510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K170308

B. Purpose for Submission:

To obtain a Substantial Equivalence Determination for the BD MAX Extended Enteric Bacterial Panel on the BD MAX System.

C. Measurand: Target DNA sequences for:

- *Plesiomonas shigelloides*
- *Vibrio (V. vulnificus, V. parahaemolyticus, and V. cholerae)*
- *Enterotoxigenic Escherichia coli (ETEC) heat-labile enterotoxin (LT)/ heat-stable enterotoxin (ST) genes*
- *Yersinia enterocolitica*

D. Type of Test:

Qualitative real-time polymerase chain reaction (PCR) assay for the amplification and detection of DNA from *Plesiomonas shigelloides, Vibrio (V. vulnificus, V. parahaemolyticus, and V. cholerae), Enterotoxigenic Escherichia coli (ETEC) heat-labile enterotoxin (LT)/ heat-stable enterotoxin (ST) genes* and *Yersinia enterocolitica* .

E. Applicant:

Becton Dickinson and Company, BD Diagnostic Systems

F. Proprietary and Established Names:

BD MAX Extended Enteric Bacterial Panel (xEBP)
BD MAX System

G. Regulatory Information:

1. Regulation section:

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

2. Classification:

Class II

3. Product code:

   PCI, PCH, OOI

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use:

   The BD MAX Extended Enteric Bacterial Panel performed on the BD MAX System, is an
   automated in vitro diagnostic test for the direct qualitative detection and differentiation of enteric bacterial pathogens. It is used in conjunction with the BD MAX Enteric Bacterial Panel as an optional Master Mix. The BD MAX Extended Enteric Bacterial Panel detects nucleic acids from

   - *Plesiomonas shigelloides*
   - *Vibrio* (*V. vulnificus, V. parahaemolyticus*, and *V. cholerae*)
   - Enterotoxigenic *Escherichia coli* (ETEC) heat-labile enterotoxin (LT)/ heat-stable
   - enterotoxin (ST) genes
   - *Yersinia enterocolitica*

   Testing is performed on soft to diarrheal unpreserved stool specimens or Cary-Blair preserved stool specimens from symptomatic patients with suspected acute gastroenteritis, enteritis or colitis. The test is performed directly on the specimen, utilizing real-time polymerase chain reaction (PCR) for the amplification of relevant gene target DNA. The test utilizes fluorogenic gene-specific hybridization probes for the detection of the amplified DNA.

   This test is intended for use, in conjunction with clinical presentation, laboratory findings, and epidemiological information, as an aid in the differential diagnosis of *Plesiomonas shigelloides*, *Vibrio* (*V. vulnificus, V. parahaemolyticus*, and *V. cholerae*), Enterotoxigenic *Escherichia coli* (ETEC) LT/ST and *Yersinia enterocolitica* infections. Results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results do not rule out coinfection with other organisms that are not detected by this test, and may not be the sole or definitive cause of patient illness. Negative results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For Prescription Use Only

4. Special instrument requirements:

   The assay is performed on the BD MAX System.

I. Device Description:

The BD MAX System and the BD MAX Extended Enteric Bacterial Panel are comprised of an instrument with associated hardware and accessories, disposable microfluidic cartridges, real-time PCR master mixes, unitized reagent strips, extraction reagents, and sample buffer tubes (SBT). The instrument automates sample preparation including target lysis, DNA extraction and concentration, reagent rehydration, and target nucleic acid amplification and detection using real-time PCR. The assay includes a Sample Processing Control (SPC) that is present in the Extraction Tube. The SPC monitors DNA extraction steps, thermal cycling steps, reagent integrity and the presence of inhibitory substances. The BD MAX System software automatically interprets test results.

Brief Explanation of the Procedure

A soft to diarrheal stool is collected and transported to the laboratory. After the stool has been homogenized, a disposable inoculating loop is used to collect a 10 µL aliquot of the stool material and the contents of the loop are dispensed into a SBT. The SBT is closed with a septum cap and vortexed. A worklist is created and the SBT, the BD MAX Enteric Bacterial Panel Unitized Reagent Strips (URS) and the BD MAX PCR cartridge are loaded onto the BD MAX System. The BD MAX System automates sample preparation including cell lysis, DNA extraction and concentration, reagent rehydration, and target nucleic acid amplification and detection using real-time PCR. Amplified targets are detected with hydrolysis probes labeled with quenched fluorophores. The amplification, detection and interpretation of the signals are done automatically by the BD MAX System.

Reagents Provided with the BD MAX Extended Enteric Bacterial Panel

- BD MAX Extended Enteric Bacterial Panel Master Mix: Dried PCR Master Mix containing DNA polymerase, nucleotides, target and Sample Processing Control-specific probe and primers.

Equipment and Materials Required But Not Provided

- BD MAX Enteric Bacterial Panel (BD Diagnostic Systems Catalog no. 442963)
- BD MAX PCR Cartridges (BD Diagnostic Systems Catalog no. 437519)
- VWR Multi-Tube Vortexer (VWR catalog no. 58816-115)
- Vortex Genie 2 or equivalent (VWR, Cat. No. 58815-234)NALGENE®
- Cryogenic Vial Holder (VWR, catalog no. 66008-783)
- Rack compatible with a multi-tube vortex mixer (e.g., Cryogenic Vial Holder or equivalent)
- Disposable 10 µL inoculating loops (BD Diagnostic Systems Catalog no. 220216)
- Lab coat and disposable gloves, powderless
- Sterile scissors (optional)
- Stopwatch or timer

J. Substantial Equivalence Information:

1. Predicate device name(s):

   BioFire Diagnostics FilmArray Gastrointestinal (GI) Panel

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

   K160459

3. Comparison with predicate:

Similarities		
Item	*BD MAX xEBP*	*FilmArray GI Panel (K160459)*
Intended Use	The BD MAX Extended Enteric Bacterial Panel performed on the BD MAX System, is an automated in vitro diagnostic test for the direct qualitative detection and differentiation of enteric bacterial pathogens. It is used in conjunction with the BD MAX Enteric Bacterial Panel as an optional Master Mix. The BD MAX Extended Enteric Bacterial Panel detects nucleic acids from • *Plesiomonas shigelloides* • *Vibrio* (*V. vulnificus, V. parahaemolyticus*, and *V. cholerae*) • Enterotoxigenic *Escherichia coli* (ETEC) heat-labile enterotoxin (LT)/ heat-stable • enterotoxin (ST) genes • *Yersinia enterocolitica* Testing is performed on soft to diarrheal unpreserved stool specimens or Cary-Blair	The FilmArray Gastrointestinal (GI) Panel is a qualitative multiplexed nucleic acid-based in vitro diagnostic test intended for use with FilmArray systems. The FilmArray GI Panel is capable of the simultaneous detection and identification of nucleic acids from multiple bacteria, viruses, and parasites directly from stool samples in Cary Blair transport media obtained from individuals with signs and/or symptoms of gastrointestinal infection. The following bacteria (including several diarrheagenic *E. coli/Shigella* pathotypes), parasites, and viruses are identified using the FilmArray GI Panel: - *Campylobacter (C. jejuni/C. coli/C. upsaliensis)* - *Clostridium difficile (C. difficile)* toxin A/B - *Plesiomonas shigelloides* - *Salmonella* - *Vibrio (V. parahaemolyticus/V. vulnificus/V. cholerae)*, including specific identification of *Vibrio cholerae* - *Yersinia enterocolitica* - Enteroaggregative *Escherichia coli* (EAEC) - Enteropathogenic *Escherichia coli* (EPEC) - Enterotoxigenic *Escherichia coli* (ETEC) lt/st

Similarities		
Item	*BD MAX xEBP*	*FilmArray GI Panel (K160459)*
	preserved stool specimens from symptomatic patients with suspected acute gastroenteritis, enteritis or colitis. The test is performed directly on the specimen, utilizing real-time polymerase chain reaction (PCR) for the amplification of relevant gene target DNA. The test utilizes fluorogenic gene-specific hybridization probes for the detection of the amplified DNA. This test is intended for use, in conjunction with clinical presentation, laboratory findings, and epidemiological information, as an aid in the differential diagnosis of *Plesiomonas shigelloides*, *Vibrio* Group (*V. vulnificus, V. parahaemolyticus*, and *V. cholerae*), Enterotoxigenic *Escherichia coli* (ETEC) LT/ST and *Yersinia enterocolitica* infections. Results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results do not rule out coinfection with other organisms that are not detected by this test, and may not be the sole or definitive cause of patient illness. Negative results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.	- Shiga-like toxin-producing *Escherichia coli* (STEC) stx1/stx2 (including specific identification of the *E. coli* O157 serogroup within STEC) - *Shigella*/Enteroinvasive *Escherichia coli* (EIEC) - *Cryptosporidium* - *Cyclospora cayetanensis* - *Entamoeba histolytica* - *Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*) - Adenovirus F 40/41 - Astrovirus - Norovirus GI/GII - Rotavirus A - Sapovirus (Genogroups I, II, IV, and V) The FilmArray GI Panel is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness and results are meant to be used in conjunction with other clinical, laboratory, and epidemiological data. Positive results do not rule out co-infection with organisms not included in the FilmArray GI Panel. The agent detected may not be the definite cause of the disease. Concomitant culture is necessary for organism recovery and further typing of bacterial agents. This device is not intended to monitor or guide treatment for *C. difficile* infection. Due to the small number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for *E. coli* O157, *Plesiomonas shigelloides, Yersinia enterocolitica, Astrovirus,* and *Rotavirus A* were established primarily with retrospective clinical specimens. Performance characteristics for *Entamoeba histolytica,* and *Vibrio (V. parahaemolyticus, V. vulnificus,* and *Vibrio cholerae)* were established primarily using contrived clinical specimens. Negative FilmArray GI Panel results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease. A gastrointestinal microorganism multiplex nucleic acid-based assay also aids in the detection and identification of acute gastroenteritis in the context of outbreaks.
Specimen Type	Cary-Blair preserved stool Unpreserved soft to diarrheal stool	Cary-Blair preserved stool. Not claimed (see Differences below)
Assay Format	Amplification: PCR Detection: fluorogenic target-specific hybridization.	Amplification: PCR Detection: non target-specific fluorescent dye
Organisms Detected	• *Plesiomonas shigelloides* • *Vibrio (V. vulnificus, V. parahaemolyticus,* and *V. cholerae)* • Enterotoxigenic *Escherichia coli* heat labile and heat stabile (LT/ST) (ETEC) • *Yersinia enterocolitica*	• *Plesiomonas shigelloides* • *Vibrio (V. parahaemolyticus/V. vulnificus/ V. cholerae)*, including specific identification of *Vibrio cholerae* • Enterotoxigenic *Escherichia coli (ETEC) lt/st* • *Yersinia enterocolitica*
Interpretation of Test Results	Automated: BD MAX System diagnostic software	Automated
Analysis Platform	BD MAX System	Film Array Instrument

Similarities		
Item	*BD MAX xEBP*	*FilmArray GI Panel (K160459)*
PCR Sample preparation	Automated: BD MAX System	Automated: Film Array Instrument
Detection Probes	TaqMan Probe	Fluorescent double stranded DNA binding dye (LC Green Plus)
Assay Controls	Sample Processing Control (SPC)	Two controls are included in each reagent pouch to control for sample processing and both stages of PCR and melt analysis.

Differences		
Item	*BD MAX xEBP*	*FilmArray GI Panel*
Specimen Type	Unpreserved soft to diarrheal stool	Not claimed
Organisms Detected	Listed in device Similarities above.	Other organisms detected: • *Campylobacter* (*C. jejuni/C. coli/C. upsaliensis*) • *Clostridium difficile* (*C. difficile*) toxin A/B • *Salmonella* • Enteroaggregative *Escherichia coli* (EAEC) • Enteropathogenic *Escherichia coli* (EPEC) • 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)

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

Class II Special Controls Guideline: Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assays for Detection and Identification of Microorganisms and Toxin Genes from Human Stool

L. Test Principle:

A stool specimen is collected and transported to the laboratory in a dry, clean container (for unpreserved specimens) or in Cary-Blair transport media. The specimen is vortexed for 15 seconds and then a 10 µL loop is used to inoculate a BD MAX Extended Enteric Bacterial Panel and a BD MAX Extended Enteric Bacterial Panel Sample Buffer Tube included in the BD MAX Enteric Bacterial Panel kit. The Sample Buffer Tube is closed with a septum cap, vortexed and transferred to the BD MAX System. A worklist is created and the Sample Buffer Tubes, the BD MAX Enteric Bacterial Panel Unitized Reagent Strip (containing both the BD MAX EBP and BD MAX xEBP master mix reagents) and the BD MAX PCR Cartridges are loaded onto the BD MAX System.

Following enzymatic bacterial cell lysis at elevated temperature, the released nucleic acids

are captured on magnetic beads. The beads, with the bound nucleic acids, are washed using Wash Buffer and the nucleic acids are eluted by heat in Elution Buffer. Eluted DNA is neutralized using Neutralization Buffer and transferred to the Master Mix tubes to rehydrate the PCR reagents. After rehydration, the BD MAX System dispenses a fixed volume of PCR-ready solution containing extracted nucleic acids into the BD MAX PCR Cartridge. Microvalves in the BD MAX PCR Cartridge are sealed by the system prior to initiating PCR to contain the amplification mixture, thus preventing evaporation and amplicon contamination.

The amplified DNA targets are detected using hydrolysis (TaqMan®) probes labeled at one end with a fluorescent reporter dye (fluorophore) and at the other end with a quencher moiety. Probes labeled with different fluorophores are used to detect the amplicons of the enteric bacterial targets (*Plesiomonas shigelloides, Vibrio* (*V. vulnificus, V. parahaemolyticus*, and *V. cholerae*), heat labile and heat stabile (LT/ST) ETEC (Enterotoxigenic *E. coli*) and *Yersinia enterocolitica*) and the Sample Processing Control amplicons in five different optical channels of the BD MAX System. When the probes are in their native state, the fluorescence of the fluorophore is quenched due to its proximity to the quencher. However, in the presence of target DNA, the probes hybridize to their complementary sequences and are hydrolyzed by the 5'–3' exonuclease activity of the DNA polymerase as it synthesizes the nascent strand along the cDNA template. As a result, the fluorophores are separated from the quencher molecules and fluorescence is emitted. The BD MAX System monitors these signals at each cycle, and interprets the data at the end of the program to report the final results.

M. Performance Characteristics:

1. Analytical performance:

   a. Precision/Reproducibility:

   Precision

   Within-laboratory precision was evaluated for the BD MAX Extended Enteric Bacterial Panel at one (1) internal site. Testing was performed over 12 days, with two runs per day (one each by 2 operators), for a total of 24 runs. The Precision panel members were divided into four (4) concentration categories, based upon organism concentration relative to the LoDs established for each of the assay targets and expected correct percent positive/negative. The panel members contained *Vibrio cholerae*, *Plesiomonas shigelloides*, ETEC and *Yersinia enterocolitica*. The following values were used as spike levels for the target organisms contained in each panel member:

   - Moderate Positive (MP): ≥2 to ≤3x LoD; positive approximately 95% of the time
   - Low Positive (LP): ≥1 to <2x LoD; positive approximately 95% of the time
   - High Negative (HN): C20-80 LoD; negative between 20 and 80% of the time

- True Negative (TN): No target; negative 100% of the time

Each panel member was spiked with negative unpreserved stool matrix. True negative samples contained no target. High negative samples were spiked with target organisms below the analytical LoD of the assay; however, the HN samples were expected to yield a positive result in approximately 20% to 80% of the replicates due the inherent sensitivity of the PCR assays. Results are summarized by target and concentration below**.**

Precision study result Using One Lot of the BD MAX Extended Enteric Bacterial Panel

Category	***Agreement with Expected Results***			
	Vibrio (95% CI)	*P. shigelloides (95% CI)*	*Y. enterocolitica (95% CI)*	*ETEC (95% CI)*
TN[a]	100 96/96 (96.2, 100)	100 96/96 (96.2, 100)	100 96/96 (96.2, 100)	100 96/96 (96.2, 100)
HN[a]	58.3 28/48 (44.3, 71.2)	45.8 22/48 (32.6, 59.7)	41.7 20/48 (28.8, 55.7)	47.9 23/48 (34.5, 61.7)
LP	100 48/48 (92.6, 100)	100 48/48 (92.6, 100)	97.9 47/48 (89.1, 99.6)	100 48/48 (92.6, 100)
MP	100 48/48 (92.6, 100)	100 48/48 (92.6, 100)	100 48/48 (92.6, 100)	97.9 47/48 (89.1, 99.6)

[a] For the True Negative (TN) and High Negative (HN) categories, the expected assay result was deemed to be negative. Therefore, percent agreement was calculated for negative results.

Site-to-site Reproducibility

The Site-to-Site reproducibility study was performed at three (3) clinical sites using one (1) reagent lot. Two (2) operators performed 2 runs per day, over five (5) distinct days (consecutive or not), for a total of 30 runs. The panels used were the same as described under the Precision heading, above.

The overall site-to-site reproducibility percent agreement was 100% for the TN category for all targets, and ranged from 30.0 to 48.9%, 97.8 to 100 % and 98.9 to 100% for the HN, LP and MP categories, respectively. Results are summarized in Table 1. The quantitative reproducibility results across sites by target are presented in Table 2**.** Ct. Score is an internal criterion used to determine final assay results and was selected as a means of assessing quantitative assay reproducibility. Mean Ct. Score and the mean Cycle EP values with variance components (SD and % CV) are shown in Table 2**.**

Table 1: Site-to-Site Reproducibility Results Using One Lot of the BD MAX Extended Enteric Bacterial Panel

Category	*Agreement with Expected Results*			
	Vibrio (95% CI)	*P. shigelloides (95% CI)*	*Y. enterocolitica (95% CI)*	*ETEC (95% CI)*
TN[a]	100 180/180 (97.9, 100)	100 180/180 (97.9, 100)	100 180/180 (97.9, 100)	100 180/180 (97.9, 100)
HN[a]	48.9 44/90 (38.8, 59.0)	30.0 27/90 (21.5, 40.1)	35.6 32/90 (26.4, 45.8)	46.7 42/90 (36.7, 56.9)
LP	100 90/90 (95.9, 100)	98.9 89/90 (94.0, 99.8)	100 90/90 (95.9, 100)	97.8 88/90 (92.3, 99.4)
MP	100 90/90 (95.9, 100)	98.9 89/90 (94.0, 99.8)	98.9 89/90 (94.0, 99.8)	100 90/90 (95.9, 100)

[a] For the True Negative (TN) and High Negative (HN) categories, the expected assay result was deemed to be
negative. Therefore, percent agreement was calculated for negative results.

Table 2: Quantitative Site-to-Site Reproducibility Results Summary

PCR Metric	***Parameter***	***Vibrio***			***P. shigelloides***			***Y. enterocolitica***			***ETEC***			***SPC***
		HN	*LP*	*MP*	*HN*	*LP*	*MP*	*HN*	*LP*	*MP*	*HN*	*LP*	*MP*	*TN*
Ct. Score	N	46	90	90	63	89	89	58	90	89	48	88	90	90
	Mean	36.9	32.1	31.7	36.0	31.5	30.7	36.3	32.9	32.7	36.8	33.7	32.4	27.2
	SD	1.18	0.45	0.56	1.50	0.90	0.74	1.06	0.78	0.64	1.37	0.80	0.72	0.40
	%CV	3.2	1.4	1.8	4.2	2.9	2.4	2.9	2.4	2.0	3.7	2.4	2.2	1.5
Cycle EP	N	46	90	90	63	89	89	58	90	89	48	88	90	90
	Mean	805.1	2725.7	3076.6	596.5	2349.7	2320.9	599.6	1191.1	1264.2	976.0	2049.6	2640.0	6020.7
	SD	350.40	812.29	485.26	284.02	416.36	630.12	296.66	317.31	388.98	577.46	529.33	598.65	688.74
	%CV	43.5	29.8	15.8	47.6	17.7	27.1	49.5	26.6	30.8	59.2	25.8	22.7	11.4

The Lot-to-lot reproducibility study was performed at one (1) site using three (3) reagent lots. Two (2) operators performed 2 runs per day, over five (5) distinct days (consecutive or not), for a total of 30 runs. The panels used were the same as described under the Precision heading, above. Results from 5 days of the accuracy and precision study were used to comprise data for one lot of reagents for the Lot-to-Lot study.

The overall Lot-to-lot reproducibility percent agreements were 100% for TN, and ranged from 23.3 to 41.1%, 97.8 to 100 % and 98.9 to 100% for the HN, LP and MP respectively. Results are summarized in Table 3. The quantitative results across lots and by target are presented in Table 8 and Table 6**.** Cycle threshold score and the Cycle EP, an internal criteria used to determine a final assay result, was selected as a means of assessing quantitative assay reproducibility. Mean Ct. Score and the mean Cycle EP values with variance components (SD and % CV) are shown in Table 4**.**

Table 3: Lot-to-lot Reproducibility Results for BD MAX Extended Enteric Bacterial Panel

Category	*Agreement with Expected Results*			
	Vibrio (95% CI)	*P. shigelloides (95% CI)*	*Y. enterocolitica (95% CI)*	*ETEC (95% CI)*
TN[a]	100 180/180 (97.9, 100)	100 180/180 (97.9, 100)	100 180/180 (97.9, 100)	100 180/180 (97.9, 100)
HN[a]	41.1 37/90 (31.5, 51.4)	28.9 26/90 (20.5, 39.0)	23.3 21/90 (15.8, 33.1)	41.1 37/90 (31.5, 51.4)
LP	100 90/90 (95.9, 100)	100 90/90 (95.9, 100)	100 90/90 (95.9, 100)	97.8 88/90 (92.3, 99.4)
MP	100 90/90 (95.9, 100)	100 90/90 (95.9, 100)	98.9 89/90 (94.0, 99.8)	100 90/90 (95.9, 100)

[a] For the True Negative (TN) and High Negative (HN) categories, the expected assay result was deemed to be negative. Therefore, percent agreement was calculated for negative results.

Table 4: Quantitative Lot-to-lot Reproducibility Results Summary

PCR Metric	***Parameter***	***Vibrio***			***P. shigelloides***			***Y. enterocolitica***			***ETEC***			***SPC***
		HN	*LP*	*MP*	*HN*	*LP*	*MP*	*HN*	*LP*	*MP*	*HN*	*LP*	*MP*	*TN*
Ct. Score	N	53	90	90	64	90	90	69	90	89	53	88	90	90
	Mean	36.6	32.2	31.9	35.8	31.3	30.7	36.1	32.4	32.3	37.0	33.6	32.4	27.2
	SD	1.18	0.51	0.64	1.42	0.61	0.90	1.29	0.94	0.93	1.06	0.78	0.87	0.30
	%CV	3.2	1.6	2.0	4.0	2.0	2.9	3.6	2.9	2.9	2.9	2.3	2.7	1.1
Cycle EP	N	53	90	90	64	90	90	69	90	89	53	88	90	90
	Mean	931.1	3124.7	3033.5	686.9	2444.1	2428.7	731.3	1339.7	1370.3	838.3	2056.8	2244.2	5544.7
	SD	369.81	242.75	236.53	337.36	491.79	416.43	296.44	158.41	165.73	332.37	419.99	458.59	633.70
	%CV	39.7	7.8	7.8	49.1	20.1	17.1	40.5	11.8	12.1	39.6	20.4	20.4	11.4

b. Linearity/assay reportable range:

Not applicable

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

Positive and Negative External Controls

External Positive Controls are intended to monitor for substantial reagent failure and External Negative Controls are used to detect reagent or environmental contamination (or carry-over) from positive specimens or nucleic acids (amplicon). External controls are not provided with the BD MAX Extended Enteric Bacterial Panel; however recommendations for control preparation and testing are provide in the package insert.

During the clinical study, external positive and negative controls were included in each run and in the case of a failure of either or both external controls, testing of all

samples included in the run were repeated from the stored SBTs. Negative external controls consisted of saline. Positive controls were prepared as organism suspensions as detailed in the table below. Each positive control was tested on a rotating basis.
BD MAX xEBP External Controls

External Negative and Positive Control Panels

Target	***ATCC Strain***	***Suspension Preparation in Saline***	***Final Concentration in Ready to use 2 mL Tubes***
Yersinia enterocolitica	9610	0.5 McF	1.5 X 10^5 CFU/mL
Vibrio cholerae *or* *Vibrio parahaemolyticus*	14033 or 17802		1.5 X 10^5 CFU/mL or 1.0 X 10^5 CFU/mL
Plesiomonas shigelloides	14029		3.0 X 10^5 CFU/mL
ETEC	35401		1.5 X 10^4 CFU/mL
Negative	-	Saline	-

Each testing site included one (1) positive external control and one (1) negative external control in each BD MAX run. A total of 186 runs were performed. Of those, 171 runs (91.9%) passed. The pass rate ranged between 85.3% and 95.7% between sites. All samples included in a run with EC failure were repeated or remained invalid if it was a final run. Runs with valid External Controls remained high throughout the trial.

Internal Specimen Processing Control:

Each BD MAX Extended Enteric Bacterial Extraction Tube contains a Sample Processing Control (SPC) which is a plasmid containing a synthetic target DNA sequence. The SPC monitors the efficiency of DNA capture, washing and elution during the sample processing steps, as well as the efficiency of DNA amplification and detection during PCR analysis. If the SPC result fails to meet the acceptance criteria, the test result of will be reported as Unresolved (UNR). An Unresolved result is indicative of sample-associated inhibition or reagent failure. The user is instructed to repeat any sample reported as Unresolved.

Specimen Stability Study: (Preservation of DNA in stool or Cary-Blair Media)

Pooled unpreserved or Cary-Blair preserved negative stool specimens were spiked with BD MAX xEBP targets and tested according to the storage conditions described in the table below. An additional storage of 3 hours at room temperature was also applied to every SBT following inoculation in order to mimic a full run of the BD MAX xEBP, representing the normal customer workflow. To further represent normal workflow, SBT septum caps were pierced before storage at the appropriate temperature (including the initial 3-hour storage period).

Storage Conditions

Condition	*In Collection Device or Transport Medium*	*In SBT*
0	No storage	
1A	24h at 25°C (Unpreserved and Cary-Blair preserved samples)	+ 48h at 25°C
1B		+ 48h + 10% at 25°C
1C		+ 120h at 2-8°C
1D		+ 120h + 20% at 2-8°C
2A	120h at 2-8°C (Unpreserved and Cary-Blair preserved samples)	+ 48h at 25°C
2B		+ 48h + 10% at 25°C
2C		+ 120h at 2-8°C
2D		+ 120h + 20% at 2-8°C

For each xEBP target and for all test conditions ≥ 95% expected results for positive and negative samples were required.

Only positive and negative results were included to determine the percentage of detection at each concentration. All non-reportable results (UNR/IND/INC) and unexpected results (ex: false positive in a channel not being evaluated) were required to be repeated from the SBT. Valid results upon repeat were included in the analysis. If the non-reportable/unexpected result did not resolve upon repeat, it was excluded from the study.

Cary-Blair Preserved Samples:

The acceptance criteria were met for positive and negative samples at all time-points analyzed (T0 to 1D) following an initial incubation time of 24h at 25°C in the collection device, except for one condition:

- ETEC negative condition (1C): *24h at 25°C plus 120h at 2-8°C* resulted in 91% (51/56). The failure was investigated and determined to be a result of poor handling while preparing the samples for the study. Specifically, it was determined that the same pipette tip that was used for manually piercing the positive tubes was used for piercing the negative tubes, resulting in contamination. These failures are not linked to assay performance as is further demonstrated with passing results at the last time point (1D) (*24h at 25°C plus 120h + 20% at 2-8°C*).

The acceptance criteria were met for positive and negative samples at all time-points analyzed (2A to 2D) following an initial incubation time of 120h at 2-8°C in the

collection device, except for two conditions, both representing buffer time point conditions:

- Plesiomonas shigelloides positive condition (2B): 120h at 2-8°C plus 48h +10% at 25°C resulted in 93% (26/28);
- Vibrio (V. cholerae, V. vulnificus and V. parahaemolyticus) positive condition (2D): 120h at 2-8°C plus 120h +20% at 2-8°C resulted in 93% (26/28).

Considering that a trend was not observed, the results for *Plesiomonas shigelloides* are likely due to the variability of quantification at low levels, resulting in insufficient low target spike and thus not indicative of stability. The method for quantification is based on plate count, for which up to 30% of variation can be expected. For *Vibrio*, if the failure was truly due to stability, it is reasonable to expect a failure at condition 2B, whereby the samples were held at a worst case room temperature condition.

Unpreserved Samples:
The acceptance criteria were met for positive and negative samples at all time-points analyzed (T0 to 1D) following an initial incubation time of 24h at 25°C in the collection device, except for one condition:

- ETEC negative condition (1C): *24h at 25°C plus 120h +20% at 2-8°C* resulted in 93% (52/56). These samples were prepared by the same operator and therefore impacted by the same handling error as described above for ETEC negative condition (1) in preserved matrix.

The acceptance criteria were met for negative and positive samples at all time-points analyzed following an initial incubation time of 120h at 2-8°C in the collection device, except for one condition:

- Vibrio (V. cholerae, V. vulnificus and V. parahaemolyticus) positive condition (2B): 120h at 2-8°C plus 48h +10% at 25°C resulted in 93% (26/28).

If the failure was truly due to stability, it is reasonable to expect a failure at condition 1B, whereby the samples were held at a worst case room temperature initial hold for 24 hours followed by room temperature storage for an additional
48 + hours.

d. Detection limit:

The analytical sensitivity (Limit of Detection or LoD) for the BD MAX Extended Enteric Bacterial Panel was determined as follows: Each target organism was prepared and quantified from culture prior to inclusion in this study. Individual inoculating loops were dipped into each organism preparation and were then transferred to a Sample Buffer Tube already containing fecal matrix (preserved or unpreserved) that was pre-determined to be negative for all the targets detected by the BD MAX Extended Enteric Bacterial Panel. Each organism was tested with a

minimum of 24 replicates per sample type (preserved or unpreserved), by 2 operators, using 3 different production lots of the BD MAX Extended Enteric Bacterial Panel. The LoD for a specific organism was confirmed by testing at least 24 additional replicates at the determined LoD concentration. Analytical sensitivity (LoD), defined as the lowest concentration at which greater than 95% of all replicates are expected to test positive, ranged from 34 to 534 CFU/SBT and 3,434 to 53,852 CFU/mL (in stool) for unpreserved specimens for both strains and 79 to 257 CFU/SBT and 7,860 to 25,712 CFU/mL (in stool) for preserved specimens.

BD MAX Extended Enteric Bacterial Panel Limit of Detection for Individual Target

	Unpreserved (1st strain)		***Unpreserved (2nd strain)***		***Cary-Blair Preserved (1st strain)***	
Target organism	***LoD[1] (CFU/SBT)***	***LoD[1] (CFU/mL in stool)***	***LoD[1] (CFU/SBT)***	***LoD[1] (CFU/mL in stool)***	***LoD[1] (CFU/SBT)***	***LoD[1] (CFU/mL in stool)***
Plesiomonas shigelloides ATCC 14029[a]; ATCC 14030[b]	458	45,752	155	15,481	257	25,712
Yersinia enterocolitica ATCC 9610[a]; CCUG 4588[b]	209	20,900	311	31,099	227	22,723
ETEC ST/LT ATCC 35401[a]; CCUG 47194[b]	34	3,434	539	53,852	137	13,706
Vibrio cholerae ATCC 14033[a]; ENF 9786[b]	149	14,942	43	4,344	252	25,238
Vibrio parahaemolyticus ATCC17802[a]; ENF 5887[b]	207	20,708	80	8,031	124	12,424
Vibrio vulnificus ATCC 27562[a] 2; ENF 10727[b]	131	13,093	80	7,959	79	7,860

[1]LoD concentrations are expressed in CFU/SBT and CFU/mL, except for *Vibrio*. for which it is expressed in cells/SBT and cells/mL.

[a]1st strain

[b]2nd strain

e. Analytical Reactivity (Inclusivity):

A variety of BD MAX Extended Enteric Bacterial Panel assay target strains were included in this study. Strain selection criteria included prevalence, serotype and geographic location, where appropriate. Sixty-nine (69) strains were tested, including strains from public collections and well-characterized clinical isolates.

Inclusivity testing included 10 strains of Plesiomonas shigelloides, 10 strains of Yersinia enterocolitica, 36 strains of Vibrio (cholerae, parahaemolyticus and vulnificus) and 13 strains of ETEC LT/ST. The strains were tested at < 3 x LoD (Limit of Detection) of the corresponding strain in unpreserved stool matrix. The BD MAX Extended Enteric Bacterial Panel correctly identified 68 of the 69 strains tested upon initial testing. One strain of ETEC ST/LT (CCUG 38088) did not meet acceptance criteria and was further evaluated. This strain was titrated and tested to determine the minimum concentration sufficient for detection. Upon repeat, the CCUG 38088 strain of ETEC ST/LT was detected at 10 x LoD.

Three replicates of each strain were tested at 2.99x LoD in unpreserved stool matrix. Three different combinations of reagent lots and 3 lots of negative unpreserved stool matrix were used.

Analytical Reactivity Results in Unpreserved Stool

SBT	***Target***	***ATCC or other collection number***	***Assay result at 2.99x***					***Non-Reportable***			***Total SBT overall***
			Yersi	*ETEC*	*Vibrio*	*Plesio*	*Strain detected*	*IND*	*UNR*	*Other*	
1	*Plesiomonas shigelloides*	ATCC 14029	0/3	0/3	0/3	3/3	Yes	0	0	0	3
2		ATCC 14030	0/3	0/3	0/3	3/3	Yes	0	0	0	3
3		ENF 9782	0/3	0/3	0/3	3/3	Yes	0	0	0	3
4		ENF 9000	0/3	0/3	0/3	3/3	Yes	0	0	0	3
5		ENF 10445	0/3	0/3	0/3	3/3	Yes	0	0	0	3
6		ENF 15744	0/3	0/3	0/3	3/3	Yes	0	0	0	3
7		CCUG 7041A	0/3	0/3	0/3	3/3	Yes	0	0	0	3
8		CCUG 9221	0/3	0/3	0/3	3/3	Yes	0	0	0	3
9		CCUG 14309	0/3	0/3	0/3	3/3	Yes	0	0	0	3
10		CCUG 14597	0/3	0/3	0/3	3/3	Yes	0	0	0	3
11	*Yersinia enterocolitica*	CCUG 4588	3/3	0/3	0/3	0/3	Yes	0	1	1	4
12		ATCC 9610	3/3	0/3	0/3	0/3	Yes	0	0	0	3
13		ATCC 27729	3/3	0/3	0/3	0/3	Yes	0	0	0	3
14		NA	3/3	0/3	0/3	0/3	Yes	0	0	0	3
15		NA	3/3	0/3	0/3	0/3	Yes	0	0	0	3
16		NA	3/3	0/3	0/3	0/3	Yes	0	0	0	3
17		NA	3/3	0/3	0/3	0/3	Yes	0	0	0	3
18		CCUG 8050	3/3	0/3	0/3	0/3	Yes	0	1	1	5
19		CCUG 8232	3/3	0/3	0/3	0/3	Yes	0	0	0	3
20		CCUG 8234	3/3	0/3	0/3	0/3	Yes	0	0	0	3
21	*Vibrio parahaemolyticus*	ATCC 17802	0/3	0/3	3/3	0/3	Yes	0	0	0	3
22		ENF 5887	0/3	0/3	3/3	0/3	Yes	0	0	0	3
23		ATCC BAA-241	0/3	0/3	3/3	0/3	Yes	0	0	0	3
24		ATCC 33847	0/3	0/3	3/3	0/3	Yes	0	0	0	3
25		ATCC 49529	0/3	0/3	3/3	0/3	Yes	0	0	0	3
26		ATCC 43996	0/3	0/3	3/3	0/3	Yes	0	0	0	3
27		ATCC 33846	0/2	0/2	2/2	0/2	Yes	1	0	1	4
28		ATCC BAA-242	0/3	0/3	3/3	0/3	Yes	0	0	1	4
29		ENF 2508	0/3	0/3	3/3	0/3	Yes	0	0	0	3
30		ENF 8159	0/3	0/3	3/3	0/3	Yes	0	0	0	3
57		CCUG 67711	0/3	0/3	2/3	0/3	Yes	2	0	0	5
58		CCUG 34902	0/3	0/3	3/3	0/3	Yes	0	0	0	3
31	*Vibrio vulnificus*	ATCC 27562	0/3	0/3	3/3	0/3	Yes	0	0	0	3
32		ATCC 29306	0/3	0/3	3/3	0/3	Yes	0	0	0	3
33		ENF 10442	0/3	0/3	3/3	0/3	Yes	0	0	0	3
34		ATCC 29307	0/3	0/3	3/3	0/3	Yes	0	1	0	4
35		NA	0/3	0/3	3/3	0/3	Yes	0	0	0	3
36		NA	0/3	0/3	3/3	0/3	Yes	0	0	0	3
37		NA	0/3	0/3	3/3	0/3	Yes	0	0	0	3
38		NA	0/3	0/3	3/3	0/3	Yes	0	0	1	4
39		ATCC 43382	0/3	0/3	3/3	0/3	Yes	0	0	0	3
40		ATCC BAA-86	0/3	0/3	3/3	0/3	Yes	0	0	0	3
59		CCUG 38297	0/3	0/3	3/3	0/3	Yes	0	0	0	3
60		CCUG 47321	0/3	0/3	3/3	0/3	Yes	0	0	0	3
41	*Vibrio cholerae*	ATCC 14033	0/3	0/3	3/3	0/3	Yes	0	0	0	3
42		ENF 9786	0/3	0/3	3/3	0/3	Yes	0	0	0	3
43		ENF 9792	0/3	0/3	3/3	0/3	Yes	0	0	0	3
44		ATCC 9458	0/3	0/3	3/3	0/3	Yes	0	0	0	3
45		ATCC 9459	0/3	0/3	3/3	0/3	Yes	0	0	0	3
46		CCUG 2573	0/3	0/3	3/3	0/3	Yes	0	0	0	3
47		CCUG 2569	0/3	0/3	3/3	0/3	Yes	0	0	0	3
48		CCUG 4070	0/3	0/3	3/3	0/3	Yes	0	0	0	3
49		CCUG 21589	0/3	0/3	3/3	0/3	Yes	0	0	0	3
61		CCUG 56875	0/3	0/3	3/3	0/3	Yes	0	0	0	3
62		CCUG 53725	0/3	0/3	3/3	0/3	Yes	0	0	0	3
63		CCUG 14542	0/3	0/3	3/3	0/3	Yes	0	0	0	3

SBT	Target	ATCC or other collection number	Assay result at 2.99x					Non-Reportable			Total SBT overall
			Yersi	ETEC	Vibrio	Plesio	Strain detected	IND	UNR	Other	
50	*Escherichia coli (ETEC)*	ATCC 35401	0/3	3/3	0/3	0/3	Yes	0	0	0	3
51		ATCC 37218	0/3	3/3	0/3	0/3	Yes	0	0	0	3
52		ATCC 43896	0/3	3/3	0/3	0/3	Yes	0	0	0	3
53		ENF 15629	0/3	3/3	0/3	0/3	Yes	0	0	0	3
54		Zeptometix 801624	0/3	3/3	0/3	0/3	Yes	0	0	0	3
55		PennState 5 2215	0/3	3/3	0/3	0/3	Yes	0	0	0	3
56		PennState 91 1633	0/3	3/3	0/3	0/3	Yes	0	0	0	3
64		PennState 5 0038	0/3	2/3	0/3	0/3	Yes	0	0	0	3
65		PennState 6 0671	0/3	2/3	0/3	0/3	Yes	0	0	0	3
66		PennState 6 1182	0/3	1/3	0/3	0/3	Yes	0	0	0	3
67		PennState 13 1186	0/3	3/3	0/3	0/3	Yes	0	0	0	3
68		CCUG 47194	0/3	3/3	0/3	0/3	Yes	0	0	0	3
69		CCUG 38088	0/3	0/3	0/3	0/3	No1	0	0	0	3

1 Further testing was performed as defined in the protocol

f. Analytical specificity:

The BD MAX Extended Enteric Bacterial Panel was performed on samples containing phylogenetically related species and other organisms (bacteria, viruses, parasites and yeast) likely to be found in stool specimens. The bacterial cells, yeasts, parasites and viruses were tested in the Sample Buffer Tube at $\geq 10^6$ CFU, cells or genome equivalents/mL in stool, or $\geq 10^5$ PFU/mL in stool or TCID50/mL in stool or equivalent amount of RNA/DNA/PCR reaction. Overall, 184 organisms were tested and are listed in the table below.

- Most of bacterial strains, yeast, parasites and viruses tested produced negative results with the BD MAX Extended Enteric Bacterial Panel.

- Two (2) strains of *Vibrio mimicus*, associated with human disease, produced positive results with the BD MAX Extended Enteric Bacterial Panel. However, no positive result was recorded at $\leq 1.0 \times 10^6$ cells/mL in stool with those two strains.

- The following 8 Vibrio species, NOT associated with infections in humans and therefore unlikely to be encountered in human stool, were detected by the BD MAX Extended Enteric Bacterial Panel: *V. brasiliensis, V. campbellii, V. harveyi, V. hispanicus, V. nereis, V. pacini, V. rotiferianus and V. tubiashii*.

Based upon in silico analysis, the following *Vibrio* species could be detected by the BD MAX Extended Enteric Bacterial Panel:

- 2 Vibrio species, NOT associated with infections in humans and therefore unlikely to be encountered in human stool: *V.*

coralliilyticus and *Moritella marina* (formerly known as *Vibrio marinus*).

- One (1) uncategorized *Vibrio* species associated with infections in humans, namely *Vibrio* HENC.

Organisms Tested to Determine the BD Max Extended Enteric Bacterial Panel Specificity

Organisms		
Abiotrophia defectiva	*Collinsella aerofaciens*	*Prevotella melaninogenica*
Acinetobacter baumannii	*Corynebacterium genitalium*	*Proteus mirabilis*
Acinetobacter lwoffii	*Cryptosporidium hominis*	*Proteus penneri*
Adenovirus 11 Slobitski	*Cryptosporidium parvum*	*Proteus vulgaris*
Adenovirus Type 1	Cytomegalovirus	*Providencia alcalifaciens*
Adenovirus Type 14	*Desulfovibrio piger*	*Pseudomonas aeruginosa*
Adenovirus Type 18	*Edwardsiella tarda*	Rotavirus
Adenovirus Type 2	*Eggerthella lenta*	*Ruminococcus bromii*
Adenovirus Type 3	*Encephalitozoon cuniculi*	*Salmonella bongori*
Adenovirus Type 31	*Encephalitozoon hellum*	*Salmonella enterica* subsp. *arizonae*
Adenovirus Type 4	*Entamoeba dispar*	*Salmonella enterica* subsp. *enterica*
Adenovirus Type 40	*Entamoeba gingivalis*	*Salmonella enterica* subsp. *enterica serovar enteriditis*
Adenovirus Type 41	*Entamoeba invadens*	*Serratia fonticola*
Adenovirus Type 5 Adenoid 75	*Entamoeba ranarum*	*Serratia liquefaciens*
Adenovirus Type 8	*Enterobacter aerogenes*	*Serratia marcescens* subsp. *marcescens*
Aeromonas caviae	*Enterobacter cloacae* subsp. *cloacae*	*Shewanella algae*
Aeromonas hydrophila	*Enterococcus faecalis*	*Shigella boydii*
Aeromonas schubertii	*Enterococcus faecium*	*Shigella dysenteriae*
Aeromonas sobria	Enterovirus 68	*Shigella flexneri*
Aeromonas veronii	Enterovirus type 69	*Shigella sonnei*
Alcaligenes faecalis subsp. *faecalis*	*Escherichia fergusonii*	*Shimwellia blattae*
Anaerococcus tetradius	*Escherichia hermannii*	*Staphylococcus aureus* subsp. *aureus*
Arcobacter butzleri	*Escherichia vulneris*	*Staphylococcus epidermidis*
Arcobacter cryaerophilus	*Fusobacterium varium*	*Stenotrophomonas maltophilia*
Astrovirus Type 4	*Gardnerella vaginalis*	*Streptococcus agalactiae*
Bacillus cereus	*Gemella morbillorum*	*Streptococcus intermedius*
Bacteroides caccae	*Giardia lamblia*	*Streptococcus pyogenes*
Bacteroides fragilis	*Giardia muris*	*Streptococcus salivarius* subsp. *salivarius*
Bacteroides stercoris	*Haemophilus influenzae*	*Streptococcus suis*
Bacteroides thetaiomicron	*Hafnia alvei*	*Trabulsiella guamensis*
Bacteroides vulgatus	*Helicobacter fennelliae*	*Trichomonas vaginalis*
Bifidobacterium adolescentis	*Helicobacter pylori*	*Veillonella parvula*
Bifidobacterium bifidum	Hepatitis A virus	*Vibrio alginolyticus*
Bifidobacterium longum subsp. *longum*	Herpes Simplex Virus Type 1	*Vibrio brasiliensis*
Blastocystis hominis	Human adenovirus 22	*Vibrio campbellii*
Bovine enterovirus Type 6	Human Adenovirus 50	*Vibrio comitans*
Bovine parvovirus	Human Coxsackievirus A 9	*Vibrio diazotrophicus*
Campylobacter coli	Human Coxsackievirus B 1	*Vibrio fluvialis*
Campylobacter fetus subsp. *fetus*	Human Coxsackievirus B 3	*Vibrio harveyi*
Campylobacter hyointestinalis	Human echovirus 1	*Vibrio hispanicus*
Campylobacter jejuni subsp. *jejuni*	Human herpesvirus 2	*Vibrio mimicus*
Campylobacter lari	Human rhinovirus 1A	*Vibrio natriegens*
Campylobacter mucosalis	*Klebsiella oxytoca*	*Vibrio nereis*
Campylobacter showae	*Klebsiella pneumoniae* subsp. *pneumoniae*	*Vibrio pacinii*
Campylobacter sputorum biovar *sputorum*	*Lactobacillus acidophilus*	*Vibrio rotiferianus*
Campylobacter ureolyticus	*Lactobacillus reuteri*	*Vibrio sinaloensis*
Cedecea davisae	*Lactococcus lactis* subsp. *lactis*	*Vibrio tubiashii*
Chlamydia trachomatis	*Leminorella grimontii*	*Yersinia bercovieri*
Citrobacter amalonaticus	*Listeria monocytogenes*	*Yersinia frederiksenii*
Citrobacter freundii	*Megamonas hypermegale*	*Yersinia intermedia*
Citrobacter koseri	*Megasphaeara elsdenii*	*Yersinia kristensenii*
Citrobacter sedlakii	*Morganella morganii* subsp. *morganii*	*Yersinia mollaretii*
Clostridium difficile	*Neisseria gonorrhoeae*	*Yersinia rohdei*
Clostridium histolyticum	Norovirus Groupe I	

Organisms	
Clostridium novyi	Norovirus Groupe II
Clostridium perfringens	*Parabacteroides merdae*
Clostridium ramosum	*Pentatrichomonas hominis*
Clostridium septicum	*Peptoniphilus asaccharolyticus*
Clostridium sordellii	*Peptostreptococcus anaerobius*
Clostridium tetani	*Photobacterium damselae* subsp. *damselae*
	Porphyromonas asaccharolytica

g. Matrix equivalence Study

N/A

h. Interference Study

Nineteen (19) biological and chemical substances that may occasionally be present in stool specimens were evaluated for potential interference with the BD MAX Extended Enteric Bacterial Panel. Included in this study was an Antibiotics Mixture, which consisted of a combination of 8 different antibiotics tested simultaneously (each antibiotic at a concentration that may be found in a stool sample).

Nystatin cream was found to interfere at levels above 3.1 mg/mL. Spermicidal lubricant and hydrocortisone cream were found to interfere at levels above 2.5 mg/mL. Vagisil was found to interfere at levels above 0.92 mg/mL. Results demonstrated no reportable interference with any other substance tested.

In addition, microorganisms that may be endogenously present in stool specimens were evaluated for potential interference with the BD MAX Extended Enteric Bacterial Panel. Ten (10) organisms were tested at high concentration (> 10^6 CFU/mL of stool). Results demonstrated no reportable interference with any microorganism tested.

Endogenous and Commercial Exogenous Substances Tested with the BD MAX Extended Enteric Bacterial Panel

Brand Name of Description	***Result***	***Brand Name of Description***	***Result***
Fecal Fat	NI	Spermicial Lubricant	P
Human DNA	NI	Diaper Rash Cream	NI
Mucus	NI	Vagisil®	P
Whole Human Blood	NI	Laxatives	NI
Hydrocortisone Cream	P	Anti-Diarrheal (liquid)	NI
Antiseptic Towelettes	NI	Anti-Diarrheal (pill)	NI
Enema	NI	Antibiotics Mixture	NI
Hemorrhoidal Gel	NI	Antacids	NI
Nystatin Cream	P	Non-Steroidal Anti-Inflamatory (NSAID)	NI
Topical Antibiotic	NI		

P: Potential interference with the BD MAX Extended Enteric Bacterial Panel at high concentrations.
NI: No reportable interference with the BD MAX Extended Enteric Bacterial Panel.

Microorganisms Tested for Interference with the BD MAX Extended Enteric Bacteria Panel

Microorganism	***Result***
Salmonella typhimurium	NI
Shigella sonnei	NI
Campylobacter coli	NI
Escherichia coli (stx1/stx2)	NI
Citrobacter amalonaticus	NI
Proteus vulgaris	NI
Bacteroides thetaiotaomicron	NI
Ruminococcus bromii	NI
Enterococcus faecalis	NI
Peptostreptococcus anaerobius	NI

NI: No reportable interference with the BD MAX Extended Enteric Bacterial Panel.

i. Fresh versus Frozen Study:

The objective of this study was to evaluate target stability after two (2) freeze/thaw cycles in target-spiked preserved and unpreserved specimens.

Individual Preserved and unpreserved negative stool specimens were used to generate the target-spiked positive specimens. A stool specimen placed into a modified Cary-Blair medium for transport and storage is defined as "preserved" matrix; a stool specimen stored in its natural state is defined as "unpreserved" matrix.

Enteric bacterial organisms representing test panel targets (V*ibrio cholera*, Enterotoxigenic *Escherichia coli* (ETEC), *Yersinia enterocolitica*, and *Plesiomonas shigelloides*) were prepared as a stock enteric organism target mix and spiked into 2 aliquots from each negative specimen in accordance with target loads illustrated in the table below**.** The concentrations were chosen to test low positive and moderate positive specimens (i.e., concentrations near the LoD; 1.99X and 3X the analytical LoD, respectively) as well as a high LoD values (6X and 9X LoD).

Target Levels and Number of Replicates per Freeze/Thaw Cycle*

Target Level (x LOD)	***Number of Replicates per Target***	
	Unpreserved	*Preserved*
1.99	24	24
3	24	24
6	24	24
9	24	24
Total # Replicates	*96*	*96*

* Testing was performed using a conditional testing sequence algorithm. This algorithm only mandated testing the lowest concentration (1.99x LoD) of replicates.

Baseline testing was performed for each individual, target-spiked sample following preparation and prior to storage or subjected to freeze/thaw cycles. Specimens were

then stored at -80 °C for one (1) month and subjected to two (2) freeze/thaw cycles. After each freeze/thaw cycle the specimens were sampled and tested.

The study was performed by four (4) operators, using three (3) lots of BD MAX xEBP reagents and eight (8) BD MAX Systems. Testing was performed using a conditional testing sequence algorithm. If >95% of the 1.99x LoD samples were gave the expected result, then no additional testing was performed.

All targets (*Vibrio cholera*, Enterotoxigenic *Escherichia coli* (ETEC), *Yersinia enterocolitica*, and *Plesiomonas shigelloides*) yielded 100% agreement with expected results, for all specimens, across the conditions tested. The BD MAX xEBP met the performance requirements for all targets with fresh and frozen specimens. Study results are shown in the table below for each freeze/thaw cycle time point

Summary of Specimen Freeze/Thaw Study Results

Condition			***Target Positive Matrix Proportion Positive (%) and number of samples tested***			
Matrix	Freeze/Thaw Cycles	Load (xLOD)	*Vibrio cholera*	ETEC	*Yersinia enterocolitica*	*Plesiomonas shigelloides*
Unpreserved	0 (fresh/baseline)	1.99	100 24/24	100 24/24	100 24/24	100 24/24
		3	100 24/24	100 24/24	100 24/24	100 24/24
		6	100 24/24	100 24/24	100 24/24	100 24/24
		9	100 24/24	100 24/24	100 24/24	100 24/24
	1	1.99	100 24/24	100 24/24	100 24/24	100 24/24
	2	1.99	100 24/24	100 24/24	100 24/24	100 24/24
Preserved	0 (fresh/baseline)	1.99	100 24/24	100 24/24	100 24/24	100 24/24
		3	100 24/24	100 24/24	100 24/24	100 24/24
		6	100 24/24	100 24/24	100 24/24	100 24/24
		9	100 23/23[a]	100 23/23[a]	100 23/23[a]	100 23/23[a]
	1	1.99	100 23/23[a]	100 23/23[a]	100 23/23[a]	100 23/23[a]
	2	1.99	100 24/24	100 24/24	100 24/24	100 24/24

[a] Proportion positive rates were calculated based on total sample number after removal of IND results, which were excluded

Based on the study results, two (2) specimen freeze/thaw cycles do not affect the performance of BD MAX xEBP. The BD MAX xEBP was able to detect 100% proportion positive for all enteric bacterial targets spiked in preserved, unpreserved, negative, clinical stool specimens before and after undergoing multiple freeze/thaw cycles.

j. Carryover / Cross-Contamination

A study was conducted to investigate within-run carryover and between-run carryover while processing specimens with high bacterial loads of *Enterotoxigenic Escherichia coli (ETEC)* in the BD MAX extended Enteric Bacterial Panel. Positive samples were prepared in SBT with an unpreserved stool ETEC was present at ~1 x 10^6 CFU/mL. The negative member did not contain any target analyte. Negative samples were prepared in SBT with negative unpreserved stool matrix.

Three different reagent lots along with three (3) lots of negative unpreserved stool matrix were used. Twelve (12) replicates of the high positive panel member and 12 replicates of the negative panel member were tested in each run by alternating negative and positive samples. Three (3) operators performed 3 consecutive runs across 3 BD MAX Instruments for a total of nine (9) runs containing 24 samples (total of 108 negative and 108 high positive samples).

Of a total of 108 ETEC positive samples and 108 ETEC negative samples, 100% and 99.1% expected assay results were obtained, respectively. One ETEC false positive result was observed among the negative samples (0.9% false positive).

k. Mixed Infection Study:

A mixed infection/competitive interference study was performed to evaluate the ability of the BD MAX Extended Enteric Bacterial Panel to detect low positive results in the presence of other targets at high concentrations. Each of the targeted organisms was evaluated at low concentrations (1.99x LoD) when present in samples containing another targeted organisms at a high concentrations (≥1x10^6 CFU/mL). Samples were prepared with unpreserved stool matrix. A total of 24 replicates were tested for each of the sample mixes described in the table below.

Mixed infection Target Combinations

Test Condition	***High Target***	***Low Load Target***
#1	*Plesiomonas shigelloides*	*Yersinia enterocolitica*
		Vibrio cholerae
		ETEC
#2	*Yersinia enterocolitica*	*Plesiomonas shigelloides*
		Vibrio cholerae
		ETEC
#3	*Vibrio cholerae*	*Plesiomonas shigelloides*
		Yersinia enterocolitica
		ETEC
#4	ETEC	*Plesiomonas shigelloides*
		Yersinia enterocolitica
		Vibrio cholerae

In the presence of high loads of *Plesiomonas shigelloides* (≥ 1 x 10^6 CFU/mL) and *Vibrio cholerae* (≥ 1 x 10^6 cells/mL), all 3 organisms corresponding to their respective

simulated mixed infection preparations were successfully detected by the BD MAX Extended Enteric Bacterial Panel. Successful detection of all 3 low target organisms by the BD MAX Extended Enteric Bacterial Panel was achieved in the presence of *Yersinia enterocolitica* at 1.0 x 10^4 CFU/mL and ETEC ST/LT at 9.44 x 10^2 CFU/mL.

Comparison studies:

a. *Method comparison with predicate device:*

N/A

b. *Matrix comparison:*

N/A

3. Clinical Study:

Clinical performance characteristics of the BD MAX Extended Enteric Bacterial Panel were determined in a multi-site investigational study. The study involved a total of six (6) geographically diverse clinical centers where stools specimens were collected as part of routine patient care, enrolled into the trial, and tested with the BD MAX Extended Enteric Bacterial Panel. Specimens were obtained from pediatric or adult patients suspected of acute bacterial gastroenteritis, enteritis or colitis, for which stool culture had been ordered by healthcare provider. The reference method for identification of *Yersinia enterocolitica, Vibrio* and *Plesiomonas shigelloides* in fresh and frozen prospective specimens, was growth on culture media to identify potential colonies based on appearance and oxidase testing followed by a PCR assay and bi-directional sequencing on potential colonies for definitive identification. For ETEC, the comparator method was two PCR assays performed on the stool specimens followed by bi-directional sequencing of the product. For retrospective specimens, the historical results were recorded at the collection site. The historical results were confirmed using a PCR assay and bi-directional sequencing as part of the composite comparator method in order to confirm the presence of the target DNA.

A total of 2264 prospective specimens (882 unpreserved and 1382 Cary-Blair preserved) and 146 retrospective specimens (87 unpreserved and 59 Cary-Blair preserved) were enrolled in the clinical evaluation for a total of 2410 specimens enrolled. All test results from the BD MAX Extended Enteric Bacterial Panel and the comparator method were single results, no coinfections were detected. Table 4 describes the number of compliant specimens enrolled by patient age and specimen type with a total of 2403 compliant specimens overall. Table 5 through 13 describe the performance characteristics of the BD MAX Extended Enteric Bacterial Panel that were observed during the clinical trial.

Compliant Specimens Tested by Age Group and Specimen Type

Age Group	Cary-Blair preserved	Unpreserved	Combined
0-1 month	6	0	6
1 month to 2 years	250	66	316
2-12	311	164	475
13-18	141	85	226
19-21	44	23	67
Over 21	671	621	1292
Unknown	16	5	21
Total	1439	964	2403

The following tables include the BD MAX Extended Enteric Bacterial Panel clinical study results stratified by prospective (fresh) and retrospective (frozen) unpreserved specimens as well as prospective and retrospective Cary-Blair preserved specimens. Assay performance for each targeted analyte is calculated as compared to the comparator method (CM) for prospective specimens and to the historical result (confirmed by alternate PCR and bi-directional sequencing) for retrospective specimens.

***Vibrio* – Overall Performance**

Specimen Type	Specimen Origin	BD MAX	Reference Method		Total
			P	N	
Cary-Blair preserved	Prospective (Fresh+Frozen)	P	2	5	7
		N	0	1351	1351
		Total	2	1356	1358
PPA (95% CI): 100% (34.2%, 100%) NPA (95% CI): 99.6% (99.1%, 99.8%)					
Cary-Blair preserved	Retrospective (Frozen)	P	2	0	2
		N	0	16	16
		Total	2	16	18
PPA (95% CI): 100% (34.2%, 100%) NPA (95% CI): 100% (80.6%, 100%)					
Unpreserved	Prospective (Fresh+Frozen)	P	0	2	2
		N	0	866	866
		Total	0	868	868
No Data for PPA Calculation NPA (95% CI): 99.8% (99.2%, 99.9%)					
Unpreserved	Retrospective (Frozen)	P	2	1	3
		N	0	45	45
		Total	2	46	48
PPA (95% CI): 100% (34.2%, 100%) NPA (95% CI): 97.8% (88.7%, 99.6%)					

Vibrio Contrived Samples Results per Specimen Type

Specimen Type	BD MAX	Expected Result		Total
		P	N	
Cary-Blair preserved	P	48	0	48
	N	0	144	144
	Total	48	144	192
PPA: 100% (92.6%, 100%) NPA: 100% (97.4%, 100%)				
Unpreserved	P	48	0	48
	N	0	144	144
	Total	48	144	192
PPA: 100% (92.6%, 100%) NPA: 100% (97.4%, 100%)				

Plesiomonas shigelloides – Overall Performance

Specimen Type	Specimen Origin	BD MAX	Reference Method		Total
			P	N	
Cary-Blair Preserved	Prospective (Fresh+Frozen)	P	0	2	2
		N	0	1355	1355
			0	1357	1357
No Data for PPA Calculation NPA: 99.9% (99.5%, 100%)					
Cary-Blair Preserved	Retrospective (Frozen)	P	4	0	4
		N	0	38	38
			4	38	42
PPA: 100% (51%, 100%) NPA: 100% (90.8%, 100%)					
Unpreserved	Prospective (Fresh+Frozen)	P	0	1	1
		N	0	863	863
			0	864	864
No Data for PPA Calculation NPA: 99.9% (99.3%, 100%)					
Unpreserved	Retrospective (Frozen)	P	3	1	4
		N	0	46	46
			3	47	50
PPA: 100% (43.9%, 100%) NPA: 97.9% (88.9%, 99.6%)					

Plesiomonas shigelloides Contrived Samples Results per Specimen Type

Specimen Type	BD MAX	Expected Result		Total
		Positive	Negative	
Cary-Blair Preserved	Positive	48	0	48
	Negative	0	144	144
	Total	48	144	192
PPA: 100% (92.6%, 100%) NPA: 100% (97.4%, 100%)				
Unpreserved	Positive	48	1[1]	49
	Negative	0	143	143
	Total	48	144	192
PPA: 100% (92.6%, 100%) NPA: 99.3% (96.2%, 99.9%)				

[1]Sample XW0007C was initially found positive for Plesiomonas shigelloides, but found negative for this target once retested from the SBT (Discrepant analysis).

Yersinia enterocolitica – Overall Performance

Specimen Type	Specimen Origin	BD MAX	Reference Method		Total
			P	N	
Cary-Blair Preserved	Prospective (Fresh+Frozen)	P	0	1	1
		N	0	1341	1341
			0	1342	1342
No Data for PPA Calculation NPA: 99.9% (99.6%, 100%)					
Cary-Blair Preserved	Retrospective (Frozen)	P	0	0	0
		N	0	32	32
			0	32	32
No Data for PPA Calculation NPA: 100% (89.3%, 100%)					
Unpreserved	Prospective (Fresh+Frozen)	P	0	0	0
		N	0	863	863
			0	863	863
No Data for PPA Calculation NPA: 100% (99.6%, 100%)					
Unpreserved	Retrospective (Frozen)	P	9	0	9
		N	0	47	47
			9	47	56
PPA: 100% (70.1%, 100%) NPA: 100% (92.4%, 100%)					

Yersinia enterocolitica Contrived Samples Results per Specimen Type

Specimen Type	BD MAX	Expected Result		Total
		Positive	Negative	
Cary-Blair Preserved	Positive	47	0	47
	Negative	1[2]	144	145
	Total	48	144	192
PPA: 97.9% (89.1%, 99.6%) NPA: 100% (97.4%, 100%)				
Unpreserved	Positive	48	0	48
	Negative	0	144	144
	Total	48	144	192
PPA: 100% (92.6%, 100%) NPA: 100% (97.4%, 100%)				

[2]Sample XW0351C was initially found negative for Yersinia enterocolitica, but found positive for this target once retested from the SBT (Discrepant analysis).

Enterotoxigenic *E. coli* (ETEC LT/ST) – Overall Performance

Specimen Type	Specimen Origin	BD MAX	Comparator Method		Total
			P	N	
Cary-Blair Preserved	Prospective (Fresh+Frozen)	P	10	3	13
		N	0	1348	1348
			10	1351	1361
PPA: 100% (72.2%, 100%) NPA: 99.8% (99.3%, 99.9%)					
Cary-Blair Preserved	Retrospective (Frozen)	P	5	0	5
		N	0	28	28
			5	28	33
PPA: 100% (56.6%, 100%) NPA: 100% (87.9%, 100%)					
Unpreserved	Prospective (Fresh+Frozen)	P	16	1	17
		N	0	818	818
			16	819	835
PPA: 100% (80.6%, 100%) NPA: 99.9% (99.3%, 100%)					
Unpreserved	Retrospective (Frozen)	P	9	1	10
		N	1	26	27
			10	27	37
PPA: 90% (59.6%, 98.2%) NPA: 96.3% (81.7%, 99.3%)					

ETEC Performance per Toxin Observed During the Clinical Trial

ETEC		LT	ST			Unknown*
			ST	STp	STh	
		PPA	PPA	PPA	PPA	PPA
Specimen Type	**Specimen Origin**	**Estimate Percent (95% CI)**	**Estimate Percent (95% CI)**	**Estimate Percent (95% CI)**	**Estimate Percent (95% CI)**	**Estimate Percent (95% CI)**
Cary-Blair Preserved	Prospective	100 (4/4) (51.0, 100)	100 (5/5) (56.6, 100)	100 (2/2) (34.2, 100)	100 (1/1) (20.7, 100)	100 (3/3) (43.9, 100)
	Retrospective	100 (4/4) (51.0, 100)	100 (5/5) (56.6, 100)	100 (4/4) (51.0, 100)	0	0
Unpreserved	Prospective	100 (4/4) (51.0, 100)	100 (7/7) (64.6, 100)	100 (3/3) (43.9, 100)	100 (2/2) (34.2, 100)	100 (7/7) (64.6, 100)
	Retrospective	100 (7/7) (64.6, 100)	80.0 (4/5) (37.6, 96.4)	100 (2/2) (34.2, 100)	100 (2/2) (34.2, 100)	0

Co-infections Observed in the Prospective Clinical Study

No co-infections were observed during the prospective clinical study.

Non-Reportable Rate

The initial unresolved rate was calculated when considering the unresolved rate of both BD MAX Enteric Bacterial Panel and BD MAX Extended Enteric Bacterial Panel assays. Of the 2264 prospective specimens initially evaluated, 3.1% of the Cary-Blair preserved and 3.9% of the unpreserved specimens initially reported as unresolved. The unresolved rate following a valid repeat test was calculated when considering BD MAX Extended Enteric Bacterial Panel only 0.1% of the prospective Cary-Blair preserved specimens and 0.3% of the prospective unpreserved specimens remained unresolved. Of all the specimens initially evaluated with both BD MAX Enteric Bacterial Panel and BD MAX Extended Enteric Bacterial Panel assays, 1.0% of the Cary-Blair preserved and 1.5% of the unpreserved initially reported as Indeterminate. Following a valid repeat test, 0.1% of the Cary-Blair preserved and none of the unpreserved remained Indeterminate. No incomplete tests were reported during this study.

Non-reportable Rates

Specimen Type	Unresolved Rate			Indeterminate Rate		Incomplete Rate		Total Rate		
	Initial xEBP (95% CI)	Initial EBP+xEBP (95% CI)	Final xEBP (95% CI)	Initial EBP+xEBP (95% CI)	Final EBP+xEBP (95% CI)	Initial EBP+xEBP (95% CI)	Final EBP+xEBP (95% CI)	Initial xEBP (95% CI)	Initial EBP+xEBP (95% CI)	Final xEBP (95% CI)
Cary-Blair Preserved	2.4% 35/1430 (1.8%, 3.4%)	3.1% 44/1430 (2.3%, 4.1%)	0.1% 1/1427 (0.0%, 0.4%)	1.0% 15/1430 (0.6%, 1.7%)	0.1% 1/1427 (0.0%, 0.4%)	0.0% 0/1430 (0.0%, 0.3%)	0.0% 0/1427 (0.0%, 0.3%)	3.5% 50/1430 (2.7%, 4.6%)	4.1% 59/1430 (3.2%, 5.3%)	0.1% 2/1427 (0.0%, 0.5%)
Unpreserved	2.2% 21/958 (1.4%, 3.3%)	3.9% 37/958 (2.8%, 5.3%)	0.3% 3/958 (0.1%, 0.9%)	1.5% 14/958 (0.9%, 2.4%)	0.0% 0/958 (0.0%, 0.4%)	0.0% 0/958 (0.0%, 0.4%)	0.0% 0/958 (0.0%, 0.4%)	3.7% 35/958 (2.6%, 5.0%)	5.3% 51/958 (4.1%, 6.9%)	0.3% 3/958 (0.1%, 0.9%)
Overall	2.3% 56/2388 (1.8%, 3.0%)	3.4% 81/2388 (2.7%, 4.2%)	0.2% 4/2385 (0.1%, 0.4%)	1.2% 29/2388 (0.8%, 1.7%)	0.0% 1/2385 (0.0%, 0.2%)	0.0% 0/2388 (0.0%, 0.2%)	0.0% 0/2385 (0.0%, 0.2%)	3.6% 85/2388 (2.9%, 4.4%)	4.6% 110/2388 (3.8%, 5.5%)	0.2% 5/2385 (0.1%, 0.5%)

4. Clinical cut-off**:**

   N/A

N. Instrument Name:

BD MAX System

O. System Descriptions:

1. Modes of Operation:

   The BD MAX System fully automates cell lysis, nucleic acid extraction, PCR set-up, target amplification and detection. The system can process and analyze up to 24 specimens in one cartridge with two cartridges running simultaneously on the instrument. The system includes external and internal barcode reading, ensuring traceability throughout extraction and PCR process. The system includes a heater module, temperature sensors, and a fluorescence detection system with six optical channels.

2. Software:

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

   Yes X or No _____

3. Specimen Identification:

   Specimens are labeled with a barcode.

4. Specimen Sampling and Handling:

   A disposable inoculating loop is used to place 10 µl of the unpreserved or Cary-Blair stool specimen into a SBT which is then vortexed and placed onto the system.

5. Calibration:

   The system is calibrated by the manufacturer on-site as part of the installation procedure as well as during biannual preventive maintenance.

6. Quality Control:

   See Section M.1c above.

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

N/A

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

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