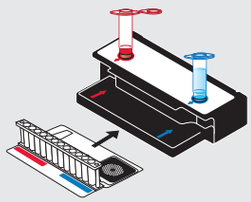
FilmArray™ Gastrointestinal Panel Quick Guide

To avoid contamination always wear gloves and work behind a protective shield.

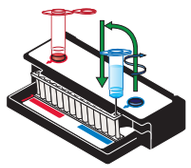
Step 1: Prepare Pouch

— Insert pouch into Pouch Loading Station.
— Place Sample Injection Vial into red well.
— Place Hydration Injection Vial into blue well.



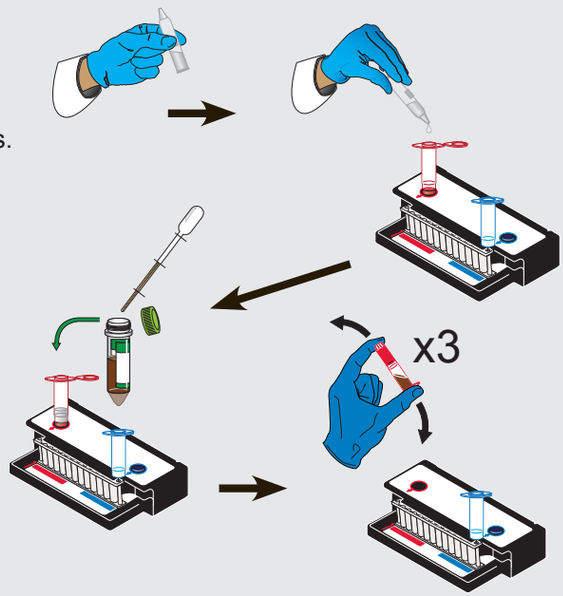
Step 2: Hydrate Pouch

— Twist off Hydration Injection Vial, leaving cap in Pouch Loading Station, and insert into pouch hydration port.
— Forcefully push down to puncture seal and wait as Hydration Solution is drawn into pouch.



Step 3: Prepare Sample Mix

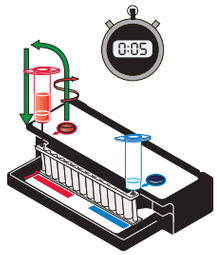
— Add Sample Buffer to Sample Injection Vial:
  • Invert Sample Buffer Ampoule so that tip is facing up.
  Note: *Do not touch the tip of the ampoule.*
  • Firmly pinch textured plastic tab on side of ampoule until seal snaps.
  • With the tip facing down, dispense Sample Buffer into Sample Injection Vial using a slow, forceful squeeze, followed by a **2nd** squeeze. Avoid generating excessive bubbles.
— Thoroughly mix stool specimen in transport media.
— Using transfer pipette, draw up specimen to **2nd** line.
— Add to Sample Injection Vial.
— Tightly close lid of Sample Injection Vial.
— Mix sample by gently inverting Sample Injection Vial **3** times.
— Return Sample Injection Vial to red well of Pouch Loading Station.



Warning: The Sample Buffer is harmful if swallowed, can cause serious eye damage and/or skin irritation.

Step 4: Load Sample Mix

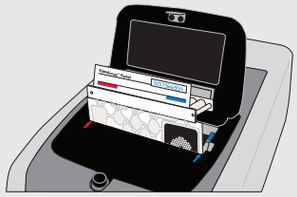
— Unscrew Sample Injection Vial from cap.
— Pause for 3-5 seconds, then remove Sample Injection Vial, leaving cap in Pouch Loading Station.
— Insert Sample Injection Vial into pouch sample port.
— Forcefully push down to puncture seal.
— Wait as Sample Mix is drawn into pouch.



Step 5: Run Pouch

— Follow instructions on computer for initiating a test.
— The pouch will click into place when properly seated.

Note: *If the pouch does not insert easily, ensure that the lid is opened completely.*



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FilmArray™ Gastrointestinal Panel Quick Guide

FilmArray™ GI Panel

BIOFIRE

www.BioFireDx.com

*The **Run Summary Section** displays information about the sample and a summary of the control and test results.*

1. Detected:
   - Names of any detected pathogens
   - If 'None', no pathogens were detected
   - If '⚠Invalid', RETEST SAMPLE
2. Controls:
   - If 'Passed', results are valid
   - If '⚠Failed', RETEST SAMPLE
   - If '⚠Invalid', RETEST SAMPLE

Run Summary			
Sample ID:	**Example**	**Run Date:**	27 Sep 2013 12:03 PM
(1.) **Detected:**	*Clostridium difficile* toxin A/B	(2.) **Controls:**	Passed

*The **Results Summary Section** lists the test results for each organism targeted by the Gastrointestinal Panel.*

3. '✓Detected', pathogen was detected
4. 'Not Detected', pathogen was not detected
5. ' ø N/A', pathogen was not reported. Refer to Instruction Booklet for more information.

Result Summary	
Bacteria	
Not Detected	*Campylobacter*
(3.) ✓ Detected	*Clostridium difficile* toxin A/B
Not Detected	*Plesiomonas shigelloides*
Not Detected	*Salmonella*
(4.) Not Detected	*Vibrio*
Not Detected	*Vibrio cholerae*
Not Detected	*Yersinia enterocolitica*
Diarrheagenic *E. coli/Shigella*	
Not Detected	Enteroaggregative *E. coli* (EAEC)
Not Detected	Enteropathogenic *E. coli* (EPEC)
Not Detected	Enterotoxigenic *E. coli* (ETEC) *lt/st*
Not Detected	Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*
(5.) ⊘ N/A	*E. coli* O157
Not Detected	*Shigella*/Enteroinvasive *E. coli* (EIEC)
Parasites	
Not Detected	*Cryptosporidium*
Not Detected	*Cyclospora cayetanensis*
Not Detected	*Entamoeba histolytica*
Not Detected	*Giardia lamblia*
Viruses	
Not Detected	Adenovirus F 40/41
Not Detected	Astrovirus
Not Detected	Norovirus GI/GII
Not Detected	Rotavirus A
Not Detected	Sapovirus

*The **Run Details Section** displays information about the pouch, instrument, run status and operator.*

6. Run Status: If Completed, run is complete.
   If Incomplete, Aborted, Instrument Communication Error, Instrument Error or Software Error, RETEST SAMPLE

Note: Contact BioFire Diagnostics Technical Support for help with error messages.

Run Details			
(6.) **Pouch:**	GI Panel	**Protocol:**	Stool FA v2.3
Run Status:	Completed	**Operator:**	**Ashley Hunter (ah)**
Serial No.:	00788640	**Instrument:**	**ITI FA "FA1115"**
Lot No.:	133813		

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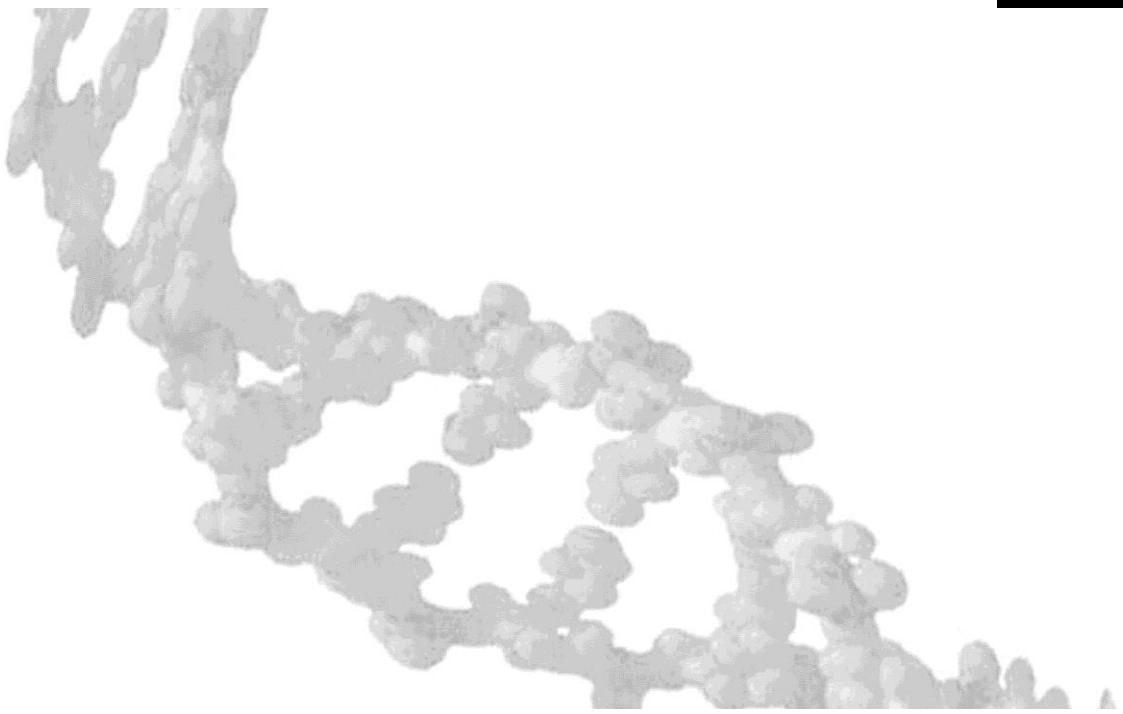
REF RFIT-ASY-0104
RFIT-ASY-0116



FilmArray™ Gastrointestinal (GI) Panel

Instruction Booklet

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 RFIT-PRT-0143-01 April 2014

TABLE OF SYMBOLS

The following symbols can be found on FilmArray GI Panel Kit components or throughout this Instruction Booklet. Use the definitions below as a guideline to interpreting the symbols.

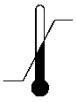
Table of Symbols					
	Manufacturer		Catalog Number		Expiry Date YYYY-MM-DD
	Consult Instructions for Use		Lot Number		Storage Temperature Limitations
	European Union Conformity		Do Not Reuse		Contains Sufficient For <n> Tests
	*In vitro* Diagnostic Medical Device		Keep Away from Sunlight		Do Not Use if Package is Damaged
	Serious eye damage, cat. 1		Acute toxicity, cat. 4 & Skin irritation, cat. 2		

TABLE OF CONTENTS

NAME AND INTENDED USE

FilmArray Gastrointestinal (GI) Panel

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.

SUMMARY AND EXPLANATION OF THE TEST

Despite advances in food safety, sanitation, and medical treatment, infectious gastroenteritis remains a significant problem in industrialized countries among all age groups. In the United States, around 76 million cases of foodborne disease, resulting in 325,000 hospitalizations and 5,000 deaths, are estimated to occur each year.[1–3] Additionally, there are over 300,000 *C. difficile* diagnoses per year in the US[4] resulting in estimated costs of at least $1 billion.[5] Globally, infectious diarrheal illness is a significant cause of mortality in young children resulting in an estimated 800,000 deaths per year in children under the age of 5.[6] In addition to this significant morbidity and mortality, diarrhea in children contributes to malnutrition, increased susceptibility to other infections, and may lead to delays in growth and intellectual development.[7,8] The FilmArray GI Panel simultaneously tests for 22 pathogens (Table 1) from stool specimens collected in Cary Blair transport medium. Results from the FilmArray GI Panel test are available within about one hour.

Table 1. Bacteria, Viruses, Diarrheagenic *E. coli*/*Shigella*, and Parasites Detected by the FilmArray GI Panel

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

Summary of Detected Organisms

Bacteria

***Campylobacter* (*C. jejuni/C. coli/C. upsaliensis*).** *Campylobacter* are gram-negative, non-spore forming, s-shaped or spiral bacteria that are usually motile. Most sporadic infections are acquired through ingestion of undercooked poultry or from cross-contamination of other foods. Outbreaks have been associated with unpasteurized dairy, contaminated water, poultry, and produce. Transmission from the stool of household pets has also been documented.[9] *C. jejuni* and *C. coli* are the species most commonly associated with diarrheal illness, followed distantly by *C. upsaliensis*. Other species such as *C. lari* and *C. fetus* are more uncommon.[10] Infection with *Campylobacter* species is common throughout the world, representing a large and perhaps under-recognized health burden.[11] *Campylobacter* are a leading cause of bacterial enteritis in the US (est. 845,000 infections annually with almost 8,500 hospitalizations[12]) and the most common cause of foodborne illness in the EU (over 220,000 confirmed cases reported by EU member states in 2011[13]). Enteric *Campylobacter* infections range from asymptomatic to severe infections characterized by bloody or non-bloody diarrhea, fever, and abdominal cramping. Infections may also lead to long-term health issues such as Guillain-Barré syndrome (GBS) and reactive arthritis.[11] *Campylobacter* infections are a notifiable disease in the US and are tracked by the European Surveillance System (TESSy).

Clostridium difficile are obligately anaerobic, gram-positive rods capable of forming hardy spores and are widespread in nature. These bacteria are acquired from the environment or transmitted via the fecal-oral route. Some *C. difficile* strains produce two enterotoxins, toxin A and toxin B, that damage the large intestine of the infected individual. *C. difficile* infection (CDI) is the major cause of hospital-acquired diarrhea and is responsible for more than 300,000 cases of diarrheal disease and 14,000 deaths annually in the US, resulting in over a billion dollars in health care costs.[14] CDI presents a similar healthcare burden in the EU.[15] Antibiotic treatment, which severely disrupts the normal gastrointestinal flora, is a major risk factor for the development of CDI. Community-acquired CDI, which has a somewhat lower association with antibiotic exposure, has also been emerging in the last few years.[16] Clinical manifestations of *C. difficile* infection range from asymptomatic carriage (estimated to occur in 3-5% of healthy adults and up to 30% of healthy neonates[17]) to pseudomembranous colitis, involving bloody diarrhea, severe abdominal pain, and fever. Due to the high asymptomatic carriage rates, especially in young children, the clinical relevance of the detection of toxigenic *C. difficile* from stool should be considered in the context of other clinical findings, patient age, and risk factors including hospitalization and antibiotic exposure.[18,19]

Plesiomonas shigelloides. *Plesiomonas shigelloides*, gram-negative rod-shaped bacteria and members of the *Enterobacteriaceae* family, are isolated from a wide range of environmental sources including freshwater and many animals, both wild and domestic. *P. shigelloides* gastroenteritis often follows consumption of seafood, as well as contaminated water used for drinking or used in preparing uncooked foods.[10] Symptoms generally include watery diarrhea, though dysenteric diarrhea can occur, and infections may be prolonged (>2 weeks duration) but are generally self-limiting.[20] Most cases reported in the US are from individuals with pre-existing health problems leading to a more severe disease outcome.[21] The incidence of *Plesiomonas* infection in the US, EU, or other regions is largely unknown.

Salmonella. *Salmonella enterica* and *S. bongori* are the sole members of the *Salmonella* genus. Greater than 2,500 different serotypes of *Salmonella* have been recognized, with the majority of pathogenic serotypes being within the *S. enterica* species.[22] These motile, rod-shaped, gram-negative, facultative bacteria are commonly recognized as a food contaminant associated with meat, poultry, produce, and manufactured products. *Salmonella* may be classified as typhoidal and non-typhoidal based on the disease that they cause. The non-typhoidal *Salmonella* are associated with intestinal illness resulting in acute, watery diarrhea, often with fever, and are a common cause of foodborne illness in the US and EU. Typhoidal *Salmonella* cause a severe, systemic disease (typhoid fever) that includes GI illness. Though rare in developed countries, it is common in the developing world (>70% of US cases are related to foreign travel).[2] In contrast, infection with non-typhoidal *Salmonella* is one of the most common causes of foodborne illness in the US and EU with greater than one million cases per year.[12,13] While large outbreaks do occur, the majority of cases are sporadic with peak in incidence in late summer/early fall. The highest incidence is seen in children aged <5 years.[23] In general *Salmonella*-related gastroenteritis is self-limiting, except in cases of severe or typhoidal illness. Salmonellosis is a notifiable disease in the US and is tracked by TESSy in the EU.

***Vibrio* (*V. parahaemolyticus/V. vulnificus/V. cholerae*).** *Vibrio* are motile, gram-negative, comma-shaped bacteria typically found in marine environments. Several species are capable of causing illness in humans, both extraintestinal (soft tissue infection, septicemia, eye and ear infections) and intestinal. Gastrointestinal illness is most commonly associated with *V. cholerae, V. parahaemolyticus, V. vulnificus, V. fluvialis*, *V.mimicus* or *V. alginolyticus* and infections are associated with consumption of contaminated food, particularly in coastal regions.[24]

V. cholerae is the only *Vibrio* species that causes endemic, epidemic, and pandemic cholera. There are three major subgroups of *V. cholerae*: *V. cholerae* O1, *V. cholerae* O139, and *V. cholerae* non-O1/non-O139. Classic cholera is characterized by passing copious amounts of watery diarrhea leading to extreme dehydration and death. Severe disease is mediated by the presence of cholera toxin (CTX). Cholera is endemic in many parts of the world and new outbreaks often follow natural disasters or social upheaval. As such, cholera remains a significant cause of morbidity and mortality in much of the world. In the US and EU, occasional cases of cholera are seen in travelers returning from overseas.

Vibriosis and cholera are notifiable diseases in the US and are tracked by the Cholera and Other *Vibrio* Illness Surveillance Network (COVIS). While *V. cholerae* infections are exceedingly rare in the US, other *Vibrio* species are estimated to cause approximately 50,000 food-borne infections per year[12,13] though only ~400 isolates recovered from stool were reported to COVIS in 2009 (the majority of which were *V. parahaemolyticus*).[24] This discrepancy between

estimated prevalence and actual detections is due to specialized testing required to recover *Vibrio* organisms from stool, leaving most cases undiagnosed. The risk of *Vibrio* infection in Europe is thought to be very low and is not tracked by TESSy.[25]

Yersinia enterocolitica are small, gram-negative bacilli, which generally appear as single cells or short chains. *Y. enterocolitica* is transmitted through ingestion of contaminated food or water, often raw undercooked meats (especially pork), and is estimated to cause almost 100,000 foodborne illnesses in the US annually (though only about 1,000 cases are laboratory-confirmed; possibly because *Y. enterocolitica* are not identified by routine enteric pathogen testing).[12] A higher incidence of Yersiniosis is observed in European countries, particularly in continental Europe[26] with nearly 7,000 confirmed cases reported in 2011.[13] The severity of the illness is based on the serotype of the infecting strain and ranges from self-limiting gastroenteritis to terminal ileitis and mesenteric lymphadenitis. Symptoms of illness mimic appendicitis and may lead to unnecessary surgery, highlighting the importance of properly identifying this organism when it is present in stool specimens. Yersiniosis is a notifiable disease in the US and is tracked by TESSy in the EU.

Diarrheagenic *Escherichia coli/Shigella*

Pathogenic *E. coli*/*Shigella* are a significant cause of diarrheal illness worldwide. There are several pathotypes of Diarrheagenic *E. coli*/*Shigella* that differ in the mechanisms and location of colonization as well as the clinical manifestations, progression, and severity of the diseases they cause. Some of these differences are attributable to the production of specific virulence factors including adhesins, invasins, and toxins. Genes encoding these virulence factors or their regulators are targeted as genetic markers by molecular assays to detect and differentiate these pathogens.[27] The five major Diarrheagenic *E. coli*/*Shigella* pathotypes are Enteroaggregative *E. coli* (EAEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Shiga-like toxin-producing *E. coli* (STEC), and *Shigella*/Enteroinvasive *E. coli* (EIEC). Each pathotype and their characteristic genetic markers are discussed below. It should be noted that these genetic markers have been shown to be horizontally transferred between strains during the evolution of these pathotypes, and more recently during the emergence of new pathotypes containing several of these genetic markers (e.g., the 2011 epidemic *E. coli* O104:H4 which contained genetic markers characteristic of both STEC and EAEC).

Enteroaggregative *E. coli* (EAEC) are defined by their "stacked brick" aggregative adherence pattern when observed on cultured cells. This phenotypic description of the pathotype results in a heterogeneous and highly divergent group of *E. coli*. Although they display a wide variety of virulence factors which are not conserved across all strains, most EAEC carry the aggregative adherence (pAA) plasmid (although genetic composition of this plasmid is variable).[28] Strains that contain *aggR* on the pAA plasmid (encoding a regulator of several virulence factors) have been classified as typical EAEC, while those that do not contain this marker are considered atypical EAEC. The *aatA* marker (an outer membrane protein) is also carried on the pAA plasmid of many EAEC strains, both typical and atypical. Transmission of EAEC is generally by the fecal-oral route via contaminated food and water. EAEC cause an inflammatory diarrheal illness characterized by watery and sometimes bloody stool, accompanied by low grade fever, vomiting, and abdominal pain. EAEC infections may also be asymptomatic. Data regarding the incidence of EAEC are limited due to the lack of widespread testing; however, based upon various studies, EAEC are suggested to be one of the most common causes of diarrheal illness in the US and EU across all age groups, a cause of persistent diarrhea in children and HIV-infected individuals, the second most common cause of travelers' diarrhea, and has been identified as the cause of large outbreaks worldwide.[29–33]

Enteropathogenic *E. coli* (EPEC) do not produce enterotoxins or Shiga-like toxins. Rather, EPEC contain additional virulence factors including those encoded by the chromosomal locus of enterocyte effacement (LEE) pathogenicity island. The adhesion protein, intimin, is encoded by the gene *eae* within the LEE pathogenicity island and is considered a definitive marker for EPEC. Strains may be further categorized as typical or atypical depending on the presence of a plasmid encoding bundle-forming pilis (*bfpA*; found in typical EPEC). Globally, EPEC are estimated to have a prevalence of 8.8% in the community setting, 9.1% in the outpatient setting, and 15.6% in the inpatient setting.[34] While typical EPEC remains a significant pathogen of young children in the developing world, atypical EPEC is more prevalent in both developing and developed countries.[27] Typical EPEC, however, has been associated with several deadly outbreaks at

hospital nurseries in developed countries in the past.[10] Outbreaks appear to peak in the warmer months of summer and early fall. Illness caused by typical EPEC is associated with acute diarrhea whereas atypical EPEC cause a prolonged, non-bloody diarrhea, and vomiting with fever.[27] When untreated in children, EPEC illness can lead to malnutrition and associated growth defects. Asymptomatic carriage of EPEC has also been documented with some studies reporting similar rates to symptomatic individuals.[27]

Enterotoxigenic *E. coli* (ETEC). The presence of heat-labile (*lt*) and/or heat-stable (*st*) enterotoxins defines Enterotoxigenic *E. coli* (ETEC). These toxins (which may be found together or separately in ETEC strains) bind to intestinal epithelial cells triggering loss of electrolytes resulting in watery diarrhea. ETEC are an important cause of diarrhea in developing countries especially among children, and are the most common bacterial cause of watery diarrhea in US and EU travelers returning from abroad (commonly referred to as travelers' diarrhea).[10,29] There were 33 documented outbreaks of ETEC in the US between 1975 and 1999.[35] ETEC are transmitted via the fecal-oral route and are becoming more common as a foodborne pathogen.[29] However, ETEC infection remains significantly under-diagnosed and underreported due to the difficulty of identification and because infected adults may not seek treatment, as infections resolve in a few days with supportive care (rehydration). ETEC may also be carried asymptomatically.[27]

Shiga-like toxin-producing *E. coli* (STEC), including *E. coli* O157. There are two main types of Shiga-like toxins, Shiga-like toxin 1 (Stx1) and Shiga-like toxin 2 (Stx2) (also known as verotoxins). Shiga-like toxin-producing *E. coli* (STEC) may contain either one or both *stx* genes. STEC are a primary cause of bloody diarrhea[10,36] and can progress to a potentially fatal condition known as hemolytic uremic syndrome (HUS; caused by Shiga-like toxin destruction of red blood cells that leads to renal failure), especially in the very young and very old. STEC are important foodborne pathogens. Infections may also be waterborne, transmitted person-to-person, or via contact with animals (especially cattle, which are a reservoir for STEC). Antimicrobial therapy for STEC may lead to an increased risk for HUS, especially in antibiotic-resistant strains, potentially by up-regulating production and thus increasing the amount of Shiga-like toxin available for absorption. Therefore, identification of Shiga-like toxin genes in a patient with gastrointestinal illness can aid in the decision of whether or not to prescribe antibiotics for patient care.

A subset of STEC contain the O157 antigen (and flagellar H7 antigen). *E. coli* O157:H7 is currently the most frequently identified diarrheagenic *E. coli* in North America. There are over 170,000 STEC infections in the US each year, of which an estimated 73,000 illnesses and 60 deaths per year are attributable specifically to *E. coli* O157.[2,12] Similar infection rates are observed in the EU.[13] Disease presentation ranges from mild, non-bloody diarrhea to hemorrhagic colitis and HUS. An estimated 4% of O157:H7 infections lead to HUS and this serotype of *E. coli* is responsible for up to 80% of all HUS illness.[37] The infectious dose is low, facilitating person-to-person transmission but most illness is caused by ingestion of contaminated ground beef, as dairy and beef cattle are often colonized with this bacterium. Although STEC O157:H7 remains the most identified serotype of STEC associated with human illness worldwide, non-O157 STEC are increasing in importance in both sporadic diarrhea and outbreaks.[27] Non-O157 STEC are likely underdiagnosed as testing methods are generally focused on detection of *E. coli* O157. STEC infections (including *E. coli* O157) are notifiable diseases in the US and are tracked by TESSy in the EU.

***Shigella*/Enteroinvasive *E. coli* (EIEC).** There are four subgroups of *Shigella* species: subgroup A (*S. dysenteriae*), subgroup B (*S. flexneri)*, subgroup C (*S. boydii)*, and subgroup D (*S. sonnei*). All *Shigella* are non-motile, gram-negative rods which are typically transferred through person-to-person contact or ingestion of contaminated food or water (humans and other primates are the only known animal reservoirs). Infections are most common where hygiene is compromised, for example institutional settings (day care, nursing homes) and may become endemic in developing societies without running water and indoor plumbing.[10] *Shigella* are responsible for multiple illnesses including shigellosis and bacillary dysentery which can result in bloody or non-bloody diarrhea.

Enteroinvasive *E. coli* (EIEC), unlike most *E. coli* do not decarboxylate lysine, and do not ferment lactose. EIEC strains contain a plasmid encoding virulence factors (such as invasion plasmid antigen *ipaH*) that allow the bacteria to invade the colon and produce a watery diarrhea syndrome that is identical to that caused by *Shigella* . EIEC is rare in the US and EU and is also less common worldwide than ETEC and EPEC.[10] *Shigella* and EIEC infections are generally treated in the same manner.

Multiple copies of the *ipaH* gene are present in all four *Shigella* species (*S. dysenteriae*, *S. flexnei, S. boydii*, and *S. sonnei*) as well as in the virulence plasmid of Enteroinvasive *E. coli* (EIEC).[38,39] *IpaH*, along with other factors encoded by the invasion plasmid, mediate entry of *Shigella* and EIEC into host cells. This is a common target for laboratory developed molecular tests.[38,39]

There are an estimated 130,000 *Shigella* infections associated with foodborne illness in the US each year[12] however no data exists for EIEC. Shigellosis is a notifiable disease in the US and is tracked by TESSy in the EU.

Parasites

Cryptosporidium is a genus of protozoa capable of causing infections of the human stomach, intestine, and biliary ducts following ingestion of chlorine-tolerant oocysts which are shed in fecal material and can contaminate drinking water, recreational water, or food. *Cryptosporidium* are among the most common parasitic causes of diarrhea in developed nations.[40] There are an estimated 60,000 illnesses every year in the US due to *Cryptosporidium* infection[12] with rates being highest in summer months.[23] At least 10 species infect humans though *C. hominis* and *C. parvum* are the most common.[10] Illness is generally characterized by short-term gastroenteritis that resolves without treatment. However, severe illness is possible in immunocompromised individuals, particularly those with AIDS, where illness resolves slowly or not at all and can be fatal. Cryptosporidiosis is a notifiable disease in the US and is tracked by TESSy in the EU.

Cyclospora cayetanensis are parasitic protozoa that cause gastroenteritis in humans, which are the only known hosts. Unsporulated oocysts are disseminated in feces. After a period of maturation (days to weeks), the oocysts become infectious and can cause illness if ingested through contaminated food or water.[10] Infections are most common in tropical, subtropical, or warm temperate regions. In the US and EU, infections are associated with travelers' diarrhea in persons returning from endemic areas. Additionally, outbreaks have been associated with consumption of contaminated food from other countries.[10,41] There are an estimated 11,000 foodborne illnesses due to *C. cayetanensis* infections annually in the US[12] but the true incidence may be underestimated due to the difficulty of diagnosing infection.[42] Illness presents as non-bloody diarrhea that may be up to several months in duration. Cyclosporiasis is a notifiable disease in the US but is not tracked by TESSy in the EU.

Entamoeba histolytica are pathogenic protozoa which are found worldwide with a particularly high prevalence in tropical and subtropical regions. *E. histolytica* cysts are generally ingested from materials contaminated with feces, such as food and water, but infection may also be transmitted sexually.[10] Humans are the primary reservoir. Most infections from *E. histolytica* appear to be asymptomatic but some infections cause invasive amebiasis which results in colitis or dysentery-like illness that can be severe and include amebic liver abscess. The epidemiology of *E. histolytica* is uncertain because it is indistinguishable from non-pathogenic *E. dispar* using the current clinical reference standard (microscopy).[43] In endemic regions, the prevalence of *Entamoeba* in stool can be as high as 50% of the general population. An estimated 500 million people world-wide are infected every year with *Entamoeba*. As *E. dispar* is thought to be 10-fold more prevalent, this translates to an estimated 50 million *E. histolytica* infections, which result in more than 100,000 deaths.[44] The FilmArray GI Panel *E. histolytica* assay demonstrates cross-reactivity with high levels of *E. dispar.*

Giardia lamblia (also referred to as *G. duodenalis* and *G. intestinalis*) are intestinal flagellate parasites found world-wide. *Giardia* are the most common intestinal parasites isolated in the US and EU and are a leading cause of parasitosis worldwide.[12,13,40] Populations with the highest risk of *G. lamblia* infection include children in day care centers, hikers, and the immunocompromised. *G. lamblia* prevalence is about 1-7% in developed countries and as high as 50% in developing countries.[10] Transmission occurs through ingestion of contaminated food or water, with approximately 77,000 foodborne illnesses in the US annually.[12] Infection rates are highest during summer months.[23] The majority of *G. lamblia* infections are asymptomatic, but those who develop illness experience nausea, fever, and watery diarrhea.[45] Infections are generally self-limiting; though symptoms are long-lasting, and some patients go on to develop chronic illness, which can lead to complications. Giardiasis is a notifiable disease in the US and is tracked by TESSy in the EU.

Viruses

Adenovirus F 40/41. Adenoviruses are double-stranded DNA viruses of the *Adenoviridae* family that cause a variety of diseases including respiratory illness and gastrointestinal illness. They are resistant to chemical and physical damage and are thus persistent in the environment, facilitating transmission. There are seven species of Adenoviruses (A-G) that are further categorized into approximately 57 serotypes, however GI illness is primarily associated with species F (which is comprised of serotypes 40 and 41). Adenovirus F 40/41 is responsible for 5 to 15% of all acute diarrheal illness in children (especially in those under two years of age).[10] Transmission is mostly through fecal-oral spread and outbreaks have been reported in hospitals and child care centers. While Adenovirus infections mostly occur in children, adults may be affected as well.[10] Illness is generally mild but of a relatively long duration (5-12 days). Immunocompromised patients may suffer chronic, prolonged diarrheal illness and other complications. Virus may be shed in stool for weeks to months following acute illness; therefore identification of infected individuals may be important for patient isolation and control of disease spread.

Astrovirus. Astroviruses (RNA viruses of the family *Astroviridae*) are named for their characteristic star-like structure and are found in a variety of animals, including birds and mammals. There are eight serotypes of human Astrovirus (HAstv 1-8) associated with gastroenteritis in both children and adults.[10] The infection route is fecal-oral and at-risk populations include children, immunocompromised adults, caregivers of sick children, military troops, and those in nursing homes. It is estimated that there are over 15,000 foodborne illnesses due to Astrovirus in the US each year[12] but diagnostic testing is limited and the true incidence is not known. Symptoms are reported to be milder than other enteric viruses and include diarrhea, vomiting, abdominal pain, and fever lasting 72 hours.[46] There is a 70-90% seroprevalence of antibodies to Astrovirus in school-aged children,[10] indicating nearly universal exposure in childhood, but the presence of antibodies and their role in immunity is not well understood.[47]

Norovirus GI/GII. Noroviruses are highly contagious members of the *Caliciviridae* family of RNA viruses and can be divided into five genogroups (GI – GV). GI, GII, and GIV have been found most commonly in humans (though GIV is very rare) where they cause moderate to severe gastroenteritis consisting primarily of nausea, vomiting, and diarrhea accompanied by fever. Transmission occurs via the fecal-oral route or through aerosolized vomitus and the infectious dose may be as low as 18 particles.[48] Symptoms of infection generally last 24-48 hours[49] and the illness is self-limiting; though immunocompromised persons may suffer chronic diarrhea and some children have been reported to develop necrotizing colitis. Outbreaks are common in closed communities such as cruise ships, hospitals, nursing homes, schools, and military installations. Norovirus infections are the leading cause of foodborne gastroenteritis in the US, causing nearly 5.4 million illnesses (and over 14,000 hospitalizations) annually[12] and are also a significant source of illness in the EU.[13] Peak infection rates occur during winter months.[50] Immunity following Norovirus illness is short lived as re-infection is possible within 6 months, even in the presence of high serum antibody titers.[51]

Rotavirus A. Rotaviruses are double stranded RNA viruses of the *Reoviridae* family and are the single most important etiologic agents of severe diarrheal illnesses in infants and young children worldwide.[52,53] Of the seven groups of Rotaviruses (A through G), Rotavirus A, B, and C infect humans, with Rotavirus A being responsible for the majority of infections.[10] Symptoms of infection may be mild and last for a few days, but prolonged illness can lead to severe dehydration in children <2 years of age and Rotavirus A infections are a considerable cause of infant mortality in the developing world.[10] Rotaviruses are shed before and after acute illness and are hardy to environmental factors, allowing them to survive on surfaces and resist inactivation. Disease rates peak during winter/spring in temperate climates and may account for up to a third of diarrheal diseases presenting to emergency rooms and outpatient clinics during this time in the US and EU.[54,55] It is estimated that 2.7 million diarrheal illnesses per year in the US are caused by Rotavirus infection.[56] Immunity is thought to be long-lasting following infection. There are two Rotavirus vaccines, Rotarix (RV1) and RotaTeq (RV5), which have been licensed worldwide and offer protection against Rotavirus A. RotaTeq was implemented in the US vaccination program in 2006[52] and has resulted in a decline in Rotavirus A infections.[57]

Sapovirus (Genogroups I, II, IV, and V). Sapovirus is a *Calciviridae* family member that is similar to Norovirus both genetically and in disease presentation. There are five genogroups (GI–GV); groups GI, GII, GIV, and GV are known to infect humans, whereas GIII causes diarrheal illness in pigs. Sapovirus causes disease mostly in children, though adults are susceptible as well, with outbreaks reported in long-term care facilities, prisons, cruise ships, and hospitals in the US

and EU.[58,59] Like Norovirus, Sapovirus is spread via the fecal-oral route and infections are highest during winter months. Symptoms primarily include vomiting and diarrhea with nausea and fever lasting 5 to 10 days.[60,61] In general, illness is self-limiting with treatment consisting of supportive care. Infections are attributed to an estimated 15,000 foodborne illnesses in the US annually,[12] however the true incidence may be much higher as there is very limited testing available.

Principle of the Procedure

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. 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 testing procedure:

1. Remove the FilmArray pouch from its vacuum-sealed package. Since solutions are drawn into the FilmArray GI pouch by vacuum, it is important to keep pouches in their protective packaging until the time of use.

2. Place the FilmArray GI pouch into the FilmArray Pouch Loading Station. The FilmArray Pouch Loading Station has been designed to prevent error by providing visual cues in the form of color-coded arrows to ensure that the pouch is properly loaded.

3. Load Hydration Solution into the FilmArray GI pouch using the Hydration Injection Vial. The vial is fitted with a blunt stainless steel cannula, which is used to deliver the solution into the pouch. Loading the pouch with Hydration Solution rehydrates the freeze-dried reagents contained in the pouch fitment.

4. Prepare the Sample Injection Vial by squeezing the contents of the Sample Buffer ampoule into the Sample Injection Vial. Thoroughly mix the stool specimen (stool in Cary Blair transport media) and transfer it to the Sample Injection Vial using the Transfer Pipette. Tightly close the lid of the Sample Injection Vial and invert at least three times to mix. The Sample Buffer contains reagents that promote binding of nucleic acids to magnetic beads for isolation.

5. Load the sample/buffer mixture into the FilmArray GI pouch using the Sample Injection Vial. When the sample mixture is loaded, a process control contained in the fitment of the pouch is introduced into the sample. The process control monitors all of the critical processes that occur in the pouch.

6. Transfer the pouch to the instrument and initiate a run. The FilmArray Instrument Control application provides on-screen animations illustrating the steps needed to start the run.

7. View results on the test report at the completion of the run.

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

1. **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.

2. **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.

3. **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.

4. **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 T_m) is consistent and predictable and the FilmArray Software automatically evaluates the data from replicate wells for each assay to report results. For a description of data interpretation and reporting see the Interpretation of Results section of this booklet.

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. The entire process takes about an hour. Additional detail can be found in the FilmArray Operator's Manual.

MATERIALS PROVIDED

Each kit contains sufficient reagents to test 30 or 6 samples (30 pouch kit or 6 pouch 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.

WARNINGS AND PRECAUTIONS

General Precautions

1. For *in vitro* diagnostic use only.
2. This device is restricted to sale by or on the order of a physician, or to a clinical laboratory; its use is restricted to, by, or on the order of a physician.
3. A trained healthcare professional should carefully interpret the results from the FilmArray GI Panel in conjunction with a patient's signs and symptoms and results from other diagnostic tests.

4. FilmArray GI pouches are only for use with the FilmArray Instrument.
5. Always check the expiration date on the pouch and do not use a pouch after its expiration date.
6. FilmArray pouches are stored under vacuum in individually-wrapped canisters. To preserve the integrity of the pouch vacuum for proper operation, be sure that a FilmArray Instrument will be available and operational before unwrapping any pouches for loading.

Safety Precautions

1. Wear appropriate Personal Protective Equipment (PPE), including (but not limited to) disposable powder-free gloves and lab coats. Protect skin, eyes and mucus membranes. Change gloves often when handling reagents or samples.
2. Handle all samples and waste materials as if they were capable of transmitting infectious agents. Observe safety guidelines such as those outlined in CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories,*[62] the CLSI Document M29-A3 *Protection of Laboratory Workers from Occupationally Acquired Infections*,[63] or other appropriate guidelines.
3. Follow your institution's safety procedures for handling biological samples.
4. Dispose of materials used in this assay, including reagents, samples, and used buffer vials, according to federal, state, and local regulations.
5. Sample Buffer is assigned the following classifications: Acute toxicity (Category 4), Serious Eye damage (Category 1), and Skin irritation (Category 2). Please refer to the FilmArray Reagent Kit Material Safety Data Sheet (SDS/MSDS) for more information.
6. Sample Buffer will form hazardous compounds and fumes when mixed with bleach or other disinfectants.

WARNING: Bleach should never be added to Sample Buffer or sample waste.

Laboratory Precautions

1. **Preventing organism contamination**

   Due to the sensitive nature of the FilmArray GI Panel, it is important to guard against contamination of the work area by carefully following the testing process outlined in this booklet, including these guidelines:

   - Stool samples may contain a high concentration of organisms. To avoid possible contamination, samples should be processed in a biosafety cabinet. If a biosafety cabinet is not used, a dead air box (e.g., AirClean PCR workstation), a splash shield (e.g., Bel-Art Scienceware Splash Shields), or a face shield should be used when preparing samples.
   - A biosafety cabinet or work station that is used for performing stool pathogen testing (e.g. culture, EIA) should not be used for sample preparation or pouch loading.
   - Prior to processing a sample, thoroughly clean both the work area and the FilmArray Pouch Loading Station 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.
   - Samples and pouches should be handled one at a time.
   - Change gloves and clean the work area between each sample.

2. **Preventing amplicon contamination**

A common concern with PCR-based assays is false positive results caused by contamination of the work area with PCR amplicon. Because the FilmArray GI pouch is a closed system, the risk of amplicon contamination is low provided that pouches remain intact after the test is completed. Adhere to the following guidelines to prevent amplicon contamination:

- Discard used pouches in an appropriate biohazard container immediately after the run has completed.
- Avoid excessive handling of pouches after test runs.
- Avoid exposing pouches to sharp edges or anything that might cause a puncture.

WARNING: If liquid is observed on the exterior of a pouch, the liquid and pouch should be immediately contained and discarded in a biohazard container. The instrument and work space must be decontaminated as described in the FilmArray Operator's Manual.

DO NOT PERFORM ADDITIONAL TESTING UNTIL THE AREA HAS BEEN DECONTAMINATED.

REAGENT STORAGE, HANDLING AND STABILITY

1. Store the test kit, including reagent pouches and buffers, at room temperature (15–25 ºC). **DO NOT REFRIGERATE.**
2. Avoid storage of any materials near heating or cooling vents or in direct sunlight.
3. Always check the expiration date and do not use reagents beyond the expiration date printed on the pouch or kit.
4. Do not remove pouches from their packaging until a sample is ready to be tested. Once the pouch packaging has been opened, the pouch should be loaded as soon as possible (within approximately 30 minutes).
5. Once a pouch has been loaded, the test run should be started as soon as possible (within 60 minutes).

SAMPLE REQUIREMENTS

This section describes the requirements for specimen collection, preparation, and handling that will help ensure accurate test results.

Stool Specimen Collection – Stool specimens should be collected in Cary Blair transport media according to manufacturer's instructions.

Minimum Sample Volume - 200 µL of sample is required for testing.

Transport and Storage - Specimens should be processed and tested as soon as possible, though they may be stored at room temperature or under refrigeration for up to four days.

PROCEDURE

Refer to the FilmArray Gastrointestinal Panel Quick Guide, the FilmArray Training Video, or the FilmArray Operator's Manual for more detail and pictorial representations of these instructions.

Gloves and other Personal Protective Equipment (PPE) should be used when handling pouches and samples. Only one FilmArray GI pouch should be prepared at a time. Once sample is added to the pouch, it should be promptly

transferred to the instrument to start the run. After the run is complete, the pouch should be discarded in a biohazard container.

Prepare Pouch

1. Thoroughly clean the work area and the FilmArray Pouch Loading Station with freshly prepared 10% bleach (or suitable disinfectant) followed by a water rinse. Note: Ensure that the Pouch Loading Station is compatible with the use of the FilmArray Injection Vials.
2. Obtain the following required materials and place in the clean hood:
    - FilmArray GI Panel pouch
    - Sample Buffer ampoule
    - Hydration Injection Vial (blue cap)
    - Sample Injection Vial (red cap)
    - Transfer pipette
3. Place a blue capped Hydration Injection Vial in the blue well of the Pouch Loading Station.
4. Place a red capped Sample Injection Vial in the red well of the Pouch Loading Station.
5. Obtain patient sample and place into hood.
6. Remove the FilmArray GI pouch from its vacuum-sealed package by tearing or cutting the notched outer packaging and opening the protective aluminum canister.

    NOTE: If the vacuum seal of the pouch is not intact, the pouch may still be used. Attempt to hydrate the pouch using the steps in the Hydrate Pouch section. If hydration is successful, continue with the run. If hydration fails, discard the pouch and use a new pouch to test the sample.

7. Slide the pouch into the Pouch Loading Station so that the red and blue labels on the pouch align with the red and blue arrows on the base of the Pouch Loading Station.

Hydrate Pouch

1. Twist the Hydration Injection Vial (blue cap), leaving cap in Pouch Loading Station, and insert the tip of the cannula into the hydration port of the pouch located directly below the blue arrow of the Pouch Loading Station. Push down forcefully in a firm and quick motion until you hear a faint “pop” and feel an ease in resistance. The correct volume of liquid will be pulled into the pouch by vacuum.
2. Verify that the pouch has been hydrated. Flip the barcode label down and check to see that fluid has entered the reagent wells (located at the base of the rigid plastic part of the pouch). Small air bubbles may be seen. If the pouch fails to hydrate (dry reagents appear as white pellets), verify that the seal of the port was broken by ensuring the vial cannula was fully inserted into the hydration port. If the pouch fails to hydrate, retrieve a new pouch and repeat from Step 2 of the Prepare Pouch section.
3. Discard the Hydration Injection Vial in a suitable puncture proof container.

Prepare Sample Mix

1. Hold the Sample Buffer ampoule so that the tip is facing up.

   NOTE: Use care to avoid touching the tip during handling, as this may introduce contamination.

2. Gently pinch the textured plastic tab on side of ampoule until the seal snaps.
3. Re-position thumb and forefinger to grip between the textured plastic tab and the bottom of the ampoule, then invert over the red Sample Injection Vial and dispense Sample Buffer using a slow, forceful squeeze, followed by a second squeeze. Avoid generating excessive bubbles.
4. Thoroughly mix the patient specimen.
5. Using the transfer pipette provided in the test kit, draw sample to the second line (approximately 0.2 mL). Add sample to the red Sample Injection Vial.

   NOTE: DO NOT use the transfer pipette to mix the sample once it is loaded into the Sample Injection Vial.

6. Tightly close the lid of the Sample Injection Vial and mix by gently inverting at least 3 times.
7. Return the Sample Injection Vial to the Pouch Loading Station.

Load Sample Mix

1. Slowly unscrew Sample Injection Vial from the cap and pause for 3-5 seconds.

   NOTE: It is important to pause after unscrewing the Sample Injection Vial to avoid sample leakage and contamination of the work area.

2. Remove Sample Injection Vial leaving cap in Pouch Loading Station and insert the cannula tip into the port in the pouch fitment located directly below the red arrow of the Pouch Loading Station. Push down forcefully in a firm and quick motion until you hear a faint “pop” and feel an ease in resistance. The correct volume of liquid will be pulled into the pouch by vacuum.
3. Verify that the sample has been loaded. Flip the barcode label down and check to see that fluid has entered the reagent well next to the sample loading port. If the pouch fails to pull sample from the Sample Injection Vial, the pouch should be discarded. Retrieve a new pouch and repeat from Step 2 of the Prepare Pouch section.
4. Discard the Sample Injection Vial in a suitable biohazard and puncture proof container. Do not re-cap the vial.
5. Record the Sample ID in the provided area on the pouch label (or affix a barcoded Sample ID) and remove the pouch from the Pouch Loading Station.

Run Pouch

The FilmArray Instrument Control Software includes a step-by-step on-screen tutor that shows each step of the test.

1. Ensure that the laptop and FilmArray Instrument have been turned on. Launch the FilmArray Instrument Control Software by double clicking on the desktop icon.
2. Open the instrument lid (if not already open).
3. Insert the FilmArray pouch into the instrument.

   Position the pouch so that the array is on the right with the film directed downward into FilmArray Instrument. The red and blue labels on the FilmArray pouch should align with the red and blue arrows on the FilmArray Instrument. The pouch will click into place. If inserted correctly, the barcode is visible and the label is readable on

the top of the pouch. The instrument and software must detect that the pouch has been inserted correctly before continuing to the next step.

NOTE: If the pouch does not slide into the instrument easily, gently push the lid of the instrument back to be sure that it is completely open.

4. Scan the barcode on the FilmArray pouch using the barcode scanner.

   Pouch identification (Lot Number and Serial Number), Pouch Type and Protocol are preprogrammed in the barcode located on the FilmArray pouch and will be automatically entered when the barcode is scanned. If it is not possible to scan the barcode, the pouch Lot Number, Serial Number, Pouch Type and Protocol can be manually entered from the information provided on the pouch label. To reduce data entry errors, is it strongly recommended that the pouch information be entered by scanning the barcode.

   NOTE: The barcode cannot be scanned prior to placing the pouch in the instrument. A "Cannot scan now" message will be displayed.

5. Enter the Sample ID.

   The Sample ID can be entered manually or scanned in by using the barcode scanner when a barcoded Sample ID is used.

6. If necessary, select a protocol from the Protocol drop down list.
7. Enter a user name and password in the Name and Password fields.
8. Close the FilmArray Instrument lid.
9. Click Start Run.

   Once the run has started, the screen displays a list of the steps being performed by the instrument and the number of minutes remaining in the run.

   NOTE: The bead-beater apparatus can be heard as a high-pitched noise (whine) during the first few minutes of operation.

10. When the run is finished, results are automatically displayed in the report section of the screen. The report is automatically saved into the database.
11. Select **Print** to print the report, or **Save** to save the report as a PDF file.
12. Follow the on-screen instructions to open the instrument and remove the pouch.
13. Immediately discard the pouch in a biohazard container.

QUALITY CONTROL

Process Controls

Two process controls are included in each pouch:

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

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

Both control assays must be positive for the test run to pass. If either control fails, the Controls field of the test report (upper right hand corner) will display Failed and all results will be listed as Invalid. If the controls fail, the sample should be retested using a new pouch.

Monitoring Test System Performance

The FilmArray Software will automatically fail the run if the melting temperature (Tm) for either the RNA Process Control or the PCR2 Control is outside an acceptable range (80.2-84.2 for the RNA Process Control and 74.1-78.1 for the PCR2 Control). If required by local, state, or accrediting organization quality control requirements, users can monitor the system by trending Tm values for the control assays and maintaining records according to standard laboratory quality control practices.[64,65] The PCR2 Control is used in all pouch types and can therefore be used to monitor the system when multiple pouch types (e.g., RP, GI and BCID) are used on the same FilmArray Instrument.

Good laboratory practice recommends running external positive and negative controls regularly. Enteric transport 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.

INTERPRETATION OF RESULTS

The FilmArray Software automatically analyzes and interprets assay results and displays the final results in a test report (see the FilmArray Gastrointestinal Panel Quick Guide to view an example of a test report). The analyses performed by the FilmArray Software and details of the test report are described below.

Assay Interpretation

When 2nd stage PCR is complete, the FilmArray Instrument performs a high resolution DNA melting analysis on the PCR products and measures the fluorescence signal generated in each well (for more information see FilmArray Operator's Manual). The FilmArray Software then performs several analyses and assigns a final assay result. The steps in the analysis are described below.

Analysis of melt curves. The FilmArray Software evaluates the DNA melt curve for each well of the 2nd stage PCR array to determine if a PCR product was present in that well. If the melt profile indicates the presence of a PCR product, then the analysis software calculates the melting temperature (Tm) of the curve. The Tm value is then compared against the expected Tm range for the assay. If the software determines that the melt curve is positive and the Tm falls inside the assay-specific Tm range, the melt curve is called positive. If the software determines that the melt curve is negative or is not in the appropriate Tm range, the melt curve is called negative.

Analysis of replicates. Once melt curves have been identified, the software evaluates the three replicates for each assay to determine the assay result. For an assay to be called positive, at least two of the three associated melt curves must be called positive, and the Tm for at least two of the three positive melt curves must be similar (within 1°C). Assays that do not meet these criteria are called negative.

Organism Interpretation

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: If four or more distinct organisms are detected in a specimen, retesting is recommended to confirm poymicrobial result.

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[18] or the Society for Healthcare Epidemiology of America and the Infectious Disease Society of America).[19]

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 support detection of 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 2 below).

Table 2. Possible Assay Results and Corresponding *Vibrio* Test Results

FilmArray GI Interpretations	*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.

Note: 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 3 below). Consequently, the FilmArray GI Panel cannot distinguish between STEC containing *eae* 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 3 below).

Note: 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 an *stx1/stx2*-negative *E. coli* O157 (see Table 3 below).

Table 3. Possible Assay Results and Corresponding Test Results for Enteropathogenic *E. coli* (EPEC) and Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*

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

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): 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.

Note: 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.

FilmArray GI Test Report

The FilmArray GI test report is automatically displayed upon completion of a run and contains three sections, the Run Summary, the Result Summary, and the Run Details (see the FilmArray Gastrointestinal Panel Quick Guide to view an example of a test report). The test report can be saved as a PDF or printed.

The **Run Summary** section of the test report provides the Sample ID, time and date of the run, control results and an overall summary of the test results. Any organism with a Detected result will be listed in the corresponding field of the summary. If all of the tests were negative then None will be displayed in the Detected field. Controls are listed as Passed, Failed or Invalid. See the Controls Field section below for detailed information about the interpretation of controls and appropriate follow-up in the case of control failures.

The **Result Summary** section of the test report lists the result for each target tested by the panel. Possible results for each organism are Detected, Not Detected, Not Applicable (N/A), or Invalid. See Results Summary section below for detailed information about interpretation of test results and appropriate follow-up for Invalid results.

The **Run Details** section provides additional information about the run including: pouch information (type, lot number, and serial number), Run Status (Completed, Incomplete, Aborted, Instrument Error, Instrument Communication Error, or Software Error), the protocol that was used to perform the test, the identity of the operator that performed the test, and the instrument used to perform the test.

Once a run has completed, it is possible to edit the Sample ID. If this information has been changed, an additional section called **Change History** will be added to the test report. This Change History section lists the field that was changed, the original entry, the revised entry, the operator that made the change, and the date that the change was made. Sample ID is the only field of the report that can be changed.

Controls Field

The Controls field on the test report will display Passed, Failed, or Invalid. The Controls field will display Passed only if the run completed successfully (no instrument or software errors) and both of the pouch control assays (RNA Process Control and PCR2 Control) were successful. The Controls field will display Failed if the run was completed successfully (no instrument or software errors) but one or both of the pouch control assays failed. If the control result is Failed, then the result for all of the tests on the panel are displayed as Invalid and the sample will need to be retested with a new pouch.

Table 4 provides a summary and explanation of the possible control results and follow-up actions.

Table 4. Interpretation of Controls Field on the FilmArray GI Test Report

Control Result	Explanation	Action Required	Outcome
Passed	The run was successfully completed AND Both pouch controls were successful.	None	Report the results provided on the test report.
Failed	The run was successfully completed BUT At least one of the pouch controls (RNA Process Control and/or PCR2 Control) failed.	Repeat the test using a new pouch.	Accept the results of the repeat testing. If the error persists, contact Technical Support for further instruction.
Invalid	The controls are invalid because the run did not complete. (Typically this indicates a software or hardware error).	Note any error codes displayed during the run and the Run Status field in the Run Details section of the report. Refer to the FilmArray Operator's Manual or contact Technical Support for further instruction. Once the error is resolved, repeat the test or repeat the test using another instrument.	Accept the valid results of the repeat testing. If the error persists, contact Technical Support for further instruction.

Results Summary – Interpretations

The Results Summary – Interpretations section provides a complete list of the test results. Possible results for each organism include Detected, Not Detected, N/A and Invalid. Table 5 provides an explanation for each interpretation and any follow-up necessary to obtain a final result.

Table 5. Reporting of Results and Required Actions

Result	Explanation	Action
Detected	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay(s) associated with the interpretation were positive based on the following requirements for at least 2 of the 3 assay replicates: -a positive melt curve, and -the Tm for the melt data were within the assay specific limits, and -the Tm for the melt data were within 1°C of each other.	None. Report results.
Not Detected	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay(s) associated with the interpretation were negative (did not meet the requirements for a positive assay described in Detected).	None. Report results.
N/A (applies to *E. coli* O157 and EPEC only)	The run was successfully completed AND The pouch controls were successful (Passed) AND For *E.* coli O157: Shiga-like toxin-producing *E. coli* was Not Detected. For EPEC: Shiga-like toxin-producing *E. coli* was Detected.	None. Report results.
Invalid	The run did not complete successfully (Aborted, Incomplete, Instrument Communication Error, Instrument Error, or Software Error) OR The pouch controls were not successful (Failed)	See Table 4, *Interpretation of Controls Field on FilmArray Report,* for instruction.

LIMITATIONS OF THE PROCEDURE

- For prescription use only.
- This product can be used only with the FilmArray Instrument.
- This test is a qualitative test and does not provide a quantitative value for the organism(s) in the sample.
- The performance of this test has only been validated with human stool collected in Cary Blair transport medium, according to the media manufacturers' instructions. It has not been validated for use with other stool transport media, raw stool, rectal swabs, endoscopy stool aspirates, or vomitus.
- This product should not be used to test stool samples in fixative (e.g., formalin or polyvinyl alcohol; PVA).
- The performance of this test has not been established for patients without signs and symptoms of gastrointestinal illness.
- Virus, bacteria, and parasite nucleic acid may persist *in vivo* independently of organism viability. Additionally, some organisms may be carried asymptomatically. Detection of organism targets does not imply that the corresponding organisms are infectious or are the causative agents for clinical symptoms.
- Results from this test must be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient. Due to high rates of asymptomatic carriage of *Clostridium difficile*, especially 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).[18,19]
- The performance of this test has not been established for monitoring treatment of infection with any of the panel organisms.
- Discrepancies between the FilmArray GI Panel and other microbial identification methods may be caused by the inability to reliably differentiate species based on standard phenotypic microbial identification methods. Examples include differentiation of *Yersinia enterocolitica* from other *Y. enterocolitica* group members such as *Y. kristensenii* or *Y. fredricksonii,* differentiation of *Entamoeba histolytica* from *E. dispar*, and differentiation of *Helicobacter pullorum* from *Campylobacter*. See Organism Interpretation section of this document for other specific examples.
- There is a risk of false negative values due to the presence of sequence variants in the gene targets of the assay, procedural errors, amplification inhibitors in specimens, or inadequate numbers of organisms for amplification.
- The identification of several diarrheagenic *E. coli* pathotypes has historically relied upon phenotypic characteristics, such as adherence patterns or toxigenicity in certain tissue culture cell lines. The FilmArray GI Panel targets genetic determinants characteristic of most pathogenic strains of these organisms but may not detect all strains having phenotypic characteristics of a pathotype. In particular, the FilmArray GI Panel will only detect Enteroaggregative *E. coli* (EAEC) strains carrying the *aggR* and/or *aatA* genes on the pAA (aggregative adherence) plasmid; it will not detect all strains exhibiting an aggregative adherence pattern.
- Target genes associated with the diarrheagenic *E. coli*/*Shigella* pathotypes are capable of horizontal transfer between strains, thus Detected results for multiple diarrheagenic *E. coli/Shigella* may be due to co-infection with multiple pathotypes or, less frequently, may be due to the presence of a single organism containing genes characteristic of multiple pathotypes. An example of the latter is the 2011 *E. coli* O104:H4 outbreak strain that contains determinants of both STEC and EAEC.
- The FilmArray GI Panel detects the heat-labile toxin (LT) and heat-stable toxin variants (ST1a and ST1b) of Enterotoxigenic *E. coli* (ETEC), which are associated with human disease. 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.

- The FilmArray GI Panel detects Enteropathogenic *E. coli* (EPEC) through targeting of the *eae* gene, which encodes the adhesin intimin. As some Shiga-like toxin-producing *E. coli* (STEC) also carry *eae* (in particular, strains identified as enterohemorrhagic *E. coli*; EHEC), the FilmArray GI Panel cannot distinguish between STEC containing *eae* and a co-infection of EPEC and STEC. Therefore, the EPEC result is not applicable (N/A) and not reported for specimens in which STEC has also been detected. Rare instances of other organisms carrying *eae* have been documented; e.g., *Aeromonas* spp., *Citrobacter* spp., *Escherichia albertii*, and *Shigella boydii*.

- *Shigella dysenteriae* possess a shiga toxin gene (*stx*) that is identical to the *stx1* gene of STEC. The detection of both *Shigella*/Enteroinvasive *E. coli* (EIEC) and STEC *stx1/stx2* analytes in the same specimen may indicate the presence of *S. dysenteriae*. Rare instances of the detection of shiga-like toxin genes in other genera/species have been reported; e.g., *Aeromonas caviae*, *Acinetobacter haemolyticus*, *Shigella sonnei*, *Enterobacter cloacae*, *Citrobacter freundii*, and *Klebsiella pneumoniae*.

- The *E. coli* O157 result is only reported in association with STEC *stx1/stx2*. While non-STEC O157 strains have been detected in human stool, their role in disease has not been established. Serotype O157 EPEC have been identified and will be detected by the FilmArray GI Panel (by the EPEC assay) due to their carriage of the *eae* gene.

- The FilmArray GI Panel cannot distinguish between infections with a single toxigenic STEC O157 or rare co-infections of STEC (non-O157) with an *stx1/stx2*-negative *E. coli* O157.

- This test only detects *Campylobacter jejuni*, *C. coli* and *C. upsaliensis* and does not differentiate between these three species of Campylobacter. Additional testing is required to differentiate between these species and to detect other Campylobacter species that may be present in stool specimens.

- The detection of organism nucleic acid is dependent upon proper sample collection, handling, transportation, storage, and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false positive and false negative results caused by improperly collected, transported, or handled specimens. The RNA process control and the PCR 2 control will not indicate whether or not nucleic acid has been lost due to inadequate collection, transport or storage of specimens.

- Due to the complex and highly variable nature of stool specimens, freezing may affect analyte integrity and subsequent test results for some specimens.

- A negative FilmArray GI Panel result does not exclude the possibility of gastrointestinal infection. Negative test results may occur from sequence variants in the region targeted by the assay, the presence of inhibitors, technical error, sample mix-up, or an infection caused by an organism not detected by the panel. Test results may also be affected by concurrent antimicrobial therapy or levels of organism in the sample that are below the limit of detection for the test. Negative results should not be used as the sole basis for diagnosis, treatment, or other management decisions.

- Organism and amplicon contamination may produce erroneous results for this test. Particular attention should be given to the Laboratory Precautions noted under the Warnings and Precautions section.

- If four or more distinct organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.

- The performance of the FilmArray GI Panel has not been established in individuals who received Rotavirus A vaccine. Recent oral administration of a Rotavirus A vaccine may cause positive results for Rotavirus A if the virus is passed in the stool.

- The effect of interfering substances has only been evaluated for those listed in the labeling. Interference by substances other than those described in the Interference section below could lead to erroneous results.

- Several organisms were shown to have the potential to cross-react with FilmArray GI Panel assays. These include *Entamoeba dispar* when present at high levels (*E. histolytica* assay); *Bifidobacterium* spp. and *Ruminococcus* spp. (*G. lamblia* assay); certain strains of *Citrobacter koseri*, *Citrobacter sedlakii*, *Hafnia alvei*, and

Cedeceae davisiae containing variants of a flagellar assembly protein (ETEC 2 assay), *E. coli* containing a variant type III secretion protein (*Salmonella* assay), *Grimontia hollisae* which was formerly classified as a *Vibrio* sp. (*Vibrio* assay), *Yersinia frederiksenii* and *Yersinia kristensenii*, which are members of the *Y. enterocolitica* group (*Y. enterocolitica* assay). Please refer to the Organism Interpretation and Analytical Specificity sections of this document for additional information.

- Cross-reactivity with organisms other than those listed above or in the Organism Interpretation or Analytical Specificity sections may lead to erroneous results.

- *Campylobacter* inclusivity testing and *in silico* analyses demonstrated that the FilmArray GI Panel may have variable detection or reduced sensitivity for some organisms detected by the *Campylobacter* assays (Note: The *Campylobacter* assays only detect *C. jejuni*, *C. coli*, and *C. upsaliensis*). *Campylobacter upsaliensis* strain ATCC 43954 and *Campylobacter jejuni* subsp. *doylei* may not be detected and *in silico* analysis indicates primer mismatches that might lead to reduced assay sensitivity or lack of reactivity with 11/138 *C. coli* sequences currently in NCBI databases.

- Empirical testing and *in silico* sequence analysis indicate that the *Vibrio* assay (*V. parahaemolyticus*/*V. vulnificus* /*V. cholerae*) may react with some less common *Vibrio* species (i.e., *V. alginolyticus*, *V. fluvialis*, and *V. mimicus*) but it is not expected to detect the rarer *Vibrio cincinnatiensis, Vibrio furnissii*, and *Vibrio metschnikovii* (Note: *Vibrio* spp. not associated with human disease were not evaluated).

- *V.cholerae* isolates with highly divergent *toxR* genes will be non-reactive with the FilmArray GI Panel *V. cholerae* assay. Additionally, very rare strains of pathogenic *V. cholerae* that do not carry that *toxR* gene will also not be detected by the Vchol assay.

- Rare isolates of *V. harveyi*, *V. mimicus*, and *V. vulnificus* that have acquired a homolog of the *toxR* gene have been reported and may show cross-reactivity with the Vchol assay.

- Based on the available sequences, a few *Cryptosporidium* species, or certain variants of species, including *C. bovis*, *C. ryanae*, and *C. xiaoi*, may not be efficiently detected by the *Cryptosporidium* assays.These species are rarely detected in human samples.

- There is a risk of false negative results due to the presence of strains with sequence variability or genetic rearrangements in the target regions of the assays. Refer to the inclusivity testing section of this document for additional information.

- Unexpected results obtained from testing isolates from culture collections (e.g., during quality control testing) may occur due to mislabeling or miscategorization of the isolate, contamination of the stock, or genetic rearrangements (including loss of virulence plasmids) during repeated passaging.

- Not all *Salmonella* serotypes were tested in validation studies; however, representatives of the 20 most prevalent serotypes recently circulating in the US (CDC National *Salmonella* Surveillance Annual Summary 2009) were evaluated. *In silico* sequence analysis supports detection of all subspecies and serotypes of *Salmonella*.

- Cross-re*activity w*ith the *Salmonella* assay may occur with certain *E. coli* strains containing variants of the cryptic ETT2 type-III secretion system (see Inclusivity for *additional* information).

- Positive and negative predictive v*alues are* highly dependent on prevalence. False negative results are more likely d*uring peak* activity when prevalence of *disease is* high. False positive results *are more* likely during periods when prevalence is moderate to low.

- The performance of this test has not been evaluated for immunocompromised individuals.

- State and local public health authorities have published guidelines for notification of reportable diseases in their jurisdictions including *Salmonella*, *Shigella*, *V. cholerae*, *E. coli* O157, Enterotoxigenic *E. coli* (ETEC) *lt*/*st*, and Shiga-like toxin-producing *E. coli* (STEC) *stx1*/*stx2* to determine necessary measures for verification of results to identify and trace outbreaks. Laboratories are responsible for following their state or local regulations for submission of clinical material or isolates on positive specimens to their state public health laboratories.

EXPECTED VALUES

In the prospective clinical evaluation of the FilmArray GI Panel, 1556 eligible specimens (stool in enteric transport medium; i.e., Cary Blair) were collected and tested at four study sites across the United States (Pacific, North Central, Great Lakes, and Northeast regions) over approximately five months (May –September 2013). The number and percentage of positive results as determined by the FilmArray GI Panel, stratified by age group, are presented in the following table. Overall, the FilmArray GI Panel detected at least one organism in 53.5% (832/1556) of the prospective specimens.

Table 6. Expected Values (as determined by the FilmArray GI Panel) Summary by Age Group for the Prospective Clinical Evaluation (May through September 2013)

FilmArray GI Panel Result	*Overall (n=1556)*	<1 year (n=121)	1-5 years (n=418)	6-12 years (n=193)	13-21 years (n=240)	22-64 years (n=411)	65+ years (n=173)
Bacteria							
Campylobacter	***58 (3.7%)***	1 (0.8%)	11 (2.6%)	12 (6.2%)	6 (2.5%)	19 (4.6%)	9 (5.2%)
Clostridium difficile toxin A/B	***204 (13.1%)***	49 (40.5%)	66 (15.8%)	18 (9.3%)	33 (13.8%)	29 (7.1%)	9 (5.2%)
Plesiomonas shigelloides	***18 (1.2%)***	0 (0.0%)	7 (1.7%)	4 (2.1%)	4 (1.7%)	3 (0.7%)	0 (0.0%)
Salmonella	***37 (2.4%)***	5 (4.1%)	7 (1.7%)	5 (2.6%)	5 (2.1%)	11 (2.7%)	4 (2.3%)
Vibrio	***2 (0.1%)***	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.5%)	0 (0.0%)
Vibrio cholerae	***1 (0.1%)***	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	0 (0.0%)
Yersinia enterocolitica	***1 (0.1%)***	1 (0.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Diarrheagenic *E. coli*/*Shigella*							
Enteroaggregative *E. coli* (EAEC)	***109 (7.0%)***	9 (7.4%)	34 (8.1%)	20 (10.4%)	17 (7.1%)	25 (6.1%)	4 (2.3%)
Enteropathogenic *E. coli* (EPEC)	***348 (22.4%)***	30 (24.8%)	155 (37.1%)	45 (23.3%)	46 (19.2%)	55 (13.4%)	17 (9.8%)
Entertoxigenic *E. coli* (ETEC) *lt/st*	***31 (2.0%)***	1 (0.8%)	5 (1.2%)	7 (3.6%)	5 (2.1%)	9 (2.2%)	4 (2.3%)
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*	***38 (2.4%)***	1 (0.8%)	24 (5.7%)	2 (1.0%)	4 (1.7%)	5 (1.2%)	2 (1.2%)
E. coli O157	***4 (0.3%)***	0 (0.0%)	3 (0.7%)	1 (0.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Shigella / Enteroinvasive *E. coli* (EIEC)[a]	***49 (3.1%)***	0 (0.0%)	31 (7.4%)	7 (3.6%)	5 (2.1%)	6 (1.5%)	0 (0.0%)
Parasites							
Cryptosporidium	***24 (1.5%)***	0 (0.0%)	9 (2.2%)	3 (1.6%)	6 (2.5%)	5 (1.2%)	1 (0.6%)
Cyclospora cayetanensis[b]	***19 (1.2%)***	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	13 (3.2%)	6 (3.5%)
Entamoeba histolytica	***0 (0.0%)***	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Giardia lamblia	***27 (1.7%)***	1 (0.8%)	6 (1.4%)	5 (2.6%)	2 (0.8%)	13 (3.2%)	0 (0.0%)
Viruses							
Adenovirus F 40/41	***55 (3.5%)***	12 (9.9%)	36 (8.6%)	5 (2.6%)	0 (0.0%)	2 (0.5%)	0 (0.0%)
Astrovirus	***8 (0.5%)***	1 (0.8%)	4 (1.0%)	0 (0.0%)	1 (0.4%)	2 (0.5%)	0 (0.0%)
Norovirus GI/GII	***70 (4.5%)***	15 (12.4%)	31 (7.4%)	5 (2.6%)	7 (2.9%)	9 (2.2%)	3 (1.7%)
Rotavirus A	***18 (1.2%)***	11 (9.1%)	2 (0.5%)	1 (0.5%)	1 (0.4%)	2 (0.5%)	1 (0.6%)
Sapovirus	***59 (3.8%)***	12 (9.9%)	31 (7.4%)	7 (3.6%)	1 (0.4%)	5 (1.2%)	3 (1.7%)

[a] 10 of 49 *Shigella*/EIEC were detected at a study site in Providence, RI, in July, 2013, during a regional *Shigella* outbreak.

[b] All 19 *C. cayetanensis* were detected at a study site in Omaha, NE, between June and July, 2013, during a multi-state *Cyclospora* outbreak.

The FilmArray Panel does not evaluate all samples for the presence of EPEC or *E. coli* O157. STEC positive samples (*stx 1*/*stx2* detected) are not evaluated for EPEC. Conversely, E. coli O157 is only evaluated in STEC positive samples (see Interpretation Section for additional explanation). The expected values for *E. coli* O157 and Enteropathogenic *E. coli* (EPEC) in association with the applicable STEC *stx1/stx2* result (Detected or Not Detected respectively) are presented in the table below.

Table 7. Expected Values (as determined by the FilmArray GI Panel) Summary for *E. coli* O157 and Enteropathogenic *E. coli* (EPEC), in Association with Applicable STEC results, for the Prospective Clinical Evaluation (May through September 2013)

FilmArray GI Panel Result (Within Applicable STEC *stx1/stx2* Result)	*Overall*	<1 year	1-5 years	6-12 years	13-21 years	22-64 years	65+ years
E. coli O157 Detected (STEC *stx1/stx2* Detected)	*4/38 (10.5%)*	0/1 (0.0%)	3/24 (12.5%)	1/2 (50.0%)	0/4 (0.0%)	0/5 (0.0%)	0/2 (0.0%)
Enteropathogenic *E. coli* (EPEC) Detected (STEC *stx1/stx2* Not Detected)	*348/1518 (22.9%)*	30/120 (25.0%)	155/394 (39.3%)	45/191 (23.6%)	46/236 (19.5%)	55/406 (13.5%)	17/171 (9.9%)

In the prospective clinical evaluation, the FilmArray GI Panel reported a total of 262 specimens with multiple organism detections (i.e., mixed infections). This represents 31.5% (262/832) of positive specimens and 16.8% of all specimens tested (262/1556). The expected values for each FilmArray GI Panel organism result in mixed infections are presented in the following table.

Table 8. Expected Values for Analytes in Mixed Infections (as determined by the FilmArray GI Panel) in the Prospective Clinical Evaluation (May through September 2013)

Analyte	Number of Specimens Containing Analyte in Mixed Infections	Prevalence of Analyte in Mixed Infections (*N* = 262)
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%

Analyte	Number of Specimens Containing Analyte in Mixed Infections	Prevalence of Analyte in Mixed Infections (*N* = 262)
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%

PERFORMANCE CHARACTERISTICS

Clinical Performance

The clinical performance of the FilmArray GI Panel was established during a multi-center study conducted at four geographically distinct U.S. study sites between May and September, 2013. A total of 1578 prospective residual stool specimens in Cary Blair transport medium 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. Table 9 provides a summary of demographic information for the 1556 specimens included in the prospective study.

Table 9. 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.

Table 10. Comparator Methods for FilmArray GI Panel Clinical Evaluation

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]	
Cyclospora cayetanensis	
Entamoeba histolytica	

[a] Because FilmArray only evaluates STEC positive samples for the presence of *E. coli* O157, the comparator method data were only used to determine the accuracy of the FilmArray determination of *E. coli* O157 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 sequence of adequate quality that matched a sequence of the expected organism/gene from 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. 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 the 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 Table 11.

Table 11. 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/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 12. *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 13. *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%)

Salmonella species/serovar	Sensitivity
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 discrepant results.

The most prevalent mixed infection was *C. difficile* with EPEC (2% 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 14. 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. 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 A, 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 is provided in Table 15 and the results of the FilmArray GI testing are presented in Table 16.

Table 15. 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 16. 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 specimens were prepared using residual specimens from the prospective clinical study that had previously tested negative for all GI panel analytes by FilmArray and comparator methods. Specimens were spiked at clinically relevant levels using five different quantified strains for each organism (or unspiked; 50 of each). The analyte status of each contrived specimen was blinded to the users analyzing the specimens. The results of the FilmArray testing are presented in Table 17.

Table 17. 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.

Limit of Detection

The analytical sensitivity or Limit of Detection (LoD) for FilmArray GI organisms was estimated with limiting dilutions as single-spiked and multi-spiked samples (up to four organisms per mix), to determine whether assay sensitivity is affected by the presence of multiple panel organisms in a single sample. The sensitivity of the assays was equivalent between single-spiked and multi-spiked samples and most confirmation testing was performed with multi-spiked samples.

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 for at least 19 of the 20 samples (19/20 = 95%) tested. LoD is defined as the lowest concentration at which the analyte is consistently detected (detection in ≥ 95% of sample tested) and the confirmed LoD for each FilmArray GI analyte is listed in Table 18.

Table 18. Limit of Detection (LoD) for FilmArray GI Panel Analytes

GI Panel Test Result	Species/Isolate Tested	LoD Concentration	Detection at LoD Concentration
BACTERIA			
Campylobacter	*Campylobacter coli* ATCC 33559	4 x 10^4 cells/mL	20/20 100%
	Campylobacter jejuni ATCC BAA-1234		20/20 100%
	Campylobacter upsaliensis ATCC BAA-1059		20/20 100%

GI Panel Test Result	Species/Isolate Tested	LoD Concentration	Detection at LoD Concentration
Clostridium difficile (toxin A/B)	*Clostridium difficile* Toxinotype 0 A+B+ ATCC 9689	4 x 10^5 cells/mL	20/20 100%
	Clostridium difficile (NAP1) Toxinotype III A+B+ Zeptometrix #801619	4 x 10^4 cells/mL	19/20 95%
Plesiomonas shigelloides	*Plesiomonas shigelloides* ATCC 14029	1 x 10^3 CFU/mL	20/20 100%
Salmonella	*Salmonella bongori* O66:H1z41:H2- SGSC RKS#3041 SarC11	1 x 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 x 10^3 CFU/mL	20/20 100%
Vibrio and *Vibrio cholerae*	*Vibrio cholerae* Ogawa serotype O:1 ATCC 14035	8 x 10^3 cells/mL	20/20 100%
	Vibrio parahaemolyticus ATCC 17802	8 x 10^4 cells/mL	20/20 100%
Yersinia enterocolitica	*Yersinia enterocolitica* Biovar1 serogroup O:8 ATCC 9610	5 x 10^4 CFU/mL	20/20 100%
DIARRHEAGENIC *E. coli*/*Shigella*			
Enteroaggregative *E. coli* (EAEC)	*Escherichia coli* JM221 O92:H33 STEC Center	1 x 10^4 CFU/mL	20/20 100%
Enteropathogenic *E. coli* (EPEC)	*Escherichia coli* E2348/69 O127:H6 STEC Center	1 x 10^3 CFU/mL	20/20 100%
Enterotoxigenic *E. coli* (ETEC) *lt/st*	*Escherichia coli* H10407 O78:H11 ATCC 35401	1 x 10^3 CFU/mL	20/20 100%
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2* *E. coli* O157	*Escherichia coli* O25:H11 ATCC BAA-2196	1 x 10^3 CFU/mL	20/20 100%
	Escherichia coli O157:H7 ATCC 43895	1 x 10^4 CFU/mL	20/20 100%
Shigella/Enteroinvasive *E. coli* (EIEC)	Escherichia *coli* O29:NM ATCC 43892	5 x 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 x 10^3 oocysts/mL [a]	20/20 100%
	Cryptosporidium hominis Clinical Specimen		20/20 100%
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%

GI Panel Test Result	Species/Isolate Tested	LoD Concentration	Detection at LoD Concentration
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 - 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*.

Analytical Reactivity (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 but that may be detected by the FilmArray GI Panel.

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 19. 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 x 10^5	3×LoD
	BEI HM-296	1.2 x 10^5	3×LoD
	ATCC43485	1.2 x 10^5	3×LoD
	ATCC 43478	1.2 x 10^5	3×LoD
	ATCC 33559[b]	4.0 x 10^4	1×LoD
Campylobacter jejuni subsp. *doylei*[c]	ATCC 49349	4.0 x 10^6	Not Detected[c]
	ATCC 49351	4.0 x 10^6	100×LoD[c]
	ATCC 49350	4.0 x 10^6	Not Detected[c]
Campylobacter jejuni subsp. *jejuni*	ATCC 43430	1.2 x 10^5	3×LoD
	ATCC BAA-1062	1.2 x 10^5	3×LoD
	ATCC BAA-1234[b]	4.0 x 10^4	1×LoD
	BEI NR-128	1.2 x 10^5	3×LoD
Campylobacter upsaliensis	ATCC BAA-1059	4.0 x 10^4	1×LoD
	CCUG 24191	1.2 x 10^5	3×LoD
	ATCC 43953	1.2 x 10^5	3×LoD

Organism	Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected
	ATCC 43954[d]	4.0 x 10^6	Not Detected[d]
	ATCC 49815	1.2 x 10^5	3×LoD
	BEI HM-297	1.2 x 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 20. 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 x 10^5	1xLoD
		ATCC BAA-1382	1.2 x 10^6	3×LoD
		ATCC 17857	1.2 x 10^6	3×LoD
		ATCC 17858	1.2 x 10^6	3×LoD
		ATCC 43255	1.2 x 10^6	3×LoD
		ATCC 43594	1.2 x 10^6	3×LoD
		ATCC 43596	1.2 x 10^6	3×LoD
		ATCC 43599	1.2 x 10^6	3×LoD
		ATCC 43600	1.2 x 10^6	3×LoD
		ATCC 51695	1.2 x 10^6	3×LoD
		ATCC 700792	1.2 x 10^6	3×LoD
	III A+B+	ATCC BAA-1805 (NAP1)	1.2 x 10^6	3×LoD
		Zeptometrix #0801619 (NAP1)[a]	4.0 x 10^4	1×LoD
	V A+B+	ATCC BAA-1875	1.2 x 10^6	3×LoD
	VIII A-B+	ATCC 43598	1.2 x 10^6	3×LoD
	X A-B+	CCUG 8864	1.2 x 10^6	3×LoD
	XII A+B+	ATCC BAA-1812	1.2 x 10^6	3×LoD
	XXII A+B (unknown)	ATCC BAA-1814	1.2 x 10^6	3×LoD

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

Table 21. 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 x 10^3	1xLoD
	CDC 16408	ATCC 14030	3.0 x 10^3	3×LoD
	Dakar, Senegal	ATCC 51572	3.0 x 10^3	3×LoD
	Unknown	ATCC 51903	3.0 x 10^3	3×LoD
	Colorado	CDPH HUM-2011019465	3.0 x 10^3	3×LoD
	Czech Republic	NIPH-Czech Republic 6300	3.0 x 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 22. 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
Salmonella enterica subsp. *indica VI*		SGSC RKS 2995	1.5×10^4	3×LoD
Salmonella enterica subsp. *enterica*	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 serovars of S*. enterica* subsp. *enterica.*

Table 23. FilmArray *Vibrio* (*V. parahaemolyticus/V. vulnificus/V. cholerae*) and *Vibrio cholerae* Inclusivity Results

Organism (species, biotype and serotype)		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 24. FilmArray *Yersinia enterocolitica* Inclusivity Results

Organism	Serotype	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 25. FilmArray Enteroaggregative *E. coli* (EAEC) Inclusivity Results

Organism	Serotype	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 26. FilmArray Enteropathogenic *E. coli* (EPEC) Inclusivity Results

Organism	Serotype	Typical/ Atypical	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 27. 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 28. 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
	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^{3c}	3×LoD	Not Detected

Organism	Serotype	*stx1*/*stx2*	Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected STEC	Multiple of LoD Detected O157
	STEC O157					
	O157:NM	+/+	STEC Center DA-26	3.0 x 10^3	3×LoD	0.3×LoD
	O157:H7	-/+	STEC Center E32511	3.0 x 10^3	3×LoD	0.3×LoD
	O157:HNT	+/+	STEC Center DA-74	3.0 x 10^3	3×LoD	0.3×LoD
	O157:H7	+/+	ATCC 43895[a]	1.0 x 10^4	10xLoD	1xLoD
	O157:H7	+/+	STEC Center A8993-CS2	3.0 x 10^4	30×LoD	3×LoD
	Non-STEC O157					
	O157:H7	-/-	ATCC 43888	3.0 x 10^4	Not Detected	N/A[d]
	O157:H45	-/-	STEC Center SC373/2	3.0 x 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 29. FilmArray *Shigella*/Enteroinvasive *E. coli* (EIEC) Inclusivity Results

Organism	Serotype (Year/Location)	Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected
Enteroinvasive *E. coli* (EIEC)	O29:NM	ATCC 43892[a]	5.0 x 10^3	1×LoD
	O29:HNM (1977)	STEC Center 1885-77	3.0 x 10^3	0.6×LoD
	O124:HNM (1978)	STEC Center 929-78	3.0 x 10^3	0.6×LoD
	O29:H27 (1979; VA, USA)	STEC Center 1827-79	3.0 x 10^3	Not Detected[b]
	O28:H- (1983, Brazil)	STEC Center LT-15	3.0 x 10^3	0.6×LoD
	O136:H- (1983, Bangladesh)	STEC Center LT-41 Strain 1111-55	3.0 x 10^3	0.6×LoD
Shigella boydii (Serogroup C)	Type 2	ATCC 8700	3.0 x 10^2	3×LoD
	Type 4	CDPH HUM-2010029296	3.0 x 10^2	3×LoD
	Type 1	ATCC 9207	3.0 x 10^2	3×LoD
	Type 20	ATCC BAA-1247	3.0 x 10^2	3×LoD
	Type 10	ATCC 12030	3.0 x 10^2	3×LoD
Shigella dysenteriae (Serogroup A)	Type 1	BEI NR-520	3.0 x 10^2	3×LoD[c]
	Type 2	CDPH PHM-2004008089	3.0 x 10^2	3×LoD
	Type 13	ATCC 49555	3.0 x 10^2	3×LoD
	Type 3	ATCC 29028	3.0 x 10^2	3×LoD
	Type 12	ATCC 49551	3.0 x 10^2	3×LoD

Organism	Serotype (Year/Location)	Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected
Shigella flexneri (Serogroup B)	Type 2a	ATCC 700930	3.0 x 10^2	3×LoD
	Type 1a	ATCC 9199	3.0 x 10^2	3×LoD
	Type 6	CDPH PHM-2006004043	3.0 x 10^2	3×LoD
	Type 2b	ATCC 12022	3.0 x 10^2	3×LoD
	Type 2a	ATCC 29903	3.0 x 10^2	3×LoD
	Unknown	STEC Center VA-6	3.0 x 10^2	3×LoD
Shigella sonnei (Serogroup D)	N/A	ATCC 29930[a]	1.0 x 10^2	1×LoD
	N/A	ATCC 11060	3.0 x 10^2	3×LoD
	N/A	CDPH HUM-2010027998	3.0 x 10^2	3×LoD
	N/A	ATCC 29031	3.0 x 10^2	3×LoD
	N/A	ATCC 25931	3.0 x 10^2	3×LoD
	N/A	ATCC 9290	3.0 x 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 30. FilmArray *Cryptosporidium* Inclusivity Results

Organism	Location/Source of Isolate or Sample	Concentration Detected (copies/mL)	Multiple of LoD Detected
Cryptosporidium canis	Peru Clinical Sample	Unknown	<LoD[a]
Cryptosporidium hominis	Scotland Clinical Sample[b]	2.1 x 10^3 [b]	1×LoD
	Scotland Clinical Sample	6.4 x 10^3	3×LoD
	Scotland Clinical Sample	6.4 x 10^3	3×LoD
	BEI NR-2520 (Purified DNA Isolate TU502)	6.4 x 10^3	3×LoD
Cryptosporidium meleagridis	BEI NR-2521 (Purified DNA Isolate TU1867)	1.8 x 10^3	3×LoD
Cryptosporidium muris	Waterborne, Inc.P104	1.5×10^4 oocycts/mL	3×LoD
Cryptosporidium parvum	Waterborne, Inc. P102C[c]	6.0 x 10^2 [c]	1×LoD
	Scotland Clinical Sample	1.8 x 10^3	3×LoD
	Scotland Clinical Sample	1.8 x 10^3	3×LoD
	BEI NR-2519 (Purified DNA Isolate Iowa)	1.8 x 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 has 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 31. 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] Specimen was used to establish the LoD for this assay.

Table 32. 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 33. 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 34. FilmArray Adenovirus F 40/41 Inclusivity Results

Organism	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 F41	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 35. 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]	~1.3×10^7	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 36. 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	Unknown	Noro2_013[a]	1.0×10^{4a}	1×LoD

Norovirus Genogroup/Genotype		Isolate ID (Clinical Samples)	Concentration Detected (copies/mL)	Multiple of LoD Detected
GII	2	NoroII.2[b]	6.0 x 10^3	0.6×LoD
	3	China-5	6.0 x 10^3	0.6×LoD
		SGB_038	6.0 x 10^3	0.6×LoD
	4	GI-PILOT-SPDRL-077	2.0 x 10^5	20×LoD[c]
		Noro2_004	2.0 x 10^5	20×LoD[c]
		Noro2_032	2.0 x 10^5	20×LoD[c]
		PCMC_025 (Sydney)	6.0 x 10^3	0.6×LoD
		PCMC_031 (Sydney)	6.0 x 10^3	0.6×LoD
	6	NYH-A	6.0 x 10^3	0.6×LoD
	7	NoroII.7[b]	6.0 x 10^3	0.6×LoD
	8	NoroII.8[b]	6.0 x 10^3	0.6×LoD
	12	NoroII.12[b]	6.0 x 10^3	0.6×LoD
	16	NoroII.16[b]	6.0 x 10^3	0.6×LoD
	20	NoroII.20c[b]	2.0 x 10^5	20×LoD[c]
		NoroII.20[b]	6.0 x 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 37. 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 x 10^3	1×LoD
	RV4 (G1P8)	NCPV 0904052v	1.2 x 10^4	3×LoD
	69M (G8P5)	NCPV 0904055v	1.2 x 10^4	3×LoD
	P (G3P1A[8])	NCPV 0904056v	1.2 x 10^4	3×LoD
	Wa (G1P1A[8])	ATCC VR-2018	1.2 x 10^4	3×LoD
	DS-1 (G2P1B[4])	ATCC VR-2550	1.2 x 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 x 10^3 copies/mL).

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

Table 38. 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 x 10^6	1×LoD
	Unknown	China_56	1.5 x 10^7	3×LoD
	Unknown	AB_SaV_03	1.5 x 10^7	3×LoD
	Unknown	PCMC_54	1.5 x 10^7	3×LoD
	Unknown	SPDRL-006	1.5 x 10^7	3×LoD
	Unknown	SPDRL-099	1.5 x 10^7	3×LoD
	Unknown	SGB-MP-11	1.5 x 10^7	3×LoD

Organism	Genogroup	Isolate ID (Clinical Samples)	Concentration Detected (copies/mL)	Multiple of LoD Detected
	GI	Sapo_03[b]	1.5 x 10^7	3×LoD
	GII	Sapo_06[b]	1.5 x 10^7	3×LoD
	GIV	Sapo_09[b]	1.5 x 10^7	3×LoD
	GV	Sapo_02[b]	1.5 x 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.

Analytical Specificity (Cross-Reactivity and Exclusivity)

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

A total of 28 on-panel organisms were tested to verify that they only react with the appropriate assay(s) on the panel. All 28 on-panel organisms gave only the expected positive results; no false positive results were reported.

A total of 174 off-panel organisms were selected for specificity testing 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 an organism of interest could not be obtained for testing, a separate organism-specific *in silico* analysis of whole genome sequence(s) directed against all GI Panel primers was attempted for reactivity predictions. Several of the off-panel organisms were selected and tested to evaluate the specificity of particular assays, while many others were tested because they are commensal or pathogenic organisms with the potential to be found at high levels in stool. All organisms were tested at a high concentration (typically ≥1.0×10^8 CFU/mL for bacteria and fungi, ≥ 1.0×10^4 cells/mL for protozoa/parasite and ≥1.0×10^5units/mL for viruses).

Table 39 lists the GI Panel assays and corresponding organisms for which cross-reactivity was identified (either observed in testing or predicted by *in silico* analyses). With the exception of *Vibrio fluvialis* and *Vibrio mimicus* detection by the *Vibrio* assay, cross-reactivity has only been observed when the cross-reactive organism was known or suspected to be present in the sample at a high level.

Table 40 contains a complete list of the off-panel bacteria, fungi, protozoa/parasites, and viruses that were tested and received the expected FilmArray GI Panel test result (negative for all assays; no cross-reactivity) or for which *in silico* analysis does not predict cross-reactivity.

Table 39. Observed or Predicted Cross-Reactivity with 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 alginolyticus* *Vibrio 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 methods.

Table 40. No Cross-Reactivity with FilmArray GI Panel Assays (Observed or Predicted by *in silico* Analysis)

BACTERIA				
Tested				
Abiotrophia defectivia	*Campylobacter mucosalis*	*Desulfovibrio piger*	*Klebsiella oxytoca*	*Ruminococcus flavefaciens*[a]
Acinetobacter baumannii	*Campylobacter rectus*	Diffusely adherent *E.coli*	*Klebsiella pneumoniae*	*Ruminococcus obeum*[a]
Acinetobacter lwoffii	*Campylobacter showae*	*Escherichia blattae*	*Lactobacillus acidophilus*	*Selenomonas ruminantium*
Aeromonas hydrophila	*Campylobacter sputorum*	*Escherichia fergusonii*	*Lactobacillus reuteri*	*Serratia liquefaciens*
Alcaligenes faecalis	*Campylobacter ureolyticus*	*Escherichia hermannii*	*Lactococcus lactis*	*Serratia marcescens*
Anaerococcus tetradius	*Cedecea davisae*[b]	*Escherichia vulneris*	*Leminorella grimontii*	*Shewanella algae*
Arcobacter butzleri	*Chlamydia trachomatis*	*Edwardsiella tarda*	*Listeria monocytogenes*	*Staphylococcus aureus*
Arcobacter cryaerophilus	*Citrobacter amalonaticus*	*Egglerthella lenta*	*Megamonas hypermegale*	*Staphylococcus epidermidis*
Bacillus cereus	*Citrobacter freundii*	*Enterobacter aerogenes*	*Megasphaeara elsdenii*	*Stenotrophomonas maltophilia*
Bacteroides fragilis	*Clostridium acetobutylicum*	*Enterobacter cloacae*	*Methanobrevibacter smithii*	*Streptococcus agalactiae*
Bacteroides thetaiotaomicron	*Clostridium botulinum*	*Enterococcus faecalis*	*Morganella morganii*	*Streptococcus intermedius*
Bacteroides vulgatus	*Clostridium difficile* non-toxigenic[c]	*Enterococcus faecium*	*Peptoniphilus asaccharolyticus*	*Streptococcus pyogenes*
Bifidobacterium adolescentis[a]	*Clostridium histolyticum*	*Eubacterium cylindroides*	*Peptostreptococcus anaerobius*	*Streptococcus salivarius*
Bifidobacterium bifidum[a]	*Clostridium methylpentosum*	*Eubacterium rectale*	*Photobacterium damselae*	*Streptococcus suis*
Bifidobacterium longum[a]	*Clostridium novyi*	*Faecalibacterium prausnitzii*	*Porphyromonas asaccharolytica*	*Trabulsiella guamensis*
Campylobacter concisus	*Clostridium perfringens*	*Fusobacterium varium*	*Prevotella melaninogenica*	*Veillonella parvula*
Campylobacter curvus	*Clostridium ramosum*	*Gardnerella vaginalis*	*Proteus mirabilis*	*Yersinia bercovieri*
Campylobacter fetus	*Clostridium septicum*	*Gemella morbillorum*	*Proteus penneri*	*Yersinia frederiksenii*[d]
Campylobacter gracilis	*Clostridium sordellii*	*Haemophilus influenzae*	*Proteus vulgaris*	*Yersinia intermedia*
Campylobacter helveticus	*Clostridium tetani*	*Hafnia alvei*[b]	*Provedencia alcalifaciens*	*Yersinia mollaretii*
Campylobacter hominis	*Collinsella aerofaciens*	*Helicobacter fennelliae*	*Pseudomonas aeruginosa*	*Yersinia pseudotuberculosis*
Campylobacter hyointestinalis	*Corynebacterium genitalium*	*Helicobacter pylori*	*Ruminococcus bromii*[a]	*Yersinia rohdei*
Campylobacter lari				

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

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

[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 in Table 39.
[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 in Table 39.
[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 in Table 39.

Cross-Contamination and Carryover

The potential for sample-to-sample carryover was evaluated by alternately testing samples containing a high concentration or organism (10^7 - 10^9 organism/mL) with samples containing no organism. No false positive results were observed during testing of five sets of a high positive sample followed directly by a negative sample; demonstrating that the system design and recommended sample handling and testing practices are effective in preventing false positive results due to carryover or cross-contamination between samples.

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) is provided in Table 41. The reproducibility of Tm for each positive assay is provided in the Table 42.

The FilmArray GI Panel provides highly accurate and reproducible test results for all analytes (15,891/15,912 = 99.87% overall agreement with a 95% confidence interval of 99.81% - 99.92%) with a Tm standard deviation of 0.5ºC or less.

Table 41. Reproducibility of the FilmArray GI Panel Test Results

Organism Tested	Concentration Tested	Expected Result	% Agreement with Expected Result[a]			
			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[b] 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)	**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[c] 100%	192/192[c] 100%	192/192[c] 100%	576/576 100% (99.4 - 100%)
Salmonella enterica[b] 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%)

Organism Tested	Concentration Tested	Expected Result	% Agreement with Expected Result[a]			
			Site A	Site B	Site C	All Sites (95% Confidence Interval)
	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%)
	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*[b]** 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, Inc. 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*[b]** (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%)
	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%)

Organism Tested	Concentration Tested	Expected Result	% Agreement with Expected Result[a] Site A	Site B	Site C	All Sites (95% Confidence Interval)
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] The expected Not Detected results were reported in all samples that were not spiked with a corresponding analyte (100% agreement with the expected results).
[b] Reproducible, but suboptimal (<95%) detection was observed at one or both concentrations in frozen contrived samples. Data presented are from samples stored at ~4°C for up to 4 days prior to testing.
[c] 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.

Table 42. Reproducibility of Tm for Positive FilmArray GI Panel Assays

Organism	Assay		Test Level	Test Site	Tm Reproducibility Mean	StDev	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)	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

Organism	Assay	Test Level	Test Site	Tm Reproducibility				
				Mean	StDev	Min	Max	(Max - Min)
			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
			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**
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, Inc. 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		Site A	71.75	± 0.28	71.29	72.31	1.02

Organism	Assay	Test Level	Test Site	Tm Reproducibility				
				Mean	StDev	Min	Max	(Max - Min)
		Moderate Positive 3xLoD 1.5x104 oocysts/mL	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
			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).

Interference

Substances that could be present in stool samples (preserved in Cary Blair medium) or introduced during sample handling were evaluated for their potential to interfere with assay performance. A potentially interfering substance was added to a contrived stool sample containing representative GI Panel organisms. Each contrived sample contained a mix of four different organisms, each present at approximately three times (3×) the limit of detection (LoD). Unspiked samples (no test substance) served as positive controls (no interference) for comparison. Spiked samples (containing the test substance) were reviewed for performance of controls and accuracy of the test results for each sample. Reproducible control failures or unexpected test results (false positive or false negative) were a sign of interference.

Of the endogenous and exogenous substances tested (Table 43), 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 43. Endogenous and Exogenous Substances Tested – No Interference

Endogenous Substances	Exogenous Substances	
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 Blair vial)	Docusate sodium	Bleach
		Ethanol

[a] Unexpected EPEC results reported due to contamination of the mucin with EPEC nucleic acid.

No inhibition or unexpected test results were obtained in the presence of high concentrations of potentially competing microorganisms (on-panel or off-panel organisms; Table 44).

Table 44. Potentially Competing Microorganisms Tested – No Interference

On-Panel Organisms	Off-Panel Organisms	
Adenovirus F41	*Aeromonas hydrophila*	Non-pathogenic *E. coli*
Enterotoxigenic *E. coli* (ETEC)	*Bacteroides vulgatus*	*Helicobacter pylori*
	Bifidobacterium bifidum	*Saccharomyces boulardii*
	Human Rhinovirus 87	

Rotavirus A reassortant strains used in the manufacturing of Rotavirus A vaccines were tested (Table 45) and Rotavirus A Detected results were reported. 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 45. Rotavirus A Vaccine Strains Tested – Rotavirus A Detected

RotaTeq Rotavirus A Vaccine Components
Rotavirus reassortant WC3:2-5, R574(9) [ATCC VR-2195]
Rotavirus reassortant WI79-4,9 [ATCC VR-2415]

Contrived stool samples prepared in various transport media, including Cary Blair (see Table 46), 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 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 46. 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) in formalin containing media.

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Safety Data Sheet (SDS/MSDS)

FilmArray™ Reagent Kit

(According to regulation (EC) 1907/2006.)
Date SDS established : 05.2014
Revision number : 04 // ASAY-PRT-0643-04

1. IDENTIFICATION OF THE SUBSTANCE / MIXTURE AND OF THE COMPANY / UNDERTAKING

1.1 PRODUCT IDENTIFIER

Product Name:	FilmArray™ Reagent Kit
Catalog #:	RFIT-ASY-0002, RFIT-ASY-0007 (RUO) or RFIT-ASY-0114 (IVD), RFIT-ASY-0008 (RUO) or RFIT-ASY-0116 (IVD), RFIT-ASY-0094, RFIT-ASY-0104, RFIT-ASY-0105 (IVD) OR RFIT-ASY-0115 (RUO), RFIT-ASY-0107, RFIT-ASY-0109, RFIT-ASY-0118
Kit Components:	FilmArray™ Respiratory Panel Pouch, BioThreat Pouch, BCID Pouch, Hydration Solution, Sample Buffer, GI Panel Pouch, ME Panel Pouch

1.2 RELEVANT IDENTIFIED USES OF THE SUBSTANCE OR MIXTURE AND USES ADVICES AGAINST

In vitro diagnostic use and for research use.

1.3 DETAILS OF THE SUPPLIER OF THE SAFETY DATA SHEET

Address:	BioFire Diagnostics, LLC, 390 Wakara Way, Salt Lake City, Utah 84108, USA
Telephone Number:	1-801-736-6354
E-mail Address:	support@BioFireDX.com

1.4 EMERGENCY TELEPHONE NUMBER

Call your local emergency center.

2. HAZARDOUS IDENTIFICATION

2.1 CLASSIFICATION OF THE MIXTURE

Sample Buffer:	Acute toxicity (Category 4)
	Serious Eye damage (Category 1)
	Skin irritation (Category 2)

The other kit components are not classified as dangerous mixtures according to regulation 1272/2008.

2.2 LABEL ELEMENTS

Labeling according Regulation (EC) No 1272/2008 [CLP]

Pictogram/Signal Word:	 Danger

Hazardous Statements	
H302	Harmful if swallowed.
H318	Causes serious eye damage.
H315	Causes skin irritation.

Precautionary Statements	
P280	Wear protective gloves/eye protection/face protection.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P301 + P312	IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.

Supplemental Hazard Statements

None

2.3 OTHER HAZARDS

None

3. COMPOSITION/INFORMATION ON INGREDIENTS

Component Name/ Hazardous Ingredient	EC nr.	Cas#	Classification acc. 1272/2008	Concentration
Sample Buffer: Guanidinium Chloride	200-0 02-3	50-01-1	Acute toxicity (Cat. 4) Eye irritation (Cat. 2) Skin irritation (Cat. 2)	50-60% w/w
Sample Buffer: Triton X-100	/	9002-93-1	Acute toxicity, Oral (Cat. 4) Serious eye damage (Cat. 1) Chronic aquatic toxicity (Cat. 2)	10-20% w/w

4. FIRST AID MEASURES

4.1 DESCRIPTION OF FIRST AID MEASURES

If Inhaled:	Remove to fresh air. Seek medical attention.
In Case of Skin Contact:	Immediately flush skin with plenty of water for at least 15 minutes. Cover irritated skin with an emollient. Remove contaminated clothing. Seek medical attention.
In Case of Eye Contact:	Check for and remove any contact lenses and immediately flush eyes with copious amounts of water. Seek medical attention.
If Swallowed:	Immediately seek medical attention. If swallowed, induce vomiting as directed to do so by medical personnel. Loosen tight clothing.

4.2 MOST IMPORTANT SYMPTOMS AND EFFECTS, BOTH ACUTE AND DELAYED

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

4.3 INDICATION OF ANY IMMEDIATE MEDICAL ATTENTION AND SPECIAL TREATMENT NEEDED

No data available

5. FIREFIGHTING MEASURES

5.1 EXTINGUISHING MEDIA

Suitable Extinguishing Media:	Use foam, carbon dioxide, water spray or dry chemical powder. Foam and water spray may cause frothing, but are still effective.
Unsuitable Extinguishing Media:	None

5.2 SPECIAL HAZARDS ARISING FROM THE SUBSTANCE OR MIXTURE

Carbon oxides, nitrogen oxides (NOx), Hydrogen chloride gas

5.3 ADVICE FOR FIREFIGHTERS

Wear self-contained breathing apparatus for fire fighting if necessary.

6. ACCIDENTAL RELEASE MEASURES

6.1 PERSONAL PRECAUTIONS, PROTECTIVE EQUIPMENT, EMERGENCY PROCEDURES

Use personal protective equipment. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation.

6.2 ENVIRONMENTAL PRECAUTIONS

Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

6.3 METHODS AND MATERIAL FOR CONTAINMENT AND CLEANING UP

Soak up with inert absorbent material and dispose of as hazardous waste. Keep in suitable, closed containers for disposal.

6.4 REFERENCE TO OTHER SECTIONS

For disposal, see section 13.

7. HANDLING AND STORAGE

7.1 PRECAUTIONS FOR SAFE HANDLING

Avoid contact with skin and eyes. Avoid formation of dust and aerosols.

7.2 CONDITIONS FOR SAFE STORAGE, INCLUDING ANY INCOMPATIBILITIES

Store at room temperature. Keep container closed and away from direct sunlight.

7.3 SPECIFIC END USE(S)

No data available

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 CONTROL PARAMETERS

Components with workplace control parameters: no data available.

8.2 EXPOSURE CONTROLS

Respiratory Protection:	Exhaust ventilation or other engineering controls.
Hand Protection:	Compatible chemical resistant gloves
Eye Protection:	Chemical safety goggles or face shield
Body Protection:	Lab coat
Other Information:	Change contaminated clothing. Wash hands after working with substances.

9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 INFORMATION ON BASIC PHYSICAL AND CHEMICAL PROPERTIES

Physical State/Form:	Colorless Liquid
Solubility in Water:	Soluble

9.2 OTHER INFORMATION

None

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10. STABILITY AND REACTIVITY

10.1 REACTIVITY

No data available

10.2 CHEMICAL STABILITY

No data available

10.3 HAZARDOUS REACTIONS

No data available

10.4 CONDITIONS TO AVOID

No data available

10.5 INCOMPATIBLE MATERIALS

Oxidizing agents

10.6 HAZARDOUS DECOMPOSITION PRODUCTS

COx and some metallic oxides

11. TOXICOLOGICAL INFORMATION

11.1 INFORMATION ON TOXICOLOGICAL EFFECTS

	Guanidinium Chloride	Triton X-100
Acute Toxicity:	LD50 Oral - rat - 475 mg/kg Remarks: Behavioral: Altered sleep time (including change in righting reflex). Behavioral: Excitement. Diarrhea LD50 Oral - mouse - 571 mg/kg Remarks: Behavioral: Altered sleep time (including change in righting reflex). Behavioral: Muscle contraction or spasticity. Behavioral: Irritability. LD50 Oral - rat – 1,120 mg/kg LC50 Inhalation - rat - 4 h - 5,3 mg/l	LD50 Oral - rat - male - 500 mg/kg LD50 Dermal - rabbit – 8,000 mg/kg
Skin Corrosion/Irritation:	Skin - rabbit - Skin irritation	No data available
Serious Eye Damage/ Eye Irritation:	Eyes - rabbit - Irritating to eyes	Eyes - rabbit - Severe eye irritation
Respiratory or Skin Sensitization:	Buehler Test - guinea pig - Did not cause sensitization on laboratory animals.	No data available
Germ Cell Mutagenicity:	Not mutagenic in Ames Test.	No data available
Carcinogenicity:	IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.	
Reproductive toxicity, Specific target organ toxicity - single exposure, Specific target organ toxicity - repeated exposure, Aspiration hazard: No data available		
Potential Health Effects:	Inhalation: May be harmful if inhaled. May cause respiratory tract irritation. Ingestion: Harmful if swallowed. Skin: May be harmful if absorbed through skin. May cause skin irritation. Eyes: Causes eye burns.	
Signs and Symptoms of Exposure:	To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.	
Additional Information:	RTECS: MF4300000	RTECS: MD0907700

12. ECOLOGICAL INFORMATION

12.1 TOXICITY

Guanidinium Chloride:	Toxicity to fish LC50 - Leuciscus idus (Golden orfe) – 1,759 mg/l
Triton X-100:	Toxicity to fish LC50 - Pimephales promelas (fathead minnow) - 8,9 mg/l - 96,0 h Toxicity to daphnia and other aquatic invertebrates. EC50 - Daphnia - 26 mg/l - 48 h

12.2 PERSISTENCE AND DEGRADABILITY

Guanidinium Chloride:	Result: - Not readily biodegradable.
Triton X-100:	Biodegradability Biotic/Aerobic Biochemical oxygen demand - Exposure time 28 d Result: 36 % - Not readily biodegradable. Method: Closed Bottle test

12.3 BIOACCUMULATIVE POTENTIAL

No data available

12.4 MOBILITY IN SOIL

No data available

12.5 RESULTS OF PBT AND VPVB ASSESSMENT

No data available

12.6 OTHER ADVERSE EFFECTS

Triton X-100:	Toxic to aquatic life with long lasting effects. Chemical Oxygen Demand (COD) 2,19 mg/g

13. DISPOSAL CONSIDERATIONS

13.1 WASTE TREATMENT METHODS

Recommendation:
Chemicals must be disposed of in compliance with the respective national regulations.

Uncleaned packaging:
Recommendation:
Disposal must be made according to official regulations.
Packagings that may not be cleansed are to be disposed of in the same manner as the product.
Recommended cleansing agents:
Water, if necessary together with cleansing agents.

14. TRANSPORT INFORMATION

No restrictions apply due to the low volumes.

15. REGULATORY INFORMATION

This safety datasheet complies with the requirements of Regulation (EC) No. 1907/2006.

15.1 SAFETY, HEALTH AND ENVIRONMENTAL REGULATIONS/LEGISLATION SPECIFIC FOR THE SUBSTANCE OR MIXTURE.

U.S. Federal Regulations:

Triton X-100:	TSCA 8(a) PAIR; TSCA 8(b) inventory; TSCA 8(d) H and S data reporting (1996). SARA 311/312 MSDS distribution – chemical inventory – hazard Identification: Immediate (Acute) and Delayed (Chronic) Health Hazard. SARA 302/304/311/312 hazardous chemicals
Guanidine:	SARA 311/312:; Acute: Yes; Chronic: No

15.2 CHEMICAL SAFETY ASSESSMENT

No data available

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16. OTHER INFORMATION

Classification and procedure used to derive the classification of the sample buffer: Calculation method.

Not for food, drug, household, agricultural or cosmetic use. The above information is correct to the best of our knowledge. The user should make independent decisions regarding completeness of the information based on all sources available. BioFire Diagnostics shall not be held liable for any damage resulting from handling or from contact with the above product.

It remains the user's own responsibility to make sure that the information is appropriate and complete for his specific use of this product. The user is also responsible for observing any laws and applicable guidelines.

Changes to previous version of the SDS: Revision date: 05.01.2014
Revised for compliance with Regulation (EC) 1907/2006 (REACH)

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