



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

I Background Information:

A 510(k) Number

K242877

B Applicant

Applied BioCode, Inc.

C Proprietary and Established Names

BioCode Gastrointestinal Pathogen Panel (GPP)

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PCH	Class II	21 CFR 866.3990 - Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assay	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for the BioCode GPP for the detection of microbial nucleic acids extracted from human stool specimens for use on the Applied BioCode MDx 3000 instrument, using an alternate sample extraction system, the KingFisher Flex.

B Measurand:

Target nucleic acid sequences of the following gastrointestinal microorganisms: *Campylobacter* (*C. jejuni*/*C. coli*), *Clostridium difficile* (*C. difficile*) toxin A/B (Fresh samples only), *Salmonella* spp., *Vibrio* (*V. parahaemolyticus*/*V. vulnificus*/ *V. cholerae*), including specific identification of *Vibrio parahaemolyticus*, *Yersinia enterocolitica*, *Enterobacteriaceae* *Escherichia coli* (EAEC), *Enterotoxigenic Escherichia coli* (ETEC) *lt/st*, *E. coli* O157 serogroup, Shiga-like toxin-producing *Escherichia coli* (STEC) *stx1/stx2*, *Shigella*/ Enteroinvasive *Escherichia coli* (EIEC), *Cryptosporidium* spp. (*C. parvum*/*C. hominis*), *Entamoeba histolytica*,

Giardia lamblia (also known as *G. intestinalis* and *G. duodenalis*), Adenovirus F 40/41, Norovirus GI/GII and Rotavirus A.

C Type of Test:

Qualitative multiplexed nucleic acid-based test

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The BioCode Gastrointestinal Pathogen Panel (GPP) is a qualitative multiplexed nucleic acid-based in vitro diagnostic test intended for use with the BioCode MDx 3000 Instrument. The BioCode GPP is capable of the simultaneous detection and identification of nucleic acids from multiple bacteria, viruses, and parasites extracted directly from unpreserved stool samples or stool preserved in Cary-Blair transport medium obtained from individuals with signs and/or symptoms of gastrointestinal infection. The following bacteria, parasites, and viruses are identified using the BioCode Gastrointestinal Pathogen Panel:

- *Campylobacter* (*C. jejuni*/*C. coli*)
- *Clostridium difficile* (*C. difficile*) toxin A/B (Fresh samples only)
- *Salmonella* spp
- *Vibrio* (*V. parahaemolyticus*/*V. vulnificus*/ *V. cholerae*), including specific identification of *Vibrio parahaemolyticus*
- *Yersinia enterocolitica*
- Enteraggative *Escherichia coli* (EAEC)
- Enterotoxigenic *Escherichia coli* (ETEC) lt/st
- *E. coli* O157 serogroup
- Shiga-like toxin-producing *Escherichia coli* (STEC) stx1/stx2
- *Shigella*/ Enteroinvasive *Escherichia coli* (EIEC)
- *Cryptosporidium* spp (*C. parvum*/*C. hominis*)
- *Entamoeba histolytica*
- *Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*)
- Adenovirus F 40/41

- Norovirus GI/GII

- Rotavirus A

The BioCode GPP is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness and results are meant to be used in conjunction with other clinical, laboratory, and epidemiological data. For In Vitro Diagnostic Use Only. For Prescription Use Only.

Positive results do not rule out co-infection with organisms not included in the BioCode GPP. The agent detected may not be the definite cause of the disease. Negative results in this setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease. Concomitant culture is necessary for organism recovery and further typing of bacterial agents. This device is not intended to monitor or guide treatment for *C. difficile* infection.

Due to the small number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for Adenovirus 40/41, *Campylobacter*, *E. coli* O157, *Shigella*/EIEC, *Yersinia enterocolitica*, and *Giardia lamblia* were established additionally with retrospective clinical specimens. Performance characteristics for *Entamoeba histolytica*, *Giardia lamblia*, *Yersinia enterocolitica* and *Vibrio* (*V. parahaemolyticus*, *V. vulnificus*, and *V. cholerae*) were established primarily using contrived clinical specimens.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

For use on the MDx 3000 instrument

IV Device/System Characteristics:

A Device Description:

The BioCode Gastrointestinal Pathogen Panel (GPP) is a multiplexed nucleic acid-based test designed to be used with the BioCode MDx 3000 system. The BioCode MDx 3000 is an automated system that integrates PCR amplification, target capture, signal generation and optical detection for multiple gastrointestinal pathogens from a single stool specimen, either unpreserved or in Cary Blair. Stool specimens are processed, and nucleic acids extracted with BioMérieux NucliSENS easyMAG, Roche MagNA Pure 96, or Thermo Fisher KingFisher Flex. Once the PCR plate is set up and sealed, all other operations are automated on MDx 3000. The BioCode MDx 3000 Gastrointestinal Infection Panel simultaneously tests for 17 pathogens from unpreserved stool specimens or stool collected in Cary-Blair transport medium. Results from the BioCode GPP test are available within less than 4 hours.

B Principle of Operation:

The principle of operation of the BioCode MDx 3000 remains unchanged from the initial clearance in K180041. Refer to the published decision summary for additional information. The BioCode MDx-3000 is an automated system that integrates PCR amplification, target capture, signal generation and optical detection for multiple gastrointestinal pathogens from a single stool specimen, either unpreserved or in Cary-Blair. Stool specimens are processed and nucleic acids are extracted with either the BioMérieux NucliSENS easyMAG, Roche MagNA Pure 96, or Thermo Fisher KingFisher Flex automated systems. Once the PCR plate is set up and sealed, all other operations are automated on the MDx 3000.

The BioCode MDx 3000 Software controls the operation of the instrument, collects and analyzes data, and automatically generates interpretation for test reports at the end of the run.

Fluorescent signals from BMBs with the same barcode are sorted and calculated to generate median fluorescence index (MFI) for each analyte. The presence or absence of a pathogen is determined relative to the validated assay cutoff by MFI. The software also analyzes the results of external and internal controls to validate the run and individual specimen results for reporting.

V Substantial Equivalence Information:

A Predicate Device Name(s):

BioCode Gastrointestinal Pathogen Panel (GPP), Biocode Gastrointestinal Pathogen Panel (GPP)

B Predicate 510(k) Number(s):

K190585

C Comparison with Predicate(s):

Device & Predicate Device(s):	<u>K242877</u>	<u>K190585</u>
Device Trade Name	BioCode Gastrointestinal Pathogen Panel (GPP)	BioCode Gastrointestinal Pathogen Panel (GPP)
General Device Characteristic Similarities		
Intended Use/Indications For Use	The BioCode Gastrointestinal Pathogen Panel (GPP) is a qualitative multiplexed nucleic acid-based in vitro diagnostic test intended for use with the BioCode MDx 3000 Instrument. The BioCode GPP is capable of the simultaneous detection and identification of nucleic acids from multiple bacteria, viruses, and parasites extracted directly from unpreserved stool samples or stool preserved in Cary-Blair transport medium obtained from individuals with signs and/or	The BioCode Gastrointestinal Pathogen Panel (GPP) is a qualitative multiplexed nucleic acid-based in vitro diagnostic test intended for use with the BioCode MDx 3000 Instrument. The BioCode GPP is capable of the simultaneous detection and identification of nucleic acids from multiple bacteria, viruses, and parasites extracted directly from unpreserved stool samples or stool preserved in Cary-Blair transport medium obtained from individuals with signs and/or

	<p>symptoms of gastrointestinal infection. The following bacteria, parasites, and viruses are identified using the BioCode Gastrointestinal Pathogen Panel:</p> <ul style="list-style-type: none"> ▪<i>Campylobacter</i> (<i>C. jejuni</i>/<i>C. coli</i>) ▪<i>Clostridium difficile</i> (<i>C. difficile</i>) toxin A/B (Fresh samples only) ▪<i>Salmonella</i> spp ▪<i>Vibrio</i> (<i>V. parahaemolyticus</i>/<i>V. vulnificus</i>/ <i>V. cholerae</i>), including specific identification of <i>Vibrio parahaemolyticus</i> ▪<i>Yersinia enterocolitica</i> ▪Enteroaggregative <i>Escherichia coli</i> (EAEC) ▪Enterotoxigenic <i>Escherichia coli</i> (ETEC) lt/st ▪<i>E. coli</i> O157 serogroup ▪Shiga-like toxin-producing <i>Escherichia coli</i> (STEC) stx1/stx2 ▪<i>Shigella</i>/ Enteroinvasive <i>Escherichia coli</i> (EIEC) ▪<i>Cryptosporidium</i> spp (<i>C. parvum</i>/<i>C. hominis</i>) ▪<i>Entamoeba histolytica</i> ▪<i>Giardia lamblia</i> (also known as <i>G. intestinalis</i> and <i>G. duodenalis</i>) ▪Adenovirus F 40/41 ▪Norovirus GI/GII ▪Rotavirus A <p>The BioCode GPP is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness and results are meant to be used in conjunction with other clinical, laboratory, and epidemiological data. For In Vitro Diagnostic Use Only. For Prescription Use Only.</p>	<p>symptoms of gastrointestinal infection. The following bacteria, parasites, and viruses are identified using the BioCode Gastrointestinal Pathogen Panel:</p> <ul style="list-style-type: none"> ▪<i>Campylobacter</i> (<i>C. jejuni</i>/<i>C. coli</i>) ▪<i>Clostridium difficile</i> (<i>C. difficile</i>) toxin A/B (Fresh samples only) ▪<i>Salmonella</i> spp ▪<i>Vibrio</i> (<i>V. parahaemolyticus</i>/<i>V. vulnificus</i>/ <i>V. cholerae</i>), including specific identification of <i>Vibrio parahaemolyticus</i> ▪<i>Yersinia enterocolitica</i> ▪Enteroaggregative <i>Escherichia coli</i> (EAEC) ▪Enterotoxigenic <i>Escherichia coli</i> (ETEC) lt/st ▪<i>E. coli</i> O157 serogroup ▪Shiga-like toxin-producing <i>Escherichia coli</i> (STEC) stx1/stx2 ▪<i>Shigella</i>/ Enteroinvasive <i>Escherichia coli</i> (EIEC) ▪<i>Cryptosporidium</i> spp (<i>C. parvum</i>/<i>C. hominis</i>) ▪<i>Entamoeba histolytica</i> ▪<i>Giardia lamblia</i> (also known as <i>G. intestinalis</i> and <i>G. duodenalis</i>) ▪Adenovirus F 40/41 ▪Norovirus GI/GII ▪Rotavirus A <p>The BioCode GPP is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness and results are meant to be used in conjunction with other clinical, laboratory, and epidemiological data. For In Vitro Diagnostic Use Only. For Prescription Use Only.</p>
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	<p>Positive results do not rule out co-infection with organisms not included in the BioCode GPP. The agent detected may not be the definite cause of the disease. Negative results in this setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease. Concomitant culture is necessary for organism recovery and further typing of bacterial agents. This device is not intended to monitor or guide treatment for <i>C. difficile</i> infection.</p> <p>Due to the small number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for Adenovirus 40/41, <i>Campylobacter</i>, <i>E. coli</i> O157, <i>Shigella</i>/EIEC, <i>Yersinia enterocolitica</i>, and <i>Giardia lamblia</i> were established additionally with retrospective clinical specimens. Performance characteristics for <i>Entamoeba histolytica</i>, <i>Giardia lamblia</i>, <i>Yersinia enterocolitica</i> and <i>Vibrio</i> (<i>V. parahaemolyticus</i>, <i>V. vulnificus</i>, and <i>V. cholerae</i>) were established primarily using contrived clinical specimens.</p>	<p>Positive results do not rule out co-infection with organisms not included in the BioCode GPP. The agent detected may not be the definite cause of the disease. Negative results in this setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease. Concomitant culture is necessary for organism recovery and further typing of bacterial agents. This device is not intended to monitor or guide treatment for <i>C. difficile</i> infection.</p> <p>Due to the small number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for Adenovirus 40/41, <i>Campylobacter</i>, <i>E. coli</i> O157, <i>Shigella</i>/EIEC, <i>Yersinia enterocolitica</i>, and <i>Giardia lamblia</i> were established additionally with retrospective clinical specimens. Performance characteristics for <i>Entamoeba histolytica</i>, <i>Giardia lamblia</i>, <i>Yersinia enterocolitica</i> and <i>Vibrio</i> (<i>V. parahaemolyticus</i>, <i>V. vulnificus</i>, and <i>V. cholerae</i>) were established primarily using contrived clinical specimens.</p>
Instrument	BioCode MDx 3000	Same
Sample Type	Unpreserved stool and stool in Cary Blair Medium	Same
Controls	Externally Sourced - Zeptomatrix	Same
Methodology	Multiplex RT-PCR and probe hybridization to biotinylated PCR product(s) followed by fluorescence detection and decoding of barcoded magnetic	Same

	beads (BMB) that are coupled to target-specific probes	
Calibrators	Internal Calibration	Same
General Device Characteristic Differences		
Sample Extraction	easyMAG, Roche MagNA Pure 96, KingFisher Flex	easyMAG, Roche MagNA Pure 96

VI Standards/Guidance Documents Referenced:

CLSI EP09c 3rd Edition, Measurement Procedure Comparison and Bias Estimation Using Patient Samples

CLSI EP12-A2, User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline - Second Edition

CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

A reproducibility study was conducted to evaluate variability associated with extraction of samples with the KingFisher Flex system. This study was designed to assess intra-assay (within run), Inter-assay (run-to-run), day-to-day and instrument-to-instrument (operator-to-operator) reproducibility. One lot of reagents was assayed at Applied BioCode on two instruments by three operators. Testing was performed including two runs per day per operator for five days (a total of 30 runs).

Organisms were diluted directly into Cary-Blair, and pools were constructed in Cary Blair media at 15X or 30X LoD. The prepared pools were then diluted 1:10 fold in pre-screened negative stool, frozen over night or longer, extracted in triplicate and assayed in singlicate. The reproducibility panel consisted of 7 contrived samples (sample 7 is a negative control) extracted in triplicate and each assayed in singlet. The samples consisted of combinations of 12 representative targets at 1.5x LoD (Low) and 3x LoD (Medium). Results from the study are shown in Table 1 below.

Table 1. Reproducibility Study Results with KingFisher Flex

Organism	Target Probe	Concentration	Expected Results	% Agreement with Expected Result			
				Instrument 1	Instrument 2	Instrument 3	All Instruments/ Operators
<i>Salmonella enterica</i> ATCC 14028	Salm	3X LoD (6.60E+03 CFU/mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		1.5X LoD (3.30E+03 CFU/mL)	Detected	30/30 (100%)	29/30 (96.7%)	30/30 (100%)	89/90 (98.9%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
<i>Clostridium difficile</i> Zeptomatrix 0801619	tcdA	3X LoD (9.21E+01 CFU/mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		1.5X LoD (4.61E+01 CFU/mL)	Detected	30/30 (100%)	29/30 (96.7%)	28/30 (93.3%)	87/90 (96.7%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
<i>Clostridium difficile</i> Zeptomatrix 0801619	tcdB	3X LoD (9.21E+01 CFU/mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		1.5X LoD (4.61E+01 CFU/mL)	Detected	30/30 (100%)	29/30 (96.7%)	30/30 (100%)	89/90 (98.9%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
<i>Giardia lamblia</i> Waterborne Inc. P101	G.lam	3X LoD (5.40E+03 cysts/mL)	Detected	30/30 (100%)	29/30 (96.7%)	30/30 (100%)	89/90 (98.9%)
		1.5X LoD (2.70E+03 cysts/mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
Adenovirus 40 Zeptomatrix 0810084CF	Adeno	3X LoD (9.90E-02 TCID ₅₀ /mL)	Detected	30/30 (100%)	29/30 (96.7%)	30/30 (100%)	89/90 (98.9%)
		1.5X LoD (4.95E-02 TCID ₅₀ /mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
<i>Shigella sonnei</i> ATCC 29930	Shig	3X LoD (1.32E+03 CFU/mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		1.5X LoD (6.60E+02 CFU/mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
ETEC ATCC 35401	LT	3X LoD	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)

Organism	Target Probe	Concentration	Expected Results	% Agreement with Expected Result			
				Instrument 1	Instrument 2	Instrument 3	All Instruments/ Operators
		(1.68E+03 CFU/mL)					
		1.5X LoD (8.42E+02 CFU/mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
ETEC ATCC 35401	ST1a	3X LoD (1.68E+03 CFU/mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		1.5X LoD (8.42E+02 CFU/mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
ETEC ATCC 35401	ST1b	3X LoD (1.68E+03 CFU/mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		1.5X LoD (8.42E+02 CFU/mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
<i>Vibrio parahaemolyticus</i> ATCC 17802	V.para	3X LoD (3.90E+01 CFU/mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		1.5X LoD (1.95E+01 CFU/mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
<i>Vibrio parahaemolyticus</i> ATCC 17802	Vib	3X LoD (3.90E+01 CFU/mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		1.5X LoD (1.95E+01 CFU/mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		None	Not Detected	147/150 (98.0%)	150/150 (100%)	150/150 (100%)	447/450 (99.3%)
<i>Yersinia enterocolitica</i> ATCC 23715	Yent	3X LoD (5.01E+02 CFU/mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		1.5X LoD (2.51E+02 CFU/mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
STEC ATCC BAA-2217	Stx2	3X LoD (2.25E+04 CFU/mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)

Organism	Target Probe	Concentration	Expected Results	% Agreement with Expected Result			
				Instrument 1	Instrument 2	Instrument 3	All Instruments/ Operators
		1.5X LoD (1.13E+04 CFU/mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	(450/450) (100%)
<i>Campylobacter coli</i> ATCC 33559	Campy	3X LoD (1.68E+02 CFU/mL)	Detected	30/30 (100%)	29/30 (96.7%)	30/30 (100%)	89/90 (98.9%)
		1.5X LoD (8.40E+01 CFU/mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
<i>Cryptosporidium Parvum</i> Waterborne, Inc. P102C	Crypto	3X LoD (9.30E+03 oocysts/mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		1.5X LoD (4.65E+03 oocysts/mL)	Detected	30/30 (100%)	29/30 (96.7%)	30/30 (100%)	89/90 (98.9%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)
Rotavirus A Zeptomatrix 0810041CF	Rota	3X LoD (6.57E+02 TCID ₅₀ /mL)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
		1.5X LoD (3.29E+02 TCID ₅₀ /mL)	Detected	30/30 (100%)	29/30 (96.7%)	30/30 (100%)	89/90 (98.9%)
		None	Not Detected	150/150 (100%)	150/150 (100%)	150/150 (100%)	450/450 (100%)

Table 2. KingFisher Flex – Negative Agreement of Results of Reproducibility Panel

Target	Negative Agreement (True Negative/True Negative+ False Positive)	95% CI
<i>Salmonella enterica</i>	450/450 (100.00)	(99.15, 100.00)
<i>Clostridium difficile</i>	450/450 (100.00)	(99.15, 100.00)
<i>Giardia lamblia</i>	450/450 (100.00)	(99.15, 100.00)
Adenovirus 40	450/450 (100.00)	(99.15, 100.00)
<i>Shigella sonnei</i>	450/450 (100.00)	(99.15, 100.00)
<i>Vibrio parahaemolyticus</i>	450/450 (100.00)	(99.15, 100.00)
ETEC	450/450 (100.00)	(99.15, 100.00)
<i>Yersinia enterocolitica</i>	450/450 (100.00)	(99.15, 100.00)
STEC	450/450 (100.00)	(99.15, 100.00)
<i>Campylobacter coli</i>	450/450 (100.00)	(99.15, 100.00)
<i>Cryptosporidium parvum</i>	450/450 (100.00)	(99.15, 100.00)
Rotavirus A	450/450 (100.00)	(99.15, 100.00)

2. Linearity:
Not applicable because the BioCode GPP is a qualitative assay.
3. Analytical Specificity/Interference:
Analytical specificity of the BioCode GPP was evaluated in the original 510(k) Premarket Notification (K180041). No additional testing was conducted.
4. Assay Reportable Range:
Not applicable because the BioCode GPP is a qualitative assay.
5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):
No modifications were made to the BioCode GPP assay or MDx 3000 instrument system. Please refer to the published decision summary for the original 510(k) submission for additional information (K180041).
6. Detection Limit:
The LoD of BioCode GPP assay was evaluated when the KingFisher Flex instrument was used for nucleic acid extraction. Contrived samples were prepared with the 21 quantified bacteria, virus, or parasite stocks (Table 3) as well as 1 high Norovirus GI-positive and 1 high Norovirus GII-positive clinical specimens. Each stock was serially diluted and extracted to challenge analytical sensitivity of the assay. LoD for each stock was the lowest concentration with $\geq 95\%$ detection of 20 replicates (19 out of 20). LoD were verified for both unpreserved stool and Cary-Blair preserved stool. To minimize the required volume of reagents and negative matrix and operator time, the LoD study was performed using dual analyte spiked samples. Side by side dilutions (initial 10-fold dilution series followed by finer dilutions) of the Norovirus GI and Norovirus GII clinical specimens was performed and assayed to demonstrate the equivalence between the cleared extraction platform EasyMag and KingFisher Flex.

Table 3. Quantitated Stocks for LoD Testing (23 total)

Species/Strain/Isolate	Source
Bacteria	
<i>E.coli</i> O157:NM CDC 92-3265	ATCC 700376
<i>Escherichia coli</i> 10C-3114 (STEC)	ATCC BAA-2217
O78:H11 <i>Escherichia coli</i> H10407 (ETEC)	ATCC 35401
O92:H33 <i>Escherichia coli</i> (EAEC)	STEC TW04440
<i>Escherichia coli</i> O29:NM (EIEC)	ATCC 43892
<i>Shigella sonnei</i>	ATCC 29930
<i>Salmonella bongori</i>	SGSC 4900
<i>Salmonella enterica subsp. enterica</i>	ATCC 14028
<i>Campylobacter jejuni subsp. jejuni</i>	ATCC 33292
<i>Campylobacter coli</i>	ATCC 33559
<i>Clostridium difficile</i> (toxigenotype 0)	ATCC 9689
<i>Clostridium difficile</i> (toxigenotype IIIA+ B+) Nap1	Zeptomatrix 0801619cf

<i>Vibrio cholerae</i>	ATCC 25870
<i>Vibrio parahaemolyticus</i>	ATCC 17802
<i>Yersinia enterocolitica</i>	ATCC 23715
Viruses	
Norovirus GI	High Positive Clinical Specimen
Norovirus GII	High Positive Clinical Specimen
Human adenovirus 40 (dugan)	Zeptomatrix 0810084CF
Human adenovirus 41 (TAK)	Zeptomatrix 0810085CF
Human rotavirus A	ATCC VR-2018
Parasites	
<i>Giardia intestinalis</i> (aka <i>G. lamblia</i>)	Waterborne Inc. P101
<i>Cryptosporidium parvum</i>	Waterborne Inc. P102
<i>Entamoeba histolytica</i> HB-301:NIH	BEI NR-176

Organism stocks were serially diluted in Cary-Blair to 10x final screening concentration and combined with prescreened negative stool or Cary-Blair stool at a 1:10 ratio at the time of extraction. Four (4) replicates of each concentration in unpreserved stool and Cary-Blair stool were extracted on the easyMAG and KingFisher Flex and tested singly with BioCode GPP assay on the BioCode MDx-3000 system to estimate LoD. The LoD was verified by extracting 20 replicates of each sample type with the easyMAG and KingFisher Flex and testing each extracted sample singly for a total of 20 replicates at or near presumptive LoD. Verification testing (20 replicates) was performed with unpreserved stool samples and Cary-Blair samples. Verification testing (20 replicates) was performed with single targets or in combination with other organism(s).

For Norovirus GI and GII targets, high positive clinical specimens were used and side by side dilutions (initial 10-fold dilution series followed by finer dilutions) was performed and assayed. Each dilution was extracted in quadruplicate with the easyMAG and KingFisher Flex and assayed singly. Twenty (20) replicates at the dilution in which all 3 replicates are detected was assayed to confirm the LoD for each of the two systems.

For Norovirus, as shown in Table 6, the LoD of the Norovirus GII in unpreserved stool was equivalent for 3 extraction systems. For Norovirus GI, the KingFisher Flex is 10-fold and 3-fold more sensitive than the easyMAG. For Cary-Blair stool, as shown in Table 7, the LoD of the Norovirus GI was equivalent for the two systems.

Table 4. LoD Study Results Comparing KingFisher Flex and EasyMag Extraction Methods – Unpreserved Stool

Strain	Source	EasyMag		KingFisher Flex	
		Unpreserved Stool LoD	Detection	Unpreserved Stool LoD	Detection
<i>Campylobacter coli</i>	ATCC 33559	5.60 x 10 ¹ CFU/mL	20/20	5.60 x 10 ¹ CFU/mL	20/20
<i>Campylobacter jejuni</i> spp. <i>jejuni</i>	ATCC 33292	2.33 x 10 ² CFU/mL	20/20	2.33 x 10 ² CFU/mL	20/20

Strain	Source	EasyMag		KingFisher Flex	
		Unpreserved Stool LoD	Detection	Unpreserved Stool LoD	Detection
<i>Clostridium difficile</i> (toxintype 0)	ATCC 9689	2.11×10^1 CFU/mL	20/20	2.11×10^1 CFU/mL	20/20
<i>Clostridium difficile</i> (toxintype III; Nap1)	Zeptomatrix 0801619cf	3.07×10^1 CFU/mL	19/20	3.07×10^1 CFU/mL	20/20
Enterotoxigenic <i>E. coli</i> O92:H33 (EAEC)	STEC TW04440	1.40×10^3 CFU/mL	20/20	1.40×10^3 CFU/mL	20/20
Enteroinvasive <i>E. coli</i> O29:NM (EIEC)	ATCC 43892	3.60×10^2 CFU/mL	20/20	3.60×10^2 CFU/mL	20/20
Enterotoxigenic <i>E. coli</i> O78:H11 H10407 (ETEC)	ATCC 35401	1.87×10^2 CFU/mL	20/20	5.61×10^2 CFU/mL	20/20
<i>Salmonella bongori</i>	SGSC 4900	1.40×10^3 CFU/mL	20/20	4.67×10^2 CFU/mL	19/20
<i>Salmonella enterica</i> ssp. <i>enterica</i>	ATCC 14028	7.33×10^2 CFU/mL	20/20	2.20×10^3 CFU/mL	19/20
Shiga-like toxin producing <i>E. coli</i> (STEC)	ATCC BAA-2217	2.50×10^3 CFU/mL	20/20	7.50×10^3 CFU/mL	20/20
<i>E. coli</i> O157	ATCC 700376	3.30×10^3 CFU/mL	20/20	3.30×10^3 CFU/mL	20/20
<i>Shigella sonnei</i>	ATCC 29930	4.40×10^2 CFU/mL	20/20	4.40×10^2 CFU/mL	20/20
<i>Vibrio cholerae</i>	ATCC 25870	1.81×10^1 CFU/mL	19/20	1.81×10^1 CFU/mL	20/20
<i>Vibrio parahaemolyticus</i>	ATCC 17802	4.33×10^0 CFU/mL	19/20	1.31×10^1 CFU/mL	20/20
<i>Yersinia enterocolitica</i> *	ATCC 23715	1.50×10^3 CFU/mL	20/20	1.67×10^2 CFU/mL	19/20
<i>Cryptosporidium parvum</i>	waterborne P102C	3.10×10^3 oocysts/mL	20/20	3.10×10^3 oocysts/mL	20/20
<i>Entamoeba histolytica</i> HB-301:NIH	BEI NR-176	3.10×10^{-1} cysts/mL	20/20	3.10×10^{-1} cysts/mL	20/20
<i>Giardia intestinalis</i> (aka <i>G. lamblia</i>)	waterborne P101	6.00×10^2 cysts/mL	20/20	1.80×10^3 cysts/mL	20/20
Adenovirus 40 (dugan)	Zeptomatrix 0810084	3.33×10^{-2} TCID ₅₀ /mL	20/20	3.33×10^{-2} TCID ₅₀ /mL	19/20
Adenovirus 41 (TAK)	Zeptomatrix 0810085	8.46×10^{-1} TCID ₅₀ /mL	20/20	8.46×10^{-1} TCID ₅₀ /mL	20/20
Rotavirus A	Zeptomatrix	2.19×10^2 TCID ₅₀ /mL	20/20	2.19×10^2 TCID ₅₀ /mL	19/20

Strain	Source	EasyMag		KingFisher Flex	
		Unpreserved Stool LoD	Detection	Unpreserved Stool LoD	Detection
	0810041C F				

Table 5. LoD Study Results Comparing KingFisher Flex and EasyMag Extraction Methods – Stool in Cary-Blair

Strain	Source	EasyMag		KingFisher Flex	
		Cary-Blair Stool LoD	Detection	Cary-Blair Stool LoD	Detection
<i>Campylobacter coli</i>	ATCC 33559	1.87 x10 ¹ CFU/mL	20/20	1.87 x10 ¹ CFU/mL	20/20
<i>Campylobacter jejuni</i> spp. <i>jejuni</i>	ATCC 33292	7.78 x10 ¹ CFU/mL	19/20	2.33 x10 ² CFU/mL	20/20
<i>Clostridium difficile</i> (toxigenotype 0)	ATCC 9689	7.04 x10 ⁰ CFU/mL	20/20	2.11 x10 ¹ CFU/mL	20/20
<i>Clostridium difficile</i> (toxigenotype III; Nap1)	Zeptomatrix 0801619cf	3.07 x10 ¹ CFU/mL	20/20	3.07 x10 ¹ CFU/mL	19/20
Enterotoxigenic <i>E. coli</i> O157:H7 (EHEC)*	STEC TW04440	1.56 x10 ² CFU/mL	20/20	1.40 x10 ³ CFU/mL	20/20
Enteroinvasive <i>E. coli</i> O29:NM (EIEC)	ATCC 43892	3.60 x10 ² CFU/mL	20/20	3.60 x10 ² CFU/mL	20/20
Enterotoxigenic <i>E. coli</i> O78:H11 H10407 (ETEC)	ATCC 35401	1.87 x10 ² CFU/mL	20/20	5.61 x10 ² CFU/mL	20/20
<i>Salmonella bongori</i>	SGSC 4900	1.40 x10 ³ CFU/mL	20/20	1.40 x10 ³ CFU/mL	20/20
<i>Salmonella enterica</i> ssp. <i>enterica</i>	ATCC 14028	7.33 x10 ² CFU/mL	20/20	7.33 x10 ² CFU/mL	20/20
Shiga-like toxin producing <i>E. coli</i> (STEC)	ATCC BAA-2217	8.33 x10 ² CFU/mL	19/20	2.50 x10 ³ CFU/mL	19/20
<i>E. coli</i> O157	ATCC 700376	1.10 x10 ³ CFU/mL	19/20	3.30 x10 ³ CFU/mL	20/20
<i>Shigella sonnei</i>	ATCC 29930	1.47 x10 ² CFU/mL	19/20	4.40 x10 ² CFU/mL	20/20
<i>Vibrio cholerae</i>	ATCC 25870	1.81 x10 ¹ CFU/mL	20/20	1.81 x10 ¹ CFU/mL	20/20
<i>Vibrio parahaemolyticus</i>	ATCC 17802	1.30 x10 ¹ CFU/mL	20/20	1.30 x10 ¹ CFU/mL	20/20
<i>Yersinia enterocolitica</i>	ATCC 23715	1.50 x10 ³ CFU/mL	20/20	5.01 x10 ² CFU/mL	20/20

Strain	Source	EasyMag		KingFisher Flex	
		Cary-Blair Stool LoD	Detection	Cary-Blair Stool LoD	Detection
<i>Cryptosporidium parvum</i>	waterborne P102C	3.10 x10 ³ oocysts/mL	20/20	1.03 x10 ³ oocysts/mL	19/20
<i>Entamoeba histolytica</i> HB-301:NIH	BEI NR-176	1.03 x10 ⁻¹ cysts/mL	20/20	1.03 x10 ⁻¹ cysts/mL	20/20
<i>Giardia intestinalis</i> (aka <i>G. lamblia</i>)	waterborne P101	6.00 x10 ² cysts/mL	20/20	1.80 x10 ³ cysts/mL	20/20
Adenovirus 40 (dugan)	Zeptomatrix 0810084	3.33 x10 ⁻² TCID ₅₀ /mL	19/20	3.33 x10 ⁻² TCID ₅₀ /mL	20/20
Adenovirus 41 (TAK)	Zeptomatrix 0810085	8.46 x10 ⁻¹ TCID ₅₀ /mL	20/20	2.54 x10 ⁰ TCID ₅₀ /mL	20/20
Rotavirus A	Zeptomatrix 0810041CF	2.19 x10 ² TCID ₅₀ /mL	19/20	7.29 x10 ¹ TCID ₅₀ /mL	20/20

Table 6. Norovirus LoD Study Results Comparing KingFisher Flex and EasyMag Extraction Methods – Unpreserved Clinical Specimen

Target	Source	Target Probe	EasyMag		KingFisher Flex	
			Unpreserved Stool Dilution	Detection	Unpreserved Stool Dilution	Detection
Norovirus GI	Clinical Sample	NoVG1	1:30,000	19/20	1:3,000	20/20
Norovirus GII	Clinical Sample	NoVG2	1:30,000	20/20	1:30,000	19/20

Table 7. Norovirus LoD Study Results Comparing KingFisher Flex and EasyMag Extraction Methods – Cary-Blair Clinical Specimen

Target	Source	Target Probe	EasyMag		KingFisher Flex	
			Cary-Blair Stool Dilution	Detection	Cary-Blair Stool Dilution	Detection
Norovirus GI	Clinical Sample ID# XTAG-24-0005574	NoVG1	1:30,000	20/20	1:30,000	20/20
Norovirus GII	Clinical Sample ID#02-0134	NoVG2	1:30,000	20/20	1:30,000	20/20

7. Assay Cut-Off:

Assay cut-off remains unchanged from the previously cleared version of the BioCode GPP panel.

B Comparison Studies:

1. Method Comparison with Predicate Device:

To support the performance of the Biocode GPP when used with the KingFisher Flex extraction system, a total of 468 remnant, de-identified clinical specimens (254 frozen unpreserved stool

and 214 inoculated Cary-Blair stool) were evaluated with both the KingFisher Flex procedure and previously cleared methods employing the easyMAG extraction platform. These remnant samples were used to support the original 510(k) clearance of the BioCode GPP device.

Fifty-four (54) frozen unpreserved samples were used for the *C. difficile* testing. In addition, a total of 120 samples were contrived at 3x LoD and 6x LoD (15 samples at 3x LoD and 15 samples at 6xLoD for each of three targets) and tested to determine the performance characteristics for *Entamoeba histolytica*, *Yersinia enterocolitica* and *Vibrio* spp. (*V. parahaemolyticus*, *V. vulnificus*, and *V. cholerae*). The demographics of the enrolled subjects in the clinical study are shown in Table 8 below.

For each specimen, the results and the validity were determined per the Interpretation Algorithm of the BioCode GPP. The PPA was calculated as $TP/(TP + FN)$. TP = true positive or positive by both the EasyMag and the Kingfisher Flex; FN = false negative or negative by the Kingfisher Flex only. The NPA was calculated as $TN/(TN + FP)$. TN= true negative or negative by the EasyMag and the KingFisher Flex; FP = false positive or positive by the KingFisher Flex only.

The PPA and NPA of the results between the KingFisher Flex and the easyMAG for individual targets for each sample type (unpreserved stool or Cary-Blair stool) and for the combined sample types plus binomial two-sided 95% confidence interval were calculated. The overall PPA and NPA of the results between the KingFisher Flex and the easyMAG for all targets combined for each sample type (unpreserved stool or Cary-Blair stool) and for the combined sample types plus binomial two-sided 95% confidence interval were calculated. The agreement for samples with co-infections were calculated separately. After discordant analysis, samples that were still invalid were excluded in the agreement calculations. The percentage of invalid samples was calculated for information only.

Table 8. Demographic Information for Clinical Study Subjects

Archived Samples	
Total Specimen Count	468
Gender	
Male	246/468 (52.6%)
Female	222/468 (47.4%)
Age Category	
≤ 5 yrs.	87/468 (18.6%)
6-21 yrs.	85/468 (18.2%)
22-59 yrs.	197/468 (42.1%)
60+yrs	99/468 (21.1%)

Table 9. Demographic Information Subjects with Freshly Collected Specimens (unpreserved)

Fresh Samples	
Total Specimen Count	54
Gender	
Male	20/54 (37.04%)
Female	34/54 (62.96%)
Age Category	
≤ 5 yrs.	0/54 (0.00%)

6-21 yrs.	2/54 (3.70%)
22-59 yrs.	24/54 (44.45%)
60+yrs	28/54 (51.85%)

Results from testing of clinical samples are summarized in Table 10 below.

**Table 10. Summary of Clinical Investigational Study Results (Archived Specimens)
Stratified by Sample Type and Storage – Comparison of KingFisher Flex to EasyMAG
results**

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
<i>Campylobacter</i> spp. ^a	Inoculated Cary-Blair	209	18/18 (100)	82.4 – 100	191/191 (100)	98.0 – 100
	Unpreserved (Frozen)	247	25/25 (100)	86.7 – 100	219/222 (98.6)	96.1 – 99.5
	All Archived	456	43/43 (100)	91.8 – 100	410/413 (99.3)	97.9 – 99.8
<i>Clostridium difficile</i> ^b	Inoculated Cary-Blair	211	35/37 (94.6)	82.3 – 98.5	173/174 (99.4)	96.8 – 99.9
	Unpreserved (Frozen)	247	31/33 (93.9)	80.4 – 98.3	213/214 (99.5)	97.4 – 99.9
	All Archived	458	66/70 (94.3)	86.2 – 97.8	386/388 (99.5)	98.1 – 99.9
<i>E. coli</i> O157 ^c	Inoculated Cary-Blair	209	5/5 (100)	56.6 – 100	204/204 (100)	98.2 – 100
	Unpreserved (Frozen)	246	8/8 (100)	67.6 – 100	238/238 (100)	98.4 – 100
	All Archived	455	13/13 (100)	77.2 – 100	442/442 (100)	99.1 – 100
Enterotoxigenic <i>E. coli</i> (EAEC) ^d	Inoculated Cary-Blair	210	30/30 (100)	88.6 – 100	180/180 (100)	97.9 – 100
	Unpreserved (Frozen)	246	20/22 (90.9)	77.2 – 97.5	224/224 (100)	98.3 – 100
	All Archived	456	50/52 (96.2)	87.0 – 98.9	404/404 (100)	99.1 – 100
Enterotoxigenic <i>E. coli</i> (ETEC) ^e	Inoculated Cary-Blair	209	14/14 (100)	78.5 – 100	195/195 (100)	98.1 – 100
	Unpreserved (Frozen)	246	11/11 (100)	74.1 – 100	235/235 (100)	98.4 – 100
	All Archived	455	25/25 (100)	86.7 – 100	430/430 (100)	99.1 – 100
Shiga toxin–producing <i>E. coli</i> (STEC) ^f	Inoculated Cary-Blair	209	12/12 (100)	75.8 – 100	197/197 (100)	98.1 – 100
	Unpreserved (Frozen)	247	20/22 (90.9)	72.2 – 97.5	224/225 (99.6)	97.5 – 99.9

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
	All Archived	456	32/34 (94.1)	80.9 – 98.4	421/422 (99.80)	98.7 – 100
<i>Salmonella</i> spp. ^g	Inoculated Cary-Blair	209	20/20 (100)	83.9 – 100	188/189 (99.4)	97.1 – 99.9
	Unpreserved (Frozen)	246	21/21 (100)	84.5 – 100	224/225 (99.6)	97.5 – 99.9
	All Archived	455	41/41 (100)	91.4 – 100	412/414 (99.5)	98.3 – 99.9
<i>Shigella</i> / EIEC ^h	Inoculated Cary-Blair	210	13/13 (100)	77.2 – 100	196/197 (99.5)	97.2 – 99.9
	Unpreserved (Frozen)	246	18/18 (100)	82.4 – 100	228/228 (100)	98.3 – 100
	All Archived	456	31/31 (100)	89.0 – 100	424/425 (99.8)	98.7 – 100
<i>Vibrio parahaemolyticus</i> ⁱ	Inoculated Cary-Blair	209	1/1 (100)	20.7 – 100	208/208 (100)	98.2 – 100
	Unpreserved (Frozen)	246	1/1 (100)	20.7 – 100	245/245 (100)	98.5 – 100
	All Archived	455	2/2 (100)	34.2 – 100	453/453 (100)	99.2 – 100
<i>Vibrio</i> spp. (not <i>parahaemolyticus</i>) ^j	Inoculated Cary-Blair	209	N/A	N/A	208/209 (99.5)	97.3 – 99.9
	Unpreserved (Frozen)	246	1/2 (50%)*	9.5 – 90.5	244/244 (100)	98.5 – 100
	All Archived	455	1/2 (50%)	9.5 – 90.5	452/453 (99.8)	98.8 – 100
<i>Yersinia enterocolitica</i> ^k	Inoculated Cary-Blair	209	3/3 (100)	43.9 – 100	204/206 (99.0)	96.5 – 99.7
	Unpreserved (Frozen)	246	3/3 (100)	43.9 – 100	243/243 (100)	98.4 – 100
	All Archived	455	6/6 (100)	61.0 – 100	447/449 (99.6)	98.4 – 99.9
<i>Cryptosporidium</i> spp. ^l	Inoculated Cary-Blair	209	9/9 (100)	70.1 – 100	200/200 (100)	98.1 – 100
	Unpreserved (Frozen)	248	18/18 (100)	82.4 – 100	230/230 (100)	98.4 – 100
	All Archived	457	27/27 (100)	87.5 – 100	430/430 (100)	99.1 – 100
<i>Entamoeba histolytica</i> ^m	Inoculated Cary-Blair	209	N/A	N/A	209/209 (100)	98.2 – 100
	Unpreserved (Frozen)	246	N/A	N/A	246/246 (100)	98.5 – 100
	All Archived	455	N/A	N/A	455/455 (100)	99.2 – 100

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
<i>Giardia lamblia</i> ⁿ	Inoculated Cary-Blair	209	4/4 (100)	51.0 – 100	205/205 (100)	98.2 – 100
	Unpreserved (Frozen)	246	11/11 (100)	74.1 – 100	235/235 (100)	98.4 – 100
	All Archived	455	15/15 (100)	79.6 – 100	440/440 (100)	99.1 – 100
Adenovirus 40/41 ^o	Inoculated Cary-Blair	209	5/5 (100)	56.6 – 100	202/204 (99.0)	96.5 – 99.7
	Unpreserved (Frozen)	246	7/8 (87.5)*	52.9 – 97.8	238/238 (100)	98.4 – 100
	All Archived	455	12/13 (92.3)	66.7 – 98.6	440/442 (99.5)	98.4 – 99.9
Norovirus (GI/GII) ^p	Inoculated Cary-Blair	210	22/23 (95.7)	79.0 – 99.2	187/187 (100)	98.0 – 100
	Unpreserved (Frozen)	247	19/20 (95.0)	76.4 – 99.1	226/227 (99.6)	97.5 – 99.9
	All Archived	457	41/43 (95.3)	84.5 – 98.7	413/414 (99.8)	98.6 – 100
Rotavirus A ^q	Inoculated Cary-Blair	210	10/10 (100)	72.2 – 100	198/200 (99.0)	96.4 – 99.7
	Unpreserved (Frozen)	246	9/9 (100)	70.1 – 100	229/237 (96.6)	93.5 – 98.3
	All Archived	456	19/19 (100)	83.2 – 100	427/437 (97.7)	95.8 – 98.8
Combined Targets	Inoculated Cary-Blair	3559	201/204 (98.5)	95.8-99.5	3345/3355 (99.7)	99.5-99.8
	Unpreserved (Frozen)	4188	223/232 (96.1)	92.8 – 97.9	3941/3956 (99.6)	99.4-99.8

*Positive agreement <90%.

Thirty-five archived samples with discordant results (between the easyMAG and KingFisher Flex were retested twice with both easyMAG and KingFisher Flex.

^a*Campylobacter* spp. One false positive continued to be positive whereas two false positives became negative by KingFisher Flex upon retesting. Twelve samples were still invalid upon retesting and not used in the agreement calculation.

^b*Clostridium difficile*. Two false positives were still positive upon retesting. Of the four false negatives, three were negative by EasyMag upon retesting whereas one remained positive. Ten samples were invalid, not used in the agreement calculation.

^c*E. coli* O157: Thirteen samples were invalid, not used in the agreement calculation.

^dEAEC: Of the two false negatives, one was negative by EasyMag and one remained positive upon retesting. Twelve samples were invalid, not used in the agreement calculation.

^eETEC: Thirteen samples were invalid, not used in the agreement calculation.

^fSTEC: Two false negatives were Negative by EasyMag upon retesting. One false positive was negative by KingFisher and positive by EasyMag upon retesting. Twelve samples were still invalid, not used in the agreement calculation.

^g*Salmonella* spp. Of the two false positives, one was negative by KingFisher Flex and one remained positive upon retesting. Thirteen samples were invalid, not used in the agreement calculation.

^h*Shigella/EIEC*. The false positive became positive by EasyMag upon retesting. Twelve samples were invalid, not used in the agreement calculation.

ⁱ*Vibrio parahaemolyticus*: Thirteen samples were invalid, not used in the agreement calculation.

^j*Vibrio* spp. One false positive was negative by KingFisher Flex upon retesting. For the false negative retested, the easyMAG had an invalid result with the 2nd run and a negative result with the 3rd run, whereas the KingFisher Flex had negative results with both runs. Thirteen samples were invalid, not used in the agreement calculation.

^k*Yersinia enterocolitica*: Of the false positives, one was negative by EasyMag upon retesting, and one remained false positive upon retesting. Thirteen samples were invalid, not used in the agreement calculation.

^l*Cryptosporidium* spp: Eleven samples were invalid, not used in the agreement calculation.

^m*Entamoeba histolytica*: Thirteen samples were invalid, not used in the agreement calculation.

ⁿ*Giardia lamblia*: Thirteen samples were invalid, not used in the agreement calculation.

^oAdenovirus 40/41: The false negative was negative by EasyMag after retesting and the false positive was positive by EasyMag upon retesting, respectively. Thirteen samples were invalid, not used in the agreement calculation.

^pNorovirus GI/GII: The false positive was negative on the KingFisher Flex upon retesting and the false negative was positive by the KingFisher Flex upon retesting. Eleven samples were invalid, not used in the agreement calculation.

^qRotavirus: Of the ten false positives, six were negatives by the KingFisher Flex upon retesting and four remained false positives upon retesting. Twelve samples were invalid, not used in the agreement calculation.

To supplement testing of archived samples, additional testing of contrived specimens were evaluated for select analytes. Results of this additional testing are shown in Table 12 below.

Table 11. Summary of Contrived Specimen Results with the KingFisher Flex

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
<i>Vibrio parahaemolyticus</i>	Unpreserved (Frozen)	115	26/28 (92.9%)	77.4-98.0%	86/87 (98.9%)	93.8-99.8%
<i>Vibrio</i> spp. (not <i>parahaemolyticus</i>)	Unpreserved (Frozen)	115	29/30 (96.7%)	83.3-99.4%	82/85 (96.5%)	90.1-98.8%
<i>Yersinia enterocolitica</i> ^a	Unpreserved (Frozen)	116	28/28 (100%)	87.9-100.0%	88/88 (100%)	95.8%-100.0%
<i>Entamoeba histolytica</i> ^b	Unpreserved (Frozen)	116	28/28 (100%)	87.9-100.0%	86/88 (97.7%)	92.1-99.4%
All other targets ^c	Unpreserved (Frozen)	1491	N/A	N/A	1491/1491 (100%)	99.7-100.0%
Combined targets	Unpreserved (Frozen)	1953	111/114 (97.4%)	92.5 – 99.1%	1833/1839 (99.7%)	99.3 – 99.9%

Note. One hundred twenty contrived samples were tested. Five samples were invalid with the easyMAG on initial testing. When the invalid samples were tested again with PCR, the samples remained invalid.

^aOf the five invalids, one of the *E. histolytica* positives was had a positive assay result for *E. histolytica* but the RNA internal control was invalid.

^bOf the five invalids, one had a positive assay result for *Yersinia enterocolitica*, but the RNA internal control was invalid.

^cOne of the negative stools being used as matrix for contriving samples exhibited weak positivity for *C. difficile* and erroneously enrolled as a negative sample. Because the *C. difficile* was not intended as part of the study, the positive agreement calculation for this target was not included.

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): None

D Clinical Cut-Off: Not applicable

E Expected Values/Reference Range:

Refer to the published decision summary for the original clearance in K180041.

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.