December 15, 2023

Biomerica, Inc.
Bonnie Qiu
Senior Director, Quality Assurance and Regulatory Affairs
17571 Von Karman Avenue
Irvine, California 92614

Re: K232892
Trade/Device Name: Hp Detect Stool Antigen ELISA
Regulation Number: 21 CFR 866.3110
Regulation Name: Campylobacter Fetus Serological Reagents
Regulatory Class: Class I, reserved
Product Code: LYR
Dated: September 15, 2023
Received: September 18, 2023

Dear Bonnie Qiu:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Natasha Griffin -S

O.B.O.
Ribhi Shawar, Ph.D. (ABMM)
Branch Chief
General Bacteriology and Antimicrobial Susceptibility Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
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Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K232892

Device Name
Hp Detect™ Stool Antigen ELISA

Indications for Use *(Describe)*
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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

A. SUBMITTER/510(K) HOLDER

Biomerica, Inc
17571 Von Karman Ave
Irvine, CA 92614

B. DEVICE NAME

Proprietary Name:	Hp Detect Stool Antigen ELISA
Common/Usual Name:	*Helicobacter pylori* stool antigen ELISA
Classification Name:	*Helicobacter pylori*

C. REGULATORY INFORMATION

Regulation Section:	21 CFR 866.3110 *Campylobacter fetus* serological reagents
Classification:	Class I
Product Code:	LYR
Panel:	83-Microbiology

D. PREDICATE DEVICE

PREMIER Platinum HpSA PLUS (K182559)

E. 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.

Polyclonal anti-*H. pylori* captures antibodies that 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

F. INDICATION FOR USE/INTENDED 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.

G. SUMMARY OF TECHNOLOGICAL CHARACTERISTICS COMPARED TO THE PREDICATE DEVICE

A summary comparison of technological characteristics, including design and materials is provided in the following Table 1.

Table 1. Comparison with Predicate Device

Item	Device: K232892	Predicate : K182559
Device Trade Name	Hp Detect Stool Antigen ELISA	PREMIER Platinum HpSA PLUS
General Device Characteristic Similarities		
Product Code	LYR	Same
Intended 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.	The PREMIER Platinum HpSA PLUS enzyme immunoassay (EIA) is an *in vitro* qualitative assay 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.
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	Positive and negative control included in the kit
Target Population	Persons suspected of having *H. pylori* infection	Same
Storage	Refrigerated (2°C – 8°C)	Same

Item	Device: K232892	Predicate : K182559
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 to -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 mixture/Monoclonal mixture
Incubation Temp	37°C ± 2°C	19-27°C
Reading Method	Spectrophotometric	Visual, Spectrophotometric

H. PERFORMANCE CHARACTERISTICS

1. Analytical Performance

a. Precision / Reproducibility

To demonstrate the reproducibility of the Hp Detect Stool Antigen ELISA, a four (4) panel of specimens were 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 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 2 below. Testing at the dual and single wavelength (450 nm) produced equivalent results.

Table 2. 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%

The Within-Lab precision/repeatability of the Hp Detect Stool Antigen ELISA was determined onsite at Biomerica using a four (4) panel of 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 3. Testing at the dual and single wavelength (450 nm) produced equivalent results.

Table 3. 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 were acceptable.

b. Linearity / Assay Reportable Range

Not Applicable

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

Traceability:

Calibrator traceability is not applicable (No calibrator is included in kit).

Sample Stability:

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 4A). 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 4B, 4C, and 4D 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 4A. 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 4B. 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 4C. 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 4D. 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 5.

Table 5. 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

d. Detection Limit (LoD)

The limit of detection (LoD) for the Hp Detect Stool Antigen ELISA was determined by using antigens from two strains of *H. pylori*, ATCC 43504 (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 6.

Table 6. 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 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.

e. Analytical Specificity

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 was assayed in duplicates. The list of microorganisms and concentrations tested for cross-reactivity and microbial interference studies are shown in Table 7 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 7. 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.

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 8 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 8. 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.

Inclusivity study

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 9. All strains were detected with Hp Detect Stool Antigen ELISA.

Table 9. 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.

f. 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.

g. 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.

2. Clinical Study

Clinical Study – Frozen Specimen

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 (west, southwest, and southeast). 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 RUT or history result and observed as follow:

(a) Four of the six discrepant false positives were either comparator 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 result and observed as follow:

(a) Four of the 13 discrepant false positive 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.

Clinical Study - Fresh Stool Specimen

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 12. Fresh Specimens - Clinical performance and statistics measured in dual and single wavelength with same results.

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.

Post –Therapy Diagnosis

The performance of the Hp Detect Stool Antigen ELISA for post-therapy diagnosis was evaluated by using 14 paired (pre-and post- therapy) frozen 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 previously shown to be positive and matched with the CRM diagnosis. The result for post-therapy was shown 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.

Clinical Cut-Off

Not Applicable.

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 independent sites in the US, each located in a different geographical region of the country – west, southwest, and southeast, and 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%

I. PROPOSED LABELING

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

J. CONCLUSIONS

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