510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K230901

B Applicant

Meridian Bioscience, Inc.

C Proprietary and Established Names

Premier HpSA Flex (619096)

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 Premier HpSA Flex by Meridian Bioscience to the predicate device. The Premier HpSA Flex assay represents a modification of the predicate device (Meridian Bioscience's PREMIER Platinum HpSA PLUS assay: K182559)— the modification being the addition of preserved stool specimen for testing with this device.

B Measurand:

Helicobacter pylori antigens.

C Type of Test:

Microwell-based enzyme immunoassay with qualitative assay outputs.

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Premier HpSA Flex enzyme immunoassay (EIA) is an *in vitro* qualitative procedure for the detection of *Helicobacter pylori* antigens in human stool. The test is intended for use with unpreserved stool specimens or preserved stool specimens in transport media. 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.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

Visual determination of Assay endpoint needs no special instrument. However, if the optional spectrophotometric determination is chosen, endpoint reading of the assay requires a generic spectrophotometric microplate reader capable of recording absorbance values at 450 nm and optionally at 630 nm for wavelength correction.

IV Device/System Characteristics:

A Device Description:

The Premier HpSA Flex assay represents a modification of the sponsor's previously FDA-cleared Premier Platinum HpSA PLUS assay (K182559). The modification is the addition of a new sample type claim (i.e., human stool specimens preserved in specific transport media, namely, Cary-Blair or Culture and Sensitivity (C&S) transport media) to the intended use of the previously cleared device (K182559). Both the subject and predicate devices are qualitative, *in vitro* diagnostic tests for the detection of *Helicobacter pylori* antigens present in human stool specimens.

B Principle of Operation:

The Premier HpSA Flex test is a microwell-based enzyme immunoassay that detects *H. pylori* antigens from human stool specimens. Stool specimen may be either unpreserved or preserved in Cary-Blair or Culture and Sensitivity (C&S) transport media. The test utilizes a plurality (mixture) of monoclonal anti-*H. pylori* capture antibodies adsorbed to microwells. Diluted patient samples and an enzyme conjugate reagent are added to the microwells and incubated for one hour at room temperature. A wash is performed to remove unbound material. Substrate is added and incubated for 10 minutes at room temperature. Color develops by reaction with the bound enzyme, indicating the presence of *H. pylori* antigen. Stop solution is added and the results are interpreted visually or spectrophotometrically. No calculations are required, and the visible change from colorless to

yellow makes the interpretation of results objective and simple. The yellow color may be read with a spectrophotometer at 450 nm wavelength with or without correction at 630 nm. In addition, the HpSA test permits assessment of established or novel anti-*H. pylori* treatment during and post-therapy to monitor for treatment effectiveness, relapse, or eradication.

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: K230901	Predicate: K182559
Device Trade Name	Premier HpSA Flex	PREMIER Platinum HpSA PLUS
Intended Use / Indications for Use	The Premier HpSA Flex enzyme immunoassay (EIA) is an *in vitro* qualitative procedure for the detection of *Helicobacter pylori* antigens in human stool. The test is intended for use with unpreserved stool specimens or *preserved stool specimens in transport media*. 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	The PREMIER Platinum HpSA PLUS is an *in vitro* diagnostic 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.
General Device Characteristic Similarities		
Measured analyte	*Helicobacter pylori* antigens	Same
Antibody / Technology / Assay format	Monoclonal / Enzyme immunoassay (EIA) / Microwell-based	Same
Type of Test	Qualitative	Same

Device & Predicate Device(s):	Device: K230901	Predicate: K182559
Controls	Positive and negative controls included in the kit	Same
Reagent storage	Refrigerated (2–8ºC)	Same
Reagent and components	Antibody Coated Microwells; Positive Control; Sample Diluent / Negative Control; Premier 20X Buffer I; Enzyme Conjugate; Premier Substrate I; Premier Stop Solution 1; Transfer pipettes; Microwell strip sealer; Wooden stick applicators	Same
Result interpretation	Visual or spectrophotometric	Same
General Device Characteristic Differences		
Device & Predicate Device(s):	Device: K230901	Predicate: K182559
Addition of specimen type	Unpreserved stool specimens and ***stool specimens preserved in Cary-Blair or C&S transport media***.	Unpreserved (raw) stool specimens

VI Standards/Guidance Documents Referenced:

None

VII Performance Characteristics:

A Analytical Performance:

Analytical performance of the subject assay (Premier HpSA Flex) was evaluated using contrived samples. Briefly, a Bulk Negative Stool (BNS) matrix was prepared by pooling unpreserved stool specimens from five (5) donors or fewer into small, pooled lots. Purified and pre-quantified *H. pylori* flagellar antigen (HpSA) was used to spike the negative stool matrix with known concentrations to create contrived positive samples. Sample matrix alone served as true negative samples. The contrived positive and negative samples were then preserved in Remel Cary Blair ("CB"; ThermoFisher, cat. #R21925) or Para-Pak Culture & Sensitivity transport media ("C&S"; MBI, cat. #900612) at a volumetric ratio of 1:4 (i.e., 1 part sample in negative stool matrix + 3 parts transport media, respectively).

1. Limit of Detection (LoD) / Analytical Sensitivity:

   Analytical sensitivity of the subject assay was estimated in two steps, (i) by estimating the Limit of Detection (LoD) of the assay using contrived positive human stool samples preserved in either CB or C&S transport media, and (ii) by determining that the performances of the assay with either medium are equivalent at near LoD antigen concentrations in samples.

Contrived samples (positives and negatives) were tested in the assay following instructions in labeling using three (3) kit lots and twenty (20) replicates per sample.

LoD was defined as the lowest concentration of HpSA (calculated as ng of antigen per mL of preserved stool) that produced positive results ≥ 95% of the time. The LoD for the subject assay was conservatively determined to be 12 ng HpSA per mL of preserved stool. The previously established LoD using unpreserved (raw) stool was 4.66 ng/mL.

Further, the equivalence between CB and C&S TM was assessed at LoD and below LoD antigen concentrations in contrived samples using three (3) kit lots with 20 replicates at each of these *Lot* × *Concentration* (total of 10 testing conditions) for each transport media. CB and C&S were determined to be equivalent for the preservation of specimens intended to be used with the subject assay, as shown below in Table 1.

Table 1: Equivalence of CB and C&S transport media for preservation of stool specimens for use in Premier HpSA Flex assay

	C&S	**CB**	**OD Difference**	**EAC***	
	Mean OD [90% CI]	**Mean OD [90% CI]**	**Mean [90% CI]**	**(Δ_{mean} OD)**	**Outcome**
All tested HpSA concentrations	0.2087 [0.131–0.286]	0.1475 [0.070–0.225]	0.0612 [0.054–0.069]	±0.1	Equivalent
At LoD (12 ng/mL)	0.2413 [0.226–0.256]	0.2141 [0.208–0.221]	0.0272 [0.011–0.043]	±0.1	Equivalent

* EAC: Equivalence Acceptance Criterion

2. Precision/Reproducibility:

A multi-site, blinded study was conducted at three (3) external clinical sites to evaluate the reproducibility and repeatability of the Premier HpSA Flex device. The testing panels of contrived stool samples were preserved in C&S transport media. Each panel consisted of negative stool matrix alone (true negative sample, TN) or matrix spiked with known concentrations of HpSA to generate high negative (<1X LoD, HN), low positive (2X LoD, LP), and moderate positive (5X LoD, MP) samples. The panels were stored at ≤ −70ºC and shipped frozen to the study sites.

Three (3) independent sites tested the panels in a blinded fashion, with each site evaluating: ten (10) panels x four (4) members/panel x three (3) replicates/member = a total of 120 samples. In total, therefore, the study evaluated 360 samples at the three sites combined. Two (2) technicians participated in the testing at each site. Along with each panel (of 24 samples), each technician tested a positive and negative control per day, for five (5) days.

Precision assessments evaluated the variabilities of consecutive measurements of the same sample input between sites, within sites, and within run, as well as the reproducibility of measurements of each sample over time and experimental conditions. Setting the expected results to be a qualitative negative or positive, total percent agreement amongst all sites was for 360 out of 360 samples, i.e., 100% (95% CI: 98.9–100%). Similarly, the total percent

agreement amongst all kit lots for 360 out of 360 samples, i.e., 100% (95% CI: 98.9–100%), as summarized in Table 2.

Table 2. Reproducibility analysis of OD values (N=360) show:

Sample type	Site (N)	Mean OD	Assay Read out	Between Site		Between Run (Within Site)		Within Run		Total (Reproducibility)	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV
TN	1 (30)	0.0037	NEG	N	N	0.002	43.7%	0.002	54.5%	0.003	69.80%
	2 (30)	0.0054	NEG	N	N	0	0.00%	0.002	29.7%	0.002	29.70%
	3 (30)	0.0024	NEG	N	N	0.003	118%	0.007	274%	0.007	298%
	ALL (90)	**0.0039**	**NEG**	**0.001**	**29.9%**	**0.002**	**48.7%**	**0.004**	**107%**	**0.005**	**121%**
HN	1 (30)	0.0056	NEG	N	N	0.003	59.5%	0.004	74.7%	0.005	95.50%
	2 (30)	0.0124	NEG	N	N	0.009	73.8%	0.005	36.6%	0.01	82.40%
	3 (30)	0.0052	NEG	N	N	0	0.00%	0.007	127%	0.007	127%
	ALL (90)	**0.0077**	**NEG**	**0.004**	**46.0%**	**0.005**	**68.90%**	**0.006**	**71.0%**	**0.008**	**109%**
LP	1 (30)	0.2699	POS	N	N	0.04	14.9%	0.08	28.5%	0.09	32.10%
	2 (30)	0.3968	POS	N	N	0.1	30.5%	0.08	20.6%	0.1	36.80%
	3 (30)	0.4455	POS	N	N	0.1	28.0%	0.07	16.1%	0.1	32.20%
	ALL (90)	**0.3707**	**POS**	**0.08**	**22.5%**	**0.1**	**27.8%**	**0.08**	**20.7%**	**0.2**	**41.30%**
MP	1 (30)	0.7005	POS	N	N	0.1	18.0%	0.1	17.3%	0.2	25.00%
	2 (30)	1.006	POS	N	N	0.2	16.8%	0.2	15.4%	0.2	22.80%
	3 (30)	1.0318	POS	N	N	0.2	15.8%	0.1	12.6%	0.2	20.20%
	ALL (90)	**0.9128**	**POS**	**0.2**	**19.3%**	**0.2**	**16.9%**	**0.1**	**14.9%**	**0.3**	**29.60%**

Considering all data points as recommended by the FDA-recognized consensus standard CLSI EP05-A3 (2014), the Within-Run % CV ranged 14.9–107% and Reproducibility (total % CV) ranged 29.6–121% across all sites. As expected, the data variability was higher at lower Ag concentrations (TN and HN). However, there was no instance recorded of unexpected conversion of a presumed negative to positive or vice versa. All concentrations tested met the predefined acceptance criteria.

3. Linearity:

Not applicable

4. Analytical Specificity/Interference:

Studies were conducted to determine whether substances that may be present in human stool specimens preserved in CB or C&S TM interfere with results generated by the subject assay (Premier HpSA Flex). Substances evaluated were those that may reasonably be expected to be present within patient samples and those that were determined to have the potential to affect test results.

True negative (negative stool matrix alone) and low positive (2X LoD) specimens were contrived in triplicate. The target interferent was added to each replicate. Where necessary, stock solutions of interferent substances were prepared in a diluent (sterile 0.85% saline) prior

to combining with stool samples; the stock concentrations were prepared such that the diluent volume did not exceed 10% of the stool matrix when combining. Specimen replicates with added interferents were preserved in C&S transport medium at a ratio of 1:4 (v/v).

The same chemical or biological substances were previously evaluated for interference with the predicate assay (K182559). Table 3 below lists the potential interferents with their target concentrations (per 500 µL of patient stool) evaluated with specimens preserved in C&S transport media.

Table 3. Interfering substances evaluated

	Substance (Active Ingredient(s))	Target Concentration (per 500 µL of Patient Stool)
1	TUMS	10 mg/500 µL
2	Mylanta (per 10 mL: Aluminum hydroxide 800 mg, Magnesium hydroxide 800 mg, Simethicone 80 mg)	11.5 mg/500 µL
3	Pepto-Bismol (Bismuth subsalicylate 525 mg/30 mL)	0.44 mg/500 µL
4	Tagamet (Cimetidine 200 mg/tablet)	1 mg/500 µL
5	Prilosec OTC (Omeprazole 20 mg/tablet)	1 mg/500 µL
6	Barium Sulfate	25 mg/500 µL
7	Whole Blood	250 µL/500 µL
8	Leukocytes (White Blood Cells)	250 µL/500 µL
9	Mucin	17 mg/500 µL
10	Hemoglobin	62.5 mg/500 µL
11	Stearic Acid (fecal fat)	5.3 mg/500 µL
12	Palmitic Acid (fecal fat)	2.65 mg/500 µL
13	NSAID, Ibuprofen	0.25mg/500 µL

Each microwell plate of samples tested was analyzed spectrophotometrically at dual wavelength ($A_{450–630nm}$). External controls (Positive and Negative) were included with each plate tested. All positive samples (preserved in C&S transport media) tested gave the expected results indicating that none of the substances evaluated in this study showed interference in the subject assay at the concentrations tested. All negative samples were also negative.

5. Analytical Specificity/Cross-reactivity:

Studies were conducted to determine whether micro-organisms that may be present human stool specimens preserved in CB or C&S transport media non-specifically reacted with the subject assay. The microorganisms (i.e., bacteria, fungus, and viruses) evaluated in this study are those that are most likely to be or are possibly present within patient stool specimens and those could potentially affect assay results.

True negative (negative stool matrix alone) and low positive (2X LoD) specimens were contrived in triplicate. Relevant bacterial organisms or fungus were tested at final concentrations of $\geq 1.0 \times 10^7$ CFU/mL, whereas viruses were tested at $\geq 1.0 \times 10^5$ $TCID_{50}$/mL. For cross-reactivity, the target micro-organisms were added to preserved true negative replicates; for microbial interference, target micro-organisms were added to preserved low positive replicates. Where necessary, microbial stock solutions were prepared in sterile 0.85% saline prior to combining with stool samples; the stock concentrations were prepared such that

the diluent volume did not exceed 10% of the stool matrix when combining. Specimen replicates with added micro-organisms were preserved in C&S transport medium at a ratio of 1:4 (v/v).

Each microwell plate of samples tested was analyzed spectrophotometrically at dual wavelength ($A_{450–630nm}$). External controls (Positive and Negative) were included with each plate tested. Table 4 below lists the micro-organisms tested in preserved stool specimens to evaluate cross-reactivity and microbial interference in the performance of the subject assay. The same microbial organisms were previously evaluated for cross-reactivity and microbial interference with the predicate assay (K182559).

Table 4. List of potentially cross-reactive organisms tested.

	Microorganism Tested	**(Strain ID)**
1	*Aeromonas hydrophila*	ATCC 35654
2	*Bacillus subtilis*	ATCC 6051
3	*Borrelia burgdorferi*	N/A
4	*Campylobacter coli*	ATCC 43478
5	*Campylobacter fetus*	ATCC 25936
6	*Campylobacter jejuni*	ATCC 33292
7	*Campylobacter lari*	ATCC BAA-1060
8	*Candida albicans*	ATCC 18804
9	*Citrobacter freundii*	ATCC 43864
10	*Clostridium difficile*	ATCC 43255
11	*Clostridium perfringens*	ATCC 12915
12	*Enterobacter cloacae*	ATCC 15337
13	*Enterococcus faecalis*	ATCC 49532
14	*Escherichia coli*	ATCC 8739
15	*Escherichia coli*	ATCC 9637
16	*Escherichia coli*	ATCC BAA-2196
17	*Escherichia coli O157:H7* (toxigenic)	ATCC 43895
18	*Escherichia fergusonii*	ATCC 35469
19	*Escherichia hermanii*	ATCC 33650
20	*Escherichia hermanii*	EMDI-64
21	*Haemophilus influenzae*	ATCC 9006
22	*Klebsiella pneumoniae*	ATCC BAA-1900
23	*Lactococcus lactis*	ATCC 49032
24	*Listeria monocytogenes*	ATCC 19115
25	*Peptostreptococcus anaerobius*	ATCC 27337
26	*Proteus vulgaris*	ATCC 6380
27	*Pseudomonas aeruginosa*	ATCC 10145
28	*Pseudomonas fluorescens*	ATCC 13525
29	*Salmonella enterica subsp. enterica serovar Dublin*	ATCC 15480
30	*Salmonella enterica subsp. enterica serovar Hilversum*	ATCC 15784
31	*Salmonella enterica subsp. enterica serovar Typhimurium*	ATCC 13311
32	*Salmonella heidelberg* (Group B)	ATCC 8326
33	*Salmonella minnesota*	ATCC 9700
34	*Serratia liquefaciens*	ATCC 27952
35	*Serratia marcescens*	ATCC 43862

	Microorganism Tested	(Strain ID)
36	*Shigella boydii*	ATCC 9207
37	*Shigella dysenteriae*	ATCC 9361
38	*Shigella flexneri*	ATCC 12022
39	*Shigella sonnei*	ATCC 25931
40	*Staphylococcus aureus*	ATCC 51153
41	*Staphylococcus aureus* (Cowan's)	ATCC 12598
42	*Staphylococcus epidermidis*	ATCC 49134
43	*Yersinia enterocolitica*	ATCC 23715
44	Adenovirus 41	Tak
45	Rotavirus RV4	

All spiked samples (preserved in C&S transport media) were observed to be negative in the assay, indicating that none of the micro-organisms evaluated in this study showed cross-reactivity or microbial interference in the subject assay at the concentrations tested. External controls (Positive and Negative) that were included also produced the expected results.

6. Assay Reportable Range:

Not Applicable.

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

Storage stability studies:
Analytical storage stability studies were performed with a panel of contrived stool specimens preserved in CB or C&S transport media to determine acceptable limits for storage and handling of such preserved specimens. For each transport media type, the panel included a true negative (TN) sample with unpreserved negative stool matrix, a moderate positive (MP) sample (5X LoD), three low positive (LP) samples (2X LoD), and a high negative (HN) sample with antigen at <1X LoD. Antigen concentration for HN sample (7.5 ng/mL) was determined by testing various dilutions until testing generated results with 10–90% positivity (i.e., 1–9 positives out of 10 replicates) at the given concentration in each transport media. Prior to the study, all panel members were preserved in CB or C&S transport media at 1:4 (v/v) ratio. Testing was performed following the instructions for use for the subject device. These analytical studies are listed below.

a) Preserved specimen storage stability:
An analytical study determined the acceptable limits for storage and handling of human stool specimens when preserved in CB or C&S transport media and tested with the subject assay. This study used the contrived and preserved specimens indicated above (i.e., TN, HN, LP, and MP samples) as a "reference" panel. A separate panel of contrived "clinical" positives was prepared using archived, preserved clinical positive stool samples with pre-quantified analyte (HpSA) levels as measured by the assay. These archived preserved stool samples were serially diluted in cognately preserved (CB or C&S) negative stool matrix and tested to arrive at assay signals that were equivalent (within ± 2 SD of mean) to those of the reference panel members LP (designated "2X") and MP (designated "5X"). Similarly, another archived preserved clinical positive specimen was serially diluted in cognately preserved negative stool matrix to create a contrived "clinical" high negative (designated "HN") sample for which replicates

generated results with 10–90% positivity (i.e., 1–9 assay positives out of 10 replicates in a set) in each transport media. Finally, archived clinical negative specimens were screened and preserved in CB or C&S transport media to create a negative (designated "NS") "clinical" sample.

After baseline testing (time 0), each preserved sample was subjected to different storage temperatures and time conditions, and the stored sample aliquots were tested in the subject assay at designated time points (~106 and 130.5 hours for refrigerated or ambient storage; 10 and 75 days for storage in −20ºC and −80ºC freezers) using a minimum of 10 replicates per sample.

When subjected to different temperature conditions as indicated above and then tested in the assay, the MP, LP, and NS preserved in CB or C&S transport media produced acceptable results at all timepoints and reliably demonstrated assay signal outputs in alignment with expectations. For preserved NS samples, baseline results met all acceptance criteria; prolonged storage at several timepoints resulted in the low analyte (HpSA antigen) content in these samples being variably detected by the subject immunoassay, as expected. Results from this study are shown in Table 5.

Table 5. Preserved Sample storage study results

Time Point	Storage temp (ºC)	Sample ID	Positive in Assay	Replicates tested	Percent Positive	Positive in Assay	Replicates tested	Percent Positive
			C&S			CB		
Base line		5X	10	10	100%	10	10	100%
		2X	10	10	100%	10	10	100%
		HN	2	10	20%	7	10	70%
		NS	10	10	0%	0	10	0%
106-108* Hours	2–8	5X	10	10	100%	10	10	100%
		2X	10	10	100%	10	10	100%
		HN	0	10	0%	7	10	70%
		NS	0	10	0%	0	10	0%
	19–27	5X	10	10	100%	10	10	100%
		2X	10	10	100%	10	10	100%
		HN	10	10	100%	10	10	100%
		NS	0	10	0%	0	10	0%
130.5 Hours	2–8	5X	10	10	100%	10	10	100%
		2X	10	10	100%	10	10	100%
		HN	0	10	0%	4	10	40%
		NS	0	10	0%	0	10	0%
	19–27	5X	10	10	100%	10	10	100%
		2X	10	10	100%	10	10	100%
		HN	4	10	40%	10	10	100%
		NS	0	10	0%	0	10	0%
10 days	−28 to −16	5X	10	10	100%	10	10	100%
		2X	10	10	100%	10	10	100%
		HN	9	10	90%	10	10	100%
		NS	0	10	0%	0	10	0%

Time Point	Storage temp (°C)	Sample ID	Positive in Assay	Replicates tested	Percent Positive	Positive in Assay	Replicates tested	Percent Positive
			C&S			CB		
	−84 to −66	5X	10	10	100%	10	10	100%
		2X	10	10	100%	10	10	100%
		HN	10	10	100%	10	10	100%
		NS	0	10	0%	0	10	0%
75 days	−28 to −16	5X	10	10	100%	10	10	100%
		2X	10	10	100%	10	10	100%
		HN	2	10	20%	10	10	100%
		NS	0	10	0%	0	10	0%
	−84 to −66	5X	10	10	100%	10	10	100%
		2X	10	10	100%	10	10	100%
		HN	10	10	100%	10	10	100%
		NS	0	10	0%	0	10	0%

***** *Note: samples were stored at 2–8°C for 108 hours and at 19–27°C for 106 hours prior to testing at these time points.*

In summary, specimen stability testing of a panel of clinical specimens diluted in negative stool matrix and preserved in CB or C&S transport media demonstrated reliably detectable assay signals when the preserved stool specimens were stored at ambient (19–27°C) or cold (2–8°C) temperatures for up to 120 hours or at frozen conditions (−20°C and/or −80°C) for up to 14 days. The collective data from this study supported the acceptable storage times and conditions and demonstrated analyte stability in frozen specimens, which served as the basis for supporting the use of frozen, archived specimens for the Method Comparison study.

b) Freeze/Thaw:

An analytical study determined the acceptable limits for the freeze / thaw cycling of human stool specimens when preserved in CB or C&S transport media for testing with the subject assay. The panel (Neg, HN, LP, and MP) of contrived, preserved stool samples was tested at baseline ("Time 0"), then subjected to a series of freeze/thaw cycles using a low temperature (−20°C) freezer (range: −28 to −16°C) or an ultralow (−80°C) freezer (range: −84 to −66°C) followed by ambient (19–27°C) thawing, and finally tested in the assay with 10–30 replicates per sample. For preserved Negative, LP (2X LoD), and MP (5X LoD) samples in either transport media and cycled up to 3 times through freeze/thaw cycles in low-temperature and ultralow freezers, all samples showed acceptable signal outputs aligned with expectations, and no significant trend was observed in the average ODs ($A_{450-630}$) compared to baseline results. For HN samples, whereas the baseline ODs remained within the sponsor's acceptance criteria of 10–90% positive signals, when subjected to up to 3 freeze/thaw cycles, the low amount (<1X LoD) of analyte present in the preserved HN samples (6.5 ng/mL of HpSA in negative stool matrix) produced only low or no signals as expected. The results are summarized in Table 6 below.

Table 6. Freeze thaw stability data.

Storage Condition	Panel Member	Positive in Assay	Replicates tested	Percent Positive	Positive in Assay	Replicates tested	Percent Positive
		C&S			CB		
Baseline	MP	10	10	100%	10	10	100%
	LP	30	30	100%	30	30	100%
	HN	9	10	90%	7	10	70%
	Neg	0	10	0%	0	10	0%
Cycle 2 (−28 to −16ºC)	MP	10	10	100%	10	10	100%
	LP	30	30	100%	30	30	100%
	HN	10	10	100%	7	10	70%
	Neg	0	10	0%	0	10	0%
Cycle 3 (−28 to −16ºC)	MP	10	10	100%	10	10	100%
	LP	30	30	100%	30	30	100%
	HN	0	10	0%	10	10	100%
	Neg	0	10	0%	0	10	0%
Cycle 2 (−84 to −66ºC)	MP	10	10	100%	10	10	100%
	LP	30	30	100%	30	30	100%
	HN	2	10	20%	0	10	0%
	Neg	0	10	0%	0	10	0%
Cycle 3 (−84 to −66ºC)	MP	10	10	100%	10	10	100%
	LP	30	30	100%	30	30	100%
	HN	0	10	0%	0	10	0%
	Neg	0	10	0%	0	10	0%

In summary, freeze/thaw stability testing of contrived specimens preserved in CB or C&S transport media demonstrated no challenges in signal acquisition in the assay when the preserved specimens were stored frozen (in −20ºC and −80ºC freezers) and thawed up to a 3rd time for testing.

Expected Values:

Studies on the epidemiology of *H. pylori* have shown that this organism is present worldwide. Gastritis caused by *H. pylori* has been shown to correlate with age, ethnic background, family size and socioeconomic class. The prevalence of *H. pylori* infection in a given population can vary from 20% to 90%. In patients diagnosed with duodenal ulcers, however, it has been shown in every age group to be approximately 80%. Currently recommended eradication treatments have an efficacy rate between 75% and 90%.

The proposed subject assay "Premier HpSA Flex" assay is intended to detect the presence of *H. pylori* antigens in unpreserved and preserved human stool specimens. Expected values for a given population should be determined for each laboratory. The rate of positivity may vary depending on geographic location, method of specimen collection, handling and transportation, test employed and general health environment of the patient population under study.

8. Assay Cut-Off:

Not Applicable

B Comparison Studies:

1. Method Comparison with Comparator Device:

A method comparison study was conducted to evaluate the agreement of the subject assay (i.e., Premier HpSA Flex) with an FDA-cleared comparator assay, for use with human stool specimens preserved in CB or C&S transport media. All specimens enrolled in this study were de-identified, left-over samples of liquid, semi-solid, or formed human stool, that were previously collected from symptomatic patients with suspicions of gastroenteritis and submitted to a clinical laboratory for *H. pylori* antigen testing under order from a prescribing healthcare provider as a part of the patients' medical care. Each specimen was sourced from one individual patient. De-identified frozen preserved clinical specimens were acceptable for use in this study since the subject device represents a modification to an existing device. Of note, originally cleared assay used fresh prospective specimens and provided support for use of frozen specimens.

From the time of receipt, the specimens were stored frozen, randomized, relabeled, and pre-screened against both the current clinical diagnostic Standard of Care (SoC) and with an FDA-cleared device. A total of 182 specimens with 50 positives and 132 negatives successfully passed pre-screening (with matching SoC and pre-screen device results) and were assigned one preservative transport medium (CB or C&S). The unpreserved (raw) specimens were transferred to the transport medium (N=90 in C&S and 92 in CB) prior to being tested with the subject assay and the comparator.

The study was carried out at a single US site using three (3) kit lots of the subject assay (Lots 601396P167, 601396P168, and 601396P169) following the instructions in the Package Insert. In parallel, the FDA-cleared comparator assay was performed with the same preserved specimens on any given day by a separate technician following the assay's Package Insert. The results are summarized in Table 7.

Table 7. Performance comparison of Premier HpSA Flex assay and comparator device.

	Comparator Assay		
Premier HpSA Flex Assay	**POSITIVE**	**NEGATIVE**	**TOTAL**
POSITIVE	49*	2	51
NEGATIVE	0	131	131
TOTAL	49	133	182
	Performance		**Wilson-Score 95% CI**
Positive Agreement	100% (49/49)		[92.7–100%]
Negative Agreement	98.5% (131/133)		[94.7–99.6%]

* One (1) sample that was positive based on SoC result and confirmed by prescreening testing gave a negative result on Comparator Method.

2. Matrix Comparison:

Not Applicable

C Clinical Studies:

1. Clinical Sensitivity:

Not Applicable

2. Clinical Specificity:

Not Applicable

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not Applicable

D Clinical Cut-Off:

Not Applicable

E Expected Values/Reference Range:

Not Applicable

VIII Proposed Labeling:

The proposed labeling included with the subject assay/device supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification (K230901) is complete and supports a substantial equivalence decision.