January 10, 2025

Applied BioCode, Inc.
Alex Chang
Regulatory Consultant
12130 Mora Drive
Unit 2
Santa Fe Springs, California 90670

Re: K242877
Trade/Device Name: BioCode Gastrointestinal Pathogen Panel (GPP)
Regulation Number: 21 CFR 866.3990
Regulation Name: Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assay
Regulatory Class: Class II
Product Code: PCH
Dated: September 20, 2024
Received: September 23, 2024

Dear Alex Chang:

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

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

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

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

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

All medical devices, including Class I and unclassified devices and combination product device constituent parts are required to be in compliance with the final Unique Device Identification System rule ("UDI Rule"). The UDI Rule requires, among other things, that a device bear a unique device identifier (UDI) on its label and package (21 CFR 801.20(a)) unless an exception or alternative applies (21 CFR 801.20(b)) and that the dates on the device label be formatted in accordance with 21 CFR 801.18. The UDI Rule (21 CFR 830.300(a) and 830.320(b)) also requires that certain information be submitted to the Global Unique Device Identification Database (GUDID) (21 CFR Part 830 Subpart E). For additional information on these requirements, please see the UDI System webpage at https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/unique-device-identification-system-udi-system.

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

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-

assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Bryan M. Grabias -S

Digitally signed by Bryan M. Grabias -S
Date: 2025.01.10 10:49:43 -05'00'

Bryan Grabias, Ph. D.
Acting Branch Chief
Bacterial Respiratory and Medical Countermeasures Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 07/31/2026
See PRA Statement below.

510(k) Number *(if known)*

K242877

Device Name
BioCode Gastrointestinal Pathogen Panel (GPP)

Indications for Use *(Describe)*
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*
- Enteroaggregative *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 the 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.

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

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

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

Prepared in accordance with the requirements of 21 CFR 807.92.

Submitter Information [807.92(a)(1)]

Submitter/ Applicant	Applied BioCode®, Inc. 12130 Mora Drive Santa Fe Springs, CA 90670 Submitter Contact: Alex Chang Phone: +1 (408) 839-5826 Email: achang@apbiocode.com
Date Prepared	September 20, 2024

Device Information [807.92(a)(2)]

Trade Name	BioCode Gastrointestinal Pathogen Panel (GPP)
Classification Name	Gastrointestinal microorganism multiplex nucleic acid-based assay
Regulation	21 CFR § 866.3990

Predicate Device [807.92(a)(3)]

K190585	BioCode Gastrointestinal Pathogen Panel (GPP)

Device Description [807.92(a)(4)]

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 easyMAG, MagNA Pure 96, KingFisher Flex and KingFisher Apex Dx. 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 (see table below) from unpreserved stool specimens or stool collected in Cary-Blair transport medium. Results from the BioCode Gastrointestinal Pathogen Panel (GPP) test are available within less than 4 hours.

Bacteria, Viruses, Diarrheagenic *E. coli/Shigella*, and Parasites
Detected by the BioCode MDx Gastrointestinal Pathogen Panel (GPP)

Bacteria	Parasites
▪ *Campylobacter* spp. (*C. jejuni, C. coli*) ▪ *Clostridium difficile* toxin A/B (Fresh samples only) ▪ Enteroaggregative *E. coli* (EAEC) ▪ Enterotoxigenic *E. coli* (ETEC): LT/ST ▪ Shiga-toxin producing *E. coli* (STEC): stx1/stx2 ▪ *E.coli* O157 ▪ *Shigella* spp. /Enteroinvasive *E.coli* (EIEC) ▪ *Salmonella* spp. ▪ *Vibrio parahaemolyticus* ▪ *Vibrio* spp *(*not *parahaemolyticus)* ▪ *Yersinia enterocolitica*	▪ *Cryptosporidium* spp. ▪ *Entamoeba histolytica* ▪ *Giardia lamblia*
	Viruses
	▪ Adenovirus 40/41 ▪ Norovirus GI/GII ▪ Rotavirus A
	RNA Internal Control

Intended Use/ Indications for Use [807.92(a)(5)]

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*
- Enteroaggregative *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 the 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.

Device Comparison [807.92(a)(6)]:

Comparison of the Applied BioCode GPP with the Predicate Device

Characteristic	Proposed Device	Predicate
Name	BioCode® Gastrointestinal Pathogen Panel (GPP)	BioCode® Gastrointestinal Pathogen Panel (GPP)
Common Name	Gastrointestinal Microorganism Multiplex Nucleic acid-based assay	Gastrointestinal Microorganism Multiplex Nucleic acid-based assay
510(k) No.	K242877	K190585
Regulation	21CFR 866.3990	21CFR 866.3990
Product Code	PCH, OOI	PCH, OOI
Device Class	II	II
Similarities		
Intended 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* ▪ Enteroaggregative *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*)	Same

Characteristic	Proposed Device	Predicate
	▪ *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 the 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.	
Instrument	Nucleic Acid Purification System BioCode MDx-3000	Same
Sample Type	Unpreserved stool and stool in Cary Blair Medium	Same
Controls	Externally Sourced - Zeptometrix	Same
Methodology	Multiplex RT-PCR and probe hybridization to biotinylated PCR product(s) followed by fluorescence detection and decoding of barcoded magnetic beads (BMB) that are coupled to target-specific probes	Same
Calibrators	Internal Calibration	Same
Differences		
Sample Extraction	easyMAG, Roche MagNA Pure 96, KingFisher Flex, KingFisher Apex Dx	easyMAG, Roche MagNA Pure 96

Summary of Performance Data [807.92(b)]
All necessary testing was conducted on BioCode Gastrointestinal Pathogen Panel (GPP) to support a determination of substantial equivalence to the predicate device.

Nonclinical testing summary [807.92(b)(1)]

REPRODUCIBILITY STUDY

A study was performed to assess the Reproducibility of the BioCode® GPP using samples extracted with the KingFisher Flex and KingFisher Apex Dx. 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 3 instruments by 3 operators, 2 runs per day per operator for 5 days (total of 30 runs).

Organisms were diluted in 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. In total 30 sets of RP pools were run in singlicate on 3 instruments by 3 operators over 5 days.

Table 1. KingFisher Flex – Positive Agreement of Results of Reproducibility Panel.

Organism	Concentration	% Agreement with Expected Result	95% CI	Mean MFI	%CV
*Salmonella enterica**	3X LoD (6.60E+03 CFU/mL)	(90/90) 100%	(95.98,100.0)	15920	10.9
	1.5X LoD (3.30E+03 CFU/mL)	**(89/90) 98.9%**	(93.96, 99.97)	16923	8.0
Clostridium difficile (tcd A)*	3X LoD (9.21E+01 CFU/mL)	(90/90) 100%	(95.98,100.0)	15693	19.7
	1.5X LoD (4.61E+01 CFU/mL)	**(89/90) 98.9%**	(93.96, 99.97)	10333	36.3
Clostridium difficile (tcd B)*	3X LoD (9.21E+01 CFU/mL)	(90/90) 100%	(95.98,100.0)	23783	12.0
	1.5X LoD (4.61E+01 CFU/mL)	**(89/90) 98.9%**	(93.96, 99.97)	18821	17.4
Giardia lamblia	3X LoD (5.40E+03 cysts/mL)	**(89/90) 98.9%**	(93.96, 99.97)	13827	28.3
	1.5X LoD (2.70E+03 cysts/mL)	(90/90) 100%	(95.98,100.0)	15071	27.6
Adenovirus 40*	3X LoD (9.90E-02 $TCID_{50}$/mL)	**(89/90) 98.9%**	(93.96, 99.97)	13061	20.6
	1.5X LoD (4.95E-02 $TCID_{50}$/mL)	(90/90) 100%	(95.98,100.0)	16742	19.3
Shigella sonnei	3X LoD (1.32E+03 CFU/mL)	(90/90) 100%	(95.98,100.0)	19328	13.9
	1.5X LoD (6.60E+02 CFU/mL)	(90/90) 100%	(95.98,100.0)	19515	14.3

Organism	Concentration	% Agreement with Expected Result	95% CI	Mean MFI	%CV
ETEC (LT)	3X LoD (1.68E+03 CFU/mL)	(90/90) 100%	(95.98,100.0)	13727	18.5
	1.5X LoD (8.42E+02 CFU/mL)	(90/90) 100%	(95.98,100.0)	13137	21.4
ETEC (ST1a)	3X LoD (1.68E+03 CFU/mL)	(90/90) 100%	(95.98,100.0)	23135	17.2
	1.5X LoD (8.42E+02 CFU/mL)	(90/90) 100%	(95.98,100.0)	25866	11.4
ETEC (ST1b)	3X LoD (1.68E+03 CFU/mL)	(90/90) 100%	(95.98,100.0)	26034	15.1
	1.5X LoD (8.42E+02 CFU/mL)	(90/90) 100%	(95.98,100.0)	27576	13.0
Vibrio parahaemolyticus (ToxR)	3X LoD (3.90E+01 CFU/mL)	(90/90) 100%	(95.98,100.0)	26590	12.1
	1.5X LoD (1.95E+01 CFU/mL)	(90/90) 100%	(95.98,100.0)	21967	20.0
Vibrio parahaemolyticus	3X LoD (3.90E+01 CFU/mL)	(90/90) 100%	(95.98,100.0)	10900	12.1
*(*Vib)	1.5X LoD (1.95E+01 CFU/mL)	(90/90) 100%	(95.98,100.0)	9295	29.7
Yersinia enterocolitica	3X LoD (5.01E+02 CFU/mL)	(90/90) 100%	(95.98,100.0)	14645	22.1
	1.5X LoD (2.51E+02 CFU/mL)	(90/90) 100%	(95.98,100.0)	13641	27.6
STEC	3X LoD (2.25E+04 CFU/mL)	(90/90) 100%	(95.98,100.0)	17774	15.0
	1.5X LoD (1.13E+04 CFU/mL)	(90/90) 100%	(95.98,100.0)	17965	18.1
*Campylobacter coli**	3X LoD (1.68E+02 CFU/mL)	**(89/90) 98.9%**	(93.96, 99.97)	22869	7.8
	1.5X LoD (8.40E+01 CFU/mL)	(90/90) 100%	(95.98,100.0)	21878	12.9
Cryptosporidium parvum*	3X LoD (9.30E+03 oocysts/mL)	(90/90) 100%	(95.98,100.0)	23202	16.9
	1.5X LoD (4.65E+03 oocysts/mL)	**(89/90) 98.9%**	(93.96, 99.97)	22292	11.9
Rotavirus A*	3X LoD (6.57E+02 $TCID_{50}$/mL)	(90/90) 100%	(95.98,100.0)	26666	24.4
	1.5X LoD (3.29E+02 $TCID_{50}$/mL)	**(89/90) 98.9%**	(93.96, 99.97)	22240	14.5

*False negatives became positives upon repeat from PCR, using the same extracts. The false negatives were due to sampling errors or PCR amplicon transfer during hybridization of MDx-3000.

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

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

Table 3. KingFisher Apex Dx– Positive Agreement of Results of Reproducibility Panel.

Organism	Concentration	% Agreement with Expected Result	95% CI	Mean MFI	%CV
Salmonella enterica	3X LoD (6.60E+03 CFU/mL)	(90/90) 100%	(95.98, 100.0)	16189	13.2
	1.5X LoD (3.30E+03 CFU/mL)	(90/90) 100%	(95.98, 100.0)	17748	18.2
Clostridium difficile (tcd A)*	3X LoD (9.21E+01 CFU/mL)	(90/90) 100%	(95.98, 100.0)	18406	18.8
	1.5X LoD (4.61E+01 CFU/mL)	**(89/90) 98.89%**	(93.96, 99.97)	12099	30.4
Clostridium difficile (tcd B)*	3X LoD (9.21E+01 CFU/mL)	(90/90) 100%	(95.98, 100.0)	26553	9.9
	1.5X LoD (4.61E+01 CFU/mL)	**(89/90) 98.89%**	(93.96, 99.97)	20579	16.5
Giardia lamblia	3X LoD (1.80E+03 cysts/mL)	(90/90) 100%	(95.98, 100.0)	14679	26.5
	1.5X LoD (9.00E+02 cysts/mL)	(90/90) 100%	(95.98, 100.0)	18652	19.5
Adenovirus 40*	3X LoD (9.90E-02 $TCID_{50}$/mL)	**(89/90) 98.89%**	(93.96, 99.97)	13952	21.4
	1.5X LoD (4.95E-02 $TCID_{50}$/mL)	(90/90) 100%	(95.98, 100.0)	19824	14.4
Shigella sonnei	3X LoD (1.32E+03 CFU/mL)	(90/90) 100%	(95.98, 100.0)	17642	17.0
	1.5X LoD (6.60E+02 CFU/mL)	(90/90) 100%	(95.98, 100.0)	18937	18.5
ETEC (LT)*	3X LoD (1.68E+03 CFU/mL)	(90/90) 100%	(95.98, 100.0)	16907	11.8
	1.5X LoD (8.42E+02 CFU/mL)	**(89/90) 98.89%**	(93.96, 99.97)	15586	15.2
ETEC (ST1a)	3X LoD (1.68E+03 CFU/mL)	(90/90) 100%	(95.98, 100.0)	22884	17.9
	1.5X LoD (8.42E+02 CFU/mL)	**(89/90) 98.9%**	(93.96, 99.97)	27127	10.6
ETEC (ST1b)*	3X LoD (1.68E+03 CFU/mL)	(90/90) 100%	(95.98, 100.0)	26896	14.3
	1.5X LoD (8.42E+02 CFU/mL)	**(89/90) 98.89%**	(93.96, 99.97)	29785	8.6
Vibrio parahaemolyticus (ToxR)*	3X LoD (1.30E+01 CFU/mL)	**(89/90) 98.89%**	(93.96, 99.97)	24510	11.9
	1.5X LoD (6.50E+00 CFU/mL)	(90/90) 100%	(95.98, 100.0)	20694	16.2
Vibrio parahaemolyticus	3X LoD (1.30E+01 CFU/mL)	**(89/90) 98.89%**	(93.96, 99.97)	9362	16.6
*(*Vib)*	1.5X LoD (6.50E+00 CFU/mL)	(90/90) 100%	(95.98, 100.0)	7110	24.2
Yersinia enterocolitica	3X LoD (1.50E+03 CFU/mL)	(90/90) 100%	(95.98, 100.0)	7245	33.6
	1.5X LoD (7.52E+02 CFU/mL)	(90/90) 100%	(95.98, 100.0)	6912	34.0

Organism	Concentration	% Agreement with Expected Result	95% CI	Mean MFI	%CV
STEC	3X LoD (2.50E+03 CFU/mL)	(90/90) 100%	(95.98, 100.0)	19069	15.0
	1.5X LoD (1.25E+03 CFU/mL)	(90/90) 100%	(95.98, 100.0)	17716	20.3
Campylobacter coli	3X LoD (1.68E+02 CFU/mL)	(90/90) 100%	(95.98, 100.0)	23830	6.2
	1.5X LoD (8.40E+01 CFU/mL)	(90/90) 100%	(95.98, 100.0)	23139	10.5
Cryptosporidium parvum	3X LoD (3.09E+03 oocysts/mL)	(90/90) 100%	(95.98, 100.0)	22639	15.5
	1.5X LoD (1.55E+03 oocysts/mL)	(90/90) 100%	(95.98, 100.0)	22527	12.5
Rotavirus A	3X LoD (6.57E+02 $TCID_{50}$/mL)	(90/90) 100%	(95.98, 100.0)	25647	20.8
	1.5X LoD (3.29E+02 $TCID_{50}$/mL)	(90/90) 100%	(95.98, 100.0)	25301	18.8

*False negatives became positives upon repeat from PCR, using the same extracts. The false negatives were due to sampling errors or PCR amplicon transfer during hybridization of MDx-3000.

Table 4. KingFisher Apex Dx– Negative Agreement of Results of Reproducibility Panel.

Target	Negative Agreement (True Negative/True Negative+ False Positive)	95% CI
Salmonella enterica	450/450 (100.00)	(99.15, 100.00)
Clostridium difficile	**448/450 (99.56)**	(98.39, 99.88)
Giardia lamblia	450/450 (100.00)	(99.15, 100.00)
Adenovirus 40	450/450 (100.00)	(99.15, 100.00)
Shigella sonnei	450/450 (100.00)	(99.15, 100.00)
Vibrio parahaemolyticus	450/450 (100.00)	(99.15, 100.00)
ETEC	450/450 (100.00)	(99.15, 100.00)
Yersinia enterocolitica	**447/450 (99.33)**	(98.06, 99.77)
STEC	450/450 (100.00)	(99.15, 100.00)
Campylobacter coli	450/450 (100.00)	(99.15, 100.00)
Cryptosporidium parvum	450/450 (100.00)	(99.15, 100.00)
Rotavirus A	450/450 (100.00)	(99.15, 100.00)

LIMIT OF DETECTION (LoD)

A study was performed to assess the performance of the BioCode GPP on the BioCode MDx-3000 at the Limit of Detection (LoD) for both unpreserved Stool and Cary-Blair specimens. In this study the GI Panel was tested with quantified bacteria, virus or parasite stocks (except norovirus which used clinical samples). For initial screening, four replicates of each concentration (near LoD for the predicate) in negative stool and Cary-Blair were extracted on the easyMAG, the KingFisher Flex and the KingFisher Apex Dx and tested in singlet with the BioCode® GPP on the BioCode MDx-3000 system. The LoD was confirmed by extracting 20 replicates of each sample type/extraction method and testing each in singlet for a total of 20 replicates at or near presumptive LoD. LoD for each stock was defined as the lowest concentration with ≥95% detection of 20 replicates (19 out of 20), and was determined separately unpreserved stool and Cary-Blair preserved stool. The LoDs determined from the quantitated stocks or clinical samples are presented below and in the labeling. The LoDs were the same or within 3-fold for each extraction system with a few notable exceptions identified in the tables with footnotes.

Table 5. Comparison of results for limit of detection testing for **Unpreserved Stool** extracted with the easyMAG, the KingFisher Apex Dx, KingFisher Flex and assayed with BioCode® GPP.

Strain	Source	EasyMag		KingFisher Flex		KingFisher Apex Dx	
		Unpreserved Stool LoD	Detection	Unpreserved Stool LoD	Detection	Unpreserved Stool LoD	Detection
Campylobacter coli	ATCC 33559	5.60×10^1 CFU/mL	20/20	5.60×10^1 CFU/mL	20/20	5.60×10^1 CFU/mL	20/20
Campylobacter jejuni spp. jejuni	ATCC 33292	2.33×10^2 CFU/mL	20/20	2.33×10^2 CFU/mL	20/20	2.33×10^2 CFU/mL	20/20
Clostridium difficile (toxinotype 0)	ATCC 9689	2.11×10^1 CFU/mL	20/20	2.11×10^1 CFU/mL	20/20	2.11×10^1 CFU/mL	20/20
Clostridium difficile (toxinotype III; Nap1)	Zeptometrix 0801619cf	3.07×10^1 CFU/mL	19/20	3.07×10^1 CFU/mL	20/20	3.07×10^1 CFU/mL	20/20
Enteroaggregative *E. coli* O92:H33 (EAEC)	STEC TW04440	1.40×10^3 CFU/mL	20/20	1.40×10^3 CFU/mL	20/20	1.40×10^3 CFU/mL	20/20
Enteroinvasive *E. coli* O29:NM (EIEC)	ATCC 43892	3.60×10^2 CFU/mL	20/20	3.60×10^2 CFU/mL	20/20	3.60×10^2 CFU/mL	20/20
Enterotoxigenic *E. coli* O78:H11 H10407 (ETEC)	ATCC 35401	1.87×10^2 CFU/mL	20/20	5.61×10^2 CFU/mL	20/20	5.61×10^2 CFU/mL	20/20
Salmonella bongori	SGSC 4900	1.40×10^3 CFU/mL	20/20	4.67×10^2 CFU/mL	19/20	1.40×10^3 CFU/mL	20/20
Salmonella enterica ssp. enterica	ATCC 14028	7.33×10^2 CFU/mL	20/20	2.20×10^3 CFU/mL	19/20	2.20×10^3 CFU/mL	20/20

Strain	Source	EasyMag		KingFisher Flex		KingFisher Apex Dx	
		Unpreserved Stool LoD	Detection	Unpreserved Stool LoD	Detection	Unpreserved Stool LoD	Detection
Shiga-like toxin producing *E. coli* (STEC)*	ATCC BAA-2217	2.50 x 10^3 CFU/mL	20/20	7.50 x10^3 CFU/mL	20/20	8.33 x10^2 CFU/mL	20/20
E. coli O157	ATCC 700376	3.30 x 10^3 CFU/mL	20/20	3.30 x 10^3 CFU/mL	20/20	3.30 x 10^3 CFU/mL	20/20
Shigella sonnei	ATCC 29930	4.40 x 10^2 CFU/mL	20/20	4.40 x 10^2 CFU/mL	20/20	4.40 x 10^2 CFU/mL	20/20
Vibrio cholerae	ATCC 25870	1.81 x 10^1 CFU/mL	19/20	1.81 x 10^1 CFU/mL	20/20	1.81 x 10^1 CFU/mL	20/20
Vibrio parahaemolyticus	ATCC 17802	4.33 x10^0 CFU/mL	19/20	1.31 x10^1 CFU/mL	20/20	4.33 x10^0 CFU/mL	20/20
Yersinia enterocolitica **	ATCC 23715	1.50 x10^3 CFU/mL	20/20	1.67 x10^2 CFU/mL	19/20	5.01 x10^2 CFU/mL	19/20
Cryptosporidium parvum	waterborne P102C	3.10 x10^3 oocysts/mL	20/20	3.10 x10^3 oocysts/mL	20/20	1.03 x10^3 oocysts/mL	20/20
Entamoeba histolytica HB-301:NIH	BEI NR-176	3.10 x10^{-1} cysts/mL	20/20	3.10 x10^{-1} cysts/mL	20/20	3.10 x10^{-1} cysts/mL	20/20
Giardia intestinalis (aka G. lamblia)	waterborne P101	6.00 x10^2 cysts/mL	20/20	1.80 x10^3 cysts/mL	20/20	6.00 x10^2 cysts/mL	20/20
Adenovirus 40 (dugan)	Zeptometrix 0810084	3.33 x10^{-2} $TCID_{50}$/mL	20/20	3.33 x10^{-2} $TCID_{50}$/mL	19/20	3.33 x10^{-2} $TCID_{50}$/mL	20/20
Adenovirus 41 (TAK)	Zeptometrix 0810085	8.46 x10^{-1} $TCID_{50}$/mL	20/20	8.46 x10^{-1} $TCID_{50}$/mL	20/20	2.82 x10^{-1} $TCID_{50}$/mL	19/20
Rotavirus A	Zeptometrix 0810041CF	2.19 x10^2 $TCID_{50}$/mL	20/20	2.19 x10^2 $TCID_{50}$/mL	19/20	2.19 x10^2 $TCID_{50}$/mL	20/20

*KingFisher Flex 9x less sensitive than KingFisher Apex Dx; **EM 9x less sensitive than KingFisher Flex

Table 6. Comparison of results for limit of detection testing for **Cary-Blair Stool** extracted with the easyMAG, the KingFisher Flex Apex Dx, and the KingFisher Flex and assayed with BioCode® GPP.

Strain	Source	EasyMag		KingFisher Flex		KingFisher Apex Dx	
		Cary-Blair Stool LoD	Detection	Cary-Blair Stool LoD	Detection	Cary-Blair Stool LoD	Detection
Campylobacter coli	ATCC 33559	1.87 x10^1 CFU/mL	20/20	1.87 x10^1 CFU/mL	20/20	1.87 x10^1 CFU/mL	19/20
Campylobacter jejuni spp. jejuni	ATCC 33292	7.78 x10^1 CFU/mL	19/20	2.33 x10^2 CFU/mL	20/20	2.33 x10^2 CFU/mL	20/20
Clostridium difficile	ATCC 9689	7.04 x10^0 CFU/mL	20/20	2.11 x10^1 CFU/mL	20/20	6.33 x10^1 CFU/mL	20/20

Strain	Source	EasyMag		KingFisher Flex		KingFisher Apex Dx	
		Cary-Blair Stool LoD	Detection	Cary-Blair Stool LoD	Detection	Cary-Blair Stool LoD	Detection
(toxinotype 0)*							
Clostridium difficile (toxinotype III; Nap1)	Zeptometrix 0801619cf	3.07 x10^1 CFU/mL	20/20	3.07 x10^1 CFU/mL	19/20	3.07 x10^1 CFU/mL	20/20
Enteroaggregative *E. coli* O92:H33 (EAEC)**	STEC TW04440	1.56 x10^2 CFU/mL	20/20	1.40 x10^3 CFU/mL	20/20	4.20 x10^3 CFU/mL	20/20
Enteroinvasive *E. coli* O29:NM (EIEC)	ATCC 43892	3.60 x10^2 CFU/mL	20/20	3.60 x10^2 CFU/mL	20/20	1.20 x10^2 CFU/mL	19/20
Enterotoxigenic *E. coli* O78:H11 H10407 (ETEC)	ATCC 35401	1.87 x10^2 CFU/mL	20/20	5.61 x10^2 CFU/mL	20/20	1.87 x10^2 CFU/mL	20/20
Salmonella bongori	SGSC 4900	1.40 x10^3 CFU/mL	20/20	1.40 x10^3 CFU/mL	20/20	1.40 x10^3 CFU/mL	20/20
Salmonella enterica ssp. enterica	ATCC 14028	7.33 x10^2 CFU/mL	20/20	7.33 x10^2 CFU/mL	20/20	2.20 x10^3 CFU/mL	20/20
Shiga-like toxin producing *E. coli* (STEC)	ATCC BAA-2217	8.33 x10^2 CFU/mL	19/20	2.50 x10^3 CFU/mL	19/20	2.50 x10^3 CFU/mL	20/20
E. coli O157	ATCC 700376	1.10 x10^3 CFU/mL	19/20	3.30 x10^3 CFU/mL	20/20	3.30 x10^3 CFU/mL	20/20
Shigella sonnei	ATCC 29930	1.47 x10^2 CFU/mL	19/20	4.40 x10^2 CFU/mL	20/20	1.47 x10^2 CFU/mL	19/20
Vibrio cholerae	ATCC 25870	1.81 x10^1 CFU/mL	20/20	1.81 x10^1 CFU/mL	20/20	1.81 x10^1 CFU/mL	20/20
Vibrio parahaemolyticus	ATCC 17802	1.30 x10^1 CFU/mL	20/20	1.30 x10^1 CFU/mL	20/20	4.33 x10^0 CFU/mL	20/20
Yersinia enterocolitica	ATCC 23715	1.50 x10^3 CFU/mL	20/20	5.01 x10^2 CFU/mL	20/20	5.01 x10^2 CFU/mL	20/20
Cryptosporidium parvum	waterborne P102C	3.10 x10^3 oocysts/mL	20/20	1.03 x10^3 oocysts/mL	19/20	1.03 x10^3 oocysts/mL	19/20
Entamoeba histolytica HB-301:NIH	BEI NR-176	1.03 x10^{-1} cysts/mL	20/20	1.03 x10^{-1} cysts/mL	20/20	1.03 x10^{-1} cysts/mL	20/20
Giardia intestinalis (aka G. lamblia)	waterborne P101	6.00 x10^2 cysts/mL	20/20	1.80 x10^3 cysts/mL	20/20	1.80 x10^3 cysts/mL	20/20

Strain	Source	EasyMag		KingFisher Flex		KingFisher Apex Dx	
		Cary-Blair Stool LoD	Detection	Cary-Blair Stool LoD	Detection	Cary-Blair Stool LoD	Detection
Adenovirus 40 (dugan)	Zeptometrix 0810084	3.33×10^{-2} $TCID_{50}$/mL	19/20	3.33×10^{-2} $TCID_{50}$/mL	20/20	3.33×10^{-2} $TCID_{50}$/mL	20/20
Adenovirus 41 (TAK)	Zeptometrix 0810085	8.46×10^{-1} $TCID_{50}$/mL	20/20	2.54×10^{0} $TCID_{50}$/mL	20/20	8.46×10^{-1} $TCID_{50}$/mL	20/20
Rotavirus A	Zeptometrix 0810041CF	2.19×10^{2} $TCID_{50}$/mL	19/20	7.29×10^{1} $TCID_{50}$/mL	20/20	7.29×10^{1} $TCID_{50}$/mL	19/20

*KingFisher Apex Dx 27x less sensitive than EM; **KingFisher Flex 9x and KingFisher Apex Dx 27x less sensitive than EM

For Norovirus GI and GII targets, positive clinical specimens were used, and serial dilutions (initial 10-fold dilution series followed by finer dilutions) were performed. Four replicates of each dilution in negative unpreserved stool and Cary-Blair stool were extracted with the easyMAG, the KingFisher Flex and KingFisher Apex Dx and tested with the BioCode® GPP on the BioCode MDx-3000 system. The LoD was confirmed by extracting 20 replicates of each sample type with each extraction method and testing at or near presumptive LoD.

For Norovirus, the LoD of the Norovirus GII in unpreserved stool was equivalent for three extraction systems. For Norovirus GI, the KingFisher Flex is 10-fold and 3-fold more sensitive than the easyMag and the KingFisher Apex Dx, respectively. For Cary-Blair stool, the LoD of the Norovirus GI was equivalent for three systems. For Norovirus GII, the KingFisher Apex Dx was 3-fold more sensitive than the EasyMag and the KingFisher Flex.

Table 7. Norovirus - Comparison of results for limit of detection testing for **Unpreserved Stool** extracted with easyMAG, KingFisher Flex, or KingFisher Apex Dx, and assayed with BioCode® GPP.

Target	Source	Target Probe	EasyMag		KingFisher Flex		KingFisher Apex Dx	
			Unpreserved Stool Dilution	Detection	Unpreserved Stool Dilution	Detection	Unpreserved Stool Dilution	Detection
Norovirus GI*	Clinical Sample ID# XTAG-24-0005574	NoVG1	1:30,000	19/20	1:3,000*	20/20	1:10,000	19/20
Norovirus GII	Clinical Sample ID#02-0134	NoVG2	1:30,000	20/20	1:30,000	19/20	1:30,000	20/20

*KF Flex 10-fold and 3-fold more sensitive than EM and KF Apex Dx.

Table 8. Norovirus - Comparison of results for limit of detection testing for **Cary-Blair Stool** extracted with the easyMAG, KingFisher Flex, or KingFisher Apex Dx, and assayed with BioCode® GPP.

Target	Source	Target Probe	EasyMag		KingFisher Flex		KingFisher Apex Dx	
			Cary-Blair Stool Dilution	Detection	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	1:30,000	20/20
Norovirus GII	Clinical Sample ID#02-0134	NoVG2	1:30,000	20/20	1:30,000	20/20	1:10,000*	20/20

*KF Apex Dx 3-fold more sensitive than EM and KF Flex

Clinical testing summary [807.92(b)(2)]

METHOD COMPARISON STUDY

A clinical investigational study was performed in which a total of 468 remnant, de-identified samples (254 frozen unpreserved stool and 214 inoculated Cary-Blair stool) that were prospectively collected for the clinical study that resulted in the K180041 BioCode® GPP FDA clearance were extracted using the KingFisher Flex, KingFisher Apex Dx, and the easyMAG, and tested on the MDx-3000 system. Fifty-four (54) freshly collected leftover 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 clinical patients and the results of the clinical investigational study are as follows:

Table 9. Demographic data for archived specimens (frozen unpreserved and inoculated Cary-Blair)

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 10. Demographic data for fresh 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%)

The Positive Percent Agreement (PPA) was calculated as TP/(TP + FN). TP = true positive or positive by both the EasyMag and the KingFisher Apex Dx or the KingFisher Flex; FN = false negative or negative by the KingFisher Apex Dx or the KingFisher Flex only. Negative Percent Agreement (NPA) was calculated as TN/(TN + FP). TN = true negative or negative by the EasyMag and the KingFisher Apex Dx or the KingFisher Flex; FP = false positive or positive by the KingFisher Apex Dx or the KingFisher Flex only. The exact binomial two-sided 95% confidence interval was calculated.

A. Method Comparison Study between EasyMag and KingFisher Flex

Table 11. KingFisher Flex: Summary of Clinical Investigational Study Results (Archived Specimens) Stratified by Sample Type and Storage

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
Campylobacter 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
Clostridium difficile [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
E. coli 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

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
Enteroaggregative *E. coli* (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 *E. coli* (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 *E. coli* (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
	All Archived	456	32/34 (94.1)	80.9 – 98.4	421/422 (99.80)	98.7 – 100
Salmonella 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
Shigella/ 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
Vibrio parahaemolyticus [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
Vibrio spp. (not *parahaemolyticus*) [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

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
Yersinia enterocolitica [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
Cryptosporidium 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
Entamoeba histolytica [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
Giardia lamblia [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

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
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 (35) archived samples with discordant results (between the easyMAG and KingFisher Flex) retested twice with both easyMAG and KingFisher Flex.

a - *Campylobacter* spp. One (1) false positive retested remained false positive whereas two (2) false positives became true negatives. Twelve (12) samples were still invalid, not used in the agreement calculation.

b - *Clostridium difficile.* Two (2) false positives retested remained false positives. Of the four (4) false negatives retested, three (3) became true negatives whereas one (1) remained false negative. Ten (10) samples were invalid, not used in the agreement calculation.

c- E. coli O157: Thirteen (13) samples were invalid, not used in the agreement calculation.

d - EAEC: Of the two (2) false negatives retested, one (1) was true negative and one (1) became true positive. Twelve (12) samples were invalid, not used in the agreement calculation.

e- ETEC: Thirteen (13) samples were invalid, not used in the agreement calculation.

f - STEC: Two (2) false negatives retested became true negatives. One (1) false positive became false negative. Twelve (12) samples were still invalid, not used in the agreement calculation.

g - *Salmonella* spp. Of the two (2) false positives retested, one (1) became true negative and one (1) remained false positive. Thirteen (13) samples were invalid, not used in the agreement calculation.

h - *Shigella/EIEC.* The false positive retested became true positive. Twelve (12) samples were invalid, not used in the agreement calculation.

i- *Vibrio parahaemolyticus*: Thirteen (13) samples were invalid, not used in the agreement calculation.

j – *Vibrio* spp. One (1) false positive retested became true negative. 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 KF Flex had negative results with both runs. Thirteen (13) samples were invalid, not used in the agreement calculation.

k – *Yersinia enterocolitica:* Of the (2) false positives retested, one (1) became true negative and one (1) remained false positive. Thirteen (13) samples were invalid, not used in the agreement calculation.

l- *Cryptosporidium* spp: Eleven (11) samples were invalid, not used in the agreement calculation.

m- *Entamoeba histolytica*: Thirteen (13) samples were invalid, not used in the agreement calculation.

n- *Giardia lamblia*: Thirteen (13) samples were invalid, not used in the agreement calculation

o - Adenovirus 40/41: The false negative and the false positive retested became true negative and true positive, respectively. Thirteen (13) samples were invalid, not used in the agreement calculation.

p – Norovirus GI/GII: The false positive and the false negative retested became true negative and true positive, respectively. Eleven (11) samples were invalid, not used in the agreement calculation.

q- Rotavirus: Of the ten (10) false positives retested, six (6) became true negatives, and four (4) remained false positives. Twelve (12) samples were invalid, not used in the agreement calculation.

The table below (**Table 12**) shows the results of retesting samples with discordant results between the easyMAG and the KingFisher Flex. Samples were retested twice with both easyMAG and/or KingFisher Flex. Consensus results of the discordant samples were not used in the calculation of the agreements but noted as the footnotes of **Table 11** above.

Table 12. KingFisher Flex: Retested Results of Discordant Specimens (Archived Specimens)

	Target	Sample Name	Sample Type	Extraction	Run 1 (Original run)	Run 2	Run 3	Consensus Result
a	*Campylobacter* spp	01-0314	Unpreserved (Frozen)	easyMAG	Ndet	Det	Ndet	Ndet
				KingFisher Flex	Det	Ndet	Det	Det
		03-0153	Unpreserved (Frozen)	easyMAG	Ndet	Ndet	Det	Ndet
				KingFisher Flex	Det	Ndet	Ndet	Ndet
		Mayo-GI_101	Unpreserved (Frozen)	easyMAG	Ndet	Det	Ndet	Ndet
				KingFisher Flex	Det	Det	Ndet	Det
b	*Clostridium difficile*	01-0019	Inoculated Cary-Blair	easyMAG	Det	Det	Ndet	Det
				KingFisher Flex	Ndet	Ndet	Ndet	Ndet
		01-0048	Inoculated Cary-Blair	easyMAG	Det	Ndet	Ndet	Ndet
				KingFisher Flex	Ndet	Ndet	Ndet	Ndet
		01-0080	Unpreserved (Frozen)	easyMAG	Det	Ndet	Ndet	Ndet
				KingFisher Flex	Ndet	Ndet	Ndet	Ndet
		02-0009	Unpreserved (Frozen)	easyMAG	Det	Ndet	Ndet	Ndet
				KingFisher Flex	Ndet	Ndet	Det	Ndet
		02-0398	Unpreserved (Frozen)	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Flex	Det	Ndet	Det	Det
		03-0353	Inoculated Cary-Blair	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Flex	Det	Det	Det	Det
c	EAEC	02-0042	Unpreserved (Frozen)	easyMAG	Det	Ndet	Ndet	Ndet
				KingFisher Flex	Ndet	Ndet	Ndet	Ndet
		SP366	Unpreserved (Frozen)	easyMAG	Det	Det	Det	Det
				KingFisher Flex	Ndet	Det	Det	Det
d	STEC	02-0211	Unpreserved (Frozen)	easyMAG	Det	Ndet	Ndet	Ndet
				KingFisher Flex	Ndet	Ndet	Ndet	Ndet
		Mayo-GI_092	Unpreserved (Frozen)	easyMAG	Ndet	Det	Det	Det
				KingFisher Flex	Det	Ndet	Ndet	Ndet
		SP228	Unpreserved	easyMAG	Det	Ndet	Ndet	Ndet

	Target	Sample Name	Sample Type	Extraction	Run 1 (Original run)	Run 2	Run 3	Consensus Result
			(Frozen)	KingFisher Flex	Ndet	Ndet	Ndet	Ndet
e	*Salmonella* spp	01-0137	Unpreserved (Frozen)	easyMAG	Ndet	Det	Ndet	Ndet
				KingFisher Flex	Det	Det	Ndet	Det
		06-0357	Cary-Blair	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Flex	Det	Ndet	Ndet	Ndet
f	*Shigella/EIEC*	01-0267	Inoculated Cary-Blair	easyMAG	Ndet	Det	Det	Det
				KingFisher Flex	Det	Det	Ndet	Det
g	*Vibrio* spp	02-0167	Unpreserved (Frozen)	easyMAG	Det	Invalid	Ndet	IND
				KingFisher Flex	Ndet	Ndet	Ndet	Ndet
		03-0396	Inoculated Cary-Blair	easyMAG	Ndet	Ndet	Invalid	Ndet
				KingFisher Flex	Det	Ndet	Ndet	Ndet
h	*Yersinia*	06-0053	Cary-Blair	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Flex	Det	Ndet	Ndet	Ndet
		06-0222	Cary-Blair	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Flex	Det	Det	Det	Det
i	Adenovirus 40/41	01-0375	Unpreserved (Frozen)	easyMAG	Det	Ndet	Ndet	Ndet
				KingFisher Flex	Ndet	Ndet	Ndet	Ndet
		SP418	Inoculated Cary-Blair	easyMAG	Ndet	Det	Det	Det
				KingFisher Flex	Det	Det	Det	Det
j	Norovirus GI/GII	01-0071	Unpreserved (Frozen)	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Flex	Det	Ndet	Ndet	Ndet
		02-0236	Unpreserved (Frozen)	easyMAG	Det	Det	Det	Det
				KingFisher Flex	Ndet	Det	Det	Det
k	Rotavirus	01-0011	Unpreserved (Frozen)	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Flex	Det	Ndet	Ndet	Ndet
		01-0023	Unpreserved (Frozen)	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Flex	Det	Ndet	Ndet	Ndet
		01-0062	Unpreserved (Frozen)	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Flex	Det	Ndet	Ndet	Ndet
		01-0150	Unpreserved (Frozen)	easyMAG	Ndet	Det	Ndet	Ndet
				KingFisher Flex	Det	Det	Det	Det
		01-0151		easyMAG	Ndet	Ndet	Ndet	Ndet

	Target	Sample Name	Sample Type	Extraction	Run 1 (Original run)	Run 2	Run 3	Consensus Result
			Inoculated Cary-Blair	KingFisher Flex	Det	Ndet	Ndet	Ndet
		CK-ST-045*	Unpreserved (Frozen)	easyMAG	Ndet	N/A	N/A	Ndet
				KingFisher Flex	Det	N/A	N/A	Det
		CK-ST-046	Unpreserved (Frozen)	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Flex	Det	Det	Ndet	Det
		ST-057	Unpreserved (Frozen)	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Flex	Det	Det	Det	Det
		ST-058	Inoculated Cary-Blair	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Flex	Det	Ndet	Ndet	Ndet
		Stool_0132	Unpreserved (Frozen)	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Flex	Det	Ndet	Ndet	Ndet

*Insufficient Volume of Sample to be retested. IND: Indeterminant; Det: Detected; Ndet: Not Detected

Twenty-seven (27) archived samples with invalid results (invalid internal control) were subjected to reflex testing per BioCode® GPP IFU. After reflex testing, fourteen (14) samples were valid, and thirteen (13) samples were still invalid, as shown in **Table 13**. Final results were used to calculate the agreements reported in **Table 11** and/or noted in the footnote of **Table 11**.

Table 13. KingFisher Flex: Results of Reflex Testing of Invalid Samples (Archived Samples)

Sample Name	Sample Type	Instrument	Run 1	Run 2	Final Result
01-0035	Unpreserved (Frozen)	easyMAG	IC Invalid	IC Valid	Valid
02-0167	Unpreserved (Frozen)	easyMAG	IC Invalid	IC Valid	Valid
03-0097	Unpreserved (Frozen)	easyMAG	IC Invalid	IC Invalid	Invalid
06-0023	Cary-Blair	easyMAG	IC Invalid	IC Invalid	Invalid
		KingFisher Flex	IC Invalid	IC Valid	Valid
06-0119	Cary-Blair	easyMAG	IC Invalid	IC Invalid	Invalid
		KingFisher Flex	IC Invalid	IC Invalid	Invalid
06-0223	Cary-Blair	easyMAG	IC Invalid	IC Invalid	Invalid
06-0258	Cary-Blair	easyMAG	IC Invalid	IC Valid	Valid
06-0331	Cary-Blair	easyMAG	IC Invalid	IC Valid	Valid
Stool_0112	Unpreserved (Frozen)	easyMAG	IC Invalid	IC Valid	Valid
Mayo-GI_107	Inoculated Cary-Blair	easyMAG	IC Invalid	IC Valid	Valid
		KingFisher Flex	IC Invalid	IC Valid	Valid
Mayo-GI_117	Inoculated Cary-Blair	easyMAG	IC Invalid	IC Valid	Valid
Mayo-GI_123	Inoculated Cary-Blair	easyMAG	IC Invalid	IC Valid	Valid
Mayo-GI_126	Inoculated Cary-Blair	easyMAG	IC Invalid	IC Valid	Valid
SP392	Inoculated Cary-Blair	easyMAG	IC Invalid	IC Valid	Valid
		KingFisher Flex	IC Invalid	IC Valid	Valid
ST-074	Inoculated Cary-Blair	easyMAG	IC Invalid	IC Valid	Valid
		KingFisher Flex	IC Invalid	IC Valid	Valid
01-0003	Unpreserved (Frozen)	KingFisher Flex	IC Invalid	IC Invalid	Invalid
01-0047	Unpreserved (Frozen)	KingFisher Flex	IC Invalid	IC Invalid	Invalid
01-0335	Unpreserved (Frozen)	KingFisher Flex	IC Invalid	IC Invalid	Invalid
02-0288	Unpreserved (Frozen)	KingFisher Flex	IC Invalid	IC Invalid	Invalid
02-0310	Unpreserved (Frozen)	KingFisher Flex	IC Invalid	IC Valid	Valid
03-0360	Unpreserved (Frozen)	KingFisher Flex	IC Invalid	IC Invalid	Invalid
06-0051	Cary-Blair	KingFisher Flex	IC Invalid	IC Valid	Valid
06-0137	Cary-Blair	KingFisher Flex	IC Invalid	IC Valid	Valid
Mayo-GI_124	Unpreserved (Frozen)	KingFisher Flex	IC Invalid	IC Invalid	Invalid
Stool_0036	Unpreserved (Frozen)	KingFisher Flex	IC Invalid	IC Invalid	Invalid

Sample Name	Sample Type	Instrument	Run 1	Run 2	Final Result
01-0073	Inoculated Cary-Blair	KingFisher Flex	IC Invalid	IC Invalid	Invalid
02-0135	Inoculated Cary-Blair	KingFisher Flex	IC Invalid	IC Invalid	Invalid

IC: Internal Control

Table 14. KingFisher Flex: Summary of Clinical Investigational Study Results of Fresh *Clostridium difficile* Positives and Negatives

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
Campylobacter spp.[a]	Unpreserved (Fresh)	53	1/1 (100.0)	20.7 - 100.0	52/52 (100.0)	93.1 - 100.0
Clostridium difficile [b]	Unpreserved (Fresh)	54	30/30 (100.0)	88.6 - 100.0	23/24 (95.8)	79.8 - 99.3
E. coli O157	Unpreserved (Fresh)	53	N/A	N/A	53/53 (100.0)	93.2 - 100.0
Enteroaggregative *E. coli* (EAEC)	Unpreserved (Fresh)	53	1/1 (100.0)	20.7 - 100.0	52/52 (100.0)	93.1 - 100.0
Enterotoxigenic *E. coli* (ETEC)	Unpreserved (Fresh)	53	N/A	N/A	53/53 (100.0)	93.2 - 100.0
Shiga toxin-producing *E. coli* (STEC)	Unpreserved (Fresh)	53	N/A	N/A	53/53 (100.0)	93.2 - 100.0
Salmonella spp.	Unpreserved (Fresh)	53	1/1 (100.0)	20.7 - 100.0	52/52 (100.0)	93.1 - 100.0
Shigella/ EIEC	Unpreserved (Fresh)	53	2/2 (100.0)	34.2 - 100.0	51/51 (100.0)	93.0 - 100.0
Vibrio parahaemolyticus	Unpreserved (Fresh)	53	N/A	N/A	53/53 (100.0)	93.2 - 100.0
Vibrio spp. (not *parahaemolyticus*)	Unpreserved (Fresh)	53	N/A	N/A	53/53 (100.0)	93.2 - 100.0
Yersinia enterocolitica	Unpreserved (Fresh)	53	N/A	N/A	53/53 (100.0)	93.2 - 100.0
Cryptosporidium spp.	Unpreserved (Fresh)	53	N/A	N/A	53/53 (100.0)	93.2 - 100.0
Entamoeba histolytica	Unpreserved (Fresh)	53	N/A	N/A	53/53 (100.0)	93.2 - 100.0
Giardia lamblia	Unpreserved (Fresh)	53	N/A	N/A	53/53 (100.0)	93.2 - 100.0
Adenovirus 40/41	Unpreserved (Fresh)	53	1/1 (100.0)	20.7 - 100.0	52/52 (100.0)	93.1 - 100.0
Norovirus (GI/GII)	Unpreserved (Fresh)	53	1/1 (100.0)	20.7 - 100.0	52/52 (100.0)	93.1 - 100.0
Rotavirus A	Unpreserved (Fresh)	53	N/A	N/A	53/53 (100.0)	93.2 - 100.0
Combined Targets	Unpreserved (Fresh)	902	37/37 (100.0)	90.6 – 100.0	864/865 (99.9)	99.3– 100.0

Note. Fifty-four (54) fresh unpreserved *C. difficile* positives and negatives were tested. *C. difficile* positive sample TRI_047A tested *C. difficile* positive but was invalid for all other targets (due to invalid

RNA IC, invalid results not calculated in negative agreement). Sample remained invalid after reflex testing per BioCode GPP IFU.
a. *C. difficile* negative sample TRI_017A had a MFI of 516 (>500 cutoff) for *Campylobacter* spp. The sample was *Campylobacter* spp negative after being retested twice with each extraction system.
b. Sample TRI_056A resulted in a false positive agreement and remained false positive after discordant analysis.

Table 15. KingFisher Flex: Summary of Contrived Specimen Results

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
Vibrio parahaemolyticus	Unpreserved (Frozen)	115	26/28 (92.9%)	77.4-98.0%	86/87 (98.9%)	93.8-99.8%
Vibrio spp. (not *parahaemolyticus*)	Unpreserved (Frozen)	115	29/30 (96.7%)	83.3-99.4%	82/85 (96.5%)	90.1-98.8%
Yersinia enterocolitica [a]	Unpreserved (Frozen)	116	28/28 (100%)	87.9-100.0%	88/88 (100%)	95.8%-100.0%
Entamoeba histolytica [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 (120) contrived samples were tested. Five (5) samples were invalid with the easyMAG on initial testing. When the invalid samples were tested again with PCR, the samples remained invalid.
a- Of the five (5) invalids, one (1) of the *E. histolytica* positives was tested E. his positive but RNA IC was invalid.
b- Of the five (5) invalids, one (1) of the *Yersinia enterocolitica* positives was tested *Yersinia enterocolitica,* but RNA IC was invalid.
c - One (1) of the negative stools being used as matrix for contriving samples was a low-positive *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.

B. Method Comparison Study between EasyMag and KingFisher Apex Dx

Table 16. KingFisher Apex Dx: Summary of Clinical Investigational Study Results (Archived Specimens) Stratified by Sample Type and Storage

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
Campylobacter spp.[a]	Inoculated Cary-Blair	210	18/18 (100)	82.4 – 100	192/192 (100)	98.0 – 100
	Unpreserved (Frozen)	249	24/25 (96.0)	80.5 – 99.3	221/224 (98.7)	96.1 – 99.5
	All Archived	459	42/43 (97.7)	87.9 – 99.6	413/416 (99.3)	97.9 – 99.8
Clostridium difficile [b]	Inoculated Cary-Blair	211	34/37 (91.9)	78.7 – 97.2	173/174 (99.4)	96.8 – 99.9
	Unpreserved (Frozen)	249	17/19 (89.5)*	68.6 – 97.1	229/230 (99.6)	97.6 – 99.9
	All Archived	460	51/56 (91.1)	80.7 – 96.1	402/404 (99.5)	98.2 – 99.9
E. coli O157 [c]	Inoculated Cary-Blair	210	4/5 (80.0)*	37.6 – 96.4	205/205 (100)	98.2 – 100
	Unpreserved (Frozen)	249	8/8 (100)	67.6 – 100	240/241 (99.6)	97.7 – 99.9
	All Archived	459	12/13 (92.3)	66.7 – 98.6	445/446 (99.8)	98.7 – 100
Enteroaggregative *E. coli* (EAEC) [d]	Inoculated Cary-Blair	211	30/30 (100)	88.6 – 100	181/181 (100)	97.9 – 100
	Unpreserved (Frozen)	249	22/22 (100)	85.1 – 100	226/227 (99.6)	97.5 – 99.9
	All Archived	460	52/52 (100)	93.1 – 100	407/408 (99.8)	98.6 – 100
Enterotoxigenic *E. coli* (ETEC) [e]	Inoculated Cary-Blair	210	14/14 (100)	78.5 – 100	194/196 (99.0)	96.4 – 99.7
	Unpreserved (Frozen)	249	11/11 (100)	74.1 – 100	237/238 (99.6)	97.7 – 99.9
	All Archived	459	25/25 (100)	86.7 – 100	431/434 (99.3)	98.0 – 99.8
Shiga toxin–producing *E. coli* (STEC) [f]	Inoculated Cary-Blair	210	12/12 (100)	75.8 – 100	198/198 (100)	98.1 – 100
	Unpreserved (Frozen)	249	20/22 (90.9)	72.2 – 97.5	226/227 (99.6)	97.5 – 99.9
	All Archived	459	32/34 (94.1)	80.9 – 98.4	424/425 (99.8)	98.7 – 100
Salmonella spp. [g]	Inoculated Cary-Blair	210	20/20 (100)	83.9 – 100	187/190 (98.4)	95.5 – 99.5

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
	Unpreserved (Frozen)	249	21/21 (100)	84.5 – 100	228/228 (100)	98.3 – 100
	All Archived	459	41/41 (100)	91.4 – 100	415/418 (99.3)	97.9 – 99.8
Shigella/ EIEC [h]	Inoculated Cary-Blair	211	13/13 (100)	77.2 – 100	194/198 (98.0)	94.9 – 99.2
	Unpreserved (Frozen)	249	17/18 (94.4)	74.2 – 99.0	231/231 (100)	98.4 – 100
	All Archived	460	30/31 (96.8)	83.8 – 99.4	425/429 (99.1)	97.6 – 99.6
Vibrio parahaemolyticus [i]	Inoculated Cary-Blair	210	1/1 (100)	20.7 – 100	208/209 (99.5)	97.3 – 99.9
	Unpreserved (Frozen)	249	1/1 (100)	20.7 – 100	248/248 (100)	98.5 – 100
	All Archived	459	2/2 (100)	34.2 – 100	456/457 (99.8)	98.8 – 100
Vibrio spp. [j] (not *parahaemolyticus*)	Inoculated Cary-Blair	210	N/A	N/A	209/210 (99.5)	97.4 – 99.9
	Unpreserved (Frozen)	249	1/2 (50%)*	9.5 – 90.5	247/247 (100)	98.4 – 100
	All Archived	459	1/2 (50%)*	9.5 – 90.5	456/457 (99.8)	98.8 – 100
Yersinia enterocolitica [k]	Inoculated Cary-Blair	210	3/3 (100)	43.9 – 100	206/207 (99.5)	97.3 – 99.9
	Unpreserved (Frozen)	249	3/3 (100)	43.9 – 100	245/246 (99.6)	97.7 – 99.9
	All Archived	459	6/6 (100)	61.0 – 100	451/453 (99.6)	98.4 – 99.9
Cryptosporidium spp. [l]	Inoculated Cary-Blair	210	9/9 (100)	70.1 – 100	201/201 (100)	98.1 – 100
	Unpreserved (Frozen)	250	17/18 (94.4)	74.2 – 99.0	231/232 (99.6)	97.6 – 99.9
	All Archived	460	26/27 (96.3)	81.7 – 99.3	432/433 (99.8)	98.7 – 100
Entamoeba histolytica [m]	Inoculated Cary-Blair	210	N/A	N/A	210/210 (100)	98.2 – 100
	Unpreserved (Frozen)	249	N/A	N/A	249/249 (100)	98.5 – 100
	All Archived	459	N/A	N/A	459/459 (100)	99.1 – 100
Giardia lamblia [n]	Inoculated Cary-Blair	210	4/4 (100)	51.0 – 100	206/206 (100)	98.2 – 100

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
	Unpreserved (Frozen)	249	10/11 (90.9)	62.3 – 98.4	238/238 (100)	98.4 – 100
	All Archived	459	14/15 (93.3)	70.2 – 98.8	444/444 (100)	99.1 – 100
Adenovirus 40/41 [o]	Inoculated Cary-Blair	210	5/5 (100)	56.6 – 100	204/205 (99.5)	97.3 – 99.9
	Unpreserved (Frozen)	249	7/8 (87.5)*	52.9 – 97.8	240/241 (99.6)	97.7 – 99.9
	All Archived	459	12/13 (92.3)	66.7 – 98.6	444/446 (99.6)	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)	250	20/20 (100)	83.9 – 100	230/230 (100)	98.4 – 100
	All Archived	460	42/43 (97.7)	87.9 – 99.6	417/417 (100)	99.1 – 100
Rotavirus A [q]	Inoculated Cary-Blair	211	10/10 (100)	72.2 – 100	201/201 (100)	98.1 – 100
	Unpreserved (Frozen)	249	9/9 (100)	70.1 – 100	240/240 (100)	98.4 – 100
	All Archived	460	19/19 (100)	83.2 – 100	441/441 (100)	99.1 – 100
Combined Targets	Inoculated Cary-Blair	3512	216/218 (99.1)	96.7 – 99.7	3280/3294 (99.6)	99.3 – 99.7
	Unpreserved (Frozen)	4235	208/218 (95.4)	91.8 – 97.5	4006/4017 (99.7)	99.5 – 99.8

*Positive agreement <90%.

Forty (40) archived samples with discordant results (between the easyMAG and KingFisher Apex Dx) retested twice with both easyMAG and KingFisher Apex Dx.

a - *Campylobacter* spp. Two (2) false positives retested remained false positives whereas another false positive became true negative. One (1) false negative became true positive. Nine (9) samples were still invalid, not used in the agreement calculation.

b - *Clostridium difficile.* Of the five (5) false positives retested, three (3) became true negatives, one (1) remained false negative, and one (1) became invalid due to invalid RNA IC with the 2nd and 3rd runs with both extraction systems. Two (2) false positives became true negatives. Eight (8) samples were still invalid, not used in the agreement calculation.

c – O157. One (1) false negative and one (1) false positive retested remained false negative and false positive.

d- EAEC: One (1) false positive retested became true negative. Eight (8) samples were still invalid, not used in the agreement calculation.

e – ETEC: Of the three (3) false positives retested, one (1) became true negative, and two (2) became true positives. Nine (9) samples were invalid, not used in the agreement calculation.

f- STEC: Of the two (2) false negatives retested, one (1) became true negative and one (1) became false positive. One (1) false positive retested remained false positive. Nine (9) samples were invalid, not used in the agreement calculation.

g - *Salmonella* spp: Of the three (3) false positives retested, two (2) became true negatives, and remained false positive. Nine (9) samples were invalid, not used in the agreement calculation.
h - *Shigella/EIEC:* Of the four (4) false positives retested, three (3) became true negatives whereas one (1) became false negative. One (1) false negative retested became true negative. Eight (8) samples were invalid, not used in the agreement calculation.
i- *Vibrio parahaemolyticus*: One (1) false positive retested became true negative. Nine (9) samples were invalid, not used in the agreement calculation.
j– *Vibrio* spp. One (1) false positive retested became true negative. 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 KF Apex Dx had negative results with both retested runs. Nine (9) samples were invalid, not used in the agreement calculation.
k – *Yersinia enterocolitica:* Of the two (2) false positives retested, one (1) became true negative and one (1) remained false positive. Nine (9) samples were invalid, not used in the agreement calculation.
l - *Cryptosporidium* spp: One (1) false positive retested became true negative. One (1) false negative retested remained false negative. Eight (8) samples were invalid, not used in the agreement calculation.
m- *Entamoeba histolytica*: Nine (9) samples were still invalid, not used in the agreement calculation.
n - *Giardia lamblia*: One (1) false negative was not retested due to insufficient volume of the sample. Nine (9) samples were invalid, not used in the agreement calculation.
o - Adenovirus 40/41: Of the wo (2) false positives retested, one (1) became true negative and one (1) became true positive. The false negative retested became true negative. Nine (9) samples were still invalid, not used in the agreement calculation.
p – Norovirus GI/GII: The false negative retested became true positive. Eight (8) samples were invalid, not used in the agreement calculation.
q- Rotavirus A: Eight (8) samples were invalid, not used in the agreement calculation.

The table below (**Table 17**) shows the results of retesting samples with discordant results between the easyMAG and the KingFisher Apex Dx. Samples were retested twice with both easyMag and KingFisher Apex Dx. Final results of the discordant samples were not used in the calculation of the agreements but noted as the footnotes of **Table 16** above.

Table 17. KingFisher Apex Dx: Retested Results of Discordant Specimens (Archived Specimens)

	Target	Sample Name	Sample Type	Extraction	Run 1 (Original Run)	Run 2	Run 3	Final Result
a	*Campylobacter spp*	01-0237	Unpreserved (Frozen)	easyMAG	Det	Det	Det	Det
				KingFisher Apex Dx	Ndet	Det	Det	Det
		03-0153	Unpreserved (Frozen)	easyMAG	Ndet	Ndet	Det	Ndet
				KingFisher Apex Dx	Det	Det	Ndet	Det
		Mayo-GI_101	Unpreserved (Frozen)	easyMAG	Ndet	Det	Ndet	Ndet
				KingFisher Apex Dx	Det	Det	Det	Det
		SP517	Unpreserved (Frozen)	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Apex Dx	Det	Ndet	Ndet	Ndet
b	*Clostridium difficile*	01-0002	Unpreserved (Frozen)	easyMAG	Det	Ndet	Ndet	Ndet
				KingFisher Apex Dx	Ndet	Det	Ndet	Ndet
		01-0019	Inoculated Cary-Blair	easyMAG	Det	Det	Ndet	Det
				KingFisher Apex Dx	Ndet	Ndet	Ndet	Ndet
		01-0048	Inoculated Cary-Blair	easyMAG	Det	Ndet	Ndet	Ndet
				KingFisher Apex Dx	Ndet	Ndet	Ndet	Ndet
		01-0256	Inoculated Cary-Blair	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Apex Dx	Det	Ndet	Ndet	Ndet
		01-0373	Unpreserved (Frozen)	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Apex Dx	Det	Ndet	Ndet	Ndet
		02-0009	Unpreserved (Frozen)	easyMAG	Det	Ndet	Ndet	Ndet
				KingFisher Apex Dx	Ndet	Ndet	Ndet	Ndet
		02-0135	Inoculated Cary-Blair	easyMAG	Det	Invalid	Invalid	Invalid
				KingFisher Apex Dx	Ndet	Invalid	Invalid	Invalid
c	O157	06-0180	Cary-Blair	easyMAG	Det	Det	Ndet	Det
				KingFisher Apex Dx	Ndet	Ndet	Det	Ndet
		SP367	Unpreserved (Frozen)	easyMAG	Ndet	Ndet	Det	Ndet
				KingFisher Apex Dx	Det	Det	Ndet	Det
d	EAEC	01-0392		easyMAG	Ndet	Ndet	Ndet	Ndet

	Target	Sample Name	Sample Type	Extraction	Run 1 (Original Run)	Run 2	Run 3	Final Result
			Unpreserved (Frozen)	KingFisher Apex Dx	Det	Ndet	Ndet	Ndet
e	ETEC	01-0374	Unpreserved (Frozen)	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Apex Dx	Det	Ndet	Ndet	Ndet
		06-0211	Cary-Blair	easyMAG	Ndet	Det	Det	Det
				KingFisher Apex Dx	Det	Det	Det	Det
		06-0305	Cary-Blair	easyMAG	Ndet	Det	Det	Det
				KingFisher Apex Dx	Det	Ndet	Det	Det
f	STEC	02-0211	Unpreserved (Frozen)	easyMAG	Det	Ndet	Ndet	Ndet
				KingFisher Apex Dx	Ndet	Ndet	Ndet	Ndet
		SP228	Unpreserved (Frozen)	easyMAG	Det	Ndet	Ndet	Ndet
				KingFisher Apex Dx	Ndet	Det	Det	Det
		SP367	Unpreserved (Frozen)	easyMAG	Ndet	Det	Ndet	Ndet
				KingFisher Apex Dx	Det	Det	Ndet	Det
g	*Salmonella spp*	01-0254	Inoculated Cary-Blair	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Apex Dx	Det	Ndet	Ndet	Ndet
		01-0302	Inoculated Cary-Blair	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Apex Dx	Det	Ndet	Ndet	Ndet
		06-0357	Cary-Blair	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Apex Dx	Det	Det	Ndet	Det
h	*Shigella/ EIEC*	01-0091	Inoculated Cary-Blair	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Apex Dx	Det	Ndet	Ndet	Ndet
		01-0267	Inoculated Cary-Blair	easyMAG	Ndet	Det	Det	Det
				KingFisher Apex Dx	Det	Ndet	Ndet	Ndet
		01-0377	Inoculated Cary-Blair	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Apex Dx	Det	Ndet	Ndet	Ndet
		02-0297	Unpreserved (Frozen)	easyMAG	Det	Det	Det	Det
				KingFisher Apex Dx	Ndet	Det	Det	Det
		03-0206	Inoculated Cary-Blair	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Apex Dx	Det	Ndet	Ndet	Ndet
l	*Vibrio parahaemolyticus*	03-0358	Inoculated Cary-Blair	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Apex Dx	Det	Ndet	Ndet	Ndet

	Target	Sample Name	Sample Type	Extraction	Run 1 (Original Run)	Run 2	Run 3	Final Result
m	*Vibrio spp*	01-0278	Inoculated Cary-Blair	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Apex Dx	Det	Ndet	Ndet	Ndet
		02-0167	Unpreserved (Frozen)	easyMAG	Det	Invalid	Ndet	IND
				KingFisher Apex Dx	Ndet	Ndet	Ndet	Ndet
n	Yersinia	02-0261	Unpreserved (Frozen)	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Apex Dx	Det	Ndet	Ndet	Ndet
		06-0222	Cary-Blair	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Apex Dx	Det	Det	Det	Det
o	*Cryptosporidium* spp	04-047	Unpreserved (Frozen)	easyMAG	Ndet	Det	Det	Det
				KingFisher Apex Dx	Det	Ndet	Det	Det
		SP399	Unpreserved (Frozen)	easyMAG	Det	Det	Ndet	Det
				KingFisher Apex Dx	Ndet	Ndet	Det	Ndet
p	*Giardia lamblia*	03-0140*	Unpreserved (Frozen)	easyMAG	Det	N/A	N/A	Det
				KingFisher Apex Dx	Ndet	N/A	N/A	Ndet
q	Adenovirus 40/41	01-0079	Unpreserved (Frozen)	easyMAG	Ndet	Ndet	Ndet	Ndet
				KingFisher Apex Dx	Det	Ndet	Ndet	Ndet
		01-0375	Unpreserved (Frozen)	easyMAG	Det	Ndet	Ndet	Ndet
				KingFisher Apex Dx	Ndet	Ndet	Ndet	Ndet
		SP418	Inoculated Cary-Blair	easyMAG	Ndet	Det	Det	Det
				KingFisher Apex Dx	Det	Det	Det	Det
r	Norovirus GI/GII	01-0290	Inoculated Cary-Blair	easyMAG	Det	Det	Ndet	Det
				KingFisher Apex Dx	Ndet	Det	Det	Det

* Insufficient Volume of Sample; IND: Indeterminant; Det: Detected; Ndet: Not Detected

Twenty-two (22) archived samples with invalid results (invalid internal control) were subjected to reflex testing per BioCode® GPP IFU. After reflex testing, thirteen (13) samples were valid, and nine (9) samples were still invalid, as shown in **Table 18** below. Final results were used to calculate the agreement reported or noted in the footnote of **Table 16**.

Table 18. KingFisher Apex Dx: Results of Reflex Testing of Invalid Samples (Archived Samples)

Sample Name	Sample Type	Instrument	Run 1	Run 2	Run 3	Final Result
01-0035	Unpreserved (Frozen)	easyMAG	IC Invalid	IC Valid	N/A	Valid
02-0167	Unpreserved (Frozen)	easyMAG	IC Invalid	IC Valid	N/A	Valid
03-0097	Unpreserved (Frozen)	easyMAG	IC Invalid	IC Invalid	N/A	Invalid
06-0023	Cary-Blair	easyMAG	IC Invalid	IC Invalid	N/A	Invalid
		KingFisher Apex Dx	IC Invalid	IC Valid	N/A	Valid
06-0119	Cary-Blair	easyMAG	IC Invalid	IC Invalid	N/A	Invalid
		KingFisher Apex Dx	IC Invalid	IC Invalid	N/A	Invalid
06-0223	Cary-Blair	easyMAG	IC Invalid	IC Invalid	N/A	Invalid
06-0258	Cary-Blair	easyMAG	IC Invalid	IC Valid	N/A	Valid
06-0331	Cary-Blair	easyMAG	IC Invalid	IC Valid	N/A	Valid
Stool_0112	Unpreserved (Frozen)	easyMAG	IC Invalid	IC Valid	N/A	Valid
Mayo-GI_107	Inoculated Cary-Blair	easyMAG	IC Invalid	IC Valid	N/A	Valid
		KingFisher Apex Dx	IC Invalid	IC Valid	N/A	Valid
Mayo-GI_117	Inoculated Cary-Blair	easyMAG	IC Invalid	IC Valid	N/A	Valid
Mayo-GI_123	Inoculated Cary-Blair	easyMAG	IC Invalid	IC Valid	N/A	Valid
Mayo-GI_126	Inoculated Cary-Blair	easyMAG	IC Invalid	IC Valid	N/A	Valid
SP392	Inoculated Cary-Blair	easyMAG	IC Invalid	IC Valid	N/A	Valid
		KingFisher Apex Dx	IC Invalid	IC Valid	N/A	Valid
ST-074	Inoculated Cary-Blair	easyMAG	IC Invalid	IC Valid	N/A	Valid
01-0003	Unpreserved (Frozen)	KingFisher Apex Dx	IC Invalid	IC Invalid	N/A	Invalid
02-0288	Unpreserved (Frozen)	KingFisher Apex Dx	IC Invalid	IC Invalid	N/A	Invalid
02-0310	Unpreserved (Frozen)	KingFisher Apex Dx	IC Invalid	IC Valid	N/A	Valid
01-0073	Inoculated Cary-Blair	KingFisher Apex Dx	IC Invalid	IC Invalid	N/A	Invalid
Stool_0163	Unpreserved (Frozen)	KingFisher Apex Dx	IC Invalid	IC Valid	N/A	Valid
01-0238	Unpreserved (Frozen)	KingFisher Apex Dx	IC Invalid	IC Invalid	N/A	Invalid
01-0379	Unpreserved (Frozen)	KingFisher Apex Dx	IC Invalid	IC Invalid	N/A	Invalid
Mayo-GI_059	Unpreserved (Frozen)	KingFisher Apex Dx	IC Invalid	IC Valid	N/A	Valid

Table 19. KingFisher Apex Dx: Summary of Clinical Investigational Study Results of Fresh *Clostridium difficile* Positives and Negatives

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
Campylobacter spp.[a]	Unpreserved (Fresh)	52	1/1 (100.0)	20.7 - 100.0	51/51 (100.0)	93.0 - 100.0
Clostridium difficile[b]	Unpreserved (Fresh)	54	29/30 (96.7)	83.3 – 99.4	24/24 (100.0)	86.2 - 100.0
E. coli O157	Unpreserved (Fresh)	52	N/A	N/A	52/52 (100.0)	93.1 - 100.0
Enteroaggregative E. coli (EAEC)	Unpreserved (Fresh)	52	1/1 (100.0)	20.7 - 100.0	50/51 (98.0)	89.7 - 99.7
Enterotoxigenic E. coli (ETEC)	Unpreserved (Fresh)	52	N/A	N/A	52/52 (100.0)	93.1 - 100.0
Shiga toxin-producing E. coli (STEC)	Unpreserved (Fresh)	52	N/A	N/A	52/52 (100.0)	93.1 - 100.0
Salmonella spp.	Unpreserved (Fresh)	52	1/1 (100.0)	20.7 - 100.0	51/51 (100.0)	93.0 - 100.0
Shigella/ EIEC	Unpreserved (Fresh)	52	2/2 (100.0)	34.2 - 100.0	50/50 (100.0)	52.9 - 100.0
Vibrio parahaemolyticus	Unpreserved (Fresh)	52	N/A	N/A	52/52 (100.0)	93.1 - 100.0
Vibrio spp. (not parahaemolyticus)	Unpreserved (Fresh)	52	N/A	N/A	52/52 (100.0)	93.1 - 100.0
Yersinia enterocolitica	Unpreserved (Fresh)	52	N/A	N/A	52/52 (100.0)	93.1 - 100.0
Cryptosporidium spp.	Unpreserved (Fresh)	52	N/A	N/A	52/52 (100.0)	93.1 - 100.0
Entamoeba histolytica	Unpreserved (Fresh)	52	N/A	N/A	52/52 (100.0)	93.1 - 100.0
Giardia lamblia	Unpreserved (Fresh)	52	N/A	N/A	52/52 (100.0)	93.1 - 100.0
Adenovirus 40/41	Unpreserved (Fresh)	52	1/1 (100.0)	20.7 - 100.0	51/51 (100.0)	93.0 - 100.0
Norovirus (GI/GII)	Unpreserved (Fresh)	52	1/1 (100.0)	20.7 - 100.0	51/51 (100.0)	93.0 - 100.0
Rotavirus A	Unpreserved (Fresh)	52	N/A	N/A	52/52 (100.0)	93.1 - 100.0
Combined Targets	Unpreserved (Fresh)	886	36/37 (97.3)	86.2 – 99.5	848/849 (99.9)	99.3 – 100.0

Note. Fifty-four (54) fresh unpreserved samples were tested. Two (2) *C. difficile* positives (TRI_047A and TRI_059A) were tested *C. difficile* positive but invalid for all other targets (due to invalid RNA IC, invalid results not calculated in negative agreement). Samples remained invalid after reflex testing per GPP IFU.

a. *C. difficile* negative sample TRI_017A had a MFI of 516 (>500 cutoff) for *Campylobacter* spp. The sample was *Campylobacter* spp negative after being retested twice with each extraction system.

b. *C. difficile* positive sample TRI_011A initially tested as false negative resulted in a true positive after retesting. The Xpert *C. difficile*-Epi test results of TRI- 011A had Ct 34.8 which indicates the sample was low positive.

Table 20. KingFisher Apex Dx: Summary of Contrived Specimen Results (Contrived at 3x LoD and 6x LoD)

Target	Specimen Type	(n)	Positive Agreement		Negative Agreement	
			PPA (%)	95% CI	NPA (%)	95% CI
Vibrio parahaemolyticus	Unpreserved (Frozen)	115	28/28 (100%)	87.9-100.0%	86/87 (98.9%)	93.8-99.8%
Vibrio spp. (not *parahaemolyticus*)	Unpreserved (Frozen)	115	29/30 (96.7%)	83.3-99.4%	84/85 (98.8%)	93.6-99.8%
Yersinia enterocolitica [a]	Unpreserved (Frozen)	116	28/28 (100%)	87.9-100.0%	88/88 (100%)	95.8%-100.0%
Entamoeba histolytica [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	1489/1491 (99.9%)	99.5-100.0%
Combined Targets	Unpreserved (Frozen)	1953	113/114 (99.1%)	95.2 – 99.8%	1833/1839 (99.7%)	99.3 – 99.9%

Note. One hundred twenty (120) contrived samples were tested. Five (5) samples were invalid with the easyMAG on initial testing. When the invalid samples were tested again with PCR, the samples remained invalid.

a- Of the five (5) invalids, one of the *E. histolytica* positives was tested E. his positive but RNA IC was invalid.

b- Of the five (5) invalids, one of the *Yersinia enterocolitica* positives was tested *Yersinia enterocolitica,* but RNA IC was invalid.

c - One (1) of negative stools being used as matrix for contriving samples was a low-positive *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.

Table 21. Clinical Co-infection Summary of Archived Samples

Co-infection summary	
+1 Target	246/466 (52.8%)
+2 Targets	78/466 (16.7%)
+3 Targets	13/466 (2.8%)

Conclusions [807.92(b)(3):

The intended use and fundamental scientific technology of the BioCode GPP using the KingFisher Flex and KingFisher Apex Dx is substantially equivalent to the predicate device. Non-clinical studies and clinical studies have established that the BioCode GPP with the KingFisher Flex and KingFisher Apex Dx is substantially equivalent to the predicate device.