510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number: K140407

B. Purpose for Submission:

To obtain clearance for the FilmArray® Gastrointestinal (GI) Panel microorganism multiplex nucleic acid-based assay.

C. Measurand:

Campylobacter (*C. jejuni*/*C. coli*/*C. upsaliensis*), *Clostridium difficile* (*C. difficile*) toxin A/B , *Plesiomonas shigelloides, Salmonella, Vibrio* (*V. parahaemolyticus/V. vulnificus/ V. cholerae*), including specific identification of *Vibrio cholerae,Yersinia enterocolitica*, Enteroaggregative *Escherichia coli* (EAEC), Enteropathogenic *Escherichia coli* (EPEC), Enterotoxigenic *Escherichia coli* (ETEC) *lt/st*, Shiga-like toxin-producing *Escherichia coli* (STEC) *stx1/stx2* (including specific identification of the *E. coli* O157 serogroup within STEC), *Shigella/* Enteroinvasive *Escherichia coli* (EIEC), *Cryptosporidium, Cyclospora cayetanensis, Entamoeba histolytica, Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*), Adenovirus F 40/41, Astrovirus, Norovirus GI/GII, Rotavirus A, Sapovirus (Genogroups I, II, IV, and V)

D. Type of Test:

A multiplexed nucleic acid-based test intended for use with the FilmArray® instrument for the qualitative *in vitro* detection and identification of multiple bacteria, viruses, and parasites. The FilmArray GI Panel assay is performed directly from stool samples in Cary Blair transport media.

E. Applicant:

BioFire Diagnostics, LLC.

F. Proprietary and Established Names:

FilmArray Gastrointestinal (GI) Panel

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3990 – Gastrointestinal microorganism multiplex nucleic acid-based assay

2. Classification:

   Class II

3. Product code:

   PCH, OOI

4. Panel:

   83 (Microbiology)

H. Intended Use:

1. Intended use(s):

   The FilmArray Gastrointestinal (GI) Panel is a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test intended for use with the FilmArray Instrument. The FilmArray GI Panel is capable of the simultaneous detection and identification of nucleic acids from multiple bacteria, viruses, and parasites directly from stool samples in Cary Blair transport media obtained from individuals with signs and/or symptoms of gastrointestinal infection. The following bacteria (including several diarrheagenic *E. coli/Shigella* pathotypes), parasites, and viruses are identified using the FilmArray GI Panel:

   - *Campylobacter* (*C. jejuni/C. coli/C. upsaliensis*)
   - *Clostridium difficile* (*C. difficile*) toxin A/B
   - *Plesiomonas shigelloides*
   - *Salmonella*
   - *Vibrio* (*V. parahaemolyticus/V. vulnificus/ V. cholerae*), including specific identification of *Vibrio cholerae*
   - *Yersinia enterocolitica*
   - Enteroaggregative *Escherichia coli* (EAEC)
   - Enteropathogenic *Escherichia coli* (EPEC)
   - Enterotoxigenic *Escherichia coli* (ETEC) *lt/st*
   - Shiga-like toxin-producing *Escherichia coli* (STEC) *stx1/stx2* (including specific identification of the *E. coli* O157 serogroup within STEC)
   - *Shigella*/ Enteroinvasive *Escherichia coli* (EIEC)
   - *Cryptosporidium*
   - *Cyclospora cayetanensis*
   - *Entamoeba histolytica*
   - *Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*)
   - Adenovirus F 40/41
   - Astrovirus
   - Norovirus GI/GII
   - Rotavirus A
   - Sapovirus (Genogroups I, II, IV, and V)

The FilmArray GI Panel 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. Positive results do not rule out co-infection with organisms not included in the FilmArray GI Panel. The agent detected may not be the definite cause of the 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 *E. coli* O157, *Plesiomonas shigelloides*, *Yersinia enterocolitica,* Astrovirus, and Rotavirus A were established primarily with retrospective clinical specimens.

Performance characteristics for *Entamoeba histolytica,* and *Vibrio* (*V. parahaemolyticus, V. vulnificus,* and *Vibrio cholerae*) were established primarily using contrived clinical specimens.

Negative FilmArray GI Panel 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.

A gastrointestinal microorganism multiplex nucleic acid-based assay also aids in the detection and identification of acute gastroenteritis in the context of outbreaks.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

For use with the FilmArray instrument

I. Device Description:

The FilmArray Gastrointestinal (GI) Panel is a multiplex nucleic acid test designed to be used with the FilmArray Instrument. The FilmArray GI pouch contains freeze-dried reagents to perform nucleic acid purification and nested, multiplex PCR with DNA melt analysis. The FilmArray Gastrointestinal (GI) Panel simultaneously conducts 22 tests for the identification of GI pathogens from stool specimens collected in Cary Blair transport

medium (see table below). Results from the FilmArray GI Panel test are available within about one hour.

Table – Organisms Detected by the FilmArray GI Panel

Bacteria	**Viruses**
Campylobacter (*C. jejuni/C. coli/C. upsaliensis*)	Adenovirus F 40/41
Clostridium difficile (toxin A/B)	Astrovirus
Plesiomonas shigelloides	Norovirus GI/GII
Salmonella	Rotavirus A
Vibrio (V. parahaemolyticus/V. vulnificus/V. cholerae)	Sapovirus (Genogroups I, II, IV, and V)
Vibrio cholera	
Yersinia enterocolitica	
Diarrheagenic *E. coli/Shigella*	**Parasites**
Enteroaggregative *E. coli* (EAEC)	*Cryptosporidium*
Enteropathogenic *E. coli* (EPEC)	*Cyclospora cayetanensis*
Enterotoxigenic *E. coli* (ETEC) *lt/st*	*Entamoeba histolytica*
Shiga toxin-producing *E. coli* (STEC) *stx1/stx2*	*Giardia lamblia*
E. coli O157	
*Shigella/*Enteroinvasive *E. coli* (EIEC)	

A test is initiated by loading Hydration Solution into one port of the FilmArray pouch and a stool sample (in Cary Blair transport medium) mixed with the provided Sample Buffer into the other port of the FilmArray GI pouch and placing it in the FilmArray Instrument. The pouch contains all of the reagents required for specimen testing and analysis in a freeze-dried format; the addition of Hydration Solution and Sample/Buffer Mix rehydrates the reagents. After the pouch is prepared, the FilmArray Software guides the user though the steps of placing the pouch into the instrument, scanning the pouch barcode, entering the sample identification, and initiating the run.

The FilmArray Instrument contains a coordinated system of inflatable bladders and seal points, which act on the pouch to control the movement of liquid between the pouch blisters. When a bladder is inflated over a reagent blister, it forces liquid from the blister into connecting channels. Alternatively, when a seal is placed over a connecting channel it acts as a valve to open or close a channel. In addition, electronically controlled pneumatic pistons are positioned over multiple plungers in order to deliver the rehydrated reagents into the blisters at the appropriate times. Two Peltier devices control heating and cooling of the pouch to drive the PCR reactions and the melt curve analysis.

Nucleic acid extraction occurs within the FilmArray pouch using mechanical and chemical lysis followed by purification using standard magnetic bead technology. After extracting and purifying nucleic acids from the unprocessed sample, the FilmArray performs a nested multiplex PCR that is executed in two stages. During the first stage, the FilmArray performs a single, large volume, highly multiplexed reverse transcription PCR (rt-PCR) reaction. The products from first stage PCR are then diluted and combined with a fresh, primer-free master mix and a fluorescent double stranded DNA binding dye (LC Green® Plus, BioFire). The solution is then distributed to each well of the array. Array wells contain sets of primers designed specifically to amplify sequences internal to the PCR

products generated during the first stage PCR reaction. The 2nd stage PCR, or nested PCR, is performed in singleplex fashion in each well of the array. At the conclusion of the 2nd stage PCR, the array is interrogated by melt curve analysis for the detection of signature amplicons denoting the presence of specific targets. A digital camera placed in front of the 2nd stage PCR captures fluorescent images of the PCR reactions and software interprets the data.

The FilmArray Software automatically interprets the results of each DNA melt curve analysis and combines the data with the results of the internal pouch controls to provide a test result for each organism on the panel.

Materials provided in each kit:

- Individually packaged FilmArray GI Panel pouches
- Single-use (1.0 mL) Sample Buffer ampoules
- Single-use pre-filled (1.5 mL) Hydration Injection Vials (blue)
- Single-use Sample Injection Vials (red)
- Individually packaged Transfer Pipettes

Materials required but not provided: FilmArray System including:

- FilmArray Instrument
- FilmArray Pouch Loading Station compatible with the use of the FilmArray Injection Vials

Note: Previous versions of Pouch Loading Station should not be used with the FilmArray Injection Vials.

For many organisms detected by the FilmArray GI Panel, the organism is considered to be detected if a single corresponding assay is positive. For example, *Plesiomonas shigelloides* will have a result of "*Plesiomonas shigelloides* Detected" if at least two of the three replicates of the one *Plesiomonas shigelloides* assay have similar positive melt peaks with Tm values that are within the assay-specific Tm range. The following organisms are detected using a single assay: toxigenic *C. difficile*, *P. shigelloides*, *Salmonella*, *Y. enterocolitica*, EAEC, *Shigella/*EIEC, Adenovirus F 40/41, Astrovirus, Sapovirus (Genogroups I, II, IV, and V), *C. cayetanensis*, *E. histolytica* and *G. lamblia*.

In contrast, the test results for several organisms rely on the combination of multiple assays. These include *Campylobacter (C. jejuni/C. coli/C. upsaliensis)*, *Vibrio* (*V. parahaemolyticus/ V. vulnificus/V. cholerae)* and *Vibrio cholerae*, *Cryptosporidium*, Norovirus GI/GII, and Rotavirus A. The test results for several Diarrheagenic *E. coli*(s) include multiple assays for genetic markers to identify various classic pathotypes of *E. coli* including EPEC, ETEC, and STEC (including O157), (as well EAEC and *Shigella*/EIEC included above). Interpretation rules for these assays are described below. Also included are summary descriptions of the assays' expected reactivity; for a full description of assay reactivity see Inclusivity.

NOTE: As polymicrobial results with four or more distinct organisms in a single sample are unusual based upon data from the prospective clinical study, confirmation of this result is recommended to rule out any unexpected error, either caused by the user's handling of the sample or the test system. Polymicrobial results of four or more organisms were detected in less than 1% (12/1556) of the FilmArray GI prospective study specimens.

Bacteria

Campylobacter (*C. jejuni/C. coli/C. upsaliensis*): The FilmArray GI Panel contains two assays (Campy 1 and Campy 2) designed to together detect, but not differentiate, the most common *Campylobacter* species associated with human gastrointestinal illness: *C. jejuni, C. coli,* and *C. upsaliensis*. These are the same three species that are identified using standard clinical laboratory practices. Other *Campylobacter* species will not be identified by the FilmArray GI Panel. Empirical testing and *in silico* sequence analysis indicates reduced sensitivity for a less common subspecies of *C. jejuni* (*C. jejuni* subsp. *doylei*). A positive result for one or both assays will give a *Campylobacter* Detected test result.

Clostridium difficile toxin A/B: The FilmArray GI Panel contains a single multiplexed assay (Cdiff) for the identification of toxigenic *C. difficile* which targets both the toxin A gene (*tcdA*) and the toxin B gene (*tcdB*). Typical toxigenic strains produce both toxins, but the presence of either is indicative of a pathogenic strain. Empirical testing and in silico sequence analysis support that the assay will detect all toxinotypes and the epidemic BI/NAP1/027 hypervirulent strain, although these will not be specifically differentiated by the assay. Detection of either or both toxin genes by this assay gives a test result for *Clostridium difficile* toxin A/B Detected. As rates of asymptomatic carriage of *C. difficile* can be high in very young children and hospitalized patients, the detection of toxigenic C. difficile should be interpreted within the context of guidelines developed by the testing facility or other experts (e.g., guidelines/policy statements published by The American Academy of Pediatrics or the Society for Healthcare Epidemiology of America and the Infectious Disease Society of America).

Plesiomonas shigelloides: The FilmArray GI Panel contains a single assay (Pshig) for detection of *P. shigelloides*, the only known species in the genus *Plesiomonas*.

Salmonella: The FilmArray GI Panel contains a single assay (Salm) designed to detect both species of *Salmonella*; *S. enterica* and *S. bongori*. Empirical testing and *in silico* sequence analysis supports the ability to detect all subspecies and serovars of *Salmonella*. Cross-reactivity may occur with certain *E. coli* strains containing variants of the cryptic ETT2 type-III secretion system (see Inclusivity for additional information).

Vibrio (*V. parahaemolyticus/V. vulnificus/V. cholerae*) and *Vibrio cholerae:* The FilmArray GI Panel contains a single assay (Vibrio) for detection of *Vibrio* species most commonly implicated in gastroenteritis (*V. parahaemolyticus, V. vulnificus,* and *V. cholerae*). Empirical testing and *in silico* sequence analysis indicate that the assay may also react with some less common *Vibrio* species (i.e., *V. alginolyticus, V. fluvialis,* and *V. mimicus*). The Vibrio assay does not indicate which species has been detected and the Vibrio assay is not expected to detect the rarer *V. cincinnatiensis, V. furnissii* and *V.*

metschnikovii. A second assay (Vchol) is also included for the specific detection of *Vibrio cholerae*. A *Vibrio cholerae* Detected result will only be reported when the *V. cholerae*-specific assay is positive, while a positive result for either assay will give a *Vibrio* Detected test result (see table below).

Table – Possible Assay Results and Corresponding Vibrio Test Results

Film Array GI Interpretation	Vibrio (Vibrio Assay)	V.cholerae (Vchol Assay)	Description
Vibrio: Not Detected *Vibrio cholerae*: Not Detected	Negative	Negative	No *Vibrio* species detected
Vibrio: Detected *Vibrio cholerae*: Not Detected	Positive	Negative	*Vibrio* species detected (not *V. cholerae*)
Vibrio: Detected *Vibrio cholerae*: Detected	Any Results	Positive	*Vibrio cholerae* detected OR *Vibrio cholerae* and one or more other *Vibrio* species detected

Yersinia enterocolitica: The FilmArray GI Panel contains a single assay (Yent) designed to detect all known serotypes/biotypes of *Y. enterocolitica*. Empirical testing and *in silico* sequence analysis indicate a potential for cross-reactivity with *Y. kristensenii* and *Y. frederiksenii* when present at high levels (>10^8 CFU/mL). These two species are in the *Y. enterocolitica* group and are difficult to differentiate from *Y. enterocolitica* by culture methods; both are suspected human pathogens.

Diarrheagenic E. coli

The FilmArray GI Panel contains multiple assays designed to detect genetic determinants associated with classic diarrheagenic *E. coli*/*Shigella* pathotypes. Horizontal transfer of these genes between organisms has been documented; therefore, Detected results for multiple diarrheagenic *E. coli*/*Shigella* may be due to the presence of multiple pathotypes or a single strain containing the characteristic determinants of multiple pathotypes. An example of this is the 2011 *E. coli* O104:H4 outbreak strain that contains determinants of both Shiga-like toxin-producing *E. coli* (STEC) and Enteroaggregative *E. coli* (EAEC).

Enteroaggregative E. coli (EAEC): The FilmArray GI Panel contains a single multiplexed assay (EAEC) for the identification of two gene targets typically associated with enteroaggregative E. coli; the *aggR* regulatory gene and the putative outer membrane protein, *aatA*, both located on the partially-conserved pAA plasmid. pAA is not present in all strains phenotypically identified as EAEC, and not all pAA plasmids carry *aggR* and *aatA* genes; therefore the FilmArray GI Panel will not detect all members of this diverse pathotype, but is likely to detect most pathogenic strains (including *E. coli* O104:H4, which was responsible for recent outbreaks in Europe).

Enterotoxigenic (ETEC) heat-labile (lt) and heat-stable (st) Enterotoxins: The FilmArray GI Panel contains three assays (ETEC 1, ETEC 2, and ETEC 3) for the detection of enterotoxins found in Enterotoxigenic *E. coli* (ETEC). The assays are designed for the detection of heat-labile (LT) enterotoxin (*ltA*) and two heat-stable (ST) enterotoxin variants

(*st1a*, also known as STp; and *st1b*, also known as STh). The reported results do not indicate which of these toxin(s) have been detected. A positive result for any combination of the three assays will give an Enterotoxigenic *E. coli* (ETEC) *lt/st* Detected test result. The variant LT-II toxin (structurally similar to LT) and the STB/ST2 toxin (structurally dissimilar to ST1) are not targeted by the ETEC assays and have not been established as important in human disease. Empirical testing and *in silico* sequence analysis indicates the potential for cross-reactivity with certain strains of *Hafnia alvei*, *C. koseri*, *C. sedlakii*, and *Cedecea davisae*.

Enteropathogenic *E. coli* (EPEC): The FilmArray GI Panel contains a single assay (Ec *eae*) for the detection of *eae*, the gene encoding the adhesin intimin. Both typical and atypical EPEC will be detected, but not differentiated. The LEE pathogenicity island, which includes the *eae* gene, is also found in some Shiga-like toxin producing *E. coli* (STEC; O157 and non-O157 strains). Therefore, the results of the *eae* assay (positive or negative) are only reported when STEC is not detected. When STEC is detected, Enteropathogenic *E. coli* (EPEC) will be reported as N/A (Not Applicable), regardless of the EPEC assay result (see table below). Consequently, the FilmArray GI Panel cannot distinguish between STEC containing the *eae* gene and a co-infection of EPEC and STEC.

Shiga-like toxin-producing *E. coli* (STEC) Shiga-like toxin genes 1 and 2 (*stx1/stx2*): The FilmArray GI Panel contains two assays (STEC 1 and STEC 2) for the detection of Shiga-like toxin 1 (*stx1*) and Shiga-like toxin 2 (*stx2*) sequences. The reported results do not indicate which of these toxin(s) have been detected. A positive result for either or both of these assays will give a Shiga-like toxin-producing *E. coli* (STEC) *stx1*/*stx2* detected test result (see table below). Shiga toxin (stx; identical to stx1 of STEC) is found in *Shigella dysenteriae*; therefore, a FilmArray GI Panel report with positive test results for Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2* and Shigella/Enteroinvasive *E. coli* (EIEC) in the same sample may indicate the presence of *S. dysenteriae*.

E. coli O157: To aid in the identification of STEC of the O157 serotype, the FilmArray GI Panel contains a single assay (Ec O157) to detect a gene target that is specific to this serotype. Strains of *E. coli* O157 that do not carry the Shiga-like toxin genes have also been identified. However, as the pathogenicity of these non-STEC strains remains undefined, the *E. coli* O157 assay result is not reported unless a Shiga-like toxin gene is also detected (STEC detected). Detection of STEC *stx1/stx2* and the *E. coli* O157 target results in a reporting of *E. coli* O157 as a qualifier to the positive STEC result. If STEC *stx1/stx2* is Not Detected, the result for *E. coli* O157 is indicated as N/A (Not Applicable). The FilmArray GI Panel cannot distinguish between infections with a single toxigenic STEC O157 or rare co-infections of STEC (non-O157) with a *stx1/stx2*-negative *E. coli* O157 (see table below).

Table - Possible Assay Results and Corresponding Test Results for Enteropathogenic *E.coli* (EPEC) and Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx*

FilmArray GI Results	EPEC (Ec eae) Assay	STEC *stx1/2* (STEC 1/ STEC 2) Assays	*E. coli* O157 (Ec O157) Assay	Description
Enteropathogenic *E. coli* (EPEC): Not Detected Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*: Not Detected *E. coli* O157: N/A	Negative	Negative	Any Result	Enteropathogenic *E. coli* (EPEC) not detected and Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2* not detected *E. coli* O157 result is not applicable when STEC is not detected
Enteropathogenic *E. coli* (EPEC): Detected Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*: Not Detected *E. coli* O157: N/A	Positive	Negative	Any Result	Enteropathogenic *E. coli* (EPEC) detected Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2* not detected *E. coli* O157 result is not applicable when STEC is not detected
Enteropathogenic *E. coli* (EPEC): N/A Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*: Detected *E. coli* O157: Not Detected	Any Result	Positive [a]	Negative	EPEC result is not applicable (detection cannot be differentiated from *eae*-containing STEC) Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2* detected, O157 serotype not detected
Enteropathogenic *E. coli* (EPEC): N/A Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*: Detected *E. coli* O157: Detected	Any Result	Positive [a]	Positive	EPEC result is not applicable (detection cannot be differentiated from *eae*-containing STEC) Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2* detected, O157 serotype detected [b]

[a] Positive results for the STEC assay(s) and the *Shigella/*Enteroinvasive *E. coli* (EIEC) assay may indicate the presence of *Shigella dysenteriae*.

[b] O157 determinant may be from the STEC or may be due to the rare possibility of a shiga-like toxin-negative *E. coli* O157 being in the same specimen with a non-O157 STEC.

*Shigella/*Enteroinvasive *E. coli* (EIEC): The FilmArray GI Panel contains a single assay (Shig) for the detection of *ipaH*, a gene specifically found in all *Shigella* species as well as Enteroinvasive *E. coli* (EIEC). It is not possible to differentiate *Shigella* from EIEC using this method, and detection of *ipaH* will result in a *Shigella*/Enteroinvasive *E. coli* (EIEC) Detected test result. Shiga toxin (*stx*; identical to *stx1* of STEC) is found in *Shigella dysenteriae*, therefore a FilmArray GI Panel report with positive test results for Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2* with *Shigella*/Enteroinvasive *E. coli* (EIEC) in the same sample may indicate the presence of *S. dysenteriae*.

Parasites

Cryptosporidium: The FilmArray GI Panel contains two assays (Crypt 1 and Crypt 2) for detection of *Cryptosporidium* species. Empirical testing and *in silico* sequence analysis support detection of approximately 23 different *Cryptosporidium*, including the most common species of human clinical relevance (i.e., *C. hominis* and *C. parvum*), as well as several less common species (e.g., *C. meleagridis*, *C. felis*, *C. canis*, *C. cuniculus*, *C. muris*, and *C. suis*). The assays do not differentiate between species and the very rare species *C. bovis*, *C. ryanae* and *C. xiaoi* may not be detected. A positive result for either or both assays will give a Cryptosporidium Detected test result.

Cyclospora cayetanensis: The FilmArray GI Panel contains a single assay (Ccayet) for the detection of *C. cayetanensis*, the only Cyclospora species implicated in human disease.

Entamoeba histolytica: The FilmArray GI Panel contains a single assay (Ehist) for the detection of *E. histolytica*, the only *Entamoeba* species implicated in gastroenteritis. This assay may cross-react with the closely related *E. dispar* when present at higher levels (approximately 10^5 oocysts/mL or greater).

Giardia lamblia: The FilmArray GI Panel contains a single assay (Glam) designed to detect *G. lamblia* (aka *G. intestinalis*, *G. duodenalis*), the only *Giardia* species infectious to humans. A very low frequency of cross-reactivity with commensal microorganisms (i.e., *Bifidobacterium* and *Ruminococcus*) was observed in the clinical evaluation.

Viruses

Adenovirus F40/41: The FilmArray GI Panel contains a single multiplexed assay (AdenoF) for the specific detection of both Adenovirus F40 and F41 (i.e., will not cross-react with respiratory non-40/41 Adenovirus species when shed in the stool). The reported results do not indicate which serotype (40 or 41) has been detected. The assay will not detect other adenovirus species, such as species B, C, and E, which are associated with respiratory infections.

Astrovirus: The FilmArray GI Panel contains a single assay (Astro) designed to detect eight subtypes (HAstV1-8) of human Astrovirus. The assay is not predicted to detect newly-identified astroviruses of the MLB and VA clades.

Norovirus GI/GII: The FilmArray GI Panel contains two assays (Noro 1 and Noro 2) that together target the Norovirus genogroups most commonly associated with human infections (GI and GII). Neither assay will detect genogroup GIV, non-human genogroups, or closely related Caliciviruses such as Sapovirus. The reported results do not indicate which genogroup(s) (GI and/or GII) have been detected. A positive result for either or both assays will produce test result of Norovirus GI/GII Detected.

Rotavirus A: The FilmArray GI Panel contains two separate Rotavirus A assays (RotaA 1 and RotaA 2) to be inclusive of all strains of Rotavirus A. *In silico* sequence analysis indicates that these assays will not cross-react with Rotavirus B and C, which are less

common in human disease, or Rotavirus D, E, and F, which have not been found in humans. Empirical testing has demonstrated that these assays will detect recombinant viruses included in Rotavirus vaccines. A FilmArray GI Panel test result of Rotavirus A Detected is reported if either or both assays are positive.

Sapovirus (Genogroups I, II, IV, and V): The FilmArray GI Panel contains a single assay (Sapo) designed to detect, but not differentiate, Sapovirus genogroups identified in human infections (I, II, IV and V). Genogroup III, a porcine pathogen will not be detected.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Luminex xTAG® Gastrointestinal Pathogen Panel (GPP)

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

   K121454

3. Comparison with predicate:

   The Luminex xTAG® Gastrointestinal Pathogen Panel (GPP) is a qualitative, multiplexed *in vitro* diagnostic assay intended to simultaneously detect and identify microorganism nucleic acids from human stool samples. Testing is performed on pre-treated human stool samples. The tables below outline the similarities and the differences between the two systems.

Similarities		
Element	**FilmArray GI Panel**	**Luminex xTAG® Gastrointestinal Pathogen Panel (GPP)**
Organisms Detected	*Campylobacter*, toxigenic *Clostridium difficile toxA/B*, *Salmonella*, Norovirus GI/GII, Rotavirus A, *Cryptosporidium*, *Giardia lamblia*, *E. coli* O157, Shiga toxin-producing *E. coli* (STEC), Enterotoxigenic *E. coli* (ETEC), and *Shigella*/Enteroinvasive *E. coli* (EIEC).	Same See below for differences
Analyte	DNA/RNA	Same
Technological Principles	Multiplex nucleic acid	Same See below for differences

Differences		
Element	**FilmArray GI Panel**	**Luminex xTAG® Gastrointestinal Pathogen Panel (GPP)**
Specimen Types	Human stool sample collected in Cary Blair transport media.	Pre-treated human stool sample.
Organisms Detected	Detects the following *Campylobacter* species: *C. jejuni/C. coli/C. upsaliensis*. Also detects additional *Cryptosporidium* species, *Plesiomonas shigelloides*, *Vibrio* (*V. parahaemolyticus/V. vulnificus/V. cholerae*), *V. cholerae*, *Yersinia enterocolitica*, Adenovirus F40/41, Astrovirus, Sapovirus (Genogroups I, II, IV, and V), *Cyclospora cayetanensis*, *Entamoeba histolytica*, Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), and Enteroaggregative *E. coli* (EAEC).	Detects the following *Campylobacter* species: *C. jejuni*, *C. coli*, and *C. lari*. Only detects the following *Cryptosporidium* species: *C. parvum* and *C. hominis.*
Technological Principles	Nested multiplex RT-PCR followed by high resolution melting analysis to confirm identity of amplified product.	Multiplex RT-PCR and multiplex TSPE followed by Fluorescence-activated sorting of labeled beads coupled to streptavidin-conjugated biotinylated products.
Instrumentation	FilmArray Instrument	Nucleic Acid Purification System PCR Thermocycler Luminex® 100/200™ or MAGPIX instruments
Time to result	Less than 1 hour	Approximately 5 hours
Reagent Storage	Room temperature	Reagents stored at 4°C and -20°C.
Sample Preparation Method	Sample Processing is automated in the FilmArray Instrument.	Up front sample processing is required to extract nucleic acid.
Test Interpretation	Automated test interpretation and report generation. User cannot access raw data.	Semi-automated test interpretation. User must review all “no call” results to determine cause and retesting strategy.
Controls	Two controls are included in each reagent pouch to control for sample processing and both stages of PCR and melt analysis.	Internal control added to each sample. External control processed with each batch of samples.

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

- Draft Guidance for Industry and Food and Drug Administration Staff - Highly Multiplexed Microbiological/Medical Countermeasure In Vitro Nucleic Acid Based Diagnostic Devices, (November 9, 2012)
- Class II Special Controls Guidance Document: Norovirus Serological Reagents. Document issued on: March 9, 2012. Document number 1767.

- Draft Guidance – Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection of *Clostridium difficile*. Document issued on November 29, 2010. Document number 1715.
- Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests, FDA Guidance Document (March 13, 2007)
- User Protocol for Evaluation of Qualitative Test Performance, Clinical and Laboratory Standards Institute **(**CLSI**)** Second Edition, EP12-A2 (January 2008)
- Interference Testing in Clinical Chemistry, CLSI Approved Guideline EP7-A2 (November 2005)
- EN ISO 14971:2012, 'Medical devices – Application of risk management to medical devices'

Software Specific

- Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices, FDA Guidance Document (May 11, 2005)
- Guidance for Industry, FDA Reviewers and Compliance on Off-The-Shelf Software Use in Medical Devices (September 9, 1999)
- General Principle of Software Validation; Final Guidance for Industry and FDA Staff (January 11, 2002)
- ISO 62304:2006, 'Medical device software – Software life-cycle processes'.

Labeling

- Use of Symbols on Labels and in Labeling of In Vitro Diagnostic Devices Intended for Professional Use, FDA Guidance Document (November 30, 2004)
- 21 CFR 809.10, Labeling for in vitro diagnostic products
- ISO 15223-1:2012, 'Medical Devices – Symbols to be used with medical device labels, labeling and information to be supplied – Part 1: General requirements'.
- EN ISO 18113-1:2011, 'In vitro diagnostic medical devices – Information supplied by the manufacturer (labeling) – Part 1: Terms, definition and general requirements'.
- EN ISO 18113-2:2011, 'In vitro diagnostic medical devices – Information supplied by the manufacturer (labeling) – Part 2: In vitro diagnostic reagents for professional use'.

L. Test Principle:

The FilmArray GI pouch is a closed system disposable that houses all the chemistry required to isolate, amplify and detect nucleic acid from multiple gastrointestinal pathogens within a single stool specimen collected in Cary Blair. The rigid plastic component (fitment) of the FilmArray GI pouch contains reagents in freeze-dried form. The flexible plastic portion of the pouch is divided into discrete segments (blisters) where the required chemical processes are carried out. The user of the FilmArray GI Panel loads the sample into the FilmArray GI pouch, places the pouch into the FilmArray Instrument, and starts the run. All other operations are automated.

The following is an overview of the operations and processes that occur during a FilmArray run:

- **Nucleic Acid Purification -** Nucleic acid purification occurs in the first three blisters of the pouch. The sample is lysed by a combination of chemical and mechanical (bead beating)

mechanisms and the liberated nucleic acid is captured, washed and eluted using magnetic bead technology. These steps require about ten minutes, and the bead-beater apparatus can be heard as a high-pitched whine during the first few minutes of operation.

- **Reverse Transcription and 1st Stage Multiplex PCR -** Since the GI Panel includes RNA viruses, a reverse transcription (RT) step is performed to convert the viral RNA into cDNA prior to amplification. The purified nucleic acid solution is combined with a preheated master mix to initiate the RT step and subsequent thermocycling for multiplex PCR. The effect of 1st stage PCR is to enrich for the target nucleic acids present in the sample.

- **2nd Stage PCR -** The products of 1st stage PCR are diluted and mixed with fresh PCR reagents containing an intercalating fluorescent DNA dye (LCGreen® Plus, BioFire Diagnostics). This solution is distributed over the 2nd stage PCR array. The individual wells of the array contain primers for different assays (each present in triplicate) that target specific nucleic acid sequences from each of the pathogens detected, as well as control template material. These primers are 'nested' or internal to the specific products of the 1st stage multiplex reaction, which enhances both the sensitivity and specificity of the reactions.

- **DNA Melting Analysis** – After 2nd stage PCR, the temperature is slowly increased and fluorescence in each well of the array is monitored and analyzed to generate a melt curve. The temperature at which a specific PCR product melts (melting temperature or Tm) is consistent and predictable and the FilmArray Software automatically evaluates the data from replicate wells for each assay to report results

The FilmArray Software controls the operation of the instrument, collects and analyzes data, and automatically generates a test report at the end of the run.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Reproducibility:

   A multicenter reproducibility study was performed to determine between-site and overall reproducibility of the FilmArray GI Panel. Reproducibility testing occurred at three test sites using a panel of contrived stool samples, each spiked with various combinations of four different GI Panel analytes. Each analyte was evaluated at three different concentrations (Negative, Low Positive and Moderate Positive).

   The study incorporated a range of potential variation introduced by 13 different operators, 4 different pouch lots, and 16 different FilmArray Instruments. Samples were stored refrigerated (4˚C) or frozen (≤-70˚C) prior to testing. Frozen samples were tested on five different days at three testing sites for 90 data points per sample and refrigerated samples were tested on four different days at three testing sites for 108 data points per sample. A summary of results (percent (%) agreement with the expected result) for each analyte (by site and overall) and the reproducibility of Tm for each positive assay is provided in the tables below.

Table - Reproducibility of the FilmArray GI Panel Test Results

Organism Tested	Concentration Tested	Expected Result	% Agreement with Expected Result			
			Site A	Site B	Site C	**All Sites** (95% Confidence Interval)
Campylobacter jejuni ATCC BAA-1234	**Moderate Positive** 3xLoD $1.2x10^5$ cells/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0 - 100%)
	Low Positive 1xLoD $4x10^4$ cells/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0 - 100%)
	None	Not Detected	192/192 100%	192/192 100%	192/192 100%	576/576 100% (99.4 - 100%)
Clostridium difficile[a] ATCC 9689	**Moderate Positive** 3xLoD $1.2x10^6$ cells/mL	Detected	36/36 100%	36/36 100%	36/36 100%	108/108 100% (96.6 - 100%)
	Low Positive 1xLoD $4x10^5$ cells/mL	Detected	36/36 100%	36/36 100%	36/36 100%	108/108 100% (96.6 - 100%)
	None	Not Detected	120/120 100%	120/120 100%	120/120 100%	360/360 100% (96.6 - 100%)
***Escherichia coli* (EPEC)** E2348/69 (STEC Center, MSU)	**Moderate Positive** 3xLoD $3x10^3$ CFU/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0 - 100%)
	Low Positive 1xLoD $1x10^3$ CFU/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0 - 100%)
	None	Not Detected	192/192[b] 100%	192/192[b] 100%	192/192[b] 100%	576/576 100% (99.4 - 100%)
Salmonella enterica[a] SarC1 (SGSC)	**Moderate Positive** 3xLoD $1.5x10^4$ CFU/mL	Detected	36/36 100%	36/36 100%	36/36 100%	108/108 100% (96.6 - 100%)
	Low Positive 1xLoD $5x10^3$ CFU/mL	Detected	36/36 100%	36/36 100%	36/36 100%	108/108 100% (96.6 - 100%)
	None	Not Detected	120/120 100%	120/120 100%	120/120 100%	360/360 100% (96.6 - 100%)
***Escherichia coli* (STEC) O157** ATCC 43895	**Moderate Positive** 3xLoD $3x10^4$ CFU/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0 - 100%)
	Low Positive 1xLoD $1x10^4$ CFU/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0 - 100%)

Organism Tested	Concentration Tested	Expected Result	% Agreement with Expected Result			
			Site A	Site B	Site C	All Sites (95% Confidence Interval)
	None	N/A	192/192 100%	192/192 100%	192/192 100%	576/576 100% (99.4 - 100%)
Shigella sonnei ATCC 29930	**Moderate Positive** 3xLoD $3x10^2$ CFU/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0 - 100%)
	Low Positive 1xLoD $1x10^2$ CFU/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0 - 100%)
	None	Not Detected	192/192 100%	192/192 100%	192/192 100%	576/576 100% (99.4 - 100%)
Vibrio parahaemolyticus[a] ATCC 17802	**Moderate Positive** 3xLoD $2.4x10^5$ cells/mL	Detected	36/36 100%	36/36 100%	36/36 100%	108/108 100% (96.6 - 100%)
	Low Positive 1xLoD $8x10^4$ cells/mL	Detected	36/36 100%	36/36 100%	36/36 100%	108/108 100% (96.6 - 100%)
	None	Not Detected	120/120 100%	120/120 100%	120/120 100%	360/360 100% (96.6 - 100%)
Cryptosporidium parvum Waterborne P102C	**Moderate Positive** 3xLoD $1.5x10^4$ oocysts/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0 - 100%)
	Low Positive 1xLoD $5x10^3$ oocysts/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0 - 100%)
	None	Not Detected	192/192 100%	192/192 100%	192/192 100%	576/576 100% (99.4 - 100%)
Giardia intestinalis[a] (syn. *Giardia lamblia*) ATCC 30957	**Moderate Positive** 3x LoD 150 cells/mL	Detected	36/36 100%	36/36 100%	36/36 100%	108/108 100% (96.6 - 100%)
	Low Positive 1xLoD 50 cells/mL	Detected	30/36 83.3%	30/36 83.3%	31/36 86.1%	91/108 84.3% (77.0 - 91.0%)
	None	Not Detected	120/120 100%	120/120 100%	120/120 100%	360/360 100% (96.6 - 100%)
Adenovirus F41 ATTC VR-930	**Moderate Positive** 3x LoD 300 $TCID_{50}$/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0 - 100%)

Organism Tested	Concentration Tested	Expected Result	% Agreement with Expected Result			
			Site A	Site B	Site C	**All Sites** (95% Confidence Interval)
	Low Positive 1xLoD 100 $TCID_{50}$/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0 - 100%)
	None	Not Detected	192/192 100%	192/192 100%	192/192 100%	576/576 100% (99.4 - 100%)
Astrovirus (Type 8) NCPV 1003071v	**Moderate Positive** 3xLoD 150 FFU/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0 - 100%)
	Low Positive 1xLoD 50 FFU/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0 - 100%)
	None	Not Detected	192/192 100%	192/192 100%	192/192 100%	576/576 100% (99.4 - 100%)
Norovirus GI Clinical Specimen	**Moderate Positive** 3xLoD $3x10^4$ copies/mL	Detected	29/30 96.7%	30/30 100%	30/30 100%	89/90 98.9% (96.0 - 100%)
	Low Positive 1xLoD $1x10^4$ copies/mL	Detected	28/30 93.3%	29/30 96.7%	30/30 100%	87/90 96.7% (96.0 - 100%)
	None	Not Detected	192/192 100%	192/192 100%	192/192 100%	576/576 100% (99.4 - 100%)

[a] Reproducible, but suboptimal (<95%) detection was observed at one or both concentrations in frozen contrived samples. Data presented are from contrived samples stored at ~4°C for up to 4 days prior to testing.
[b] Includes N/A results for 60 samples (180 for all sites) spiked with STEC O157. When an STEC is detected, N/A is reported for the EPEC test result, regardless of the status of the EPEC assay.

The reproducibility of Tm for each positive assay was also evaluated and a summary is provided in the following TABLE:

Table - Reproducibility of Tm for Positive FilmArray GI Panel Assays

Organism	Assay		Test Level	Test Site	Tm Reproducibility				
					Mean	StDev Tm	Min	Max	(Max - Min)
Bacteria and (Including Diarrheagenic *E. coli*)									
Campylobacter jejuni ATCC BAA-1234	**Campy 1**		**Moderate Positive 3xLoD $1.2x10^5$ cells/mL**	**Site A**	78.38	± 0.27	77.86	79.01	1.15
				Site B	78.28	± 0.21	77.87	78.59	0.72
				Site C	78.04	± 0.29	77.60	78.59	0.99
				All Sites	78.23	± 0.30	77.60	79.01	1.41
			Low Positive 1xLoD $4x10^4$ cells/mL	**Site A**	78.60	± 0.33	77.73	79.47	1.74
				Site B	78.65	± 0.19	78.28	79.01	0.73
				Site C	78.21	± 0.26	77.73	78.72	0.99
				All Sites	78.48	± 0.33	77.73	79.47	1.74
Clostridium difficile ATCC 9689	**Cdiff[a]**	**Tm 1**	**Moderate Positive 3xLoD $1.2x10^6$ cells/mL**	**Site A**	76.01	± 0.34	75.30	76.99	1.69
				Site B	75.79	± 0.40	74.71	76.59	1.88
				Site C	75.60	± 0.34	75.02	77.09	2.07
				All Sites	75.80	± 0.39	74.71	77.09	2.38
			Low Positive 1xLoD $4x10^5$ cells/mL	**Site A**	76.18	± 0.43	75.45	77.15	1.70
				Site B	75.94	± 0.43	75.09	76.74	1.65
				Site C	75.73	± 0.28	75.29	76.45	1.16
				All Sites	75.95	± 0.43	75.09	77.15	2.06
		Tm 2	**Moderate Positive 3xLoD $1.2x10^6$ cells/mL**	**Site A**	78.84	± 0.26	78.44	79.56	1.12
				Site B	78.61	± 0.30	77.86	79.17	1.31
				Site C	78.40	± 0.22	78.01	79.02	1.01
				All Sites	78.62	± 0.32	77.86	79.56	1.70
			Low Positive 1xLoD $4x10^5$ cells/mL	**Site A**	78.94	± 0.31	78.45	79.61	1.16
				Site B	78.67	± 0.30	78.02	79.17	1.15
				Site C	78.48	± 0.24	78.02	79.02	1.00
				All Sites	78.70	± 0.34	78.02	79.61	1.59
***Escherichia coli* (EPEC)** E2348/69 (STEC Center, MSU)	**Ec eae**		**Moderate Positive 3xLoD $3x10^3$ CFU/mL**	**Site A**	80.53	± 0.24	80.16	81.04	0.88
				Site B	80.39	± 0.20	79.86	80.74	0.88
				Site C	80.38	± 0.17	80.01	80.61	0.60
				All Sites	80.43	± 0.22	79.86	81.04	1.18
			Low Positive 1xLoD $1x10^3$ CFU/mL	**Site A**	80.59	± 0.24	80.15	81.18	1.03
				Site B	80.46	± 0.20	79.87	80.73	0.86
				Site C	80.42	± 0.14	80.15	80.72	0.57
				All Sites	80.49	± 0.21	79.87	81.18	1.31
Salmonella enterica SarC1 (SGSC)	**Salm**		**Moderate Positive 3xLoD $1.5x10^4$ CFU/mL**	**Site A**	82.17	± 0.20	81.86	82.59	0.73
				Site B	81.88	± 0.26	81.30	82.32	1.02
				Site C	81.78	± 0.25	81.44	82.17	0.73

Organism	Assay	Test Level	Test Site	Tm Reproducibility				
				Mean	StDev Tm	Min	Max	(Max - Min)
			All Sites	81.95	± 0.29	81.30	82.59	1.29
		Low Positive 1xLoD $5x10^3$ CFU/mL	**Site A**	82.21	± 0.27	81.74	82.77	1.03
			Site B	81.96	± 0.26	81.31	82.39	1.08
			Site C	81.83	± 0.25	81.45	82.31	0.86
			All Sites	82.00	± 0.30	81.31	82.77	1.46
***Escherichia coli* (STEC) O157** ATCC 43895	**O157**	**Moderate Positive 3xLoD $3x10^4$ CFU/mL**	**Site A**	83.23	± 0.22	82.58	83.77	1.19
			Site B	83.20	± 0.19	82.85	83.60	0.75
			Site C	82.96	± 0.29	82.59	83.44	0.85
			All Sites	83.13	± 0.26	82.58	83.77	1.19
		Low Positive 1xLoD $1x10^4$ CFU/mL	**Site A**	83.26	± 0.24	82.80	83.88	1.08
			Site B	83.20	± 0.20	82.73	83.59	0.86
			Site C	83.01	± 0.29	82.46	83.60	1.14
			All Sites	83.16	± 0.26	82.46	83.88	1.42
	STEC 1	**Moderate Positive 3xLoD $3x10^4$ CFU/mL**	**Site A**	82.85	± 0.25	82.16	83.48	1.32
			Site B	82.80	± 0.19	82.28	83.17	0.89
			Site C	82.52	± 0.28	82.16	83.02	0.86
			All Sites	82.72	± 0.28	82.16	83.48	1.32
		Low Positive 1xLoD $1x10^4$ CFU/mL	**Site A**	82.89	± 0.24	82.44	83.31	0.87
			Site B	82.78	± 0.18	82.44	83.17	0.73
			Site C	82.55	± 0.28	82.03	83.17	1.14
			All Sites	82.74	± 0.27	82.03	83.31	1.28
	STEC 2	**Moderate Positive 3xLoD $3x10^4$ CFU/mL**	**Site A**	84.99	± 0.22	84.44	85.49	1.05
			Site B	84.90	± 0.19	84.43	85.31	0.88
			Site C	84.68	± 0.30	84.30	85.16	0.86
			All Sites	84.86	± 0.27	84.30	85.49	1.19
		Low Positive 1xLoD $1x10^4$ CFU/mL	**Site A**	84.98	± 0.22	84.58	85.45	0.87
			Site B	84.92	± 0.19	84.45	85.30	0.85
			Site C	84.72	± 0.28	84.31	85.32	1.01
			All Sites	84.88	± 0.26	84.31	85.45	1.14
Shigella sonnei ATCC 29930	**Shig**	**Moderate Positive 3xLoD $3x10^2$ CFU/mL**	**Site A**	86.58	± 0.25	86.01	87.05	1.04
			Site B	86.38	± 0.19	85.87	86.61	0.74
			Site C	86.44	± 0.17	86.16	86.75	0.59
			All Sites	86.47	± 0.22	85.87	87.05	1.18
		Low Positive 1xLoD $1x10^2$ CFU/mL	**Site A**	86.57	± 0.22	86.29	87.18	0.89
			Site B	86.52	± 0.24	86.02	87.01	0.99
			Site C	86.26	± 0.24	85.87	86.73	0.86
			All Sites	86.45	± 0.27	85.87	87.18	1.31

Organism	Assay	Test Level	Test Site	Tm Reproducibility				
				Mean	StDev Tm	Min	Max	(Max - Min)
Vibrio parahaemolyticus ATCC 17802	**Vibrio**	**Moderate Positive 3xLoD 2.4x10^5 cells/mL**	**Site A**	81.96	± 0.23	81.59	82.42	0.83
			Site B	81.69	± 0.24	81.02	82.03	1.01
			Site C	81.57	± 0.27	81.17	82.16	0.99
			All Sites	81.74	± 0.30	81.02	82.42	1.40
		Low Positive 1xLoD 8x10^4 cells/mL	**Site A**	82.03	± 0.17	81.73	82.42	0.69
			Site B	81.74	± 0.23	81.29	82.17	0.88
			Site C	81.60	± 0.22	81.30	82.02	0.72
			All Sites	81.79	± 0.28	81.29	82.42	1.13
Protozoa								
Cryptosporidium parvum Waterborne P102C	**Crypt 1**	**Moderate Positive 3xLoD 1.5x10^4 oocysts/mL**	**Site A**	78.99	± 0.23	78.58	79.46	0.88
			Site B	78.95	± 0.24	78.29	79.58	1.29
			Site C	78.83	± 0.15	78.57	79.16	0.59
			All Sites	78.92	± 0.22	78.29	79.58	1.29
		Low Positive 1xLoD 5x10^3 oocysts/mL	**Site A**	79.00	± 0.26	78.59	79.61	1.02
			Site B	78.94	± 0.21	78.29	79.31	1.02
			Site C	78.88	± 0.18	78.43	79.17	0.74
			All Sites	78.95	± 0.23	78.29	79.61	1.32
	Crypt 2	**Moderate Positive 3xLoD 1.5x104 oocysts/mL**	**Site A**	71.75	± 0.28	71.29	72.31	1.02
			Site B	71.74	± 0.20	71.15	72.15	1.00
			Site C	71.50	± 0.20	71.28	72.15	0.87
			All Sites	71.67	± 0.26	71.15	72.31	1.16
		Low Positive 1xLoD 5x10^3 oocysts/mL	**Site A**	71.81	± 0.35	71.29	72.43	1.14
			Site B	71.81	± 0.16	71.43	72.16	0.73
			Site C	71.59	± 0.21	71.28	72.14	0.86
			All Sites	71.74	± 0.27	71.28	72.43	1.15
Giardia intestinalis (syn. *G. lamblia*) ATCC 30957	**Glam**	**Moderate Positive 3xLoD 150 cells/mL**	**Site A**	91.52	± 0.24	91.04	92.08	1.04
			Site B	91.19	± 0.25	90.47	91.59	1.12
			Site C	91.12	± 0.29	90.62	91.74	1.12
			All Sites	91.28	± 0.31	90.47	92.08	1.61
		Low Positive 1xLoD 50 cells/mL	**Site A**	91.57	± 0.21	91.17	91.91	0.74
			Site B	91.24	± 0.22	90.75	91.62	0.87
			Site C	91.10	± 0.30	90.60	91.61	1.01
			All Sites	91.30	± 0.31	90.60	91.91	1.31
Viruses								
Adenovirus F41 ATTC VR-930	**AdenoF**	**Moderate Positive 3xLoD 300 TCID$_{50}$/mL**	**Site A**	86.71	± 0.23	86.01	87.35	1.34
			Site B	86.61	± 0.18	86.28	87.03	0.75
			Site C	86.36	± 0.31	85.87	86.87	1.00

Organism	Assay	Test Level	Test Site	Tm Reproducibility				
				Mean	StDev Tm	Min	Max	(Max - Min)
			All Sites	86.56	± 0.28	85.87	87.35	1.48
		Low Positive 1xLoD 100 $TCID_{50}$/mL	**Site A**	86.85	± 0.27	86.37	87.48	1.11
			Site B	86.70	± 0.20	86.30	87.16	0.86
			Site C	86.47	± 0.29	86.02	87.03	1.01
			All Sites	86.67	± 0.30	86.02	87.48	1.46
Astrovirus (Type 8) NCPV 1003071v	**Astro**	**Moderate Positive 3xLoD 150 FFU/mL**	**Site A**	85.62	± 0.25	85.17	86.06	0.89
			Site B	85.48	± 0.18	85.01	85.88	0.87
			Site C	85.51	± 0.21	85.02	85.90	0.88
			All Sites	85.54	± 0.22	85.01	86.06	1.05
		Low Positive 1xLoD 50 FFU/mL	**Site A**	85.67	± 0.26	85.17	86.19	1.02
			Site B	85.54	± 0.22	85.01	86.01	1.00
			Site C	85.55	± 0.16	85.29	85.89	0.60
			All Sites	85.59	± 0.22	85.01	86.19	1.18
Norovirus GI Clinical Specimen	**Noro 1**	**Moderate Positive 3xLoD $3x10^4$ copies/mL**	**Site A**	83.69	± 0.23	83.14	84.07	0.93
			Site B	83.46	± 0.20	82.92	83.76	0.84
			Site C	83.43	± 0.20	83.02	83.87	0.85
			All Sites	83.52	± 0.24	82.92	84.07	1.15
		Low Positive 1xLoD $1x10^4$ copies/mL	**Site A**	83.62	± 0.24	83.22	84.15	0.93
			Site B	83.59	± 0.21	83.18	83.98	0.80
			Site C	83.30	± 0.24	82.93	83.79	0.86
			All Sites	83.50	± 0.27	82.93	84.15	1.22

[a] A characteristic double melt profile is observed when both *C. difficile* toxin genes (tcdA and tcdB) are present in a sample and two different Tm values are reported (Tm1 and Tm2).

b. Linearity/assay reportable range:

Not applicable, qualitative assay.

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

Internal Controls:

The following internal controls are included in each FilmArray GI Panel pouch:

- DNA Process Control: The DNA Process Control assay targets DNA from the yeast *Schizosaccharomyces pombe*. The yeast is present in the pouch in a freeze-dried form and is hydrated and introduced into the test when the sample is loaded. The control material is carried through all stages of the test process, including lysis, nucleic acid purification, 1st stage PCR, dilution, 2nd stage PCR, and DNA melting. A positive control result indicates that all steps carried out in the pouch were successful.

- PCR2 Control: The PCR2 Control assay detects a DNA target that is dried into the wells of the array along with the corresponding primers. A positive result indicates that the 2nd stage PCR was successful.

Both internal control assays must be positive for the test run to pass. When either control fails, the Controls field of the test report will display "Failed" and all results will be listed as Invalid. If the controls fail, the user is instructed to repeat the test using a new pouch. Of the nine pouch control failures observed in the prospective clinical study, seven were attributed to the RNA process control and two were due to the PCR2 control. There were no instances of both RNA process and PCR2 control failure in the same pouch.

Recommended External Controls:

External controls are not provided with the FilmArray GI Panel, but are recommended in the package insert. Cary Blair media can be used as an external negative control. Previously characterized positive stool samples or negative samples spiked with well characterized organisms can be used as external positive controls. External controls should be used in accordance with the appropriate accrediting organization requirements, as applicable.

External Controls used in clinical studies:

Daily external control mixes (ECMs) and weekly swab testing for contamination monitoring was performed during the clinical evaluation of the FilmArray GI Panel. External controls used in the clinical study consisted of four different organism mixes and a negative control provided to the clinical sites as frozen single use aliquots. (Three control mixes consisted of Cary Blair media containing whole organisms, one contained nucleic acid template (for organisms that could not be obtained in sufficient quantities), and one contained Cary Blair media alone. A valid ECM was required for each day of testing. All FilmArray GI Assay targets were represented by the four external control mixes. Testing of external controls was rotated, with one of the five controls tested each day. No external contamination was found when following assay instructions (i.e., prior to processing samples, both the work area and the FilmArray Pouch Loading Station were thoroughly cleaned using a suitable cleaner such as freshly prepared 10% bleach or a similar disinfectant. To avoid residue build-up and potential PCR inhibition, wipe disinfected surfaces with water.)

A total of 234 control mix runs were attempted, all of which completed. Of these, 225 (96.2%; 225/234) were successful while nine (3.8%) did not return the correct organism results either due to the detection of an extra analyte (n=3) and/or the failure to detect one or more spiked analytes (n=6).

PCR Comparator Controls:

An inhibition control was performed as a separate assay (primers + template) on the nucleic acid extract of each specimen prior to running any other assay. There were no inhibition control failures.

Specimen Stability:

To determine whether FilmArray GI Panel test results are obtained from stool samples in Cary-Blair stored under the time and temperature conditions recommended for use with transport media (e.g. up to 96 hours (4 days) at room temperature or refrigerated (4 days), 10 different stool-Cary Blair specimens were spiked with representative panel organisms. Samples were prepared by spiking organism mixes at concentrations approximately 3-fold higher (3×) than the established LoD for each individual organism into 10 unique stool samples in Cary Blair collected from asymptomatic donors. Each mix contained four different representative panel organisms for a total of 12 organisms.

'Fresh' or non-stored sample test results (Day 0) were established by testing samples with the FilmArray GI Panel within an hour of when the samples were prepared. The remaining sample was aliquoted and stored at the desired storage temperatures (room temperature or under refrigeration). At designated time-points (i.e., days 1-4), an aliquot from each sample was tested with the FilmArray GI Panel.

Transport and storage conditions were considered acceptable for use with the FilmArray GI Panel if accurate test results (equivalent to the non-stored samples (Day 0)) were obtained for at least nine of the ten aliquots evaluated at that condition. Negative results were compiled from specimens that had not been spiked with a particular organism/analyte, such that for every storage temperature/time-point, 20-30 negative FilmArray test results were expected. All analytes with unexpected negative test results were investigated and resolved by testing two additional aliquots of the same sample at the same condition.

Table - Test Results for Contrived Samples Stored at Room Temperature for 0-4 Days

Test Result	Day 0		Day 1		Day 2		Day 3		Day 4	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
Campylobacter	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20
Clostridium difficile	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20
Plesiomonas shigelloides		30/30		30/30		30/30		30/30		30/30
Salmonella	10/10	20/20	9/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20
Vibrio		30/30		30/30		29/30[d]		30/30		30/30
V. cholerae		30/30		30/30		29/30[d]		30/30		30/30
Yersinia enterocolitica		30/30		30/30		30/30		30/30		30/30
EAEC		30/30		30/30		29/30[d]		30/30		30/30
EPEC		30/30		30/30		30/30		30/30		30/30
ETEC		30/30		30/30		30/30		30/30		30/30
STEC	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20
O157	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20
Shigella/EIEC		30/30		30/30		30/30		30/30		30/30
Cryptosporidium	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20
Cyclospora cayetanensis		30/30		30/30		30/30		30/30		30/30
Entamoeba histolytica	10/10	20/20	9/10	20/20	10/10	20/20	9/10	20/20	10/10	20/20
Giardia lamblia	10/10	20/20	10/10	20/20	10/10	20/20	9/10	20/20	9/10	20/20
Adenovirus F40/41	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20
Astrovirus	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20
Norovirus	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20
Rotavirus	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20
Sapovirus		30/30		30/30		30/30		30/30		30/30

For refrigerated samples, the expected results (at least 9/10 positive) were observed for each spiked analyte of *Clostridium difficile* on Day 4 (8/10 positive). All analytes with unexpected negative test results were investigated and resolved by testing two additional aliquots of the same sample at the same condition.

Table - Test Results for Contrived Samples Stored Refrigerated for 0-4 Days

Test Result	Day 0		Day 1		Day 2		Day 3		Day 4	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
Aeromonas	10/10	20/20	8/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20
Campylobacter	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20
Clostridium difficile	10/10	20/20	9/10	20/20	10/10	20/20	10/10	20/20	8/10	20/20
Plesiomonas shigelloides		30/30		30/30		30/30		30/30		30/30
Salmonella	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20	9/10	20/20
Vibrio		30/30		30/30		30/30		30/30		30/30
V. cholerae		30/30		30/30		30/30		30/30		30/30
Yersinia enterocolitica		30/30		30/30		30/30		30/30		30/30
EAEC		30/30		30/30		30/30		30/30		30/30
EPEC		30/30		30/30		30/30		30/30		30/30
ETEC		30/30		30/30		30/30		30/30		30/30
STEC	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20
O157	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20
Shigella/EIEC		30/30		30/30		30/30		30/30		30/30
Cryptosporidium	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20
Cyclospora cayetanensis		30/30		30/30		30/30		30/30		30/30
Entamoeba histolytica	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20
Giardia lamblia	10/10	20/20	10/10	20/20	10/10	20/20	9/10	20/20	10/10	20/20
Adenovirus F40/41	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20	9/10	20/20
Astrovirus	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20
Norovirus	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20
Rotavirus	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20	10/10	20/20
Sapovirus		30/30		30/30		30/30		30/30		30/30

The results of this study support the recommendations for sample handling and storage. Stool specimens in Cary Blair can be stored for up to four days at room temperature or in the refrigerator without affecting the accuracy of FilmArray GI Panel test results.

A total of 325 runs were initiated throughout the course of this study and 318 completed with valid results (318/325 = 97.8%). A total of 5 runs (5/325; 1.5%) were invalid due to a control failure, one run did not complete due to a software error (1/325; 0.3%) and another one run (1/325; 0.3%) did not complete due to an instrument error.

d. Detection limit:

A study was performed to determine the analytical sensitivity, or limit of detection (LoD), of the FilmArray GI Panel for each test result included in the panel. LoD (or LoD_{95}) is defined as the lowest concentration of organism that can be consistently detected (≥ 95% of samples test positive) in the defined sample type (stool in Cary Blair transport medium). The LoD for each organism was estimated with limiting dilutions as single-spiked and multi-spiked samples (up to four organisms per mix), to provide an estimated LoD concentration, and to determine whether assay sensitivity is affected by the presence of

multiple panel organisms in a single sample. Confirmation of LoDs was performed by spiking organism (single or multi-spike) at the LoD estimate determined by the dilutions series, into 20 independent stool samples. LoD was confirmed when the correct organism/assay results were obtained from at least 19 of the 20 samples (19/20 = 95%) tested.

Table - Confirmed Limit of Detection (LoD) for GI Panel Analytes

GI Panel Test Result	Species/Isolate Tested	Confirmed LoD Concentration	Detection at LoD Concentration
BACTERIA			
Campylobacter	*Campylobacter coli* ATCC 33559	4×10^4 cells/mL	20/20 100%
	Campylobacter jejuni ATCC BAA-1234		20/20 100%
	Campylobacter upsaliensis ATCC BAA-1059		20/20 100%
Clostridium difficile (toxin A/B)	*Clostridium difficile* Toxinotype 0 A+B+ ATCC 9689	4×10^5 cells/mL	20/20 100%
	Clostridium difficile (NAP1) Toxinotype III A+B+ Zeptometrix #801619	4×10^4 cells/mL	19/20 95%
Plesiomonas shigelloides	*Plesiomonas shigelloides* ATCC 14029	1×10^3 CFU/mL	20/20 100%
Salmonella	*Salmonella bongori* O66:H1z41:H2- SGSC RKS#3041 SarC11	1×10^4 CFU/mL	20/20 100%
	Salmonella enterica ssp. *enterica* Serovar Typhimurium O1,4,[5],12:H1i:H21,2 SGSC RKS#4194 SarC1	5×10^3 CFU/mL	20/20 100%
Vibrio and *Vibrio cholerae*	*Vibrio cholerae* Ogawa serotype O:1 ATCC 14035	8×10^3 cells/mL	20/20 100%
	Vibrio parahaemolyticus ATCC 17802	8×10^4 cells/mL	20/20 100%
Yersinia enterocolitica	*Yersinia enterocolitica* ATCC 9610 Biovar1 serogroup O:8	5×10^4 CFU/mL	20/20 100%
DIARRHEAGENIC *E. coli*/*Shigella*			
Enteroaggregative *E. coli* (EAEC)	*Escherichia coli* O92:H33 STEC Center # JM221	1×10^4 CFU/mL	20/20 100%
Enteropathogenic *E. coli* (EPEC)	*Escherichia coli* E2348/69 O127:H6 STEC Center	1×10^3 CFU/mL	20/20 100%
Enterotoxigenic *E. coli* (ETEC) *lt/st*	*Escherichia coli* H10407 O78:H11 ATCC 35401	1×10^3 CFU/mL	20/20 100%
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*	*Escherichia coli* O25:H11 ATCC BAA-2196	1×10^3 CFU/mL	20/20 100%
E. coli O157	*Escherichia coli* O157:H7 ATCC 43895	1×10^4 CFU/mL	20/20 100%
Shigella/Enteroinvasive *E. coli* (EIEC)	Escherichia *coli* O29:NM ATCC 43892	5×10^3 CFU/mL	20/20 100%
	Shigella sonnei ATCC 29930	100 CFU/mL	20/20 100%
PARASITES			
Cryptosporidium [a]	*Cryptosporidium parvum* *Iowa* isolate (Harley Moon) Waterborne, Inc. P102C	5×10^3 oocysts/mL	20/20 100%
	Cryptosporidium hominis Clinical Specimen		20/20 100%

GI Panel Test Result	Species/Isolate Tested	Confirmed LoD Concentration	Detection at LoD Concentration
Cyclospora cayetanensis	*Cyclospora cayetanensis* Clinical Specimen	180 genome equivalents (GE)/mL	20/20 100%
Entamoeba histolytica	*Entamoeba histolytica* HM-1:IMSS ATCC 30459	2 x 10^3 cells/mL	19/20 95%
Giardia lamblia	*Giardia intestinalis* (aka *G. lamblia*) ATCC 30957	50 cells/mL	20/20 100%
VIRUSES			
Adenovirus F 40/41	Adenovirus F40 ATCC VR-931	1 $TCID_{50}$/mL	20/20 100%
	Adenovirus F41 ATCC VR-930	100 $TCID_{50}$/mL	20/20 100%
Astrovirus	Astrovirus - Type 8 NCPV#1003071v	50 FFU/mL	20/20 100%
Norovirus GI/GII	Norovirus GI Clinical Specimen	1 x 10^4 RNA copies/mL	19/20 95%
	Norovirus GII Clinical Specimen		20/20 100%
Rotavirus A	Rotavirus A - Type G4 [P6] NCPV#0904053v	1 x 10^5 FFU/mL	20/20 100%
Sapovirus	Sapovirus (Genogroup I) Clinical Specimen	1.1 x 10^7 RNA copies /mL	20/20 100%

[a] Limited testing with a clinical specimen containing *Cryptosporidium meleagridis* indicates that the LoD for this species is similar to that of *C. parvum* and *C. hominis*.

e. Inclusivity

The analytical reactivity (inclusivity) of the FilmArray GI Panel was evaluated with a collection of 270 isolates that represent the diversity of the FilmArray GI Panel analytes. Isolates were selected to represent relevant subspecies or serotypes and selection was biased toward more common species and known human pathogens. When possible, *in silico* analysis of sequence data was used to make predictions of assay reactivity for less common species, strains, serovars, or serotypes that were not tested.

Organisms were tested at concentrations near the limit of detection (LoD). If a sample containing a particular strain was positive (detected) at the initial test level, no further testing was required. If a strain was not detected, the strain was retested at the same level (up to five additional times) and if necessary, additional testing was performed at 10- and 100-fold higher concentrations to determine if the strain can be detected by the GI Panel. Based upon predicted assay reactivity, a few select isolates were initially tested at a high concentration, followed by evaluation at lower concentrations if detection was observed. Results are provided below for each FilmArray GI Panel test result.

Table - FilmArray *Campylobacter* Inclusivity Results (*C. coli/C. jejuni/C. upsaliensis*)

Organism	Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected
Campylobacter coli[a]	ATCC BAA-1061	1.2×10^5	3×LoD
	BEI HM-296	1.2×10^5	3×LoD
	ATCC43485	1.2×10^5	3×LoD
	ATCC 43478	1.2×10^5	3×LoD
	ATCC 33559[b]	4.0×10^4	1×LoD
Campylobacter jejuni subsp. *doylei*[c]	ATCC 49349	4.0×10^6	Not Detected[c]
	ATCC 49351	4.0×10^6	100×LoD[c]
	ATCC 49350	4.0×10^6	Not Detected[c]
Campylobacter jejuni subsp. *jejuni*	ATCC 43430	1.2×10^5	3×LoD
	ATCC BAA-1062	1.2×10^5	3×LoD
	ATCC BAA-1234[b]	4.0×10^4	1×LoD
	BEI NR-128	1.2×10^5	3×LoD
Campylobacter upsaliensis	ATCC BAA-1059	4.0×10^4	1×LoD
	CCUG 24191	1.2×10^5	3×LoD
	ATCC 43953	1.2×10^5	3×LoD
	ATCC 43954[d]	4.0×10^6	Not Detected[d]
	ATCC 49815	1.2×10^5	3×LoD
	BEI HM-297	1.2×10^5	3×LoD

[a] *In silico* analysis indicates primer mismatches that might lead to reduced assay sensitivity or lack of reactivity with 11/138 *C. coli* sequences.
[b] Isolate was used to establish the LoD for this assay.
[c] *In silico* analysis indicates primer mismatches that might lead to reduced assay sensitivity for this subspecies.
[d] Sequencing under the primers identified an insertion/deletion in the primer binding region of the target gene.

Table - FilmArray *Clostridium difficile* toxin A/B Inclusivity Results

Organism	Toxinotype	Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected
Clostridium difficile	0 A+B+	ATCC 9689[a]	4.0×10^5	1xLoD
		ATCC BAA-1382	1.2×10^6	3×LoD
		ATCC 17857	1.2×10^6	3×LoD
		ATCC 17858	1.2×10^6	3×LoD
		ATCC 43255	1.2×10^6	3×LoD
		ATCC 43594	1.2×10^6	3×LoD
		ATCC 43596	1.2×10^6	3×LoD
		ATCC 43599	1.2×10^6	3×LoD
		ATCC 43600	1.2×10^6	3×LoD

Organism	Toxinotype	Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected
		ATCC 51695	1.2×10^6	3×LoD
		ATCC 700792	1.2×10^6	3×LoD
	III A+B+	ATCC BAA-1805 (NAP1)	1.2×10^6	3×LoD
		Zeptometrix #0801619 (NAP1)[a]	4.0×10^4	1×LoD
	V A+B+	ATCC BAA-1875	1.2×10^6	3×LoD
	VIII A-B+	ATCC 43598	1.2×10^6	3×LoD
	X A-B+	CCUG 8864	1.2×10^6	3×LoD
	XII A+B+	ATCC BAA-1812	1.2×10^6	3×LoD
	XXII A+B (unknown)	ATCC BAA-1814	1.2×10^6	3×LoD

[a] This isolate was used to establish the LoD for this assay.

Table - FilmArray *Plesiomonas shigelloides* Inclusivity Results

Organism	Geographic Isolation	Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected
Plesiomonas shigelloides	CDC 3085-55	ATCC 14029[a]	1.0×10^3	1xLoD
	CDC 16408	ATCC 14030	3.0×10^3	3×LoD
	Dakar, Senegal	ATCC 51572	3.0×10^3	3×LoD
	Unknown	ATCC 51903	3.0×10^3	3×LoD
	Colorado	CDPH HUM-2011019465	3.0×10^3	3×LoD
	Czech Republic	NIPH-Czech Republic 6300	3.0×10^3	3×LoD

[a] This isolate was used to establish the LoD for this assay. The organism was quantified in CFU/mL by plate enumeration.

Table FilmArray *Salmonella* Inclusivity Results

Organism (species, subspecies, and serovar)	Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected
Salmonella bongori	SGSC RKS 3041[a]	1.0×10^4	1xLoD
	NCTC 10946	3.0×10^4	3×LoD
	SGSC RKS 3044	3.0×10^4	3×LoD
Salmonella enterica subsp. *salamae II*	SGSC RKS 2985	1.5×10^4	3×LoD
Salmonella enterica subsp. *arizonae IIIa*	SGSC RKS 2980	1.5×10^4	3×LoD
Salmonella enterica subsp. *diarizonae IIIb*	SGSC RKS 2978	1.5×10^4	3×LoD
Salmonella enterica subsp. *houtenae IV*	SGSC RKS 3027	1.5×10^4	3×LoD

Organism (species, subspecies, and serovar)		Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected
Salmonella enterica subsp. *indica VI*		SGSC RKS 2995	1.5×10^4	3×LoD
Salmonella enterica subsp. *enterica*[b]	Typhimurium	SGSC RKS 4194[a]	5.0×10^3	1xLoD
	Enteritidis	ATCC BAA-708	1.5×10^4	3×LoD
	Newport	ATCC 27869	1.5×10^4	3×LoD
	Javiana	ATCC 10721	1.5×10^4	3×LoD
	Heidelberg	ATCC 8326	1.5×10^4	3×LoD
	Montevideo	ATCC BAA-710	1.5×10^4	3×LoD
	I 4,[5],12:i:-	Cornell CU0580	1.5×10^4	3×LoD
	Oranienburg	ATCC 9239	1.5×10^4	3×LoD
	Saintpaul	ATCC 9712	1.5×10^4	3×LoD
	Muenchen	ATCC 8388	1.5×10^4	3×LoD
	Braenderup	ATCC 700136	1.5×10^4	3×LoD
	Infantis	ATCC BAA-1675	1.5×10^4	3×LoD
	Thompson	ATCC 8391	1.5×10^4	3×LoD
	Mississippi	Cornell CU0633	1.5×10^4	3×LoD
	Paratyphi B var. L(+) tartrate+ (formerly java)	CCUG 9561	1.5×10^4	3×LoD
	Typhi (Purified DNA)[b]	ATCC 700931D-5	1.5×10^4	3×LoD
	Agona	ATCC 51957	1.5×10^4	3×LoD
	Schwarzengrund	CCUG 21280	1.5×10^4	3×LoD
	Bareilly	ATCC 9115	1.5×10^4	3×LoD
	Hadar	ATCC 51956	1.5×10^4	3×LoD

[a] This isolate was used to establish the LoD for this assay. The organism was quantified in CFU/mL by plate enumeration.
[b] Purified DNA was quantified in GE/mL by spectrophotometer.

Note: In addition to those evaluated in this study, *in silico* sequence analysis indicates the FilmArray *Salmonella* assay should react with all species and subspecies of *Salmonella*, including all serotypes of S. *enterica* subsp. *enterica.*

Table - FilmArray *Vibrio* (*V. parahaemolyticus/V. vulnificus/V. cholerae*) and *Vibrio cholerae* Inclusivity Results

Organism species, biotype and serotype)		Source/Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected
Vibrio cholerae	O:1 Ogawa	ATCC 14035[a]	8.0×10^3	1xLoD
	O:1 Inaba, Biotype El Tor	BEI NR-147	2.4×10^4	3xLoD
	O:1 Ogawa, Biotype El Tor	BEI NR-148	2.4×10^4	3xLoD
	non-O:1,non-O:139 (O:2)	BEI NR-149	2.4×10^4	3xLoD
	non-O:1,non-O:139 (O:7)	BEI NR-152	2.4×10^4	3xLoD
	O:1 Inaba, Biotype El Tor	ATCC 25870	2.4×10^4	3xLoD
Vibrio parahaemolyticus		ATCC 17802[a]	8.0×10^4	1xLoD
		ATCC BAA-242	2.4×10^5	3xLoD
		ATCC 27969	2.4×10^5	3xLoD
		ATCC 33845	2.4×10^5	3xLoD
		BEI NR-21990	2.4×10^5	3xLoD
		BEI NR-21992	2.4×10^5	3xLoD
Vibrio vulnificus		ATCC 29306	2.4×10^5	3xLoD
		ATCC 33817	2.4×10^5	3xLoD
		ATCC BAA-88	2.4×10^5	3xLoD
		ATCC 27562	2.4×10^4	0.3xLoD
		ATCC BAA-86	2.4×10^4	0.3xLoD

[a] Isolate was used to establish the LoD for this assay.

Note: In the clinical evaluation, a *Vibrio* carrying a variant *toxR* sequence was not detected by the Vchol assay and very rare strains of pathogenic *V. cholerae* that do not carry that *toxR* gene will also not be detected by the Vchol assay.

Table - FilmArray *Yersinia enterocolitica* Inclusivity Results

Organism	Serotype	Source/Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected
Yersinia enterocolitica	O:8	ATCC 9610[a]	5.0×10^4	1xLoD
		ATCC 23715	1.5×10^5	3xLoD
		BEI NR-207	1.5×10^5	3xLoD
	O:5, 27	NCTC 10463	1.5×10^5	3xLoD
	O:3	ATCC 700822	1.5×10^5	3xLoD
		BEI NR-212	1.5×10^5	3xLoD
	O:9	ATCC 55075	1.5×10^5	3xLoD

[a] Isolate was used to establish the LoD for this assay. The organism was quantified in CFU/mL by plate enumeration.

Note: In addition to those evaluated in this study, *in silico* sequence analysis indicates the FilmArray *Yersinia enterocolitica* assay should react with all strains/serotypes of *Y. enterocolitica* (including O:1, 2a, 3; O:2a,3; O:4,32; O:12,25; O:13a,13b; O:19; O:20; and O:21).

Table - FilmArray Enteroaggregative *E. coli* (EAEC) Inclusivity Results

Organism	Serotype	Source/Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected
Enteroaggregative *E. coli* (EAEC)	O92:H33	STEC Center JM221[a]	1.0×10^4	1xLoD
	O162:NM	Penn State 92.0148	3.0×10^4	3xLoD
	O17:H6	Penn State 92.0142	3.0×10^4	3xLoD
	O4:H7	Penn State 92.0144	3.0×10^4	3xLoD
	O51:H11	Penn State 92.0143	3.0×10^4	3xLoD
	O68:NM	Penn State 92.0154	3.0×10^4	3xLoD
	O7:NM	Penn State 92.0151	3.0×10^3	0.3xLoD
	O44:H18	STEC Center O42	3.0×10^3	0.3xLoD
	O104:H4 (Purified DNA)[b]	2011 European Outbreak strain[c]	3.0×10^3	0.3xLoD
	Ond:H10[d]	STEC Center 101-1	1.5×10^8	Not Detected[d]

[a] Isolate was used to establish the LoD for this assay. The organism was quantified in CFU/mL by plate enumeration.
[b] Purified DNA was quantified in GE/mL by spectrophotometer.
[c] Isolate has genetic characteristics consistent with STEC and EAEC.
[d] Phenotypic EAEC but known to not carry the marker(s) detected by the FilmArray GI Panel EAEC assay.

Table - FilmArray Enteropathogenic *E. coli* (EPEC) Inclusivity Results

Organism	Serotype	Typical/ Atypical	Source/Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected
Enteropathogenic *E. coli* (EPEC)	O127:H6	Typical	STEC Center E2348/69[a]	1.0×10^3	1×LoD
	O128:H2	Atypical	STEC Center DEC11a	3.0×10^3	3×LoD
	111a:NM	Unknown	STEC Center Stoke W	3.0×10^3	3×LoD
	O142:H6	Typical	STEC Center E851/71	3.0×10^3	3×LoD
	O55:H7	Atypical	STEC Center DEC5A	3.0×10^3	3×LoD
	O114:H2	Typical	STEC Center 3448-87	3.0×10^3	3×LoD
	O119:H+	Unknown	STEC Center RN410/1	3.0×10^3	3×LoD
	O96:H	Unknown	STEC Center HSP19/4	3.0×10^3	3×LoD
	O86:Hnm	Unknown	STEC Center E990	3.0×10^3	3×LoD
	O55:H-	Unknown	STEC Center MA551/1	3.0×10^3	3×LoD

[a] Isolate was used to establish the LoD for this assay. The organism was quantified in CFU/mL by plate enumeration.

Table-FilmArray Enterotoxigenic *E. coli* (ETEC) *lt/st* Inclusivity Results

Organism	Serotype	ST/LT	Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected
Enterotoxigenic *E. coli* (ETEC)	O78:H11	STA (+)/LT (+)	ATCC 35401[a]	1.0×10^3	1×LoD
	O175:H15	STA (-)/LT (+)	Penn State 6.0671	3.0×10^3	3×LoD
	O149:H5	STA (-)/LT (+)	Penn State 6.1182	3.0×10^3	3×LoD
	O84:H28	STA (-)/LT (+)[b]	Penn State 7.1493	3.0×10^3	Not Detected[b]
	H5	STA (+)/LT (-)	Penn State 10.0049	3.0×10^3	3×LoD
	O168	STA (+)/LT (-)	Penn State 9.1809	3.0×10^3	3×LoD
	O145:H25	STA (+)/LT (-)	Penn State 10.0136	1.0×10^4	100xLoD[c]
	O78	STA (+)/LT (+)	Penn State 2.1507	3.0×10^3	3×LoD
	O19:H5	STA (+)/LT (+)	Penn State 5.0038	3.0×10^3	3×LoD
	H14	STA (+)/LT (-)	Penn State 10.045	3.0×10^3	3×LoD
	O141	STA (+)/LT (+)	Penn State 93.0045	3.0×10^3	3×LoD
	Unknown	STB (+)[d] STA(-)/LT(-)	Penn State 8.2425	1.5×10^9	Not Detected[d]
	Unknown	STB (+)[d] STA(-)/LT(-)	Penn State 9.1179	1.5×10^9	Not Detected[d]

[a] Isolate was used to establish the LoD for this assay. The organism was quantified in CFU/mL by plate enumeration.
[b] Secondary PCR assay could not confirm the presence of the target gene(s) – plasmid/gene loss suspected.
[c] Sequencing of the target gene(s) identified sequence variation leading to reduced sensitivity for STA in this isolate.
[d] The FilmArray GI Panel will not detect phenotypic ETEC that that express only heat-stable toxin ST2/STB or heat-labile toxin LT-II.

Table -FilmArray Shiga-like toxin producing *E. coli* (STEC) *stx1/stx2* and *E. coli* O157 Inclusivity Results

Organism	Serotype	*stx1/stx2*	Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected STEC	Multiple of LoD Detected O157
Shiga-like toxin producing *E. coli* (STEC)	**STEC (non-O157)**					
	O25:H11	+/+	ATCC BAA-2196[a]	1.0×10^3	1×LoD	Not Detected
	O113:H21	+/+	ATCC BAA-177	3.0×10^3	3×LoD	Not Detected
	O45:H2	Unknown	STEC Center DEC11C	3.0×10^3	3×LoD	Not Detected
	O103:H2	+/Unknown	STEC Center 107-226	3.0×10^3	3×LoD	Not Detected
	O104:H21	-/+	STEC Center G5506	3.0×10^3	3×LoD	Not Detected
	O111:NM	+/+	STEC Center 95-3208	3.0×10^3	3×LoD	Not Detected
	O111:H2	-/+	STEC Center RD8	3.0×10^3	3×LoD	Not Detected
	O111:H8	+/+	STEC Center DEC8B	3.0×10^3	3×LoD	Not Detected
	O121:H19	Unknown	STEC Center F6173	3.0×10^3	3×LoD	Not Detected

Organism	Serotype	*stx1/stx2*	Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected STEC	Multiple of LoD Detected O157
	O26:NM	+/-	STEC Center DA-22	3.0×10^3	3×LoD	Not Detected
	O26:H11	+/-	STEC Center H19	3.0×10^3	3×LoD	Not Detected
	O145:NM	+/-	STEC Center GS G5578620	3.0×10^3	3×LoD	Not Detected
	0104:H4[b] (Purified DNA)[c]	-/+	ATCC BAA-2326D-5[b]	3.0×10^{3}[c]	3×LoD	Not Detected
	STEC O157					
	O157:NM	+/+	STEC Center DA-26	3.0×10^3	3×LoD	0.3×LoD
	O157:H7	-/+	STEC Center E32511	3.0×10^3	3×LoD	0.3×LoD
	O157:HNT	+/+	STEC Center DA-74	3.0×10^3	3×LoD	0.3×LoD
	O157:H7	+/+	ATCC 43895[a]	1.0×10^4	10xLoD	1xLoD
	O157:H7	+/+	STEC Center A8993-CS2	3.0×10^4	30×LoD	3×LoD
	Non-STEC O157					
	O157:H7	-/-	ATCC 43888	3.0×10^4	Not Detected	N/A[d]
	O157:H45	-/-	STEC Center SC373/2	3.0×10^4	Not Detected	N/A[d]

[a] Isolate was used to establish the LoD. The organism was quantified in CFU/mL by plate enumeration.
[b] 2011 European Outbreak Strain. Isolate has genetic characteristics consistent with STEC and EAEC.
[c] Purified DNA was quantified in GE/mL by spectrophotometer.
[d] *E. coli* O157 N/A results reported due to lack of positive results for STEC.

Note: Based on *in silico* analysis, *stx2* subtypes e and f are predicted to be detected with reduced sensitivity or not detected by the FilmArray GI Panel STEC assays.

Table - FilmArray Shigella/Enteroinvasive *E. coli* (EIEC) Inclusivity Results

Organism	Serotype (Temporal/Geographic Isolation)	Source/Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected
Enteroinvasive *E. coli* (EIEC)	O29:NM	ATCC 43892[a]	5.0×10^3	1×LoD
	O29:HNM (1977)	STEC Center 1885-77	3.0×10^3	0.6×LoD
	O124:HNM (1978)	STEC Center 929-78	3.0×10^3	0.6×LoD
	O29:H27 (USA, VA; 1979)	STEC Center 1827-79	3.0×10^3	Not Detected[b]
	O28:H- (Brazil, 1983)	STEC Center LT-15	3.0×10^3	0.6×LoD
	O136:H- (Bangladesh, 1983)	STEC Center LT-41 Strain 1111-55	3.0×10^3	0.6×LoD
Shigella boydii (Serogroup C)	Type 2	ATCC 8700	3.0×10^2	3×LoD
	Type 4	CDPH HUM-2010029296	3.0×10^2	3×LoD

Organism	Serotype (Temporal/Geographic Isolation)	Source/Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected
	Type 1	ATCC 9207	3.0×10^2	3×LoD
	Type 20	ATCC BAA-1247	3.0×10^2	3×LoD
	Type 10	ATCC 12030	3.0×10^2	3×LoD
Shigella dysenteriae (Serogroup A)	Type 1	BEI NR-520	3.0×10^2	3×LoD[c]
	Type 2	CDPH PHM-2004008089	3.0×10^2	3×LoD
	Type 13	ATCC 49555	3.0×10^2	3×LoD
	Type 3	ATCC 29028	3.0×10^2	3×LoD
	Type 12	ATCC 49551	3.0×10^2	3×LoD
Shigella flexneri (Serogroup B)	Type 2a	ATCC 700930	3.0×10^2	3×LoD
	Type 1a	ATCC 9199	3.0×10^2	3×LoD
	Type 6	CDPH PHM-2006004043	3.0×10^2	3×LoD
	Type 2b	ATCC 12022	3.0×10^2	3×LoD
	Type 2a	ATCC 29903	3.0×10^2	3×LoD
	Unknown	STEC Center VA-6	3.0×10^2	3×LoD
Shigella sonnei (Serogroup D)	N/A	ATCC 29930	1.0×10^{2}[a]	1×LoD
	N/A	ATCC 11060	3.0×10^2	3×LoD
	N/A	CDPH HUM-2010027998	3.0×10^2	3×LoD
	N/A	ATCC 29031	3.0×10^2	3×LoD
	N/A	ATCC 25931	3.0×10^2	3×LoD
	N/A	ATCC 9290	3.0×10^2	3×LoD

[a] Isolate was used to establish the LoD for this assay. The organism was quantified in CFU/mL by plate enumeration.
[b] Secondary PCR assay could not confirm the presence of the target gene(s) – plasmid/gene loss suspected.
[c] This isolate gave the expected STEC Detected and *Shigella*/EIEC Detected results due to the presence of *stx* in *Shigella dysenteriae*.

Table - FilmArray *Cryptosporidium* Inclusivity Results

Organism	Geographic Source/ Isolate	Concentration Detected (cells/mL)	Multiple of LoD Detected
Cryptosporidium canis	Peru Clinical Sample	Unknown	<LoD[a]
Cryptosporidium hominis	Scotland Clinical Sample[b]	2.1×10^{3} [b]	1×LoD
	Scotland Clinical Sample	6.4×10^3	3×LoD
	Scotland Clinical Sample	6.4×10^3	3×LoD
	BEI NR-2520 (Purified DNA Isolate TU502)	6.4×10^3	3×LoD
Cryptosporidium meleagridis	BEI NR-2521 (Purified DNA Isolate TU1867)	1.8×10^3	3×LoD

Organism	Geographic Source/ Isolate	Concentration Detected (cells/mL)	Multiple of LoD Detected
Cryptosporidium muris	Waterborne P104	1.5×10^4 oocycts/mL	3×LoD
Cryptosporidium parvum	Waterborne P102C[c]	6.0×10^2 [c]	1×LoD
	Scotland Clinical Sample	1.8×10^3	3×LoD
	Scotland Clinical Sample	1.8×10^3	3×LoD
	BEI NR-2519 (Purified DNA Isolate Iowa)	1.8×10^3	3×LoD
Cryptosporidium ubiquitum	Scotland Purified DNA from Clinical Sample	Unknown	<LoD[a]
	Scotland Purified DNA from Clinical Sample	Unknown	<LoD[a]

[a] Quantification by qPCR indicated that these purified samples have an analyte concentration that is lower than the assay LoD.
[b] This *C. hominis* sample was used to establish the LoD for *C. hominis* (LoD of 5.0×10^3 oocysts/mL was determined to be equivalent to 2.1×10^3 copies/mL).
[c] This *C. parvum* isolate was used to establish the LoD for *C. parvum* (LoD of 5.0×10^3 oocysts/mL was determined to be equivalent to 6.0×10^2 copies/mL).

Note: *In silico* sequence analysis indicates the FilmArray *Cryptosporidium* assay(s) should react with approximately 23 different *Cryptosporidium* species (including those evaluated in this study) as well as sequences not assigned to specific species. *In silico* analysis predicts that the *Cryptosporidium* assay(s) may not react with the rare or non-human species *C. bovis*, *C. ryanae* and *C. xiaoi*.

Table - FilmArray *Cyclospora cayetanensis* Inclusivity Results

Organism	Location/Sample		Concentration Detected (GE/mL)	Multiple of LoD Detected
Cyclospora cayetanensis	Nebraska	Clinical Specimen[a]	180	1×LoD
		Clinical Specimen	540	3×LoD
		Clinical Specimen	540	3×LoD
	Peru	Clinical Specimen	540	3×LoD
		Clinical Specimen	540	3×LoD
		Clinical Specimen	540	3×LoD
		Clinical Specimen	540	3×LoD

[a] Isolate was used to establish the LoD for this assay.

Table - FilmArray *Entamoeba histolytica* Inclusivity Results

Organism	Strain Designation	Location/Year of Isolation	Isolate ID	Concentration Detected (copies/mL)	Multiple of LoD Detected
Entamoeba histolytica	HM-1:IMSS	Mexico City 1967	ATCC 30459[a]	~1.2 x 10^5	1×LoD
	EntaHB-301:NIH	Burma 1960	BEI NR-176	3.6 x 10^5	3×LoD
	Rahman	England 1972	BEI NR-179	3.6 x 10^5	3×LoD
	HU-21:AMC	Arkansas 1970	BEI NR-2589	3.6 x 10^5	3×LoD
	IP:1182:2	Honduras 1982	BEI NR-20088	3.6 x 10^5	3×LoD
	SAW 408 RR, Clone A	Mexico	BEI NR-20090	3.6 x 10^5	3×LoD

[a] Isolate was used to establish the LoD for this assay (LoD of 2.0×10^3 cells/mL was determined to be equivalent to ~1.2×10^5 copies/mL).

Table - FilmArray *Giardia lamblia* Inclusivity Results

Organism	Location/Year of Isolation	Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected
Giardia lamblia (aka *G. intestinalis* or *G. duodenalis*)	New Orleans, LA 1985	ATCC 50137	150	3×LoD
	Portland, OR 1971	ATCC 30888	150	3×LoD
	Bethesda, MD 1979	ATCC 30957[a]	50	1×LoD
	Unknown	Waterborne P101	150	3×LoD
	Egypt	ATCC PRA-243	150	3×LoD
	United States	ATCC PRA-247	150	3×LoD

[a] Isolate was used to establish the LoD for this assay.

Table - FilmArray Adenovirus F 40/41 Inclusivity Results

Organism	Source/ Isolate ID	Concentration Detected (copies/mL)	Multiple of LoD Detected
Adenovirus F 40	ATCC VR-931[a]	~2.8×10^5	1×LoD
	Clinical Sample E239	8.4×10^5	3×LoD
	NCPV 0101141v (Dugan)	8.4×10^5	3×LoD
	Zeptometrix 0810084CF	8.4×10^5	3×LoD
Adenovirus F 41	ATCC VR-930 (Tak)[a]	~3.0×10^4	1×LoD
	Zeptometrix #0810085CF (Tak)[b]	9.0×10^4	10×LoD[b]
	UIRF/Zeptometrix 305571	9.0×10^4	3×LoD
	Clinical Sample 762	9.0×10^4	3×LoD
	Clinical Sample 976	9.0×10^4	3×LoD
	Clinical Sample Chn81	9.0×10^4	3×LoD

[a] Isolate was used to establish the LoD for this assay. For ATCC VR-9310, the LoD of 1 $TCID_{50}$/mL was determined to be equivalent to 2.8×10^5copies/mL and for ATCC VR-930, the LoD of 100 $TCID_{50}$/mL was determined to be equivalent to 3.0×10^4copies/mL.

[b] Same strain as ATCC VR-930 (which was detected at 1× LoD) but obtained from a different source.

Table - FilmArray Astrovirus Inclusivity Results

Organism	Type	Location/Source/Isolate ID	Concentration Detected (copies/mL)	Multiple of LoD Detected
Human Astrovirus	1	China Clinical Sample	3.9×10^7	10×LoD
		China Clinical Sample	3.9×10^7	3×LoD
	2	USA Clinical Sample	3.9×10^7	3×LoD
	3	University of Barcelona Spain	3.9×10^7	3×LoD
	4	NCPV #1002072v	3.9×10^7	3×LoD
	5	USA Clinical Sample	3.9×10^7	3×LoD
		USA Clinical Sample	3.9×10^7	3×LoD
	6	University of Barcelona Spain	3.9×10^7	3×LoD
	7	University of Barcelona Spain	3.9×10^7	3×LoD
	8	NCPV #1003071v[a]	$\sim1.3\times10^7$	1×LoD

[a] Isolate was used to establish the LoD for this assay (LoD of 50 FFU/mL was determined to be equivalent to1.3×10^7 copies/mL).

Table - FilmArray Norovirus GI/GII Inclusivity Results

Norovirus Genogroup/Genotype		Isolate ID (Clinical Samples)	Concentration Detected (copies/mL)	Multiple of LoD Detected
Norovirus GI	3	Noro1_036[a]	1.0×10^4	1×LoD
	2	Noro1_002	6.0×10^3	0.6×LoD
	3	Noro1_003	6.0×10^3	0.6×LoD
		Noro1_012	6.0×10^3	0.6×LoD
		Noro1_030	6.0×10^3	0.6×LoD
	4	Noro1_031	6.0×10^3	0.6×LoD
	6	Noro1_021	1.0×10^5	10×LoD
	7	Noro1_009	2.0×10^5	20×LoD[c]
		Noro1_029	6.0×10^3	0.6×LoD
		Noro1_034	6.0×10^3	0.6×LoD
	8	Noro G1.8[b]	6.0×10^4	6×LoD
Norovirus GII	Unknown	Noro2_013[a]	1.0×10^4	1×LoD
	2	NoroII.2[b]	6.0×10^3	0.6×LoD
	3	China-5	6.0×10^3	0.6×LoD
		SGB_038	6.0×10^3	0.6×LoD
	4	GI-PILOT-SPDRL-077	2.0×10^5	20×LoD[c]
		Noro2_004	2.0×10^5	20×LoD[c]
		Noro2_032	2.0×10^5	20×LoD[c]
		PCMC_025 (Sydney)	6.0×10^3	0.6×LoD
		PCMC_031 (Sydney)	6.0×10^3	0.6×LoD
	6	NYH-A	6.0×10^3	0.6×LoD

Norovirus Genogroup/Genotype		Isolate ID (Clinical Samples)	Concentration Detected (copies/mL)	Multiple of LoD Detected
	7	NoroII.7[b]	6.0×10^3	0.6×LoD
	8	NoroII.8[b]	6.0×10^3	0.6×LoD
	12	NoroII.12[b]	6.0×10^3	0.6×LoD
	16	NoroII.16[b]	6.0×10^3	0.6×LoD
	20	NoroII.20c[b]	2.0×10^5	20×LoD[c]
		NoroII.20[b]	6.0×10^3	0.6×LoD

[a] Isolate was used to establish the LoD for this assay.
[b] Isolate obtained as RNA extract from a clinical sample. Genotype provided by the source laboratory.
[c] Noroviruses are genetically diverse. *In silico* analysis predicts that most strains of all genotypes will be detected, though some variant strains may be detected with reduced sensitivity or may not be detected due to inefficient amplification or exclusion by melt analysis.

Table - FilmArray Rotavirus A Inclusivity Results

Organism	Strain Designation (Serotype)	Isolate ID	Concentration Detected (copies/mL)	Multiple of LoD Detected
Rotavirus A	ST3 (G4P6)	NCPV 0904053v[a]	3.9×10^3	1×LoD
	RV4 (G1P8)	NCPV 0904052v	1.2×10^4	3×LoD
	69M (G8P5)	NCPV 0904055v	1.2×10^4	3×LoD
	P (G3P1A[8])	NCPV 0904056v	1.2×10^4	3×LoD
	Wa (G1P1A[8])	ATCC VR-2018	1.2×10^4	3×LoD
	DS-1 (G2P1B[4])	ATCC VR-2550	1.2×10^4	3×LoD

[a] Isolate was used to establish the LoD for this assay (LoD of 1.0×10^5 FFU/mL was determined to be equivalent to 3.9×10^3 copies/mL).

Note: The Rotavirus A assay will also detect reassortant viruses used in vaccine production.

Table - FilmArray Sapovirus Inclusivity Results

Organism	Genogroup	Isolate ID (Clinical Samples)	Concentration Detected (copies/mL)	Multiple of LoD Detected
Sapovirus	GI	AB_SaV_14[a]	5.0×10^6	1×LoD
	Unknown	China_56	1.5×10^7	3×LoD
	Unknown	AB_SaV_03	1.5×10^7	3×LoD
	Unknown	PCMC_54	1.5×10^7	3×LoD
	Unknown	SPDRL-006	1.5×10^7	3×LoD
	Unknown	SPDRL-099	1.5×10^7	3×LoD
	Unknown	SGB-MP-11	1.5×10^7	3×LoD
	GI	Sapo_03[b]	1.5×10^7	3×LoD
	GII	Sapo_06[b]	1.5×10^7	3×LoD
	GIV	Sapo_09[b]	1.5×10^7	3×LoD
	GV	Sapo_02[b]	1.5×10^7	3×LoD

[a] Clinical Sample was used to establish the LoD for this assay.
[b] Isolate obtained as RNA extract from a clinical sample, genogroup information provided by source laboratory.

f. Analytical specificity (Exclusivity):

The potential for cross-reactivity between assays contained in the FilmArray GI Panel was evaluated by testing high concentrations of analyte in contrived stool samples. The organisms/viruses tested consisted of on-panel (identified by the GI Panel assays) and off-panel (not identified by the GI Panel assays) organisms/viruses.

On-panel organisms were tested to verify that they only react with the appropriate assays on the panel. On-panel exclusivity testing included 28 analytes were selected to evaluate the potential for cross-reactivity with other panel assays. This group of organisms was chosen such that at least one positive result would be obtained for each assay in the FilmArray GI Panel. Each organism was tested at a high concentration to show analytical specificity with all FilmArray GI Panel assays.

Organisms for off-panel testing were selected based on a combination of several factors including (1) relatedness to specific species detected by the GI Panel (near-neighbors), (2) clinical relevance, (3) likelihood of being present in stool specimens and (4) genetic similarity to GI Panel assay primers, as determined by *in silico* analyses during assay design. When empirical testing of these organisms was not performed, a separate organism-specific *in silico* analysis of whole genome sequence(s) directed against all GI Panel primers was attempted for reactivity predictions.

Results are presented for all organisms/viruses that were tested and received the expected FilmArray GI Panel test result(s) (no cross-reactivity, table below)

BACTERIA		
Wet Tested		
Abiotrophia defectivia	*Clostridium novyi*	*Megamonas hypermegale*
Acinetobacter baumannii	*Clostridium perfringens*	*Megasphaeara elsdenii*
Acinetobacter lwoffii	*Clostridium ramosum*	*Methanobrevibacter smithii*
Aeromonas hydrophila	*Clostridium septicum*	*Morganella morganii*
Alcaligenes faecalis	*Clostridium sordellii*	*Peptoniphilus asaccharolyticus*
Anaerococcus tetradius	*Clostridium tetani*	*Peptostreptococcus anaerobius*
Arcobacter butzleri	*Collinsella aerofaciens*	*Photobacterium damselae*
Arcobacter cryaerophilus	*Corynebacterium genitalium*	*Porphyromonas asaccharolytica*
Bacillus cereus	*Desulfovibrio piger*	*Prevotella melaninogenica*
Bacteroides fragilis	Diffusely adherent *E.coli*	*Proteus mirabilis*
Bacteroides thetaiotaomicron	*Escherichia blattae*	*Proteus penneri*
Bacteroides vulgatus	*Escherichia fergusonii*	*Proteus vulgaris*
Bifidobacterium adolescentis[a]	*Escherichia hermannii*	*Provedencia alcalifaciens*
Bifidobacterium bifidum[a]	*Escherichia vulneris*	*Pseudomonas aeruginosa*
Bifidobacterium longum[a]	*Edwardsiella tarda*	*Ruminococcus bromiia*
Campylobacter concisus	*Egglerthella lenta*	*Ruminococcus flavefaciensa*
Campylobacter curvus	*Enterobacter aerogenes*	*Ruminococcus obeuma*
Campylobacter fetus	*Enterobacter cloacae*	*Selenomonas ruminantium*
Campylobacter gracilis	*Enterococcus faecalis*	*Serratia liquefaciens*

Campylobacter helveticus	*Enterococcus faecium*	*Serratia marcescens*
Campylobacter hominis	*Eubacterium cylindroides*	*Shewanella algae*
Campylobacter hyointestinalis	*Eubacterium rectale*	*Staphylococcus aureus*
Campylobacter lari	*Faecalibacterium prausnitzii*	*Staphylococcus epidermidis*
Campylobacter mucosalis	*Fusobacterium varium*	*Stenotrophomonas maltophilia*
Campylobacter rectus	*Gardnerella vaginalis*	*Streptococcus agalactiae*
Campylobacter showae	*Gemella morbillorum*	*Streptococcus intermedius*
Campylobacter sputorum	*Haemophilus influenzae*	*Streptococcus pyogenes*
Campylobacter ureolyticus	*Hafnia alveib*	*Streptococcus salivarius*
Cedecea davisae[b]	*Helicobacter fennelliae*	*Streptococcus suis*
Chlamydia trachomatis	*Helicobacter pylori*	*Trabulsiella guamensis*
Citrobacter amalonaticus	*Klebsiella oxytoca*	*Veillonella parvula*
Citrobacter freundii	*Klebsiella pneumoniae*	*Yersinia bercovieri*
Clostridium acetobutylicum	*Lactobacillus acidophilus*	*Yersinia frederiksenii*[d]
Clostridium botulinum	*Lactobacillus reuteri*	*Yersinia intermedia*
Clostridium difficile non-toxigenic[c]	*Lactococcus lactis*	*Yersinia mollaretii*
Clostridium histolyticum	*Leminorella grimontii*	*Yersinia pseudotuberculosis*
Clostridium methylpentosum	*Listeria monocytogenes*	*Yersinia rohdei*

[a] Though not observed in this study, cross-reactivity of the *Giardia lamblia* assay with one or more *Bifidobacterium* and *Ruminococcus* species was observed in the clinical evaluation. *Bifidobacterium* and *Ruminococcus* species are listed as potential cross-reacting organisms.

[b] Though not observed in this study, possible cross-reactivity of the ETEC 2 assay with *Hafnia alvei* and *Cedeceae davisiae* was observed in the clinical evaluation or predicted by *in silico* analysis. *Halfnia alvei* and *Cedeceae davisiae* are also listed as potentially cross-reactive organisms.

[c] Two isolates of this species were tested for analytical specificity.

[d] Though not observed in this study, i*n silico* analysis indicates that cross-reactivity between *Yersinia fredricksenii* and the *Yersinia entercolitica* assay is possible at high concentrations. *Y. fredricksenii* is also listed as potentially cross-reactive organism.

Table - No Cross-Reactivity with FilmArray GI Panel Assays Observed (Tested) or Predicted by *in silico* Analysis

PROTOZOA/PARASITES		**FUNGI**
Wet Tested	***In silico* Analysis Only**	**Tested**
Babesia microti	*Ancylostoma duodenale*	*Aspergillus fumigatus*
Blastocystis hominis	*Ascaris lumbricoides*	*Candida albicans*
Conidiobolus lachnodes	*Balantidium coli*	*Candida catenulate*
Conidiobolus lobatus	*Chilomastix mesnili*	*Penicillium marneffei*
Encephalitozoon hellem	*Dientamoeba fragilis*	*Saccharomyces boulardi*
Encephalitozoon intestinalis	*Endolimax nana*	*Saccharomyces cerevisiae*
Entamoeba gingivalis	*Entamoeba coli*	
Entamoeba moshkovskii	*Entamoeba hartmanni*	
Giardia muris	*Entamoeba polecki*	
Pentatrichomonas hominis	*Enterobius vermicularis*	
Schistosoma mansoni	*Enteromonas hominis*	
Toxoplasma gondii	*Isospora belli*	
Trichomonas tenax	*Necator americanus*	

Table - No Cross-Reactivity with FilmArray GI Panel Assays Observed (Tested) or Predicted by *in silico* Analysis

VIRUSES		
Wet Tested		***In silico* Analysis Only**
Adenovirus A:31	Coronavirus 229E	Adenovirus G52
Adenovirus B:34	Coxsackievirus B3	Norovirus GIV
Adenovirus C:2	Cytomegalovirus (CMV)	Rotavirus B
Adenovirus D-37	Enterovirus 6	Rotavirus C
Adenovirus E:4a	Enterovirus 68	
Astrovirus variant VA1	Hepatitis A	
Astrovirus variant MLB	Herpes Simplex Type 2	
Bocavirus Type 1	Rhinovirus 1A	

Table - Observed or Predicted Cross-Reactivity between GI Panel Assays and Off-Panel Organisms

FilmArray GI Panel Test Result	Cross-Reactive Organism(s)
Entamoeba histolytica	*Entamoeba dispar*
Giardia lamblia	*Bifidobacterium* spp.[a]
	Ruminococcus spp.[a]
Enterotoxigenic *E.coli* (ETEC) *It/st* [ETEC 2 assay]	*Citrobacter koseri*
	Citrobacter sedlakii
	Hafnia alvei[a]
	Cedeceae davisiae[a]
Salmonella [b]	*E. coli* with variant type III secretion protein[b]
Vibrio (*V. parahaemolyticus/V. vulnificus/ V. cholerae*)	*Vibrio alginolyticusVibrio fluvialis*[c] *Vibrio mimicus*[c] *Grimontia (*formerly *Vibrio) hollisae*
Yersinia enterocolitica	*Yersinia frederiksenii*[a,d]
	Yersinia kristensenii[d]

[a] Cross-reactivity was not observed when tested at high concentration (1.5×10^9 cells/mL). However, cross reactivity was suspected or confirmed in clinical specimens and/or the potential for cross-reactivity is supported by *in silico* predictions.
[b] Cross-reactivity resulting in false positive *Salmonella* results has not been observed in analytic or clinical testing. However, non-specific amplification products with Tm values close to the assay specific Tm range have been observed and the potential for false positive *Salmonella* test results exists.
[c] Detected at concentrations near the *Vibrio* assay LoD.
[d] *Y. kristensenii* and *Y. fredericksenii* are difficult to distinguish from *Y. entercolitica* by standard laboratory method

g. Assay cut-off:

The FilmArray GI Panel Melt Detector software determines whether a FilmArray GI assay result is positive or negative using a predefined algorithm that includes Tm values, fluorescence values, and analysis of melting curves.

The FilmArray GI Melt Detector software performs a set of basic calculations on the melt data to determine if a PCR reaction occurred in each well. If the melt profile indicates that a PCR product is present, then the analysis software calculates one or two Tm values, depending on the number of melt curves present in the data, and the Tm values are compared against an expected melt range for the associated assay. If the software determines that the melt is positive and the melt curve falls inside the assay's specific melt range, then the curve is called positive. If the software determines that the melt is negative or that it is not in the appropriate range,

then the curve is called negative. All assays have three replicates. The melt curve for each replicate is given a positive or negative melt call by the Melt Detector.

For individual melt curves, the observed sensitivity and specificity, as compared to expert annotation, of the Melt Detector is greater than 98.92% and 99.99%, respectively. For the Analysis Software, the observed sensitivity and specificity, as compared to expert annotation, of the assay calls are greater than 99.34% and 99.99%, respectively.

h. Fresh versus Frozen Study:

This study was performed to support the use of frozen specimens in the FilmArray GI Panel archived specimen and reproducibility testing; the test is not intended for use on frozen samples.

Stool specimens in Cary Blair were stored at <-70°C. The test panel contained at least one specimen for every organism on the panel (with the exception of *Entamoeba histolytica*). Specimens from all four prospective clinical study sites were included in the study. The median time interval between testing fresh and retesting from frozen was 60 days (Range: 10-90 days). Detection was 100% for most analytes but 90-94% for five analytes (*Campylobacter, C. difficile, P. shigelloides*, EPEC, and *Cryptosporidium*), and 54-83% for four analytes (*Shigella*/EIEC, *G. lamblia*, Norovirus) Based on study results, specific analytes for study testing were performed with fresh or frozen samples as appropriate.

A total of 203 FilmArray runs were attempted in this evaluation of 201 frozen specimens, 201 of which were completed (99%; 201/203). There was one run failure each for a software error (0.5%) and a control failure (0.5%).

i. Interference

Substances that could be present in stool samples (preserved in Cary Blair transport medium) or introduced during sample handling were evaluated for their potential to interfere with assay performance. A potentially interfering substance (see tables below) was added to a contrived stool sample by spiking representative GI Panel organisms into negative sample matrix (individual or pooled donor stool in transport medium). Each contrived sample contained a mix of four different organisms, each present at approximately three times (3×) the limit of detection (LoD). Contrived samples without added potentially interfering substances served as positive controls.

Of the endogenous and exogenous substances tested, only the bovine-derived mucin gave unexpected results (EPEC was reported in samples that were not spiked with EPEC). An investigation found bacterial nucleic acid in the bovine-derived mucin used as the test substance, and it was determined that the unexpected results were due to EPEC contamination in the commercially prepared mucin.

Table - Potentially Interfering Endogenous and Exogenous Substances Tested

Endogenous Substances	Exogenous Substances (including laboratory disinfectants)	
Human Whole Blood	Bacitracin	Glycerin
Triglycerides	Doxycycline	Hydrocortisone
Cholesterol	Nystatin	Loperamide hydrochloride
Fatty acids (palmitic acid)	Metronidazole	Magnesium hydroxide
Fatty acids (stearic acid)	Naproxen sodium	Mineral oil
Bovine Mucin [a]	Bisacodyl	Phenylephrine hydrochloride
Human Bile	Bismuth subsalicylate	Sodium phosphate
Human Urine	Calcium carbonate	Nonoxynol-9
Human stool (overfill of Cary	Docusate sodium	Bleach
Blair vial)		Ethanol

[a] Unexpected EPEC detected results reported. The presence of EPEC nucleic acid in test material was confirmed by independent PCR assays, indicating the unexpected results were caused by contamination of the mucin with EPEC.

No inhibition or unexpected test results were obtained in the presence of high concentrations of potentially competing microorganisms (on-panel or off-panel organisms; see table below). However, Rotavirus A Detected results were reported when Rotavirus A reassortant strains used in the manufacturing of Rotavirus A vaccines were tested (see table below). Rotavirus A vaccine may be shed in stool following oral administration and Rotavirus A will be detected by the FilmArray GI Panel if vaccine is present in the test sample.

Table- Potentially Interfering or Competing Organisms and Vaccine Material Tested

Off-Panel Organisms	On-Panel Organisms
Aeromonas hydrophila	Adenovirus F41
Bacteroides vulgatus	Enterotoxigenic *E. coli* (ETEC)
Bifidobacterium bifidum	
Human Rhinovirus 87	
Non-pathogenic *E. coli*	**RotaTeq Rotavirus A Vaccine Components**
Helicobacter pylori	Rotavirus reassortant WC3:2-5, R574(9) [ATCC VR-2195][a]
Saccharomyces boulardii	Rotavirus reassortant WI79-4,9 [ATCC VR-2415][a]

[a] Reactivity with the FilmArray Rotavirus A assay expected.

Contrived stool samples prepared in various enteric and fixative-containing transport media, including Cary Blair (see table below), were evaluated for the potential of different media to interfere with the accuracy of FilmArray GI Panel test results. No interference was observed for samples collected in Protocol Cary Blair or other brands of enteric transport media (Para-Pak Enteric Plus and Para-Pak C&S media; performance of the FilmArray GI Panel has not been established in these media). However, accurate detection of analytes was impaired (false negative results) for samples prepared in media containing fixatives, particularly those containing formalin.

Table Transport Media Tested

Enteric Transport Media – No Interference Observed		
PROTOCOL™ Cary Blair	Para-Pak Enteric Plus[a]	Para-Pak C&S[a]
Fixative-containing Transport Media – Interference Observed[a]		
Modified (Cu) PVA Fixative	Para-Pak 10% Formalin Fixative[b]	Para-Pak SAF Fixative[a]
Para-Pak ECOFIX Fixative	Para-Pak LV-PVA Fixative	Para-Pak Zn-PVA Fixative

[a]Performance has not been established in these media
[b]Impaired detection of analytes (false negative results) observed in formalin containing media.

j. Carryover Study:

To demonstrate sample-to-sample carryover does not occur when the recommended testing and cleaning procedures are followed, five sets of samples were tested with the FilmArray GI Panel over several days such that testing of one positive, high analyte level sample (*Plesiomonas shigelloides*, *Escherichia coli* (STEC), *Yersinia enterocolitica*, *Giardia intestinalis*, or Adenovirus F41) was followed directly by testing of a negative sample (containing no analyte).

In accordance with recommended procedures, each sample was prepared and loaded into a GI pouch one at a time using the provided pouch loading implements. Samples were loaded in a work area (biosafety cabinet or hood) that was separated from the location of the FilmArray instrument(s) and the loading block was cleaned between each pouch. For each set of samples, the negative sample was loaded in the same workspace, using the same Pouch Loading Station and tested directly after the high positive sample using the same FilmArray instrument. No false positive results were observed during testing of five sets of a high positive sample followed directly by a negative sample

k. Analysis of Pouch Hydration

The FilmArray GI pouch is manufactured to contain a vacuum that draws in the required amount of Hydration Solution and Sample/Buffer Mix when the seals are broken by the cannula-tipped cartridges in each of the pouch ports. If either the Hydration Solution or the Sample/Buffer Mix is not drawn into the pouch, the operator is instructed to discard the faulty pouch and obtain a new pouch to test the specimen. A total of 1591 pouches were used to achieve successful results for the 1556 valid specimens (note: this includes 14 pouches used for retests after initial pouch/instrument failures). Twenty two (22; 1.4%) of 1591 pouches failed to hydrate or pull in sample/buffer mix and were discarded; successful hydration was achieved on the subsequent pouch. Overall, 98.6% (1569/1591) of pouches used to test study specimens loaded successfully. No specimens were lost due to pouch hydration failures.

Table - Analysis of pouch hydration

Total Distinct Pouches Used	Loaded Successfully	Failure to Hydrate/Pull Sample
1591	1569	22
	98.6%	1.4%

2. Comparison studies:

   a. Method comparison with predicate device: N/A

   b. Matrix comparison: N/A

3. Clinical studies:

   The clinical performance of the FilmArray GI Panel was established during a multi-center study conducted at four geographically distinct U.S. study sites (Pacific, North Central, Great Lakes, and Northeast regions) between May and September, 2013. A total of 1578 prospective residual stool specimens in Cary Blair transport media were acquired for the clinical study; 22 of these were excluded. The most common reasons for exclusion were that a valid external control was not completed on the day of testing, that the specimen was not plated to all of the appropriate bacterial culture media required for the reference method, or that the specimen was beyond four days from the date of collection. The final data set consisted of 1556 specimens.

   The tables below provides a summary of the number of clinician ordered tests per site, the types of test ordered per site and the demographic information for the 1556 specimens included in the prospective study.

Table - Clinician Ordering Practices: number of tests ordered*

Number of additional tests	Site 1 (*n=253)*	Site 2 (*n*=366)	Site 3 (*n*=793)	Site 4 (*n*=144)	Total (*n*=1556)
0	72	43	219	28	362
1	118	137	189	48	492
2	59	150	230	39	478
3	4	18	117	18	157
4	-	11	36	10	54
5	-	2	2	1	5
6	-	5	-	-	5

**Excludes standard testing: Yersinia at Site 3 and Shiga toxin at sites 1, 3, and 4.*

Table - Clinician ordering practices: types of tests ordered*

Test Ordered	Site 1 (*n=253)*	Site 2 (*n*=366)	Site 3 (*n*=793)	Site 4 (*n*=144)	Total (*n*=1556)
Vibrio Culture	2	14	1	10	27
Yersinia Culture	1	51	-	10	62
Shiga Toxin	-	0	-	-	0
O&P Exam	72	12	373	91	548
Crypto/Giardia DFA	2	236	368	50	656
Virology	16	19	61	0	96
Toxigenic *C. difficile*	155	243	265	45	708
Other	0	0	86	19	105

**Excludes standard testing: Yersinia at Site 3 and Shiga toxin at sites 1, 3, and 4.*

Table - Demographic Summary for Prospective FilmArray GI Panel Clinical Evaluation

Prospective Study Specimens	
Total Specimens	1556
Sex	**Number of Specimens (%)**
Male	718 (46%)
Female	838 (54%)
Age Group	**Number of Specimens (%)**
<1 year	121 (8%)
1-5 years	418 (27%)
6-12 years	193 (12%)
13-21 years	240 (15%)
22-64 years	411 (26%)
65+ years	173 (11%)
Status	**Number of Specimens (%)**
Outpatient	1350 (87%)
Hospitalized	164 (11%)
Emergency	42 (3%)

The performance of the FilmArray GI Panel was evaluated by comparing the FilmArray GI Panel test result for each member of the panel with the appropriate comparator/reference methods shown in the table below. For each comparator PCR assay, each extracted sample was first tested with an inhibition control.

Table -FilmArray GI Panel Study Comparator/Reference Methods

FilmArray Test Results	Reference/Comparator Method
Campylobacter	Stool culture[b] (Blood agar, Blood agar with Ampicillin, MacConkey agar, Sorbitol-MacConkey agar, GN broth + Hektoen enteric agar, Campylobacter agar, Cefsulodin-Irgasan™-Novobiocin agar, and Thiosulfate Citrate Bile Salts agar) with standard manual and automated microbiological/biochemical identification methods
E. coli O157[a]	
Plesiomonas shigelloides	
Salmonella	
Vibrio and *V. cholerae*	
Yersinia enterocolitica	
STEC (*stx1/2*)	PCR with Bi-directional Sequencing[h]
ETEC	
EPEC[c]	
EIEC/*Shigella*[d]	
EAEC	
Adenovirus F 40/41	
Astrovirus	
Norovirus GI/GII[e]	
Rotavirus A	
Sapovirus[f]	
Clostridium difficile toxin A/B	
Cryptosporidium	
Giardia lamblia[g]	

FilmArray Test Results	Reference/Comparator Method
Cyclospora cayetanensis	
Entamoeba histolytica	

[a] The *E. coli* O157 comparator method data were only used to determine the accuracy of the FilmArray determination of *E. coli* O157 detected or not detected for specimens in which FilmArray detected STEC.

[b] Any bacteria isolated from stool culture that could not be identified to the species level by laboratory methods were sequenced using an assay capable of providing species information (e.g., 16S).

[c] A result for EPEC is only reported in the absence of STEC (same algorithm as FilmArray).

[d] *Shigella* may be identified by routine culture methods; however, culture detection will be reported for informational purposes only.

[e] CDC Calicinet assays (non-sequenceable) were used for the comparator method for Norovirus.

[f] Sapovirus comparator assays consisted of one well-validated, sequenceable assay and one published assay that was not sequenceable.

[g] *G. lamblia* comparator assays consisted of one well-validated, sequenceable assay and one published assay that was not sequenceable.

[h] PCR assays were designed to amplify different sequences than those targeted by FilmArray GI. Positive results for sequenceable assays required a sequencing result of adequate quality to match a sequence of the expected organism/gene deposited in the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov), with an acceptable E-value.

A total of 1556 specimens were evaluated in this study. Of these specimens, 832 (53.5%) were positive for at least one analyte. Clinical sensitivity or positive percent agreement (PPA) was calculated as 100% x (TP / (TP + FN)). True positive (TP) indicates that both the FilmArray GI Panel and reference/comparator method had a positive result for this specific analyte, and false negative (FN) indicates that the FilmArray result was negative while the comparator result was positive. Specificity or negative percent agreement (NPA) was calculated as 100% x (TN / (TN + FP)). True negative (TN) indicates that both the FilmArray GI Panel and the reference/comparator method had negative results, and a false positive (FP) indicates that the FilmArray GI Panel result was positive but the comparator result was negative. The exact binomial two-sided 95% confidence interval was calculated. The results are summarized in the table below.

Table - FilmArray GI Clinical Performance Summary

Bacteria	Sensitivity/PPA[a]			Specificity/NPA[a]		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Campylobacter (*C. jejuni/C. coli/C. upsaliensis*)	34/35[b]	97.1	85.1-99.9	1497/1521[b]	98.4	97.7-99.0
Clostridium difficile toxin A/B[a]	163/165[c]	98.8	95.7-99.9	1350/1391[c]	97.1	96.0-97.9
Plesiomonas shigelloides	3/3	100	29.2-100	1538/1553[d]	99.0	98.4-99.5
Salmonella	31/31	100	88.8-100	1519/1525[e]	99.6	99.1-99.9
Vibrio (*V. parahaemolyticus/V. vulnificus/V. cholerae*)	0/0	-	-	1554/1556[f]	99.9	99.5-100
Vibrio cholerae	0/0	-	-	1555/1556[g]	99.9	99.6-100
Yersinia enterocolitica	1/1	100	N/A	1555/1555	100	99.8-100
Diarrheagenic *E. coli/Shigella*	**Positive Percent Agreement (PPA)[a]**			**Negative Percent Agreement (NPA)[a]**		
	TP/(TP + FN)	**%**	**95% CI**	**TN/(TN + FP)**	**%**	**95% CI**
Enteroaqggregative *E. coli* (EAEC)	82/83	98.8	93.5-100	1446/1473[h]	98.2	97.3-98.8
Enteropathogenic *E.* coli (EPEC)	314/317	99.1	97.3-99.8	1167/1201[i]	97.2	96.1-98.0
Enterotoxigenic *E. coli* (ETEC) *lt/st*	22/22	100	84.6-100	1525/1534[j]	99.4	98.9-99.7
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*	33/33	100	89.4-100	1518/1523[k]	99.7	99.2-99.9
E. coli O157[a]	3/3	100	29.2-100	34/35[l]	97.1	85.1-99.9
*Shigella/*Enteroinvasive *E. coli* (EIEC)	47/49	95.9	86.0-99.5	1505/1507	99.9	99.5-100
Parasites	**Positive Percent Agreement (PPA)[a]**			**Negative Percent Agreement (NPA)[a]**		
	TP/(TP + FN)	**%**	**95% CI**	**TN/(TN + FP)**	**%**	**95% CI**
Cryptosporidium	18/18	100	81.5-100	1532/1538[m]	99.6	99.2-99.9
Cyclospora cayetanensis	19/19	100	82.4-100	1537/1537	100	99.8-100
Entamoeba histolytica	0/0	-	-	1556/1556	100	99.8-100
Giardia lamblia	20/20	100	83.2-100	1529/1536[n]	99.5	99.1-99.8

Viruses	Positive Percent Agreement (PPA)[a]			Negative Percent Agreement (NPA)[a]		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Adenovirus F 40/41	42/44[o]	95.5	84.5-99.4	1499/1512[o]	99.1	98.5-99.5
Astrovirus	7/7	100	59.0-100	1548/1549[p]	99.9	99.6-100
Norovirus GI/GII	52/55[q]	94.5	84.9-98.9	1483/1501[q]	98.8	98.1-99.3
Rotavirus A	6/6	100	54.1-100	1538/1550[r]	99.2	98.7-99.6
Sapovirus (Genogroups I, II, IV, and V)	46/46	100	92.3-100	1497/1510[s]	99.1	98.5-99.5

[a] *C. difficile* performance is reported as positive percent agreement and negative percent agreement, and *E. coli* O157 performance is reported as sensitivity/specificity, in contrast to the headings of their respective sections. The performance measures of sensitivity and specificity only refer to those analytes for which the gold-standard bacterial culture was used as the reference method; *Campylobacter*, *E. coli* O157, *Plesiomonas shigelloides*, *Salmonella*, *Vibrio*, *Vibrio cholerae*, and *Yersinia enterocolitica*. Performance measures of positive percent agreement (PPA) and negative percent agreement (NPA) refer to all other analytes, for which PCR/sequencing assays were used as comparator methods.

[b] *Campylobacter jejuni* subsp. *doylei* was identified in the single false negative specimen using bi-directional sequence analysis. *Campylobacter* was detected in 19/24 false positive specimens using bi-directional sequence analysis.

[c] *C. difficile* was detected in 1/2 false negative specimens and 41/41 false positive specimens using bi-directional sequence analysis.

[d] *P. shigelloides* was detected in 15/15 false positive specimens using bi-directional sequence analysis.

[e] *Salmonella* was detected in 6/6 false positive specimens using bi-directional sequence analysis.

[f] *Vibrio* was detected in 2/2 false positive specimens using bi-directional sequence analysis.

[g] *V. cholerae* was detected in the single false positive specimen using bi-directional sequence analysis.

[h] EAEC was detected in 27/27 false positive specimens using bi-directional sequence analysis.

[i] EPEC was detected in 23/34 false positive specimens using bi-directional sequence analysis.

[j] ETEC was detected in 6/9 false positive specimens using bi-directional sequence analysis. The three remaining false positive results were determined to have been caused by cross-reactivity with *Citrobacter koseri* (2 instances), and *Hafnia alvei* (1 instance). These bacteria contain a variant of the *fliP* gene with sequence similarity to assay primers.

[k] STEC was detected in 5/5 false positive specimens using bi-directional sequence analysis.

[l] *E. coli* O157 was detected in the single false positive specimen using bi-directional sequence analysis.

[m] *Cryptosporidium* was detected in 6/6 false positive specimens using bi-directional sequence analysis.

[n] *G. lamblia* was detected in 4/7 false positive specimens using bi-directional sequence analysis. Two false positive results appear to be caused by cross-reactivity with *Bifidobacterium longum* and *Ruminococcus callidus*.

[o] Adenovirus was detected in 1/2 false negative specimens and 11/13 false positive specimens using bi-directional sequence analysis

[p] Astrovirus was detected in the single false positive specimen using bi-directional sequence analysis.

[q] The FilmArray GI system detected Norovirus in 1/3 false negative specimens when retested. Norovirus was detected in 1/2 remaining false negative specimens and 8/18 false positive specimens using bi-directional sequence analysis.

[r] Rotavirus A was detected in 11/12 false positive specimens using bi-directional sequence analysis.

[s] Sapovirus was detected in 12/13 false positive specimens using bi-directional sequence analysis.

FilmArray GI reports genus level (or multiple species group) results for three bacterial analytes; i.e., *Campylobacter* (*C. jejuni/C. coli/C. upsaliensis*), *Salmonella*, and *Vibrio* (*V. parahaemolyticus/V. vulnificus/V. cholerae*). Standard laboratory methods identified various species/serovars within each of these groups during the clinical evaluation. Where

standard methods did not provide a species identification, bi-directional sequencing was used to identify the species of the isolate. Stratification of performance by species/serovar is presented below.

For *Vibrio*, no organisms were isolated by the culture methods; however, bi-directional sequencing from the original specimens identified one *V. parahaemolyticus* and one *V. cholerae*.

Table –*Campylobacter* Clinical Performance Stratified by Species

***Campylobacter* species**[a]	**Sensitivity**
C. jejuni[b]	31/31 (100%)
C. coli	2/2 (100%)
C. jejuni subsp. *Doylei*	0/1 (0%)
C. upsaliensis	1/1 (100%)
Overall *Campylobacter*	**34/35 (97.1%)** **95%CI = 81.3-99.3%**

[a] Fifteen (15) *Campylobacter* were not speciated by the source laboratory and were subject to sequencing of the *cadF* gene. This method identified 11 *C. jejuni*, two *C. coli*, one *C. jejuni* subsp. *doylei*, and one *C. upsaliensis.*

[b] Two *C. jejuni* were originally identified by the source lab as "*Campylobacter* species". Sequencing of the isolates provided by the laboratory identified them as *C. jejuni*. However, molecular testing of the specimen from which the isolates were obtained also detected the presence of *C. upsaliensis*, representing co-infection by these two species.

Table - *Salmonella* Clinical Performance Stratified by Species/Serovar

***Salmonella* species/serovar**	**Sensitivity**
S. enterica ser. Enteritidis	7/7 (100%)
S. enterica ser. Typhimurium (i:-)	7/7 (100%)
S. enterica ser. Typhimurium	3/3 (100%)
S. enterica ser. Javiana	2/2 (100%)
S. enterica ser. Newport	2/2 (100%)
S. enterica ser. Agbeni	1/1 (100%)
S. enterica ser. Berta	1/1 (100%)
S. enterica ser. Ealing	1/1 (100%)
S. enterica ser. Gaminara	1/1 (100%)
S. enterica ser. Infantis	1/1 (100%)
S. enterica ser. Mbandaka	1/1 (100%)
S. enterica ser. Miami	1/1 (100%)
S. enterica ser. Muenchen	1/1 (100%)
S. enterica ser. Paratyphi B var L-Tartrate	1/1 (100%)
S. enterica ser. Thompson	1/1 (100%)
Overall *Salmonella*	**31/31 (100%)** **95%CI = 88.8-100%**

The FilmArray GI Panel reported multiple organism detections (i.e., mixed infections) for a total of 262 specimens. This represents 31.5% of positive specimens (262/832) and 16.8% of all specimens (262/1556). The majority of multiple detections (199/262; 76.0%) contained two organisms, while 19.1% (50/262) contained three organisms, 3.4% (9/262)

contained four organisms, 1.1% (3/262) contained five organisms, and 0.4% (1/262) contained six organisms. The three organisms that were most prevalent in co-infections were also the three most prevalent organisms in the study as a whole (i.e., EPEC, *C. difficile*, and EAEC). Out of the 262 specimens with multiple detections, 144 specimens (55.0%; 144/262) were concordant with the reference methods. One hundred eighteen specimens (45.0%; 118/262) contained one or more organisms that had not been detected by the reference/comparator methods (i.e., 139 false positive results); however, bi-directional sequence analysis confirmed the presence of the analyte for 88.5% (123/139) of the discordant results.

Table - Prevalence of Analytes in Mixed Infections as determined by the FilmArray GI Panel

Analyte	**Prevalence in Mixed Infections *N* = 262**	
	No.	**%**
Bacteria		
Campylobacter	30	11.5%
Clostridium difficile toxin A/B	109	41.6%
Plesiomonas shigelloides	16	6.1%
Salmonella	15	5.7%
Vibrio	1	0.4%
Vibrio cholerae	1	0.4%
Yersinia enterocolitica	1	0.4%
Diarrheagenic *E. coli/Shigella*		
Enteroaggregative *E. coli* (EAEC)	67	25.6%
Enteropathogenic *E. coli* (EPEC)	159	60.7%
Enterotoxigenic *E. coli* (ETEC) *lt/st*	26	9.9%
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*	13	5.0%
E. coli O157	1	0.4%
Shigella / Enteroinvasive *E. coli* (EIEC)	17	6.5%
Parasites		
Cryptosporidium	11	4.2%
Cyclospora cayetanensis	2	0.8%
Entamoeba histolytica	0	0%
Giardia lamblia	14	5.3%
Viruses		
Adenovirus F 40/41	34	13.0%
Astrovirus	4	1.5%
Norovirus GI/GII	43	16.4%
Rotavirus A	10	3.8%
Sapovirus	33	12.6%

The most prevalent mixed infection was *C. difficile* toxinA/B with EPEC (2.0% of all specimens; 32/1556) followed by EAEC with EPEC (1% of all specimens; 15/1556); as previously stated

these were the most prevalent organisms detected in the study. Mixed infections were observed for all combinations of analyte classes (e.g. bacteria with viruses, diarrheagenic *E. coli/Shigella* with parasites) and co-infections were observed within classes (e.g. three diarrheagenic *E. coli/Shigella* combined; ETEC, EAEC, and STEC).

Table - Most Prevalent Multiple Detection Combinations (≥5 instances) as Determined by the FilmArray GI Panel

Multiple Detection Combination	Number of Specimens
C. difficile toxin A/B + EPEC	32
EAEC + EPEC	15
Campylobacter + EPEC	11
EPEC + Sapovirus	10
Adenovirus + EPEC	9
EPEC + Norovirus GI/GII	9
C. difficile toxin A/B + EAEC	7
C. difficile toxin A/B + Norovirus GI/GII	6
C. difficile toxin A/B + STEC *stx1/stx2*	5
EPEC + ETEC *lt/st*	5
EPEC + *G. lamblia*	5
EPEC + *Shigella/EIEC*	5

The overall success rate for initial specimen tests in the prospective study was 99.4% (1544/1557). Four tests were incomplete due to software errors (3) or a user aborted run (1), and nine tests were invalid due to pouch control failures. Of the nine pouch control failures observed in the prospective clinical study, seven were attributed to the RNA process control and two were due to the PCR2 control. There were no instances of both RNA process and PCR2 control failure in the same pouch. All specimens but one were retested within four days of specimen collection and were successful after a single retest, for a final success rate of 99.9% (1556/1557).

Testing of Preselected Archived Specimens

Several analytes were either not encountered or had a low prevalence in the clinical study. To supplement the results of the prospective clinical study, an evaluation of 222 preselected archived specimens was performed. These specimens were archived clinical specimens that were selected because they had previously tested positive for one of the following analytes: *E. coli* O157, *P. shigelloides*, *Y. enterocolitica*, *Vibrio*, Astrovirus, Rotavirus, and *E. histolytica*, or had been negative in previous laboratory testing. Prior to testing with the FilmArray GI Panel, the presence (or absence for negative specimens) of the expected analytes was verified in each specimen using analyte-specific PCR followed by bi-directional sequencing.

The specimens were organized into “test panels” and randomized such that the users performing the FilmArray GI Panel testing were blinded as to the expected test result. A summary of the available demographic information of the tested samples and the results of the FilmArray GI testing are presented in the tables below

.

Table - Demographic Summary for Preselected Archived Specimens

Preselected Archived Specimens	
Total Specimens	222
Sex	**Number of Specimens (%)**
Male	57 (25.7%)
Female	48 (21.6%)
Unknown	117 (52.7%)
Age Group	**Number of Specimens (%)**
<1 year	12 (5.4%)
1-5 years	36 (16.2%)
6-12 years	15 (6.8%)
13-21 years	11 (5%)
22-64 years	18 (8.1%)
65+ years	4 (1.8%)
Unknown	126 (56.8%)

Table - FilmArray GI Panel Archived Specimen Performance Data Summary

Analyte	Positive Percent Agreement (PPA)			Negative Percent Agreement (NPA)		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Bacteria						
Plesiomonas shigelloides	12/12	100	73.5-100	107/107	100	96.6-100
Vibrio	1/1	100	N/A	127/127	100	97.1-100
Yersinia enterocolitica	8/8	100	63.1-100	117/117	100	96.9-100
Diarrheagenic *E. coli/Shigella*						
(STEC) *E. coli* O157[a]	19/19	100	82.4-100	0/0	-	-
Parasites						
Cryptosporidium	29/30	96.7	82.8-99.9	66/66	100	94.6-100
Entamoeba histolytica	2/2	100	15.8-100	123/123	100	97.0-100
Giardia lamblia	26/26	100	86.8-100	66/66	100	94.6-100
Viruses						
Astrovirus	31/32	96.9	83.8-99.9	91/91	100	96.0-100
Rotavirus A	29/29	100	88.1-100	65/65	100	94.5-100

[a] No non-O157 STEC were included in the data set; therefore, negative percent agreement (NPA) could not be calculated for *E. coli* O157.

Testing of Contrived Specimens

Several analytes, such as *Entamoeba histolytica*, are so rare that both prospective and archived testing efforts were insufficient to demonstrate system performance. To supplement the prospective and archived data, an evaluation of contrived specimens was performed. Surrogate clinical specimens were prepared using residual specimens from the prospective clinical study that had previously tested negative for all GI panel analytes by FilmArray GI and comparator methods. Specimens were spiked at clinically relevant levels using five different quantified strains for each organism (or unspiked; at least 50 of each). The analyte status of each contrived specimen was blinded to the users analyzing the specimens, and the specimens were randomized before testing. The results of the FilmArray GI testing are presented in the table below:

Table - FilmArray GI Panel Performance using Contrived Specimens

Analyte	Positive Percent Agreement (PPA)			Negative Percent Agreement (NPA)		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Entamoeba histolytica	44/50	88.0	75.7-95.5	75/75	100	95.2-100
Plesiomonas shigelloides	70/70	100	94.9-100	105/105	100	96.5-100
Vibrio[a]	112/115	97.4	92.6-99.5	60/60	100	94.0-100
V. cholerae[b]	55/65	84.6	73.5-92.4	110/110	100	96.7-100
Yersinia enterocolitica	65/65	100	94.5-100	110/110	100	96.7-100

[a] Includes 64/65 *V. cholerae* (five different strains were used in spiking; one specimen spiked near the assay limit of detection was not detected) and 48/50 non-*V. cholerae* (four *V. parahaemolyticus* strains and one *V. vulnificus* strain were used in spiking; two specimens spiked with *V. parahaemolyticus* near the assay limit of detection were not detected).

[b] Ten (10) of these specimens were spiked with an isolate which was found to have a highly divergent *toxR* gene that was not present in the NCBI database and non-reactive with the FilmArray GI Panel *V. cholerae* assay. The FilmArray GI Panel *Vibrio* assay was positive for nine of these specimens.

4. Clinical cut-off**:**

N/A

5. Expected values/Reference range:

The prevalence of detected analytes has been stratified in the tables below.

Table - Expected Values for *E. coli* O157 in presence of STEC

	Overall (n=38)		<1 (n=1)		1-5 (n=24)		6-12 (n=2)		13-21 (n=4)		22-64 (n=5)		65+ (n=2)	
	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV
E. coli O157 in presence of STEC	4	10.5%	0	0.0%	3	12.5%	1	50%	0	0.0%	0	0.0%	0	0.0%

Table - Expected Values for EPEC in absence of STEC

	Overall (n=1518)		<1 (n=120)		1-5 (n=394)		6-12 (n=191)		13-21 (n=236)		22-64 (n=406)		65+ (n=171)	
	n	EV	n	EV	n	EV	n	EV	n	EV	n	EV	n	EV
EPEC in absence of STEC	348	22.9%	30	25.0%	155	39.3%	45	23.6%	46	19.5%	55	13.5%	17	9.9%

Table - Prevalence of Detected Analytes Stratified by Age Group (n# = Number; EV= Expected Value)

	Overall (n=1556)		<1 (n=121)		1-5 (n=418)		6-12 (n=193)		13-21 (n=240)		22-64 (n-411)		65+ (n=173)	
	n	EV	n	EV	n	EV	n	EV	n	EV	n	EV	n	EV
Campylobacter	58	3.70%	1	0.80%	11	2.60%	12	6.20%	6	2.50%	19	4.60%	9	5.20%
C. difficile toxin A/B	204	13.10%	49	40.50%	66	15.80%	18	9.30%	33	13.80%	29	7.10%	9	5.20%
Plesiomonas shigelloides	18	1.20%	0	0%	7	1.70%	4	2.10%	4	1.70%	3	0.70%	0	0%
Salmonella	37	2.40%	5	4.10%	7	1.70%	5	2.60%	5	2.10%	11	2.70%	4	2.30%
Vibrio	2	0.10%	0	0%	0	0%	0	0%	0	0%	2	0.50%	0	0%
Vibrio Cholerae	1	0.10%	0	0%	0	0%	0	0%	0	0%	1	0.20%	0	0%
Yersinia enterocolitica	1	0.10%	1	0.80%	0	0%	0	0%	0	0%	0	0%	0	0%
Enteroaggregative *E. coli* (EAEC)	109	7.00%	9	7.40%	34	8.10%	20	10.40%	17	7.10%	25	6.10%	4	2.30%
Enteropathogenic *E. coli* (EPEC)	348	22.40%	30	24.80%	155	37.10%	45	23.30%	46	19.20%	55	13.40%	17	9.80%
Entertoxigenic *E. coli (*ETEC)	31	2.00%	1	0.80%	5	1.20%	7	3.60%	5	2.10%	9	2.20%	4	2.30%
Shiga-like toxin-producing *E. coli* (STEC)	38	2.40%	1	0.80%	24	5.70%	2	1.00%	4	1.70%	5	1.20%	2	1.20%
E. coli O157	4	0.30%	0	0%	3	0.70%	1	0.50%	0	0%	0	0%	0	0%
Shigella / Enteroinvasive *E. coli* (EIEC)	49	3.10%	0	0%	31	7.40%	7	3.60%	5	2.10%	6	1.50%	0	0%
Cryptosporidium	24	1.50%	0	0%	9	2.20%	3	1.60%	6	2.50%	5	1.20%	1	0.60%
Cyclospora cayetanensis	19	1.20%	0	0%	0	0%	0	0%	0	0%	13	3.20%	6	3.50%
Entamoeba histolytica	0	0.00%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Giardia lamblia	27	1.70%	1	0.80%	6	1.40%	5	2.60%	2	0.80%	13	3.20%	0	0%
Adenovirus F 40/41	55	3.50%	12	9.90%	36	8.60%	5	2.60%	0	0%	2	0.50%	0	0%
Astrovirus	8	0.50%	1	0.80%	4	1.00%	0	0%	1	0.40%	2	0.50%	0	0%
Norovirus GI/GII	70	4.50%	15	12.40%	31	7.40%	5	2.60%	7	2.90%	9	2.20%	3	1.70%
Rotavirus A	18	1.20%	11	9.10%	2	0.50%	1	0.50%	1	0.40%	2	0.50%	1	0.60%
Sapovirus	59	3.80%	12	9.90%	31	7.40%	7	3.60%	1	0.40%	5	1.20%	3	1.70%

A study of 100 asymptomatic volunteers was conducted. The FilmArray GI Panel gave a negative result for 67 (67%) of specimens. Of the 33 positive specimens, 27 (81.8%) were positive for one analyte and six (18.2%) were positive for two analytes.

Table - 95% Confidence Intervals for Prevalence Rates in the Prospective Clinical Study and Asymptomatic Volunteers Stratified

Observation	Prospective Study (*n* = 1556)			Asymptomatic Volunteers (*n* = 100)		
	Obs %	CI95 lower bound	CI95 upper bound	Obs %	CI95 lower bound	CI95 upper bound
Total Positives	54.3	51.8	56.8	33	23.9	43.1
Clostridium difficile toxin A/B	13.1	11.5	14.9	5	1.6	11.3
Plesiomonas shigelloides	1.2	0.7	1.8	1	0.02	5
EAEC	7	5.8	8.4	7	2.8	13.9
EPEC*	22.4	20.3	24.5	18	11	27
Giardia lamblia	1.7	1.2	2.5	3	0.06	8.5
Adenovirus F 40/41	3.5	2.7	4.6	2	0.02	7
Rotavirus A	1.2	0.7	1.8	1	0.02	5
Sapovirus	3.8	2.9	4.9	2	0.02	7

***** Asymptomatic carriage of EPEC has also been documented with some studies reporting similar rates to symptomatic individuals

Table – FilmArray GI Detections in Asymptomatic Volunteers Stratified by Age Range

Analyte	< 1	1-5	6-12	13-21	22-64	65+
All Negative	5 (62.5%)	17 (58.6%)	11 (73.3%)	8 (57.1%)	20 (74.1%)	6 (85.7%)
Single Infection	0 (0%)	11 (37.9%)	3 (20%)	5 (35.7%)	7 (25.9%)	1 (14.3%)
Co-Infections	3 (37.5%)	1 (3.4%)	1 (6.7%)	1 (7.1%)	0 (0%)	0 (0%)
Clostridium difficile toxin A/B	1 (12.5%)	2 (6.9%)	1 (6.7%)	0 (0%)	1 (3.7%)	0 (0%)
Plesiomonas shigelloides	0 (0%)	0 (0%)	1 (6.7%)	0 (0%)	0 (0%)	0 (0%)
EAEC	1 (12.5%)	2 (6.9%)	1 (6.7%)	1 (7.1%)	1 (3.7%)	1 (14.3%)
EPEC	1 (12.5%)	6 (20.7%)	2 (13.3%)	4 (28.6%)	5 (18.5%)	0 (0%)
Giardia lamblia	0 (0%)	1 (3.4%)	0 (0%)	2 (14.3%)	0 (0%)	0 (0%)
Adenovirus F 40/41	2 (25%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Rotavirus A	1 (12.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Sapovirus	0 (0%)	2 (6.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

N. Instrument Name:

FilmArray® Instrument

O. System Descriptions:

1. Modes of Operation:

   See Device Description (Section I) above

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes X or No _____

3. Specimen Identification:

   The Sample ID can be entered manually or scanned in by using the FilmArray barcode scanner.

4. Specimen Sampling and Handling:

   See Section I Device Description.

5. Calibration:

   N/A

6. Quality Control:

   See Analytical Section *M.1c -Traceability, Stability, Expected values (controls, calibrators, or methods)*

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not Applicable

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10 and 21 CFR 801.109(b)(1).

R. Conclusion:

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