BIOFIRE® FILMARRAY® Gastrointestinal (GI) Panel

Instructions for Use	https://www.biofiredx.com/e-labeling/ITI0030
Quick Guide	https://www.biofiredx.com/e-labeling/ITI0011
Safety Data Sheet (SDS)	https://www.biofiredx.com/e-labeling/ITI0009
Pouch Module	https://www.biofiredx.com/e-labeling/ITIFA20GI21

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Rx Only

Customer and Technical Support Information *For more information on how to contact Customer and Technical Support, refer to Appendix B.	Phone: 1-800-682-2666 (toll free) Email: BioFireSupport@biomerieux.com Website: www.biofiredx.com Or contact the local bioMérieux sales representative or an authorized distributor.

INTENDED PURPOSE

Intended Use

The BIOFIRE® FILMARRAY® Gastrointestinal (GI) Panel is a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test intended for use with BIOFIRE® FILMARRAY® Systems. The BIOFIRE 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 BIOFIRE 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 BIOFIRE 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 BIOFIRE 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 BIOFIRE 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.

Intended User and Use Environment

The BIOFIRE GI Panel is intended for use by trained medical and laboratory professionals in a laboratory setting or under the supervision of a trained laboratory professional.

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 BIOFIRE GI Panel simultaneously tests for 22 pathogens (Table 1) from stool specimens collected in Cary Blair transport medium. Results from the BIOFIRE GI Panel test are available within about one hour.

Table 1. Bacteria, Viruses, Diarrheagenic *E. coli*/*Shigella*, and Parasites Detected by the BIOFIRE 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 (re-classified as Clostridioides 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 pili (*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. flexneri, 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 BIOFIRE 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 at least ten genogroups (GI – GX) and many genotypes and variants within each genogroup based on VP1 sequences.[48] Genogroups 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.[49] Symptoms of infection generally last 24-48 hours[50] 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.[51] Immunity following Norovirus illness is short lived as re-infection is possible within 6 months, even in the presence of high serum antibody titers.[52]

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.[53,54] 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.[55,56] It is

estimated that 2.7 million diarrheal illnesses per year in the US are caused by Rotavirus infection.[57] 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[53] and has resulted in a decline in Rotavirus A infections.[58]

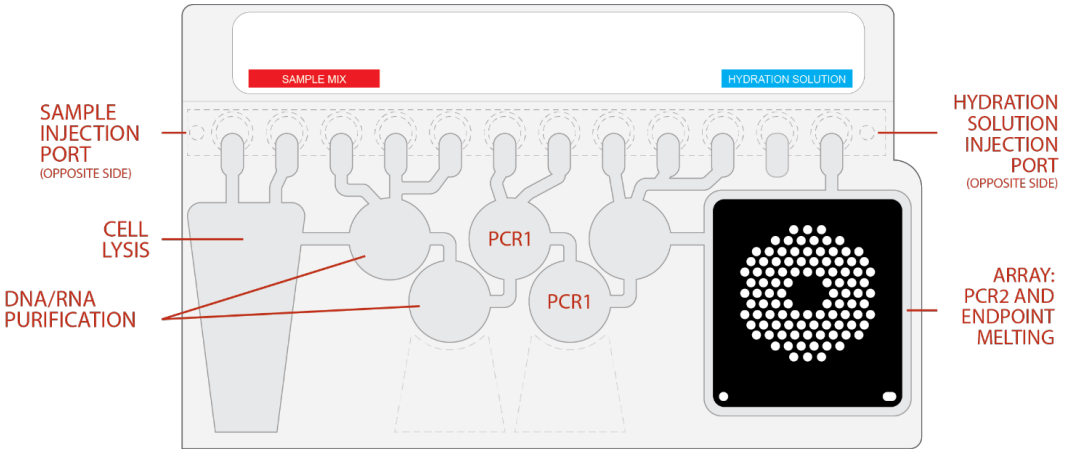
Sapovirus (Genogroups I, II, IV, and V). Sapovirus is a *Calciviridae* family member that is similar to Norovirus both genetically and in disease presentation. There are many Sapovirus genogroups of which groups GI, GII, GIV, and GV are known to infect humans. Sapovirus causes disease mostly in children, though adults are susceptible as well, with outbreaks reported in long-term care facilities, prisons, cruise ships, and hospitals in the US and EU.[59,60] 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.[61,62] 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 BIOFIRE GI Panel pouch is a closed system disposable that stores all the necessary reagents for sample preparation, reverse transcription, polymerase chain reaction (PCR), and detection in order to isolate, amplify, and detect nucleic acid from multiple gastrointestinal pathogens from a single stool specimen. After sample collection, the user injects Hydration Solution and sample combined with Sample Buffer into the pouch, places the pouch into a BIOFIRE® FILMARRAY® System module, and starts a run. The entire run process takes about one hour. Additional detail can be found in the appropriate BIOFIRE System Operator's Manual.

During a run, the BIOFIRE System:

- Lyses the sample by agitation (bead beating).
- Extracts and purifies all nucleic acids from the sample using magnetic bead technology.
- Performs nested multiplex PCR by:
    - First performing reverse transcription and a single, large volume, massively multiplexed reaction (PCR1), and
    - Then performing multiple singleplex second-stage PCR reactions (PCR2) to amplify sequences within the PCR1 products.
- Uses endpoint melting curve data to detect and generate a result for each target on the BIOFIRE GI Panel array.



MATERIALS PROVIDED

The BIOFIRE GI Panel contains materials consisting of primers, buffers, dNTPs, polymerase, molecular grade water, guanidinium chloride (50 - < 60%), Triton-X 100 (10 - < 20%), and LCGreen® Plus.

Each kit contains sufficient reagents to test 30 or 6 samples (30 test kit: RFIT-ASY-0116 or 6 test kit: RFIT-ASY-0104):

- Individually packaged BIOFIRE GI Panel pouches
- Single-use Sample Buffer ampoules
- Single-use pre-filled Hydration Injection Vials (blue)
- Single-use Sample Injection Vials (red)
- Individually packaged Transfer Pipettes
- BIOFIRE GI Panel Pouch Module Software

  This software is required to run the BIOFIRE GI Panel and can be downloaded at https://www.biofiredx.com/e-labeling/ITIFA20GI21 if not already installed on the BIOFIRE® FILMARRAY® 2.0 or BIOFIRE® FILMARRAY® TORCH Systems.

NOTE: The most current revision of the BIOFIRE GI Panel Pouch Module software and the BIOFIRE System Software provides customers with the option to enable selective reporting of *Clostridium difficile* (*C. difficile*), Norovirus GI/GII, and/or both. This is an optional feature. Please refer to the appropriate BIOFIRE System Operator's Manual for more information.

The Norovirus GI/GII selective reporting feature may not be available in all regions. Please contact your local bioMérieux sales representative or an authorized distributor for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

- BIOFIRE System including:
  - BIOFIRE 2.0 or BIOFIRE TORCH Systems including accompanying system-specific core software
  - BIOFIRE® Pouch Loading Station
- 10% bleach solution or similar disinfectant

WARNINGS AND PRECAUTIONS

General Precautions

1. For *in vitro* diagnostic use only.
2. False positives and false negatives can be the result of a variety of sources and causes. A trained healthcare professional should carefully interpret the results from the BIOFIRE GI Panel in conjunction with a patient's signs and symptoms, results from other diagnostic tests, and relevant epidemiological information.

3. BIOFIRE GI Panel pouches are only for use with BIOFIRE 2.0 and BIOFIRE TORCH Systems.
4. Performance characteristics of the BIOFIRE GI Panel have only been determined with stool specimens in Cary Blair transport media.
5. Always check the expiration date on the kit. Do not use kit components or a pouch after its expiration date.
6. BIOFIRE pouches are stored under vacuum in individually wrapped canisters. To preserve the integrity of the pouch vacuum for proper operation, be sure that a BIOFIRE instrument module 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 are capable of transmitting infectious agents. Observe safety guidelines such as those outlined in:
    - CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories,*[63]
    - CLSI Document M29 *Protection of Laboratory Workers from Occupationally Acquired Infections*,[64]
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 ampoules, according to federal, state, regional, and local regulations.
5. Sample Buffer contains Guanidinium chloride and Triton X100.

   The following statements apply:

   - Health Hazards
       - Acute Toxicity, oral (Category 4)
           - H302 – Harmful if swallowed.
       - Skin corrosion/irritation (Category 2)
           - H315 - Causes skin irritation.
       - Serious eye damage/eye irritation (Category 1)
           - H318 - Causes serious eye damage.
   - Environment Hazards
       - Hazardous to the aquatic environment, acute aquatic hazard (Category 1)
           - H400 - Very toxic to aquatic life.
       - Hazardous to the aquatic environment, long-term aquatic hazard (Category 1)
           - H410 - Very toxic to aquatic life with long lasting effects.
   - Precautionary Statements
       - Prevention
           - P273 – Avoid release to the environment.
           - P280 – Wear protective gloves/protective clothing/eye protections/face protection.
   - Response
           - P332 + P313 - If skin irritation occurs: Get medical advice/attention.

- 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 CENTRE/doctor if you feel unwell.
- P337 + P313 - If eye irritation persists: Get medical advice/attention.

Please refer to the BIOFIRE GI Panel Safety Data Sheet (SDS) for more information: https://www.biofiredx.com/e-labeling/ITI0009.

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.

7. Bleach, a recommended disinfectant, is corrosive and may cause severe irritation or damage to eyes and skin. Vapor or mist may irritate the respiratory tract. Bleach is harmful if swallowed or inhaled. The following first aid measures are recommended.
    - Eye contact: Hold eye open and rinse with water for 15-20 minutes. Remove contact lenses after the first 5 minutes and continue rinsing eye. Seek medical attention.
    - Skin contact: Immediately flush skin with plenty of water for at least 15 minutes. If irritation develops, seek medical attention.
    - Ingestion: Do not induce vomiting. Drink a glassful of water. If irritation develops, seek medical attention. Please refer to the appropriate Safety Data Sheet (SDS) for more information.

Laboratory Precautions

1. **Preventing organism contamination**

    Due to the sensitive nature of the BIOFIRE 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:

    - Laboratory personnel may carry or shed common gastrointestinal pathogens asymptomatically and can inadvertently contaminate the specimen while it is being processed. Stool samples may also contain a high concentration of organisms. Careful adherence to the sample processing steps described in this document is recommended to avoid possible contamination. Samples should be processed in a clean biosafety cabinet if available, or according to local laboratory guidelines. 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 can be used when preparing samples instead.
    - It is recommended to avoid handling specimens or pouches in an area used to routinely process stool pathogen testing (e.g., culture, EIA).
    - Prior to processing a sample, thoroughly clean both the work area and the 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.
    - Use clean gloves to remove materials from packaging bags and, where applicable, reseal packaging bags when not in use.
    - 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 BIOFIRE GI Panel 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.
- Change gloves after handling a used pouch.
- 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 module and workspace must be decontaminated as described in the appropriate BIOFIRE Operator's Manual.

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

3. **Cary Blair media may contain non-viable organisms and/or nucleic acids at levels that can be detected by the BIOFIRE GI Panel.**

   The presence of non-viable organisms and/or nucleic acids in Cary Blair may lead to false positive test results. False positive results due to non-viable *Yersinia enterocolitica* and *Vibrio* species have been previously identified in various media types; the organism involved, and frequency of such occurrences may change in the future. In cases where results from the BIOFIRE GI Panel are not concordant with other clinical, laboratory, and epidemiological data, the BIOFIRE GI Panel results should be confirmed.

Precaution Related to Public Health Reporting in the United States

Local, state, federal, and country regulations for notification of reportable disease are continually updated and include a number of organisms for surveillance and outbreak investigations[65,66]. The U.S. Centers for Disease Control (CDC) recommends that when pathogens from reportable diseases are detected by a culture independent diagnostic test (CIDT), the laboratory should facilitate obtaining the isolate or clinical materials for submission to the appropriate public health laboratory to aid in outbreak detection and epidemiological investigations. Laboratories are responsible for following their state, local, and/or country regulations and should consult their local and/or state public health laboratories for isolate and/or clinical sample submission guidelines.

Precaution Related REACH Regulation (EC 1907/2006)

This statement only applies to countries within the European Union (EU) with regard to the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation (EC 1907/2006):

It is recommended that all material associated with the test, including the material used to clean up spills, contaminated packaging, and/or unused and expired IVD tests, is incinerated. Please ensure that you follow local regulations regarding disposal.

REAGENT STORAGE, HANDLING AND STABILITY

1. All kit components should be stored and used together at room temperature (15–25 ºC), including reagent pouches and buffers. Do not use components from one kit with those of another kit.
2. Avoid storage of any materials near heating or cooling vents or in direct sunlight.
3. Always check the expiration date on the kit. 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 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	0.2 mL (200 µL) of sample is required for testing
Transport and Storage	Specimens should be tested with the BIOFIRE GI Panel as soon as possible.[a] If storage is required, specimens can be held: • At room temperature for up to 4 days • Refrigerated for up to 4 days Note: refer to Cary Blair transport media manufacturer's instructions for any additional transport and storage recommendations.

[a] The performance validation included the evaluation of clinical specimens that were frozen at -70°C or lower for up to 90 days. However, longer frozen storage may be acceptable. Please follow your institutions rules and protocols regarding sample storage validation.

PROCEDURE

Refer to the BIOFIRE GI Panel Quick Guide or the appropriate BIOFIRE System Operator's Manual for more detail and pictorial representations of these instructions.

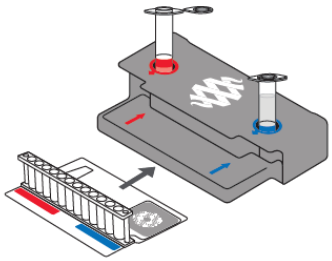
Gloves and other Personal Protective Equipment (PPE) should be used when handling pouches and samples. Only one BIOFIRE GI Panel pouch should be prepared at a time. Once sample is added to the pouch, it should be promptly transferred to the module to start the run. After the run is complete, the pouch should be discarded in a biohazard container.

Step 1: Prepare Pouch

1. Thoroughly clean the work area and the Pouch Loading Station with freshly prepared 10% bleach (or suitable disinfectant) followed by a water rinse.
2. Remove the BIOFIRE GI Panel pouch from its vacuum-sealed package by tearing or cutting the notched outer packaging and opening the protective canister. Use caution when opening the vacuum-sealed package as edges may be sharp.

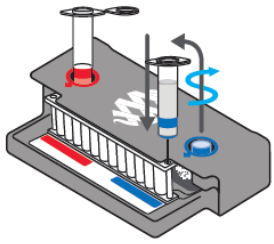
NOTE: The pouch may still be used even if the vacuum seal of the pouch is not intact. Attempt to hydrate the pouch using the steps in the Hydrate Pouch section. If hydration is successful, continue with the run. If hydration fails, discard the pouch and use a new pouch to test the sample.

3. Check the expiration date on the pouch. Do not use expired pouches.
4. Insert the pouch into the Pouch Loading Station, aligning the red and blue labels on the pouch with the red and blue arrows on the Pouch Loading Station.
5. Place a blue-capped Hydration Injection Vial in the blue well of the Pouch Loading Station.
6. Place a red-capped Sample Injection Vial in the red well of the Pouch Loading Station.



Step 2: Hydrate Pouch

1. Unscrew the Hydration Injection Vial from the blue cap.
2. Remove the Hydration Injection Vial, leaving the blue cap in the Pouch Loading Station.
3. Insert the cannula tip of the Hydration Injection Vial into the pouch hydration port located directly below the blue arrow of the Pouch Loading Station.
4. Forcefully push down in a firm and quick motion to puncture seal until a faint "pop" is heard and there is an ease in resistance. Wait as the correct volume of Hydration Solution is pulled into the pouch by vacuum.



   - If the hydration solution is not automatically drawn into the pouch, repeat Step 2 to verify that the seal of the pouch hydration port was broken. If hydration solution is again not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch*.
5. 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), repeat Step 2 to verify that the seal of the pouch hydration port was broken. If Hydration Solution is still not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch.*

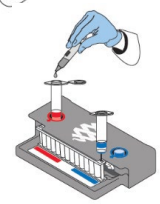
Step 3: Prepare Sample Mix

1. Add Sample Buffer to the Sample Injection Vial.
    - Hold the Sample Buffer ampoule with the tip facing up.

    NOTE: Avoid touching the ampoule tip during handling, as this may introduce contamination.

    - To open the Sample Buffer ampoule:
        - If the ampoule has a plastic tab on the tip: Gently twist and remove tab at the tip of the Sample Buffer ampoule.
        - If the ampoule does not have a plastic tab on the tip: Firmly pinch the textured plastic tab on the side of the ampoule until the seal snaps.

    - Invert the ampoule over the red-capped Sample Injection Vial and dispense Sample Buffer using a slow, forceful squeeze, followed by a second squeeze.



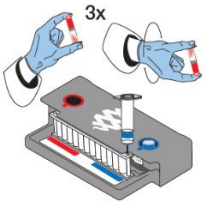
NOTE: Avoid squeezing the ampoule additional times. This will generate foam, which should be avoided.

WARNING: The Sample Buffer is harmful if swallowed and can cause serious eye damage and skin irritation.

2. Thoroughly mix the patient specimen.
3. Use the Transfer Pipette provided in the test kit to draw specimen to the second line (approximately 0.2 mL) of the Transfer Pipette.
4. Add the specimen to the Sample Buffer in the Sample Injection Vial.

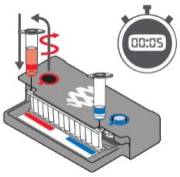
NOTE: DO NOT use the Transfer Pipette to mix the sample once it is loaded into the Sample Injection Vial.

5. Tightly close the lid of the Sample Injection Vial and discard the transfer pipette in a biohazard waste container.
6. Remove the Sample Injection Vial from the Pouch Loading Station and gently invert the vial at least 3 times to mix.
7. Return the Sample Injection Vial to the red well of the Pouch Loading Station.



Step 4: Load Sample Mix

1. Slowly twist to unscrew the Sample Injection Vial from the red cap so it loosens from its red cap, and wait for 5 seconds with the vial resting in the cap.



NOTE: Waiting 5 seconds decreases the risk of dripping and contamination from the sample.

2. Lift the Sample Injection Vial, leaving the red cap in the well of the Pouch Loading Station, and insert the Sample Injection Vial cannula tip into the pouch sample port located directly below the red arrow of the Pouch Loading Station.
3. Forcefully push down in a firm and quick motion to puncture the seal (a faint “pop” is heard) and the sample is pulled into the pouch by vacuum.
4. 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 1: Prepare Pouch.*
5. Discard the Hydration Injection Vial and Sample Injection Vial in an appropriate biohazard sharps container.
6. 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.

Step 5: Run Pouch

The BIOFIRE® FILMARRAY® Software includes step-by-step on-screen instructions that guide the operator through performing a run. Brief instructions for BIOFIRE 2.0 and BIOFIRE TORCH Systems are given below. Refer to the appropriate BIOFIRE System Operator’s Manual for more detailed instructions.

BIOFIRE 2.0

1. Ensure that the system (instrument and computer) is powered on and the software is launched.
2. Follow on-screen instructions and procedures described in the Operator’s Manual to place the pouch in a module, enter pouch, sample, and operator information.
3. Pouch identification (Lot Number and Serial Number), Pouch Type, and Protocol information 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 into the appropriate fields. To reduce data entry errors, it is strongly recommended that the pouch information be entered by scanning the barcode.

NOTE: When selecting a Pouch Type manually, ensure that the Pouch Type matches the label on the BIOFIRE GI Panel pouch.

4. 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.
5. If necessary, select and/or confirm a protocol from the Protocol for your sample type from the Protocol drop down list.
6. Enter a user name and password in the Name and Password fields.

NOTE: The font color of the username is red until the username is recognized by the software.

7. Review the entered run information on the screen. If correct, click the Start Run button on the screen.

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

NOTE: The bead-beater apparatus can be heard as a high-pitched noise during the first minute of operation.

8. When the run is finished, follow the on-screen instructions to remove the pouch, then immediately discard the pouch in a biohazard container.
9. The run file is automatically saved in the BIOFIRE Software database and the test report can be viewed, printed, and/or saved as a PDF file.

BIOFIRE TORCH

1. Ensure that the BIOFIRE TORCH system is on.
2. Select an available Module on the touch screen or scan the barcode on the BIOFIRE pouch using the barcode scanner.
3. Pouch identification (Lot Number and Serial Number), Pouch Type, and Protocol are preprogrammed in the rectangular barcode located on the BIOFIRE pouch. The information 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 into the appropriate fields. To reduce data entry errors, it is strongly recommended that the pouch information be entered by scanning the barcode.

NOTE: When selecting a Pouch Type manually, ensure that the Pouch Type matches the label on the BIOFIRE GI Panel pouch.

4. 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.
5. Insert the pouch into the Module.

   Ensure that the pouch fitment label is lying flat on top of pouch and not folded over. As the pouch is inserted, the Module will grab onto the pouch and pull it into the chamber.
6. If necessary, select and/or confirm a protocol from the protocol drop down list.
7. Enter operator user name and password, then select Next.

NOTE: The font color of the username is red until the username is recognized by the software.

8. Review the entered run information on the screen. If correct, select Start Run.

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

NOTE: The bead-beater apparatus can be heard as a high-pitched noise during the first minute of operation.

9. At the end of the run, remove the partially ejected pouch, then immediately discard it in a biohazard waste container.
10. The run file is automatically saved in the BIOFIRE Software database, and the test report can be viewed, printed, and/or saved as a PDF file.

QUALITY CONTROL

Process Controls

Two process controls are included in each pouch:

1. **RNA Process Control**
   The RNA Process Control assay targets an RNA sequence 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 pouch were successful.
2. **PCR2 Control**
   The PCR2 Control assay detects synthetic DNA 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 BIOFIRE 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 maintain records according to standard laboratory quality control practices.[67,68] The PCR2 Control is used in many pouch types and can therefore be used to monitor the system when multiple pouch types are used on the same BIOFIRE System.

External Controls

External controls should be used in accordance with laboratory protocols and the appropriate accrediting organization requirements, as applicable. Cary Blair 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. Commercial external control materials may be available from other manufacturers; these should be used in accordance with the manufacturers' instructions and appropriate accrediting organization requirements, as applicable.

Note: Contamination can cause unexpected positive results in negative or positive external controls. If unexpected positive results are observed, thoroughly clean and decontaminate the workspace and contact customer support if the unexpected results persist.

INTERPRETATION OF RESULTS

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

Assay Interpretation

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

Analysis of melt curves. The BIOFIRE 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 BIOFIRE GI Panel, the organism is reported as 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 Pshig 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, Vibrio cholerae, Y. enterocolitica*, EAEC, *Shigella/*EIEC, Adenovirus F 40/41, Astrovirus, Sapovirus (Genogroups I, II, IV, and V), *C. cayetanensis, E. histolytica* and *G. lamblia*.

Test results for several other organisms rely on the combination of multiple assays. These include *Campylobacter (C. jejuni/C. coli/C. upsaliensis), Vibrio* (*V. parahaemolyticus/ V. vulnificus/V. 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; see also the Analytical Reactivity (Inclusivity) section.

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

Bacteria

Campylobacter (C. jejuni/C. coli/C. upsaliensis)

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

Salmonella

The BIOFIRE 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 Analytical Reactivity (Inclusivity) for additional information).

Vibrio (V. parahaemolyticus/V. vulnificus/V. cholerae) and Vibrio cholerae

The BIOFIRE 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*) and with *Grimontia* (formerly *Vibrio) hollisae* (see Analytical Specificity (Cross-Reactivity and Exclusivity)). The Vibrio assay does not indicate which species has been detected and the Vibrio assay is not expected to detect the more rare species *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

BIOFIRE 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 Result	Positive	*Vibrio cholerae* detected OR *Vibrio cholerae* and one or more other *Vibrio* species detected

Yersinia enterocolitica

The BIOFIRE 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 BIOFIRE 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 BIOFIRE 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 BIOFIRE 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 *E. coli* (ETEC) heat-labile (lt) and heat-stable (st) Enterotoxins

The BIOFIRE GI Panel contains three assays (ETEC 1, ETEC 2, and ETEC 3) for the detection of genes encoding enterotoxins found in Enterotoxigenic *E. coli* (ETEC). The assays are designed for the detection of the genes encoding 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 gene(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 genes encoding 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 BIOFIRE 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 BIOFIRE 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 BIOFIRE 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 BIOFIRE 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 BIOFIRE 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 BIOFIRE GI Panel cannot distinguish between infections with a single toxigenic STEC O157 or rare co-infections of STEC (non-O157) with a *stx1/stx2*-negative *E. coli* O157 (see Table 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*

BIOFIRE GI Panel 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

BIOFIRE GI Panel 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 BIOFIRE 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 BIOFIRE 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 BIOFIRE GI Panel contains two assays (Crypt 1 and Crypt 2) for detection of *Cryptosporidium* species. Empirical testing and/or *in silico* sequence analysis support detection of approximately 23 different *Cryptosporidium* species, 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 BIOFIRE GI Panel contains a single assay (Ccayet) for the detection of *C. cayetanensis*, the only *Cyclospora* species implicated in human disease.

Entamoeba histolytica

The BIOFIRE 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 BIOFIRE 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 BIOFIRE 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 BIOFIRE 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 BIOFIRE 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). 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 a test result of Norovirus GI/GII Detected. The assay(s) will also detect Norovirus GIX.1, which was formerly classified as GII.15.[48] Cross-reactivity of the Norovirus assay(s) with sequences linked to a variety of commensal microorganisms (e.g. *Mediterraneibacter gnavus*, *Prevotella sp*.) has been identified by investigation of specimens in clinical studies and via post-market monitoring (see Analytical Specificity (Cross-Reactivity and Exclusivity)).

Rotavirus A

The BIOFIRE 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 BIOFIRE 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 BIOFIRE 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.

BIOFIRE GI Panel Test Report

The BIOFIRE GI Panel 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 BIOFIRE GI 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 module 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 BIOFIRE 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 Customer Technical Support for further instruction.

Control Result	Explanation	Action Required	Outcome
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 appropriate BIOFIRE Operator's Manual or contact Technical Support for further instruction. Once the error is resolved, repeat the test or repeat the test using another module.	Accept the valid results of the repeat testing. If the error persists, contact Customer 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 BIOFIRE Report,* for instruction.

LIMITATIONS OF THE PROCEDURE

1. For prescription use only.
2. The BIOFIRE GI Panel is intended for use only on the BIOFIRE 2.0 and BIOFIRE TORCH systems. Performance of the panel was established on the BIOFIRE FILMARRAY System (no longer manufactured or distributed), BIOFIRE 2.0 System, and BIOFIRE TORCH System. This test is a qualitative test and does not provide a quantitative value for the organism(s) in the sample.
3. 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.
4. This product should not be used to test stool samples in fixative (e.g., formalin or polyvinyl alcohol; PVA).
5. The performance of this product has not been established for the screening of stool for stool transplants.
6. The performance of this test has not been established for patients without signs and symptoms of gastrointestinal illness.
7. False positive and false negative results can be the result of a variety of sources and causes, it is important that these results be used in conjunction with other clinical, epidemiological, or laboratory information.
8. 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.
9. 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 *C. 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]
10. The performance of this test has not been established for monitoring treatment of infection with any of the panel organisms.
11. Discrepancies between the BIOFIRE 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. frederiksenii,* differentiation of *Entamoeba histolytica* from *E. dispar*, and differentiation of *Helicobacter pullorum* from *Campylobacter*. Also refer to the Organism Interpretation section of this document.
12. There is a risk of false negative results 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.
13. 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 BIOFIRE 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 BIOFIRE 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.
14. 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.
15. The BIOFIRE 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.

16. The BIOFIRE 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 BIOFIRE 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. In rare cases, STEC may be reported as EPEC when an STEC carrying *eae* (EHEC) is present in a specimen below the LoD of the STEC assay(s), or the strain carries an *stx* variant that is not detected well by the STEC assay(s) (e.g. *stx2* variant f). Rare instances of other organisms carrying *eae* have been documented; e.g., *Aeromonas* spp., *Citrobacter* spp., *Escherichia albertii*, and *Shigella boydii*.
17. *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*.
18. 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 BIOFIRE GI Panel (by the EPEC assay) due to their carriage of the *eae* gene.
19. The BIOFIRE GI Panel cannot distinguish between infections with a single toxigenic STEC O157 or rare co-infections of STEC (non-O157) with a *stx1/stx2*-negative *E. coli* O157.
20. 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.
21. The LoD values for *Giardia intestinalis* and *Entamoeba histolytica* were determined in units of cells/mL based on microscopic examination. However, parasites have a variable number of nuclei per cell in different life cycle stages (e.g. 1-2 nuclei per trophozoite and 4 (or more) nuclei per mature cyst). Because there is not a constant ratio of nucleic acid copy number per cell, detection by the BIOFIRE GI Panel may be variable when testing at an LoD measured in cells/mL.
22. 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 or no result. 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.
23. A negative BIOFIRE 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.
24. Cary Blair transport medium may contain non-viable organisms and/or nucleic acid at levels that can be detected by the BIOFIRE GI Panel and lead to false positive results.
25. Due to the complex and highly variable nature of stool specimens, freezing may affect analyte integrity and subsequent test results for some specimens.
26. Organism, nucleic acid, 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.
27. If four or more distinct organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.
28. The performance of the BIOFIRE 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.

29. 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.
30. Several organisms were shown to have the potential to cross-react with BIOFIRE 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), atypical sequences of unculturable/ uncharacterized *Prevotella* species, some sequences of *Anaerostipes hadrus*, *Enterobacter hormaechei* and *Parabacteroides* species, and *Mediterraneibacter* (*Ruminococcus*) *gnavus* (Noro 1 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 (Cross-Reactivity and Exclusivity) sections of this document.
31. Cross-reactivity with organisms other than those listed above or in the Organism Interpretation or Analytical Specificity sections may lead to erroneous results.
32. *Campylobacter* inclusivity testing and *in silico* analyses demonstrated that the BIOFIRE 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 evaluated from the NCBI database.
33. 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).
34. *V. cholerae* isolates with highly divergent *toxR* genes will be non-reactive with the BIOFIRE 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.
35. 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.
36. 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.
37. There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay.
38. 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.
39. 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.
40. Not all *Salmonella* serotypes were tested in validation studies; however, representatives of 20 prevalent serotypes 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*.
41. Cross-reactivity with the *Salmonella* assay may occur with certain *E. coli* strains containing variants of the cryptic ETT2 type-III secretion system (see Analytical Reactivity (Inclusivity) for additional information).
42. Positive and negative predictive values are highly dependent on prevalence. False negative results are more likely during peak activity when prevalence of disease is high. False positive results are more likely during periods when prevalence is moderate to low.

43. The performance of this test has not been evaluated for immunocompromised individuals.
44. 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 BIOFIRE GI Panel, 1556 eligible specimens (stool in Cary Blair transport medium) 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 BIOFIRE GI Panel, stratified by age group, are presented in the following table. Overall, the BIOFIRE GI Panel detected at least one organism in 53.5% (832/1556) of the prospective specimens.

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

BIOFIRE 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%)

BIOFIRE 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)
Norovirus GI/GII[c]	***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.
[c] Refer to Table 9 below for additional Norovirus GI/GII Expected Values results.

The BIOFIRE GI 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 BIOFIRE 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)

BIOFIRE 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 BIOFIRE 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 BIOFIRE 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 BIOFIRE 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%

Analyte	Number of Specimens Containing Analyte in Mixed Infections	Prevalence of Analyte in Mixed Infections (*N* = 262)
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*	13	5.0%
E. coli O157	1	0.4%
Shigella / Enteroinvasive *E. coli* (EIEC)	17	6.5%
Parasites		
Cryptosporidium	11	4.2%
Cyclospora cayetanensis	2	0.8%
Entamoeba histolytica	0	0%
Giardia lamblia	14	5.3%
Viruses		
Adenovirus F 40/41	34	13.0%
Astrovirus	4	1.5%
Norovirus GI/GII	43	16.4%
Rotavirus A	10	3.8%
Sapovirus	33	12.6%

In 2023, bioMérieux conducted a new prospective clinical evaluation to assess norovirus performance using the most recent version of the U.S. Centers for Disease Control and Prevention (CDC) CaliciNet Norovirus GI/GII assay. This assay was updated with new probe designs and quantification cycle cutoff thresholds compared to the version that was used in the original clinical evaluation (2013, above).

The prospective clinical evaluation for norovirus detection on the BIOFIRE GI Panel included 872 eligible specimens (stool in Cary Blair transport medium) that were collected and tested at three study sites across the United States over approximately four months (April – July 2023). The number and percentage of positive norovirus results as determined by the BIOFIRE GI Panel, stratified by age group, are presented in the following table.

Table 9. Expected Values (EV) (as determined by the BIOFIRE GI Panel) by Age Group for the Prospective Clinical Evaluation (April through July 2023)

BIOFIRE GI Panel Result	***Overall (n=872)***		<1 year (n=70)		1-5 years (n=120)		6-12 years (n=64)		13-21 years (n=127)		22-64 years (n=283)		65+ years (n=208)	
	#	***EV***	**#**	**EV**	**#**	**EV**	**#**	**EV**	**#**	**EV**	**#**	**EV**	**#**	**EV**
Norovirus GI/GII	***63***	***7.2%***	13	18.6%	16	13.3%	7	10.9%	11	8.7%	12	4.2%	4	1.9%

PERFORMANCE CHARACTERISTICS

NOTE: BIOFIRE GI Panel performance was initially established on the first generation BIOFIRE FILMARRAY System (REF: FLM1-ASY-0001). The BIOFIRE FILMARRAY System is no longer being manufactured or distributed, but the performance characteristics established on that system are relevant to the BIOFIRE GI Panel and remain in this Instructions for Use. Comparison studies have established that BIOFIRE GI Panel performance characteristics are equivalent between BIOFIRE FILMARRAY System, BIOFIRE 2.0, and BIOFIRE Torch systems.

Clinical Performance (2013)

The clinical performance of the BIOFIRE 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 10 provides a summary of demographic information for the 1556 specimens included in the prospective study.

Table 10. Demographic Summary for Prospective BIOFIRE 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 BIOFIRE GI Panel was evaluated by comparing the BIOFIRE GI Panel test result for each member of the panel with the appropriate comparator/reference methods shown in the table below.

Table 11. Comparator Methods for BIOFIRE GI Panel Clinical Evaluation

BIOFIRE FILMARRAY System 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 BIOFIRE FILMARRAY System 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 BIOFIRE FILMARRAY System determination of *E. coli* O157 for specimens in which BIOFIRE FILMARRAY System 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 BIOFIRE FILMARRAY System).
[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 BIOFIRE 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 BIOFIRE GI Panel and reference/comparator method had a positive result for the specific analyte, and false negative (FN) indicates that the GI Panel 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 BIOFIRE GI Panel and the reference/comparator method had negative results, and a false positive (FP) indicates that the BIOFIRE 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 12.

Table 12. BIOFIRE 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
Enteroaggregative *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 BIOFIRE GI Panel 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. Refer to "Clinical Performance (2023)" section below for additional Norovirus GI/GII performance results.
[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.

BIOFIRE GI Panel 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 13. *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 14. *Salmonella* Clinical Performance Stratified by Species/Serovar

***Salmonella* species/serovar**	**Sensitivity**
S. enterica ser. Enteritidis	7/7 (100%)
S. enterica ser. Typhimurium (i:-)	7/7 (100%)
S. enterica ser. Typhimurium	3/3 (100%)
S. enterica ser. Javiana	2/2 (100%)
S. enterica ser. Newport	2/2 (100%)
S. enterica ser. Agbeni	1/1 (100%)
S. enterica ser. Berta	1/1 (100%)
S. enterica ser. Ealing	1/1 (100%)
S. enterica ser. Gaminara	1/1 (100%)
S. enterica ser. Infantis	1/1 (100%)
S. enterica ser. Mbandaka	1/1 (100%)
S. enterica ser. Miami	1/1 (100%)
S. enterica ser. Muenchen	1/1 (100%)

Salmonella species/serovar	Sensitivity
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 BIOFIRE 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 15. Most Prevalent Multiple Detection Combinations (≥5 instances)

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 99.2% (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).

Clinical Performance (2023)

The clinical performance of norovirus detection on the BIOFIRE GI Panel was assessed using the most recent version of the CDC CaliciNet Norovirus GI/GII assay with specimens from a multi-center study conducted at three geographically distinct U.S. study sites between April and July 2023. A total of 872 prospective residual stool specimens in Cary Blair transport medium were eligible for this evaluation; none of these were excluded. Table 16 provides a summary of the demographic information for the 872 specimens included in this prospective clinical evaluation.

Table 16. Demographic Summary for the BIOFIRE GI Panel Prospective Clinical Evaluation (April through July 2023)

Study Specimens	
Total Specimens	872
Sex	**Number of Specimens (%)**
Male	394 (45.2%)
Female	478 (54.8%)
Age Group	**Number of Specimens (%)**
< 1 year	70 (8.0%)
1-5 years	120 (13.8%)
6-12 years	64 (7.3%)
13-21 years	127 (14.6%)
22-64 years	283 (32.5%)
65+ years	208 (23.9%)
Status	**Number of Specimens (%)**
Outpatient	368 (42.2%)
Hospitalized	204 (23.4%)
Emergency	84 (9.6%)
Unknown	216 (24.8%)

The performance of the BIOFIRE GI Panel Norovirus GI/GII assay was evaluated by comparing the BIOFIRE GI Panel test result with an updated version of the comparator method used in the original (2013) prospective clinical evaluation: the U.S. Centers for Disease Control and Prevention (CDC) CaliciNet Norovirus GI/GII assay (updated in 2017).

A total of 872 specimens were evaluated. Positive percent agreement (PPA) was calculated as 100% × (TP / (TP + FN)). True positive (TP) indicates that both the BIOFIRE GI Panel and the comparator method had positive results, and a false negative (FN) indicates that the BIOFIRE GI Panel result was negative while the comparator result was positive. Negative percent agreement (NPA) was calculated as 100% × (TN / (TN + FP)). True negative (TN) indicates that both the BIOFIRE GI Panel and the comparator method had negative results, and a false positive (FP) indicates that the BIOFIRE GI Panel result was positive, but the comparator result was negative. The exact binomial two-sided 95% confidence interval was calculated. The results are shown in Table 17.

Table 17. BIOFIRE GI Panel Norovirus GI/GII Performance in the Prospective Clinical Evaluation (April through July 2023)

BIOFIRE GI Panel Result	Positive Percent Agreement (PPA)			Negative Percent Agreement (NPA)		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Norovirus GI/GII	34/35[a]	97.1	85.1-99.9%	808/837[a]	96.5	95.1-97.7%

[a] Norovirus was detected in the single FN specimen using bi-directional sequencing analysis. Norovirus was detected in 3/29 false positive specimens using bi-directional sequencing analysis. Twenty (20) of the remaining false positive results appear to have been caused by cross-reactivity; refer to the Analytical Specificity (Cross-Reactivity and Exclusivity) section for cross-reactive organisms.

The overall success rate for initial specimen tests in the prospective clinical evaluation was 99.0% (863/872). Nine specimens required repeat testing. Eight tests were incomplete due to instrument errors and one test was invalid due to a pouch control failure. All specimens were retested and were successful after a single retest (eight specimens) or two retests (one specimen), for a final success rate of 100% (872/872).

Clinical Comparison on the BIOFIRE 2.0

Clinical and non-clinical studies have established that the performance characteristics of the BIOFIRE GI Panel, including LoD (see Limit of Detection section below), positive percent agreement and negative percent agreement, and reproducibility are equivalent on the previously sold BIOFIRE FILMARRAY System and current BIOFIRE 2.0 systems. Non-clinical studies also demonstrate similar performance characteristics on BIOFIRE TORCH systems.

NOTE: BIOFIRE TORCH Modules are BIOFIRE 2.0 modules that have been re-configured into a stacked system for higher throughput in a smaller workspace.

A clinical comparison study of the BIOFIRE FILMARRAY System and BIOFIRE 2.0 was performed using specimens previously obtained during the BIOFIRE GI Panel prospective clinical evaluation and supplemented with other archived specimens collected from external medical facilities and reference laboratories to increase the number of specimens being tested for low prevalence analytes. Contrived clinical specimens were also used for GI analytes which are extremely rare and for which no clinical specimens were available (*Entamoeba histolytica, Vibrio* spp., and *V. cholerae*). A total of 104 specimens were selected such that each analyte was represented 3-5 times. Each specimen was thawed or contrived and tested using the BIOFIRE FILMARRAY System and BIOFIRE 2.0 systems. Overall positive percent agreement (PPA) between systems was 96.4% with the lower bound of the two-sided 95% confidence interval (95% CI) at 91.0%. Overall negative percent agreement (NPA) was 99.4% with the lower bound of the two-sided 95% CI at 98.9%.

Table 18. Analyte Results from BIOFIRE FILMARRAY System Clinical Comparison Study

Analyte	BIOFIRE 2.0 / BIOFIRE FILMARRAY System					
	PPA	%	95% CI	NPA	%	95% CI
Bacteria						
Campylobacter	5/5	100%	47.8-100%	96/97	99%	94.4-100%
Clostridium difficile toxin A/B	5/5	100%	47.8-100%	95/97	97.9%	92.7-99.7%
Plesiomonas shigelloides	3/3	100%	29.2-100%	99/99	100%	96.3-100%
Salmonella	5/5	100%	47.8-100%	97/97	100%	96.3-100%
Vibrio	6/7	85.7%	42.1-99.6%	94/95	98.9%	94.3-100%
Vibrio cholerae	3/3	100%	29.2-100%	98/99	99%	94.5-100%
Yersinia enterocolitica	4/4	100%	39.8-100%	98/98	100%	96.3-100%
Diarrheagenic *E. coli* / *Shigella*						
Enteroaggregative *E. coli* (EAEC)	8/8	100%	63.1-100%	94/94	100%	96.2-100%
Enteropathogenic *E. coli* (EPEC)	11/12	91.7%	61.5-99.8%	84/84	100%	95.7-100%
Enterotoxigenic *E. coli* (ETEC)	5/5	100%	47.8-100%	96/97	99%	94.4-100%
Shiga-like toxin-producing *E. coli* (STEC)	6/6	100%	54.1-100%	96/96	100%	96.2-100%
Escherichia coli O157	3/3	100%	29.2-100%	3/3	100%	29.2-100%
Shigella/Enteroinvasive *E. coli* (EIEC)	6/6	100%	54.1-100%	96/96	100%	96.2-100%
Parasites						

Analyte	BIOFIRE 2.0 / BIOFIRE FILMARRAY System					
	PPA	%	95% CI	NPA	%	95% CI
Cryptosporidium	6/6	100%	54.1-100%	96/96	100%	96.2-100%
Cyclospora cayetanensis	4/4	100%	39.8-100%	98/98	100%	96.3-100%
Entamoeba histolytica	5/5	100%	47.8-100%	97/97	100%	96.3-100%
Giardia lamblia	6/6	100%	54.1-100%	96/96	100%	96.2-100%
Viruses						
Adenovirus F 40/41	7/9	77.8%	40-97.2%	90/93	96.8%	90.9-99.3%
Astrovirus	5/5	100%	47.8-100%	97/97	100%	96.3-100%
Norovirus GI/GII	4/4	100%	39.8-100%	96/98	98%	92.8-99.8%
Rotavirus A	4/4	100%	39.8-100%	98/98	100%	96.3-100%
Sapovirus	5/5	100%	47.8-100%	97/97	100%	96.3-100%
Overall agreement	***116/120***	***96.7%***	***91.7-99.1%***	***2011/2022***	***99.5%***	***99.0-99.7%***

System performance for testing of 104 specimens on each platform was calculated. For the BIOFIRE FILMARRAY System, a total of 105 runs were attempted, 104 of which were completed (99.0%; 104/105). One run was aborted by the user (0.9%). There were no control failures. For the BIOFIRE 2.0, a total of 104 runs were attempted, all of which completed (100%; 104/104). There was one control failure.

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 BIOFIRE 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 BIOFIRE 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 19 and the results of the BIOFIRE GI testing are presented in Table 20.

Table 19. 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 20. BIOFIRE 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 BIOFIRE GI Panel. 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 BIOFIRE GI Panel testing are presented in Table 21.

Table 21. BIOFIRE 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 BIOFIRE GI Panel *V. cholerae* assay. The BIOFIRE GI Panel *Vibrio* assay was positive for nine of these specimens.

Limit of Detection

The Limit of Detection (LoD) for BIOFIRE GI Panel analytes was estimated with dilutions of single-spiked and multi-spiked samples (up to four organisms per sample). Detection was equivalent between single-spiked and multi-spiked samples, and LoD confirmation testing was performed by spiking one or more organism(s) into stool samples at the estimated LoD concentration and testing 20 replicates per sample. The LoD concentrations listed in Table 22 were confirmed on the BIOFIRE 2.0 and BIOFIRE TORCH systems with analyte detection in at least 19/20 replicates (≥95%).

Table 22. Limit of Detection (LoD) for BIOFIRE GI Panel Analytes

GI Panel Test Result	Species/Isolate Tested	LoD Concentration
BACTERIA		
Campylobacter	*Campylobacter coli* ATCC 33559	4 x 10^4 cells/mL
	Campylobacter jejuni ATCC BAA-1234	
	Campylobacter upsaliensis ATCC BAA-1059	
Clostridium difficile (toxin A/B)	*Clostridium difficile* Toxinotype 0 A+B+ ATCC 9689	4 x 10^5 cells/mL
	Clostridium difficile (NAP1) Toxinotype III A+B+ Zeptometrix #801619	4 x 10^4 cells/mL
Plesiomonas shigelloides	*Plesiomonas shigelloides* ATCC 14029	1 x 10^3 CFU/mL
Salmonella	*Salmonella bongori* O66:H1z41:H2- SGSC RKS#3041 SarC11	1 x 10^4 CFU/mL
	Salmonella enterica ssp. *enterica* Serovar Typhimurium O1,4,[5],12:H1i:H21,2 SGSC RKS#4194 SarC1	5 x 10^3 CFU/mL
Vibrio and *Vibrio cholerae*	*Vibrio cholerae* Ogawa serotype O:1 ATCC 14035	8 x 10^3 cells/mL
	Vibrio parahaemolyticus ATCC 17802	8 x 10^4 cells/mL
Yersinia enterocolitica	*Yersinia enterocolitica* Biovar1 serogroup O:8 ATCC 9610	5 x 10^4 CFU/mL
DIARRHEAGENIC *E. coli*/*Shigella*		
Enteroaggregative *E. coli* (EAEC)	*Escherichia coli* JM221 O92:H33 STEC Center	1 x 10^4 CFU/mL
Enteropathogenic *E. coli* (EPEC)	*Escherichia coli* E2348/69 O127:H6 STEC Center	1 x 10^3 CFU/mL
Enterotoxigenic *E. coli* (ETEC) *lt/st*	*Escherichia coli* H10407 O78:H11 ATCC 35401	1 x 10^3 CFU/mL
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2* *E. coli* O157	*Escherichia coli* O26:H11 ATCC BAA-2196	1 x 10^3 CFU/mL
	Escherichia coli O157:H7 ATCC 43895	1 x 10^4 CFU/mL

GI Panel Test Result	Species/Isolate Tested	LoD Concentration
Shigella/Enteroinvasive *E. coli* (EIEC)	Escherichia *coli* O29:NM ATCC 43892	5 x 10^3 CFU/mL
	Shigella sonnei ATCC 29930	100 CFU/mL
PARASITES[a]		
Cryptosporidium [a]	*Cryptosporidium parvum* *Iowa* isolate (Harley Moon) Waterborne, Inc. P102C	5 x 10^3 oocysts/mL [a,b]
	Cryptosporidium hominis Clinical Specimen	
Cyclospora cayetanensis	*Cyclospora cayetanensis* Clinical Specimen	180 genome equivalents (GE)/mL
Entamoeba histolytica	*Entamoeba histolytica* HM-1:IMSS ATCC 30459	2 x 10^3 cells/mL[b]
Giardia lamblia	*Giardia intestinalis* (aka *G. lamblia*) ATCC 30957	50 cells/mL [b]
VIRUSES		
Adenovirus F 40/41	Adenovirus F40 ATCC VR-931	1 $TCID_{50}$/mL
	Adenovirus F41 ATCC VR-930	100 $TCID_{50}$/mL
Astrovirus	Astrovirus - Type 8 NCPV#1003071v	50 FFU/mL
Norovirus GI/GII	Norovirus GI Clinical Specimen	1 x 10^4 RNA copies/mL
	Norovirus GII Clinical Specimen	
Rotavirus A	Rotavirus A - G4[P6] NCPV#0904053v	1 x 10^5 FFU/mL
Sapovirus	Sapovirus (Genogroup I) Clinical Specimen	1.1 x 10^7 RNA copies/mL

[a] Limited testing with a clinical specimen containing *Cryptosporidium meleagridis* indicates that the LoD for *C. meleagridis* is similar to that of *C. parvum* and *C. hominis*.
[b] Note that parasites will have variable numbers of nuclei per cell (up to 16) in different stages of reproduction and maturation (e.g. cyst versus trophozoite). Therefore, LoD established in units of cells/mL or oocysts/mL may not be reproducible, due to variable DNA concentration in each cell/cyst/trophozoite in the sample or culture tested.

Analytical Reactivity (Inclusivity)

The analytical reactivity (inclusivity) of the BIOFIRE GI Panel was evaluated with a collection of more than 250 isolates that represent the diversity of the BIOFIRE 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 BIOFIRE 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 BIOFIRE 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 BIOFIRE GI Panel test result.

Table 23. *Campylobacter* Inclusivity Results (*C. coli*/*C. jejuni*/*C. upsaliensis*)

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

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

Table 24. *Clostridium difficile* toxin A/B Inclusivity Results

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

Organism	Toxinotype	Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected
		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 25. *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 26. *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 x 10^4	1xLoD
	NCTC 10946	3.0 x 10^4	3×LoD
	SGSC RKS 3044	3.0 x 10^4	3×LoD
Salmonella enterica subsp. *salamae II*	SGSC RKS 2985	1.5 x 10^4	3×LoD
Salmonella enterica subsp. *arizonae IIIa*	SGSC RKS 2980	1.5 x 10^4	3×LoD
Salmonella enterica subsp. *diarizonae IIIb*	SGSC RKS 2978	1.5 x 10^4	3×LoD
Salmonella enterica subsp. *houtenae IV*	SGSC RKS 3027	1.5 x 10^4	3×LoD

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

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

Table 27. *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 x 10^3	1xLoD
	O:1 Inaba, Biotype El Tor	BEI NR-147	2.4 x 10^4	3xLoD
	O:1 Ogawa, Biotype El Tor	BEI NR-148	2.4 x 10^4	3xLoD
	non-O:1,non-O:139 (O:2)	BEI NR-149	2.4 x 10^4	3xLoD
	non-O:1,non-O:139 (O:7)	BEI NR-152	2.4 x 10^4	3xLoD
	O:1 Inaba, Biotype El Tor	ATCC 25870	2.4 x 10^4	3xLoD
Vibrio parahaemolyticus		ATCC 17802[a]	8.0 x 10^4	1xLoD
		ATCC BAA-242	2.4 x 10^5	3xLoD

Organism (species, biotype and serotype)	Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected
	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 28. *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 BIOFIRE GI Panel should react with all strains/serotypes of *Y. enterocolitica*.

Table 29. 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

Organism	Serotype	Isolate ID	Concentration Detected (cells/mL)	Multiple of LoD Detected
	O104:H4 (Purified DNA)[b]	2011 European Outbreak strain[c]	3.0 x 10^3	0.3xLoD
	Ond:H10[d]	STEC Center 101-1	1.5 x 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 (GE = genomic equivalents).
[c] Isolate has genetic characteristics consistent with STEC and EAEC.
[d] Phenotypic EAEC but known to not carry the marker(s) detected by the BIOFIRE GI Panel EAEC assay.

Table 30. 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 x 10^3	1×LoD
	O128:H2	Atypical	STEC Center DEC11a	3.0 x 10^3	3×LoD
	111a:NM	Unknown	STEC Center Stoke W	3.0 x 10^3	3×LoD
	O142:H6	Typical	STEC Center E851/71	3.0 x 10^3	3×LoD
	O55:H7	Atypical	STEC Center DEC5A	3.0 x 10^3	3×LoD
	O114:H2	Typical	STEC Center 3448-87	3.0 x 10^3	3×LoD
	O119:H+	Unknown	STEC Center RN410/1	3.0 x 10^3	3×LoD
	O96:H	Unknown	STEC Center HSP19/4	3.0 x 10^3	3×LoD
	O86:Hnm	Unknown	STEC Center E990	3.0 x 10^3	3×LoD
	O55:H-	Unknown	STEC Center MA551/1	3.0 x 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 31. 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 x 10^3	1×LoD
	O175:H15	STA (-)/LT (+)	Penn State 6.0671	3.0 x 10^3	3×LoD
	O149:H5	STA (-)/LT (+)	Penn State 6.1182	3.0 x 10^3	3×LoD
	O84:H28	STA (-)/LT (+)[b]	Penn State 7.1493	3.0 x 10^3	Not Detected[b]
	H5	STA (+)/LT (-)	Penn State 10.0049	3.0 x 10^3	3×LoD
	O168	STA (+)/LT (-)	Penn State 9.1809	3.0 x 10^3	3×LoD
	O145:H25	STA (+)/LT (-)	Penn State 10.0136	1.0 x 10^4	100xLoD[c]
	O78	STA (+)/LT (+)	Penn State 2.1507	3.0 x 10^3	3×LoD
	O19:H5	STA (+)/LT (+)	Penn State 5.0038	3.0 x 10^3	3×LoD
	H14	STA (+)/LT (-)	Penn State 10.045	3.0 x 10^3	3×LoD
	O141	STA (+)/LT (+)	Penn State 93.0045	3.0 x 10^3	3×LoD
	Unknown	STB (+)[d] STA(-)/LT(-)	Penn State 8.2425	1.5 x 10^9	Not Detected[d]
	Unknown	STB (+)[d] STA(-)/LT(-)	Penn State 9.1179	1.5 x 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 BIOFIRE GI Panel will not detect phenotypic ETEC that that express only heat-stable toxin ST2/STB or heat-labile toxin LT-II.

Table 32. 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)**					
	O26: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
	STEC O157					
	O157:NM	+/+	STEC Center DA-26	3.0×10^3	3×LoD	0.3×LoD
	O157:H7	-/+	STEC Center E32511	3.0×10^3	3×LoD	0.3×LoD
	O157:HNT	+/+	STEC Center DA-74	3.0×10^3	3×LoD	0.3×LoD
	O157:H7	+/+	ATCC 43895[a]	1.0×10^4	10xLoD	1xLoD
	O157:H7	+/+	STEC Center A8993-CS2	3.0×10^4	30×LoD	3×LoD
	Non-STEC O157					
	O157:H7	-/-	ATCC 43888	3.0×10^4	Not Detected	N/A[d]
	O157:H45	-/-	STEC Center SC373/2	3.0×10^4	Not Detected	N/A[d]

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

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

Table 33. *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]	5.0×10^3	1×LoD
	O29:HNM (1977)	STEC Center 1885-77	3.0×10^3	0.6×LoD
	O124:HNM (1978)	STEC Center 929-78	3.0×10^3	0.6×LoD
	O29:H27 (1979; VA, USA)	STEC Center 1827-79	3.0×10^3	Not Detected[b]
	O28:H- (1983, Brazil)	STEC Center LT-15	3.0×10^3	0.6×LoD
	O136:H- (1983, Bangladesh)	STEC Center LT-41 Strain 1111-55	3.0×10^3	0.6×LoD
Shigella boydii (Serogroup C)	Type 2	ATCC 8700	3.0×10^2	3×LoD
	Type 4	CDPH HUM-2010029296	3.0×10^2	3×LoD
	Type 1	ATCC 9207	3.0×10^2	3×LoD
	Type 20	ATCC BAA-1247	3.0×10^2	3×LoD
	Type 10	ATCC 12030	3.0×10^2	3×LoD
Shigella dysenteriae (Serogroup A)	Type 1	BEI NR-520	3.0×10^2	3×LoD[c]
	Type 2	CDPH PHM-2004008089	3.0×10^2	3×LoD
	Type 13	ATCC 49555	3.0×10^2	3×LoD
	Type 3	ATCC 29028	3.0×10^2	3×LoD
	Type 12	ATCC 49551	3.0×10^2	3×LoD
Shigella flexneri (Serogroup B)	Type 2a	ATCC 700930	3.0×10^2	3×LoD
	Type 1a	ATCC 9199	3.0×10^2	3×LoD
	Type 6	CDPH PHM-2006004043	3.0×10^2	3×LoD
	Type 2b	ATCC 12022	3.0×10^2	3×LoD
	Type 2a	ATCC 29903	3.0×10^2	3×LoD
	Unknown	STEC Center VA-6	3.0×10^2	3×LoD
Shigella sonnei (Serogroup D)	N/A	ATCC 29930[a]	1.0×10^2	1×LoD
	N/A	ATCC 11060	3.0×10^2	3×LoD
	N/A	CDPH HUM-2010027998	3.0×10^2	3×LoD
	N/A	ATCC 29031	3.0×10^2	3×LoD
	N/A	ATCC 25931	3.0×10^2	3×LoD
	N/A	ATCC 9290	3.0×10^2	3×LoD

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

Table 34. *Cryptosporidium* Inclusivity Results[a]

Organism	Location/Source of Isolate or Sample	Concentration Detected (copies/mL)	Multiple of LoD Detected
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[d]
	Scotland Purified DNA from Clinical Sample	Unknown	≤LoD[d]

[a] Testing also included a clinical sample containing *Cryptosporidium canis* at a very low or non-amplifiable concentration (based on evaluation with a commercial quantitative real-time PCR (qPCR) assay). *Cryptosporidium* was not detected in the sample, but sequence analysis predicts that the species *C. canis* will be reliably detected when present in a sample at a concentration equal to or greater than the LoD of the Cryptosporidium assay(s) (i.e., no limitation on reactivity with *C. canis*).
[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 based on qPCR).
[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 based on qPCR).
[d] *C. ubiquitum* DNA purified from clinical samples was detected by the BIOFIRE GI Panel Cryptosporidium assay(s), though the DNA concentration tested was estimated (by qPCR) to be at or below the 95% LoD.

Note: *In silico* sequence analysis indicates the *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 (see also the Organism Interpretation section above). *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 35. *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 36. *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 based on qPCR).

Table 37. *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 38. 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]	3.0×10^5	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 based on qPCR.
[b] Same strain as ATCC VR-930 (which was detected at 1× LoD) but obtained from a different source.

Table 39. Astrovirus Inclusivity Results

Organism	Type	Location/Source/Isolate ID	Concentration Detected (copies/mL)	Multiple of LoD Detected
Human Astrovirus	1	China Clinical Sample	1.3×10^8	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

Organism	Type	Location/Source/Isolate ID	Concentration Detected (copies/mL)	Multiple of LoD Detected
	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 based on qRT-PCR).

Table 40. Norovirus GI/GII[a] Inclusivity Results

Norovirus Genogroup/Genotype		Isolate ID (Clinical Samples)	Concentration Detected (copies/mL)	Multiple of LoD Detected
Norovirus GI	3	Noro1_036[b]	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[d]
		Noro1_029	6.0×10^3	0.6×LoD
		Noro1_034	6.0×10^3	0.6×LoD
	8	Noro G1.8[c]	6.0×10^4	6×LoD
Norovirus GII	Unknown	Noro2_013[b]	1.0×10^{4a}	1×LoD
	2	NoroII.2[b]	6.0×10^3	0.6×LoD
	3	China-5	6.0×10^3	0.6×LoD
		SGB_038	6.0×10^3	0.6×LoD
	4	GI-PILOT-SPDRL-077	2.0×10^5	20×LoD[d]
		Noro2_004	2.0×10^5	20×LoD[d]
		Noro2_032	2.0×10^5	20×LoD[d]
		PCMC_025 (Sydney)	6.0×10^3	0.6×LoD
		PCMC_031 (Sydney)	6.0×10^3	0.6×LoD
	6	NYH-A	6.0×10^3	0.6×LoD
	7	NoroII.7[c]	6.0×10^3	0.6×LoD
	8	NoroII.8[c]	6.0×10^3	0.6×LoD
	12	NoroII.12[c]	6.0×10^3	0.6×LoD
	16	NoroII.16[c]	6.0×10^3	0.6×LoD
	20	NoroII.20c[c]	2.0×10^5	20×LoD[d]
		NoroII.20[c]	6.0×10^3	0.6×LoD

[a] Sequence analysis indicates the Norovirus assays(s) will also detect Norovirus GIX.1 (formerly classified as genogroup GII.15).
[b] Isolate was used to establish the LoD for this assay.
[c] Isolate obtained as RNA extract from a clinical sample. Genotype provided by the source laboratory.
[d] 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 41. Rotavirus A Inclusivity Results

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

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

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

Table 42. Sapovirus Inclusivity Results

Organism	Genogroup	Isolate ID (Clinical Samples)	Concentration Detected (copies/mL)	Multiple of LoD Detected
Sapovirus	GI	AB_SaV_14[a]	1.1×10^7	1×LoD
	Unknown	China_56	3.3×10^7	3×LoD
	Unknown	AB_SaV_03	3.3×10^7	3×LoD
	Unknown	PCMC_54	3.3×10^7	3×LoD
	Unknown	SPDRL-006	3.3×10^7	3×LoD
	Unknown	SPDRL-099	3.3×10^7	3×LoD
	Unknown	SGB-MP-11	3.3×10^7	3×LoD
	GI	Sapo_03[b]	3.3×10^7	3×LoD
	GII	Sapo_06[b]	3.3×10^7	3×LoD
	GIV	Sapo_09[b]	3.3×10^7	3×LoD
	GV	Sapo_02[b]	3.3×10^7	3×LoD

[a] Clinical Sample was used to establish the LoD for this assay. LoD copies/mL is based on qRT-PCR of RNA extracted from the sample(s).
[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 BIOFIRE 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.

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

Over 175 off-panel organisms were selected for specificity testing based on a combination of several factors including (1) relatedness to specific species detected by the BIOFIRE GI Panel (near-neighbors), (2) clinical relevance, (3) likelihood of being present in stool specimens, and (4) genetic similarity to BIOFIRE 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 BIOFIRE 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/parasites, and ≥1.0×10^{5}units/mL for viruses).

Table 43 lists the 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 44 contains a complete list of the off-panel bacteria, fungi, protozoa/parasites, and viruses that were tested or for which *in silico* analysis was performed. Species with cross-reactivity observed in analytical testing are in bold font. Species without bold font received the expected BIOFIRE GI Panel test result (negative for all assays; no cross-reactivity) or for which *in silico* analysis does not predict cross-reactivity.

Table 43. Observed or Predicted Cross-Reactivity with Off-Panel Organisms

BIOFIRE GI Panel Test Result	Cross-Reactive Organism(s)
Entamoeba histolytica	*Entamoeba dispar*
Giardia lamblia	*Bifidobacterium spp* [a] *Ruminococcus spp* [a]
Enterotoxigenic *E.coli* (ETEC) *lt/st* [ETEC 2 assay]	*Citrobacter koseri* *Citrobacter sedlakii* *Hafnia alvei* [a] *Cedecea davisiae* [a]
Norovirus GI/GII [Noro 1 assay] [b]	*Prevotella* spp. (sequences from unculturable/uncharacterized species) [c] *Mediterraneibacter (Ruminococcus) gnavus* *Parabacteroides spp. (P. merdae, P. acidifaciens* [d]*, P. distasonis* [e]*)* *Anaerostipes hadrus (select sequences)* [f] *Enterobacter hormaechei (select sequences)* [g]
Salmonella	*E. coli* with variant type III secretion protein [h]
Vibrio (*V. parahaemolyticus/V. vulnificus/ V. cholerae*)	*Vibrio alginolyticus* *Vibrio fluvialis* [i] *Vibrio mimicus* [i] *Grimontia (*formerly *Vibrio) hollisae*
Yersinia enterocolitica	*Yersinia frederiksenii*[a,j] *Yersinia kristensenii* [j]

[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 was identified by post-market investigation of suspected false positive Norovirus GI/GII results in clinical specimens. Cross-reactivity with the species listed was confirmed by analytical testing at high concentration (>2.4x10^8 cells/mL) and/or is supported by sequence analysis.

[c] Cross-reactive sequences are inconsistent with other *Prevotella* sequence data, suggesting non-specific interaction with atypical or uncharacterized species and/or sequences.

[d] *P. acidifaciens* was not tested but was determined by sequence analysis to have a similar risk of cross-reactivity as *P. merdae*.

[e] Norovirus GI/GII Not Detected was reported when *P. distasonis* was tested at high concentration (3.1x10^9 cells/mL). However, non-specific amplification products with Tm values close to the assay specific Tm range have been observed and the potential for false positive Norovirus GI/GII test results exists.

[f] The risk of false positive Norovirus GI/GII results due to cross-reactivity with *A. hadrus* is associated with only a subset of *A. hadrus* RefSeq genome sequences (<35% as of June 2024).

[g] The risk of false positive Norovirus GI/GII results due to cross-reactivity with *E. hormaechei* is associated with only a subset of *E. hormaechei* RefSeq genome sequences (<25% as of June 2024).

[h] Cross-reactivity resulting in false positive *Salmonell*a results has not been observed in analytical 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.

[i] Detected at concentrations near the *Vibrio* assay LoD.

[j] *Y. kristensenii* and *Y. fredericksenii* are difficult to distinguish from *Y. enterocolitica* by standard laboratory methods.

Table 44. Off-Panel Organisms Tested or Evaluated by in silico Analysis for BIOFIRE GI Panel Analytical Specificity

Species with cross-reactivity observed in analytical testing are in bold font. On-panel species were also tested at high concentration (not shown).

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

PROTOZOA/PARASITES				FUNGI
Tested		***In silico* Analysis Only**		**Tested**
Babesia microti	*Entamoeba gingivalis*	*Ancylostoma duodenale*	*Entamoeba hartmanni*	*Aspergillus fumigatus*
Blastocystis hominis	*Entamoeba moshkovskii*	*Ascaris lumbricoides*	*Entamoeba polecki*	*Candida albicans*
Conidiobolus lachnodes	*Giardia muris*	*Balantidium coli*	*Enterobius vermicularis*	*Candida catenulate*
Conidiobolus lobatus	*Pentatrichomonas hominis*	*Chilomastix mesnili*	*Enteromonas hominis*	*Penicillium marneffei*
Encephalitozoon hellem	*Schistosoma mansoni*	*Dientamoeba fragilis*	*Isospora belli*	*Saccharomyces boulardi*
Encephalitozoon intestinalis	*Toxoplasma gondii*	*Endolimax nana*	*Necator americanus*	*Saccharomyces cerevisiae*
Entamoeba dispar	*Trichomonas tenax*	*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] *Anerostipes hadrus* isolates (DSM 23942 and ATCC 29173) were tested at >2.4×10^8 cells/mL. Norovirus GI/GII Detected results were only observed with DSM 23942. The ATCC 29173 isolate does not carry the cross-reactive sequence.

[b] Though not observed in analytical testing, cross-reactivity of the *Giardia lamblia* assay with one or more *Bifidobacterium* and *Ruminococcus* species was observed in the clinical evaluation (see Table 43).

[c] Though not observed in analytical testing, possible cross-reactivity of the ETEC 2 assay with *Hafnia alvei* and *Cedecea davisiae* was observed in the clinical evaluation or predicted by in silico analysis (see Table 43).

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

[e] *Enterobacter hormaechei* isolates (ATCC BAA-2082 and ATCC 49162) were tested at >5.0×10^8 cells/mL. Norovirus GI/GII Detected results were only observed with ATCC 49162. The ATCC BAA-2082 sequence is not predicted to be cross-reactive by in silico analysis.

[f] Though not observed in analytical testing, cross-reactivity of the Noro 1 assay with a sequence identified in roughly half (~50%) of the *P. distasonis* genomes evaluated could occur at high concentration.

[g] A similar risk of cross-reactivity was identified with sequences annotated as *Parabacteroides* sp. and *P. acidifaciens*.

[h] No cross-reactivity with high concentrations of various *Prevotella* species (commensal and pathogenic) was observed in analytical testing, but the potential for weak cross-reactivity between the Noro 1 assay and unique variant sequences annotated as unculturable *Prevotella* sp. has been identified via investigation of discrepant results in clinical specimens.

[i] Though not observed in analytical testing, in silico analysis indicates that, similar to *Y. kristensenii*, cross-reactivity between the *Yersinia entercolitica* assay and *Yersinia fredericksenii* is possible at high concentrations (see Table 43).

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 BIOFIRE GI Panel. Reproducibility testing occurred at three test sites using a panel of contrived stool samples, each spiked with various combinations of four different BIOFIRE 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 BIOFIRE modules. 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 45. The BIOFIRE GI Panel provided 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%).

Table 45. Reproducibility of the BIOFIRE GI Panel Test Results on the BIOFIRE FILMARRAY System

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%)

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

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

[a] 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.

Similar reproducibility studies were also performed to determine between-site/system and overall reproducibility of the GI Panel on BIOFIRE 2.0 and BIOFIRE TORCH systems. For the BIOFIRE 2.0 study, testing occurred at three test sites using contrived stool samples, each spiked with various combinations of five different BIOFIRE GI Panel analytes representing the types of organisms detected by the panel (bacteria (including diarrheagenic *E. coli*), parasites, DNA viruses and RNA viruses). Each analyte was evaluated at three different concentrations (Negative, Low Positive and Moderate Positive). Negative results for each assay were obtained from samples that were not spiked with a corresponding organism (analyte not in the sample).

The data include 108 replicates per analyte and incorporate a range of potential variation introduced by 9 different operators, 3 different pouch lots, and 9 different BIOFIRE 2.0 modules configured on 3 different multi-instrument systems. Similar to the reproducibility of the BIOFIRE GI Panel on the BIOFIRE FILMARRAY System (Table 45 above), percent (%) agreement with the expected Detected, Not Detected or N/A result was 99.1% or better (Table 46).

The reproducibility of the BIOFIRE GI Panel results on the BIOFIRE TORCH system showed agreement with the expected Detected results in 97.0% of replicates for various representative analytes at 1×LoD and agreement with the expected Not Detected or N/A results was ≥99.0% (not shown).

Note: *Giardia lamblia* was tested in the BIOFIRE FILMARRAY System and BIOFIRE TORCH Reproducibility studies and was not detected in ≥95% of replicates tested at the 1× LoD concentration in either study (84.3% on BIOFIRE FILMARRAY System and 91.7% on BIOFIRE TORCH). It was subsequently determined that the integrity of the stock culture used to prepare the contrived samples had been compromised (requires storage in liquid nitrogen). Re-testing of 20 replicates of a sample prepared from a new stock culture resulted in the expected ≥95% detection at the 1× LoD concentration when tested on the on the BIOFIRE FILMARRAY System, BIOFIRE 2.0, and BIOFIRE TORCH systems.

Table 46. Reproducibility of the BIOFIRE GI Panel Test Results on the BIOFIRE 2.0 System

Organism Tested	Concentration Tested	Expected Result	% Agreement with Expected Result			
			Site/System			Total (95% Confidence Interval)
			A	B	C	
Clostridium difficile (toxinotype 0 A+B+) ATCC 9689	**Moderate Positive** 3× LoD 1.2x10^6 CFU/mL	Detected	36/36 100%	36/36 100%	36/36 100%	108/108 100% (96.6-100%)
	Low Positive 1× LoD 4.0x10^5 CFU/mL	Detected	35/36 97.2%	36/36 100%	36/36 100%	107/108 99.1% (95.0-100%)
	Negative	Not Detected	36/36 100%	36/36 100%	36/36 100%	108/108 100% (96.6-100%)
Shiga-toxin producing *Escherichia coli* O157 (STEC O157) ATCC 43895	**Moderate Positive** 3× LoD 3.0x10^4 CFU/mL	Detected	36/36 100%	36/36 100%	36/36 100%	108/108 100% (96.6-100%)
	Low Positive 1× LoD 1.0x10^4 CFU/mL	Detected	36/36 100%	36/36 100%	36/36 100%	108/108 100% (96.6-100%)
	Negative	Not Detected (STEC) and N/A (O157)	36/36 100%	36/36 100%	36/36 100%	108/108 100% (96.6-100%)
Cryptosporidium parvum Waterborne P102C	**Moderate Positive** 3× LoD 1.5x10^4 oocysts/mL	Detected	36/36 100%	36/36 100%	36/36 100%	108/108 100% (96.6-100%)
	Low Positive 1× LoD 5.0x10^3 oocysts/mL	Detected	35/36 97.2%	36/36 100%	36/36 100%	107/108 99.1% (95.0-100%)
	Negative	Not Detected	36/36 100%	36/36 100%	36/36 100%	108/108 100% (96.6-100%)
Adenovirus F41 ATCC VR-930	**Moderate Positive** 3× LoD 300 $TCID_{50}$/mL	Detected	36/36 100%	36/36 100%	36/36 100%	108/108 100% (96.6-100%)
	Low Positive 1× LoD 100 $TCID_{50}$/mL	Detected	36/36 100%	36/36 100%	36/36 100%	108/108 100% (96.6-100%)
	Negative	Not Detected	36/36 100%	36/36 100%	36/36 100%	108/108 100% (96.6-100%)
Astrovirus NCPV 10037071v	**Moderate Positive** 3× LoD 150 FFU/mL	Detected	36/36 100%	36/36 100%	36/36 100%	108/108 100% (96.6-100%)
	Low Positive 1× LoD 50 FFU/mL	Detected	36/36 100%	36/36 100%	36/36 100%	108/108 100% (96.6-100%)
	Negative	Not Detected	36/36 100%	36/36 100%	36/36 100%	108/108 100% (96.6-100%)

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 BIOFIRE 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 47), 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 47. 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 48).

Table 48. 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 49) 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 BIOFIRE GI Panel if vaccine is present in the test sample.

Table 49. 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 50), were evaluated for the potential of different media to interfere with the accuracy of BIOFIRE 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 50. 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.

APPENDIX A

Symbols Glossary

ISO 15223-1 Medical devices - Symbols to be used with medical devices labels, labeling and information to be supplied					
5.1.1	Manufacturer	5.1.2	Authorized representative in the European Community	5.1.4	Use-By date (YYYY-MM-DD)
5.1.5	Batch Code (Lot Number)	5.1.6	Catalog Number	5.1.7	Serial Number
5.2.8	Do Not Use if Package Is Damaged	5.3.2	Keep Away from Sunlight	5.3.7	Temperature Limit
5.4.2	Do Not Reuse	5.4.3	Consult Instructions for Use	5.5.1	*In vitro* Diagnostic Medical Device
5.5.5	Contains Sufficient For <n> Tests		5.7.10	Unique Device Identifier	
Use of Symbols in Labeling – 81 FR 38911, Docket No. (FDA-2013-N-0125)					
Rx Only	Prescription Use Only				
United Nations Globally Harmonized System of Classification and Labeling of chemicals (GHS) (ST/SG/AC.10/30)					
	Serious eye damage, Category 1		Acute toxicity, oral, Category 4 & Skin corrosion, irritation, Category 2		Acute aquatic hazard, Category 1 & Long-term aquatic hazard, Category 1
European *In Vitro* Diagnostic Regulation (IVDR 2017/746)			**UK Medical Devices Regulation 2002**		
	European Union Conformity			UKCA – UK Conformity Assessed	
Manufacture Symbols (BIOFIRE Diagnostics, LLC)					
	European Union Product Importer			A product in the BIOFIRE GI Panel family	
	BIOFIRE GI Panel				

APPENDIX B

Contact and Legal Information

Customer and Technical Support	
Reach Us on the Web http://www.BioFireDX.com **Reach Us by Email** BioFireSupport@biomerieux.com **Reach Us by Mail** 515 Colorow Drive Salt Lake City, UT 84108 USA	**Reach Us by Phone** 1-800-682-2666 – Toll Free (801) 736-6354 – Utah **Reach Us by Fax** (801) 588-0507
Customer and Technical Support outside of the U.S.	
Contact the local bioMérieux sales representative or an authorized distributor for technical support.	

Note: A paper copy of this Instructions for Use is available upon request by contacting Customer Support.

BioFire Diagnostics, LLC
515 Colorow Drive
Salt Lake City, UT 84108
USA

EC REP

QbD RePs BV
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2610 Wilrijk, Belgium

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376, Chemin de l'Orme
69280 Marcy l'Etoile-
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UKRP

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Basingstoke RG24 8NA
UK

NOTE FOR CUSTOMERS WITHIN THE EUROPEAN UNION (EU): Any serious incident that has occurred in relation to the device must be reported to BioFire Diagnostics, LLC or local bioMérieux sales representative and the competent authority of the Member State in which the user and/or the patient is established.

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Warranty Information

Product warranty information is available online at:

http://www.biofiredx.com/support/documents/

For warranty information for customers outside the United States, contact the local bioMérieux sales representative or an authorized distributor.

APPENDIX C

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REVISION HISTORY

Version	Revision Date	Description of Revision(s)
01-04	N/A	Previous Revisions
05	May 2021	Additions: • Revision history table • REACH regulation statement for EU customers • Adverse event reporting notice for EU customers in the EU • Intended Purpose, Intended User, and Use Environment statement • The following limitations were added: o The performance of this product has not been established for the screening of stool for stool transplants. o False positive and false negative results can be the result of a variety of sources and causes, it is important that these results be used in conjunction with other clinical, epidemiological, or laboratory information. o Cary Blair transport medium may contain non-viable organisms and/or nucleic acid at levels that can be detected by the BioFire GI Panel. o There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay. o Added to cross-reactivity limitation a risk of non-specific interaction with atypical *Prevotella* sequences. Updates to: • Symbols Glossary • E-labeling links • Fix typographical errors and formatting issues • Branding and logo • EC REP Address • Principle of the Procedure, Sample Requirements, Materials, Warnings, Precautions, External Controls, and Limitations sections to align with other BioFire Panels and post-market input. • Analytical Reactivity section to correct the test concentration for all Sapovirus isolates, one Adenovirus F41 isolate, and one Human Astrovirus isolate. • Analytical Specificity section to include testing of *Prevotella* species, risk of cross-reactivity with atypical *Prevotella* sequences, and include taxonomy updates. • Reproducibility section to address reproducibility on the BioFire 2.0 and Torch systems. Removals: • BioFire FilmArray (1st generation system) references and operation. Please refer to revision 04 for information on the operation of the BioFire GI Panel with the BioFire FilmArray.
06	March 2023	Additions: • CE updated to CE2797 on cover • UKCA symbol added to symbols glossary and cover • UK Authorized Rep Address added • UDI symbol added to symbols glossary • GI symbol added to symbols glossary • Additional Sample Buffer ampoule steps • Additional component information added to “Materials Provided” section Updates to: • EC updated to EU in front of importer information • Organization of IFU content including creation of Appendices A, B, and C • Updated Table 34 (*Cryptosporidium* Inclusivity Results) footnote a to indicate that sequence analysis predicts that *C. canis* will be reliably detected when present, but the clinical sample containing *Cryptosporidium canis* was at a concentration estimated (by qPCR) to be below the LoD • Detection summary for Torch Reproducibility study (adjusted from ≥99% to >97%) • Branding Removals • Removed first line (*Cryptosporidium canis*) of Table 34 – replaced with a footnote description of the testing condition and result. • “DO NOT REFRIGERATE” statement removed from the “Reagent Storage, Handling and Stability” section. • Rx symbol (not applicable to OUS customers)

07	August 2023	Additions: • Note in MATERIALS PROVIDED section regarding the new selective reporting option for *Clostridium difficile*. • Rx symbol (applicable to US customers) Updates to: • Customer Technical Support Email • Minor typographical errors and formatting Removals • Removal of volumes in MATERIALS PROVIDED section
08	November 2024	Additions: • Additional clinical data from 2023 related to post-marketing performance follow up study, including change in specificity for Norovirus result. Updates to: • Minor typographical errors and formatting • Clinical updates: Table 16. new demographic summary table for 2023 prospective clinical evaluation. Table 17. new GI/GII performance table for 2023 prospective clinical evaluation. • Analytical specificity (cross-reactivity) updates: Table 43. updated to include *Mediterraneibacter* (*Ruminococcus*) *gnavus*, *Parabacteroides spp*. (*P. merdae*, *P. acidifaciens*, *P. distasonis*), *Anaerostipes hadrus* (select sequences), and *Enterobacter hormaechei* (select sequences) as cross reactive with the Noro 1 assay. Table 44. updated to include all Off-panel testing and indicate which tested species had cross-reactivity confirmed in analytical testing (in bold). • Customer Support phone number. • Limitation #24 language updated to include false positive results. • Updated General Laboratory Precaution #3 to include more information for false positive test results. Removals • Removal of reference to instructions that explain how to use the BIOFIRE FILMARRAY GI Panel with the legacy BIOFIRE FILMARRAY Instrument. The legacy instrument is no longer manufactured or supported by bioMérieux. • IVDD Regulation from symbols glossary.
09	July 2025	Updates to: • EU Authorized Representative name and address • Procedure Section to align with other Panel documentation • Corrections of minor typographical errors Additions: • Note in the ‘Materials Provided’ section updated to include Norovirus.

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