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

A. 510(k) Number:

K183573

B. Purpose for Submission:

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

C. Measurand:

H. pylori antigen

D. Type of Test:

Qualitative immunochromatographic assay

E. Applicant:

Panion & BF Biotech Inc.

F. Proprietary and Established Names:

Vstrip H. pylori Antigen Rapid Test

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3110 *Campylobacter fetus* serological reagents

2. Classification:

   Class I

3. Product code:

   LYR

4. Panel:

83-Microbiology

H. Intended Use:

1. Intended use(s):

   Vstrip H. pylori Antigen Rapid Test is a single use immunochromatographic assay for the qualitative detection of *H. pylori* antigen in unpreserved human stool specimens. Test results are intended to aid in the initial diagnosis and treatment of *H. pylori* infection. Test results should be taken into consideration by the physician in conjunction with the patient history and symptoms.

2. Indication(s) for use:

   Same as Intended use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   None

I. Device Description:

The Vstrip H. pylori Antigen Rapid Test is a qualitative immunochromatographic assay which employs antibody-coated latex beads to detect *Helicobacter pylori* (*H. pylori*) antigens in stool specimens from patients suspected of *H. pylori* infection. Each assay kit contains the materials needed for 20 tests, and includes the test cassettes, sample preparation tubes, positive control reagent, and the package insert and instructions. The test cassette houses the diagnostic strip which incorporates a pair of *H. pylori*-specific monoclonal antibodies for detecting *H. pylori* antigens in stool. Results are visualized in the test cassette "Result Window". The sample preparation tube contains sample diluent buffer, and it incorporates a sampler tool attached to the underside of the tube cap and a dispenser tool attached to the top of the tube. The positive control reagent contains inactivated *H. pylori*.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   ImmunoCard STAT! HpSA

2. Predicate 510(k) number(s):

   K032222

3. Comparison with predicate:

Similarities		
Item	**Device: Vstrip H. pylori Antigen Rapid Test (K183573)**	**Predicate: ImmunoCard STAT! HpSA (K032222)**
Intended use	Vstrip H. pylori Antigen Rapid Test is a single use immunochromatographic assay for the qualitative detection of *H. pylori* antigen in unpreserved human stool specimens. Test results are intended to aid in the initial diagnosis and treatment of *H. pylori* infection. Test results should be taken into consideration by the physician in conjunction with the patient history and symptoms.	ImmunoCard STAT! HpSA is a rapid in vitro qualitative assay for the detection of *Helicobacter pylori* antigen (HpSA) in human stool. The stool antigen detection is intended to aid in the diagnosis of *H. pylori* infection and to demonstrate loss of *H. pylori* stool antigen following treatment. Conventional medical practice recommends that testing by any method to confirm the loss of antigen be done at least four weeks following completion of therapy.
Technology	Immunochromatographic	Same
Type of test	Qualitative	Same
Result interpretation	Manual end point visualization of colored bands.	Same
Measurand	*H. pylori* antigen	Same
Controls	Internal Control Line Positive and negative controls included in kit	Same
Test line capture antibody	Monoclonal anti-*H. pylori*	Same
Conjugate	Red latex particle conjugated anti-*H. pylori*	Same

Differences		
Item	**Device: Vstrip H. pylori Antigen Rapid Test (K183573)**	**Predicate: ImmunoCard STAT! HpSA (K032222)**
Time to read	10 minutes	5 minutes
Kit storage temperature	15 – 30 ℃	2 - 8 ℃
Specimen type	Formed stool, semi-solid stool only	Formed stool, semi-solid stool, and liquid stool
Use	Aid in initial diagnosis	Aid in initial diagnosis and monitor antigen loss after treatment

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

Not applicable

L. Test Principle:

To perform the test, a small portion of stool sample is diluted and mixed in the sample preparation tube, and the diluted sample is added to the test cassette. The sample flows through a labeled pad containing red-colored antibody-coated latex particles. If the sample contains *H. pylori* antigen, the antigen will bind to the antibody to form antigen-antibody-latex complexes which flow through the nitrocellulose membrane by capillary action. The complexes bind *H. pylori* specific antibodies at the test line position to form a pink-red line. A blue line at the control line position serves as an internal control to show that adequate flow of the sample has occurred, and that active components have been employed during a test run. For a positive result, a pink-red test line (next to the letter T) along with a blue control line (next to the letter C) must be visible in the "Result Window". If *H. pylori* antigen is not present or is present at very low levels in the stool sample, only a blue control line will be visible, and the test result is negative. If the blue control line does not develop within 10 minutes, the test result is invalid.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

A reproducibility study was conducted at three laboratory sites in the US. Three lots of the Vstrip H. pylori Antigen Rapid Test were used. Two trained operators per site tested a coded and randomized sample panel once per day over five days. The panels consisted of 12 frozen samples contrived from pooled negative stool matrix spiked with known amounts of cultured *H. pylori*, and a true negative sample. Positive samples at different levels included three replicates each of medium positive (4X

LoD), low positive (1-2X LoD, near C_{95} concentration expected to be positive approximately 95% of the time), high negative (near C_5 concentration expected to be negative approximately 95% of the time). In total 90 data points were collected for each panel member. Across all sites and operators, agreement with the expected result was 97.8% (88/90) for the moderate positive panel member, 97.8% (88/90) for the low positive panel member, 98.9% (89/90) for the high negative panel member, and 100% (90/90) for the true negative panel member. The reproducibility study results were acceptable.

b. Linearity/assay reportable range:

Not applicable

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

Controls:
Internal controls. Each test strip contains a blue internal control line to confirm correct sample flow and active reagents for the test run. If the blue control line is not visible, the result is invalid and the test should be repeated. The background color of the strip should be white and should not interfere with the reading of the test result. If the background strip color interferes with line reading, it is recommended to repeat the test.

External controls. The kit instructions recommend that external positive and negative controls should be tested with each new kit shipment. One vial of positive control reagent is provided with each test kit, and the kit sample diluent buffer serves as the negative control. The positive and negative controls are intended to ensure that the kit is functional to produce the expected results. The kit should not be used if either external control fails to deliver the correct test results.
External positive and negative controls were run during clinical and reproducibility studies, and all controls passed.

Sample stability:
Studies were conducted to examine the stability of stool samples when stored refrigerated at 2-8º C or frozen at -20º C. Negative stool samples and contrived positive samples were stored either refrigerated or frozen/thawed and tested over time with the Vstrip H. pylori Antigen Rapid Test. The positive samples consisted of *H. pylori* organisms spiked into negative stool matrix at moderate positive (5X LoD), low positive (1-2X LoD), and high negative (below 1X LoD) concentrations. The study results supported the recommendations that stool samples can be stored for up to seven days refrigerated at 2-8º C, up to 20 days frozen at -20º C, and can undergo up to three freeze/thaw cycles.

d. Detection limit:

The limit of detection (LoD) was determined using negative stool matrix spiked with

serial dilutions of two strains of cultured *H. pylori* (ATCC 43504 and ATCC 51653). The initial LoD was established by two operators testing at least ten replicates of each dilution. The initial LoD concentrations were confirmed by testing an additional 20 replicates at the target concentration by two additional operators. The LoD was defined as the lowest concentration at which 95% or more of the replicates were positive. An assay cutoff value was defined as the concentration that produced a 50% positive rate. The LoD was determined to be $8x10^5$ CFU/mL for *H. pylori* strain ATCC 43504 and $4.5x10^4$ CFU/mL for *H. pylori* strain ATCC 51653.

Analytical inclusivity (reactivity):
In addition to the *H. pylori* strains assessed in the LoD study, analytical inclusivity of the Vstrip H. pylori Antigen Rapid Test was also demonstrated with three additional strains. All strains were detected 100% of the time at the concentrations listed (Table 1).

Table 1. Inclusivity

Strain	Origin	CFU/mL
ATCC 700392 (BCRC 17219)	UK	3.8×10^5
ATCC 51110 (BCRC 17025)	N/A	1.9×10^5
Taiwan (BCRC 17132)	Taiwan	3.8×10^6

e. *Analytical specificity:*

Cross reactivity:
The potential cross reactivity and microbial interference of the Vstrip H. pylori Antigen Rapid Test was assessed by testing high concentrations of non-target microorganisms in triplicate in the presence or absence of *H. pylori* (2-3X LoD) in negative stool matrix (Table 2). The non-target microorganisms were spiked into the samples at $\geq 10^6$ CFU/mL for bacteria and at $\geq 10^5$ $TCID_{50}$/mL for viruses. No false positive or false negative Vstrip H. pylori Antigen Rapid Test results were observed in the presence of the non-target microorganisms.

Table 2. Cross Reactivity Panel

Microorganism	Source	Microorganism	Source
Aeromonas hydrophila	ATCC 43414	*Streptococcus* Group A	ATCC 19615
Bacillus sp.	ATCC 31006	*Streptococcus* Group B	ATCC 12386
Borrelia burgdorferi	ATCC 35210	*Streptococcus* Group C	ATCC 12388
Campylobacter jejuni	ATCC 33292	*Streptococcus* Group F	ATCC 12392
Candida albicans	ATCC 18804	*Streptococcus* Group G	ATCC 43492
Citrobacter freundii	ATCC 8090	*Streptococcus mutans*	ATCC 25175
Clostridium difficile	ATCC 43596	*Streptococcus pneumoniae*	ATCC 6301
Clostridium perfringens	ATCC 13124	*Streptococcus sanguis*	ATCC 49295
Enterobacter cloacae	ATCC 13047	*Vibrio cholerae*	NCTC 8021
Escherichia coli	ATCC 23815	*Yersinia enterocolitica*	ATCC 23715
Haemophilus influenzae	ATCC 9007	*Listeria innocua*	ATCC 33091
Klebsiella pneumoniae	ATCC 13883	*Salmonella hilversum*	ATCC 15784
Proteus vulgaris	ATCC 8427	*Salmonella paratyphi*	ATCC 9281
Pseudomonas aeruginosa	ATCC 31156	*Salmonella typhimurium*	ATCC 13311
Salmonella enteriditis 16	ATCC 13076	Adeno virus type 7	Chang Gung University

Microorganism	Source	Microorganism	Source
Salmonella typhi 17	ATCC 6539	Adeno virus type 41*	Chang Gung University
Serratia marcescens18	ATCC 21074	Coxsackie type B2	Chang Gung University
Shigella boydii	ATCC 35966	Coxsackie type B6	Chang Gung University
Shigella dysenteriae	ATCC 13313	Echovirus Type 11	Chang Gung University
Shigella flexneri	ATCC 12022	Rotavirus (VR-2104)	ATCCVR-2104
Shigella sonnei	ATCC 25931	Rotavirus (VR-2272)	ATCCVR-2272
Staphylococcus aureus	ATCC 12715		
Staphylococcus epidermidis	ATCC 12228		

* Tested at 1.0 x 10^3 $TCID_{50}$/mL

Interfering substances

An interfering substances study was conducted to assess the potential interference of exogenous and exogenous substances on the performance of the Vstrip H. pylori Antigen Rapid Test (Table 3). High concentrations of the substances were tested in negative stool matrix in the presence or absence of *H. pylori* spiked at two to three times the LoD. The samples were tested in triplicate. No false positive or false negative Vstrip H. pylori Antigen Rapid Test results were observed for the substances at the concentrations tested.

Table 3. Interfering Substances Panel

Interfering substance	Concentration
Aspirin	3 mg/mL
Barium Sulfate	2 % (w/v)
Bilirubin	0.25 mg/mL
Cimetidine	5 mg/mL
Hemoglobin	12.5 % (w/v)
Leukocytes	50 % (v/v)
Metronidazole	0.25 mg/mL
Mylanta	5% (w/v)
Omeprazole	5 mg/mL
Palmitic acid	4 % (w/v)
Pepto-Bismol	5% (v/v)
Stearic acid	4 % (w/v)
Tums Antacid	5mg/mL
Whole blood	40 % (v/v)
Mucin	3.4 % (w/v)

High-dose hook effect/prozone:

A high-dose hook effect (prozone) study was conducted to evaluate potential interference from high concentrations of *H. pylori* antigen on test performance. Cultures of five *H. pylori* strains were tested in triplicate at concentrations ranging from 10^6 – 10^7 CFU/mL (10 – 100X LoD). No false negative results suggestive of a high-dose hook effect were observed.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

The performance of the Vstrip H. pylori Antigen Rapid Test was evaluated in a multi-site prospective clinical study. Three sites (one site in the US and two sites in Taiwan) used freshly collected, leftover stool samples from patients suspected of *H. pylori* infection and tested the samples with the Vstrip H. pylori Antigen Rapid Test.

The Vstrip H. pylori Antigen Rapid Test results were compared to results from an FDA cleared *H. pylori* stool antigen ELISA. Performance of the FDA cleared comparator ELISA was previously established in comparison to an endoscopy biopsy composite reference method (i.e., culture, histology, and RUT) for initial *H. pylori* diagnosis with demonstrated sensitivity and specificity point estimates greater than or equal to 95%, and a lower bound of the two-sided 95% confidence intervals (CI) greater than 89% for each. The performance of the Vstrip H. pylori Antigen Rapid Test was reported as positive percent agreement (PPA) and negative percent agreement (NPA) with the FDA cleared comparator ELISA results.

A total of 333 fresh stool samples were tested in the study (150 collected in the US and 183 collected in Taiwan). The study population was 70% (232) female, 30% (101) male, and the ages ranged from four to 86 years old (Table 4).

Table 4. Study Population Demographics

Age	Sex		
	F	M	Total
4-18	13	6	19
19-60	153	60	213
>60	63	34	97
Unknown	3	1	4
Grand Total	**232**	**101**	**333**

No invalid results were observed for the Vstrip H. pylori Antigen Rapid Test or the comparator ELISA.

The Vstrip H. pylori Antigen Rapid Test demonstrated a positive percent agreement of 96.7% [58/60; 95% CI 88.6% - 99.1%] and a negative percent agreement of 98.2% [268/273; 95% CI 95.8% - 99.2%] with the comparator ELISA (Table 5).

Table 5. Clinical Study Performance

		FDA-Cleared *H. pylori* Antigen ELISA		
		Positive	**Negative**	**Total**
Vstrip H. pylori Antigen Rapid Test Result	**Positive**	58	5	63
	Negative	2	268	270
	Total	60	273	333

			95% CI
PPA	96.7%	(58/60)	88.6% to 99.1%
NPA	98.2%	(268/273)	95.8% to 99.2%

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

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

Not applicable

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

Not applicable

N. Proposed Labeling:

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

O. Conclusion:

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