievirus Type B3	

*Bacteria were tested at 1×10^7 CFU/mL (except for *Clostridium perfringens* at 1×10^6 CFU/mL) and *Candida albicans* at 1×10^6 CFU/mL. Viruses were tested at 1×10^5 $TCID_{50}$/mL except for Echovirus and Rotavirus which were tested at $TCID_{50}$/mL of 1×10^4.

The results for cross-reactivity and microbial interference with the Hp Detect Stool Antigen ELISA were acceptable.

B. Interfering substances:

The Hp Detect Stool Antigen ELISA was evaluated for interference with the potential interfering substances. A contrived positive matrix was prepared by spiking *H. pylori* purified antigen (ATCC strain 43504) at 2.2 × LoD (5.61 ng/mL) into the negative fecal matrix. Potentially interfering substances were spiked into either the contrived positive sample or negative fecal matrix. Positive and negative controls were included in each run. List of potential interfering substances and their concentrations tested for cross-reactivity and interference studies were shown in Table 4 below. No Interference was observed when the listed interfering substances were tested at the given concentrations. Assays were performed in both dual (450/620 or 450/630 nm) and single wavelength (450 nm). All three-wavelength measurements produced equivalent results.

Table 4. Interfering substance concentrations tested.

Interfering Substances	**Concentration tested**
Barium Sulfate	5% m/v
Human Hemoglobin	3 mg/mL
Metronidazole	701 µmol/L
Mucin	13.4 mg/mL
Palmitic Acid (Fecal Fat)	15.8 mg/mL
Polyethylene Glycol 3350	14.3 g/L
Steric Acid (Fecal Fat)	15.8 mg/mL
Vancomycin Hydrochloride	69 µmol/L
Whole Blood	200 µL/mL
Over the Counter (OTC) Medications	
Imodium AD (Loperamide)	5%v/v
Kaopectate (Bismuth Subsalicylate)	0.70 mg/mL
Mylanta (Aluminum Hydroxide & Magnesium Hydroxide or Calcium Carbonate)	1.68 mg/mL
Pepto Bismol (Bismuth Subsalicylate)	0.70 mg/mL
Prilosec (Omeprazole) OTC	5 µg/mL
Simethicone	25 mg/mL
Tagamet (Cimetidine)	5 µg/mL
TUMS (Calcium Carbonate)	50 µg/mL

The results for interfering substances study with the Hp Detect Stool Antigen ELISA were acceptable.

C. Inclusivity:

A total of six (6) *H. pylori* strains (whole cells) and one purified *H. pylori* antigen were evaluated for inclusivity study. Samples were prepared by diluting each strain (whole cells) and antigen in negative stool matrix at the level of 1-3 × LoD (using LoD of 1.69×10^3 CFU/mL whole cells for strain ATCC 43504 and 5.86 ng/mL antigen for strain ATCC 49503). Samples were tested for reactivity in triplicate with the Hp Detect Stool Antigen ELISA measuring by both dual (450/620 or 450/630 nm) and single wavelength (450 nm). Results for inclusivity are shown in Table 5. All strains were detected with Hp Detect Stool Antigen ELISA.

Table 5. List of *H. pylori* strains tested for inclusivity study.

***H. pylori* Strain**	**Concentration**	**Positive/Tested (% Detection)**
ATCC 43526	3.70×10^3 CFU/mL	3/3 (100%)
ATCC 43579	3.48×10^3 CFU/mL	3/3 (100%)
ATCC 49396	3.42×10^3 CFU/mL	3/3 (100%)
ATCC 700392	3.85×10^3 CFU/mL	3/3 (100%)
ATCC 700824	3.96×10^3 CFU/mL	3/3 (100%)
ATCC BAA-945	3.78×10^3 CFU/mL	3/3 (100%)
ATCC 49503	6.33 ng/mL Antigen	3/3 (100%)

The Inclusivity results were acceptable.

5. Assay Reportable Range:

Not applicable.

6. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Sample Stability – Contrived Specimens:

The sample stability for contrived specimens was evaluated for both refrigerated and frozen storage conditions including freeze and thaw. Samples were prepared by spiking *H. pylori* antigen (ATCC strain 43504) into a negative pooled fecal matrix. The sample panel was made up of 10 members of *H. pylori* antigen concentrations ranging from 0.47 to 5.42 × LoD tested in triplicates. After establishing the baseline measurement (time zero), each of the 10 panel members were aliquoted and stored at three storage conditions: 2°C to 8°C, ≤-12°C, and ≤-60°C and tested for each time point and temperature range (shown in Table 6A). All the stability tests were measured in both dual (450/620 or 450/630 nm) and single wavelength (450 nm) with equivalent results. Results shown in Table 6B, 6C, and 6D are for 10 panel sample members grouped into 3 high negatives (<1 × LoD), 4 low positives (1-2 × LoD), and 3 moderate positives (3-5 × LoD) measured in dual wavelengths.

Table 6A. Stability Testing Temperatures and Time Points

Stability Condition	**Temperature Range (°C)**	**Time Point (Days)**
Refrigerated	2°C to 8°C	0, 3, 5

Frozen	≤-12°C	0, 8, 15
Deep Frozen	≤-60°C	0, 8, 15

Table 6B. Results for storage of specimens at refrigerated conditions (2 – 8°C)

Sample ID	**Positive/Tested in refrigerated (2-8°C) storage period**		
	0 day	**3 days**	**5 days**
High Negative (<1 × LoD)	0/9	0/9	0/9
Low Positive (1-2 × LoD)	12/12	12/12	12/12
Moderate Positive (3-4 × LoD)	9/9	9/9	9/9

Table 6C. Results for storage of specimens at frozen conditions (≤ -12°C)

Sample ID	**Positive/Tested in frozen (≤ -12°C) storage period**		
	0 day	**8 days**	**15 days**
High Negative (<1 × LoD)	0/9	0/9	0/9
Low Positive (1-2 × LoD)	12/12	12/12	12/12
Moderate Positive (3-4 × LoD)	9/9	9/9	9/9

Table 6D. Results for storage of specimens at deep frozen conditions (≤ -60°C)

Sample ID	**Positive/Tested in frozen (≤ -60°C) storage period**		
	0 day	**8 days**	**15 days**
High Negative (<1 × LoD)	0/9	0/9	0/9
Low Positive (1-2 × LoD)	12/12	12/12	12/12
Moderate Positive (3-4 × LoD)	9/9	9/9	9/9

The results demonstrated no change in the performance interpretation from time zero throughout all the test points at each storage condition.

A freeze/thaw study was also conducted to evaluate the antigen stability after two (2) freeze-thaw cycles. The freeze/thaw study used the same 10 panels members as described in the specimen stability study. Following the baseline measurement (time zero) each sample was frozen at ≤-12°C and ≤-60°C and tested after one (1) and two (2) freeze/thaw cycle respectively. Test results demonstrate no change in the performance interpretation from time zero throughout each of the two freeze-thaw cycles. The recommended storage conditions for stool samples are summarized in Table 7.

Table 7. Recommended Storage Conditions

Stability Condition	**Temperature Range (°C)**	**Recommended Storage Time**
Refrigerated	2°C to 8°C	5 Days
Frozen	≤-12°C	15 Days
Deep Frozen	≤-60°C	15 Days
Freeze-Thaw	≤-12°C and ≤-60°C	2 Freeze-Thaw Cycles

7. Detection Limit:

The limit of detection (LoD) for the Hp Detect Stool Antigen ELISA was determined by using antigens from two strains of *H. pylori*, ATCC43504 (Primary strain) and ATCC 49503 (Secondary strain). Detergent solubilized whole cell lysate of *H. pylori* was used to purify antigens. For each strain, three positive sample panels were prepared by spiking purified *H. pylori* antigen into a negative stool matrix. Each positive sample was then used to prepare its initial series of six dilutions. For Strain ATCC 43504 additional four dilutions were made (total 10 dilutions) and for Strain ATCC 43503 additional 5 dilutions were made (total 11 dilutions) to ensure that at least 3 dilutions are within 0.10 to 0.90 (10% to 90%) hit rate for Probit analysis. To determine LoD, each dilution was tested in seven replicates across three days producing 63 data points per dilution per strain (3 positive samples × 7 replicates × 3 days = 63 data points). Testing was conducted using two reagent lots and measuring by both dual (450/620 or 450/630 nm) and single wavelength (450 nm). A Probit analysis was performed to estimate LoD which is defined as the concentration of *H. pylori* antigen that yields a positive result 95% of the time. LoD values determined for the Hp Detect Stool Antigen ELISA test are shown in Table 9.

Table 9. LoD values for Hp Detect Stool Antigen ELISA

ATCC Strain	ng/mL (95%CI)	Quantity/Test
Strain 43504	2.53 (2.48 to 2.60)	0.38 ng
Strain 49503	5.86 (5.56 to 6.37)	0.88 ng

The Limit of Detection (LoD) for the Hp Detect Stool Antigen ELISA was established at 2.53 ng/mL (0.38 ng/test) for strain ATCC 43504 and 5.86 ng/mL (0.88 ng/test) for strain ATCC 49503, measured in dual wavelength.

The correlation between antigen amount (ng/mL) and bacterial count (CFU/mL) was established for strain ATCC 43504 by dermining the LoD in bacterial count (CFU/mL). The LoD of bacterial concentration determined for *H. pylori* Strain ATCC 43504 is 1.69×10^3 CFU/mL. Therefore, *H. pylori* strain ATCC 43504 has a LoD of 2.53 ng/mL of antigen concentration correlates to a LoD of 1.69×10^3 CFU/mL of bacteria concentration when compared to side to side (data not shown).

The study to determine LoD for the Hp Detect Stool Antigen ELISA is acceptable and these values (in CFU/mL and ng/mL) were used to guide the analytical studies as appropriate.

Prozone / Hook Effect

To ensure that a high concentration of *H. pylori* antigen does not interfere with a positive reaction in the Hp Detect Stool Antigen ELISA, a high positive sample was prepared by spiking *H. pylori* antigen (ATCC strain 43504) into negative fecal matrix. The sample was serially diluted at concentrations ranging from 20,000 ng/mL to 0.25 ng/mL. Each of the serial dilutions were tested in 8 replicates measuring by both dual (450/620 or 450/630 nm) and single wavelength (450 nm) settings. Positive and negative controls were included in each run. The reading value of the plate reader reached to maximum level at 800 ng/ml of *H. pylori* antigen and remained at maximum level up to 20,000 ng/ml. No high-dose hook effect was observed for the assay at antigen concentrations of up to 20,000 ng/mL.

8. Assay Cut-Off:
Assay cut-off for Hp Detect Stool Antigen ELISA was determined for both spectrophotometric measurement of Dual Wavelength (450/620 nm or 450/630 nm) and Single Wavelength (450 nm). A panel of 227 specimens, of which 83 were positive and 144 were negative by the predicate device were evaluated with the Hp Detect Stool Antigen ELISA. Receiver operating characteristic (ROC) curve was constructed and the cut-off values for spectrophotometric single and dual wavelength reading methods were selected to achieve optimum diagnostic sensitivity and specificity:

Spectrophotometric Dual Wavelength (450/620 nm or 450/630 nm)

Negative: <0.100

Positive: ≥0.100

Spectrophotometric Single Wavelength (450 nm)

Negative: <0.140

Positive: ≥0.140

A positive result indicates that *H. pylori* antigens were detected. A negative result indicates that no *H. pylori* antigens were detected, or that the antigen levels are below what can be detected by the assay.

B Comparison Studies:

1. Method Comparison with Predicate Device:
Not applicable.

2. Matrix Comparison:
Not applicable.

C Clinical Studies:

1. Clinical Performance - Frozen Specimens:
The performance of the Hp Detect Stool Antigen ELISA for frozen specimens was evaluated by using method comparison study with an FDA cleared device. A total of 433 frozen and de-identified fecal samples were tested in which 355 specimens were collected from Italy and 78 specimens collected from three geographically different regions of the USA. The clinical specimens were collected from the patients presenting with dyspepsia, and would be undergoing a routine endoscopy and biopsy, not on antibiotics, bismuth, or proton-pump inhibitors, and those excluded if they had been treated for *H. pylori* withing the past 6 months. Specimens were distributed into three testing sites located within the USA which include two external sites and one internal (Biomerica) site. Results were evaluated by reading absorbance at dual wavelength (450/620 or 450/630 nm) and single wavelength (450 nm). The performance and statistics are shown in Table 10 for dual wavelength and Table 11 for single wavelength.

Table 10. Frozen Specimen - Clinical performance and statistics measured in dual wavelength.

Hp Detect Stool Antigen ELISA	Comparator: FDA Cleared Device		
	Positive	Negative	Total
Positive	111	6[a]	117
Negative	1[b]	315	316
Total	112	321	433

Statistics	Performance	95% Confidence Interval
Positive Percent Agreement (PPA)	99.11%	95.12 – 99.84 %
Negative Percent Agreement (NPA)	98.13 %	95.98 – 99.14 %

The discrepant results were further analyzed by chart review and determined to have a rapid urease test (RUT) or history result and observed as follow:
(a) Four of the six discrepant false positives were positive by RUT or histology.
(b) The discrepant false negative was negative by RUT or histology.

Table 11. Clinical performance and statistics measured in single wavelength.

Hp Detect Stool Antigen ELISA	Comparator: FDA Cleared Device		
	Positive	Negative	Total
Positive	111	13[a]	124
Negative	1[b]	308	309
Total	112	321	433

Statistics	Performance	95% Confidence Interval
Positive Percent Agreement (PPA)	99.11%	95.12 – 99.84 %
Negative Percent Agreement (NPA)	95.95 %	93.20 – 97.62 %

The discrepant results were further analyzed by chart review and determined to have a RUT or history and observed as follow:
(a) Four of the 13 discrepant false positives were positive by RUT or histology.
(b) The discrepant false negative was negative by RUT or histology.

The performance results for frozen specimen clinical study are acceptable.

2. Clinical Performance - Fresh Specimens

The performance of the Hp Detect Stool Antigen ELISA for fresh specimens (never frozen) was evaluated by using method comparison study with an FDA cleared device. A total of 142 fresh, de-identified, fecal specimens from patients with physician's medical evaluation for symptoms of *H. pylori* infection were collected through multiple biospecimen vendor and clinical laboratories. The specimens were tested in the collection centers with the comparator device and specimen aliquots were shipped to Biomerica (internal site) and tested using Hp Detect Stool Antigen ELISA using both dual wavelength (450/620 or 450/630 nm) and single wavelength (450 nm) settings.

Table 13. Fresh Specimens - Clinical performance and statistics measured in dual and single wavelength with same result.

Hp Detect Stool Antigen ELISA	Comparator: FDA Cleared Device		
	Positive	Negative	Total
Positive	20	2	22
Negative	0	120	120
Total	20	122	142

Statistics	Performance	95% Confidence Interval
Positive Percent Agreement (PPA)	100.00%	83.89 – 100%
Negative Percent Agreement (NPA)	98.36%	94.22 – 99.55%

The Hp Detect Stool Antigen ELISA demonstrated the clinical performance of 100% positive percent agreement and 98.4% negative percent agreement for fresh stool specimens using both dual and single wavelength configurations. The performance results for fresh specimen clinical study are acceptable.

3. Post-Therapy Performance

The performance of the Hp Detect Stool Antigen ELISA for post-therapy diagnosis was evaluated by using 14 paired (pre-and post- therapy) retrospective specimens from Italy. All subjects initially tested positive for *H. pylori* by composite reference method (CRM) consisting of histology and Rapid Urease Test and all completed eradication therapy. Post-therapy fecal samples were collected at a minimum of 4 weeks post completion of eradication therapy. All 14 pre-therapy samples were shown to be positive and matched with the CRM diagnosis. The result for post-therapy was show in Table 13.

Table 13. Post-Therapy Performance with composite reference method

Hp Detect Stool Antigen ELISA	Post-Therapy CRM		
	Positive	Negative	Total
Positive	10	0	10
Negative	0	4	4
Total	10	4	14

Statistics	Performance	95% Confidence Interval
Sensitivity	100%	72.25 – 100 %
Specificity	100 %	51.02 – 100 %

The results for post-therapy performance show that the Hp Detect Stool Antigen ELISA exhibited a 100% sensitivity and 100% specificity when compared to the composite reference method. The performance results for post-therapy study are acceptable and support the claim for the test to be used in assessing *H. pylori* status after treatment.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

The Hp Detect Stool Antigen ELISA assay detects the presence of *H. pylori* antigen in human feces. The clinical study included in this submission had results from 433 frozen specimens collected from three sites in different geographic regions in the US and one site outside the US – Italy. The observed prevalence of *H. pylori* in this study was 27.0% (117/433). *H. pylori* detection and prevalence stratified by specimen origin are shown in Table 14 and categorized by gender is shown in Table 15.

Table 14: *H. pylori* detection and prevalence by specimen origin

Specimen Origin	**Hp Detect Stool Antigen ELISA Result**		**Total**	**Prevalence (% Positive)**
	Negative	**Positive**		
US - Southeast	26	8	34	23.5%
US - Southwest	13	3	16	18.8%
US - West	19	9	28	32.1%

US (All Sites)	58	20	78	25.6%
Italy	258	97	355	27.3%
Grand Total	316	117	433	27.0%

Table 15: *H. pylori* detection and prevalence by gender

Gender	Hp Detect Stool Antigen ELISA Result		Total	Prevalence (% Positive)
	Negative	Positive		
Females	205	76	281	27.0%
Males	111	41	152	26.9%
Total	316	117	433	27.0%

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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