DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
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Silver Spring, MD 20993-0002

May 2, 2017

BECTON, DICKINSON AND COMPANY
LAURA STEWART
REGULATORY AFFAIRS SPECIALISTS
7 LOVETON CIRCLE
SPARKS MD 21152

Re: K170308
Trade/Device Name: BD Max Extended Enteric Bacterial Panel, BD Max System
Regulation Number: 21 CFR 866.3990
Regulation Name: Gastrointestinal microorganism multiplex nucleic acid-based assay.
Regulatory Class: II
Product Code: PCH
Dated: January 31, 2017
Received: February 1, 2017

Dear Ms. Stewart:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

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

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

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Kristian M. Roth -S

For: Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K170308

Device Name
BD MAX™ Extended Enteric Bacterial Panel

Indications for Use *(Describe)*

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 co-infection 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.

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

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

CONTINUE ON A SEPARATE PAGE IF NEEDED

5. 510(k) Summary

BD MAX™ Extended Enteric Bacterial Panel (xEBP)
Summary Preparation Date:
1/30/2017

Submitted by:
BD Diagnostic Systems
Becton, Dickinson and Company
7 Loveton Circle
Sparks, Maryland 21152

Contact:
Laura Stewart
Regulatory Affairs Specialist

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Proprietary Names:
For the instrument:
BD MAX™ System
For the assay:
BD MAX™ Extended Enteric Bacterial Panel (xEBP)

Common Names:
For the instrument:
Bench-top molecular diagnostics workstation
For the assay:
Gastrointestinal Bacterial Panel Multiplex Nucleic Acid-Based Assay System
Enteric Bacterial Panel
Enteric Bacterial Nucleic Acid Test
Enteric Bacterial identification and differentiation system
Enteric assay
Enteric test

Regulatory Information
Regulation section:
866.3990 – Gastrointestinal microorganism multiplex nucleic acid-based assay.

Classification:
Class II

Panel:
Microbiology (83)

Product Code(s):
PCI- Gastrointestinal Bacterial Panel Multiplex Nucleic Acid-Based Assay System
PCH- Gastrointestinal Pathogen Panel Multiplex Nucleic Acid-Based Assay System
OOI- Real Time Nucleic Acid Amplification System

Predicate Device
BioFire Diagnostics FilmArray Gastrointestinal (GI) Panel [510(k) K160459]

Device Establishment
GeneOhm Sciences Canada, Inc. (BD Diagnostics)
2555 Boul. du Parc-Technologique
Quebec, QC G1P 4S5
Canada

Registration Number:
3007420875

Performance Standards
Class II Special Controls Guideline: Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assays for Detection and Identification of Microorganisms and Toxin Genes from Human Stool Specimens, November 2, 2015.

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 co-infection 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.

Special Conditions for Use Statement: For prescription use.

Special Instrument Requirements: BD MAX™ System

Device Description

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 unpreserved or Cary-Blair preserved soft to diarrheal stool specimens from symptomatic patients with suspected acute gastroenteritis, enteritis or colitis.

The BD MAX™ Extended Enteric Bacterial Panel (BD MAX™ xEBP) is composed of a single Master Mix that must be used in conjunction with the BD MAX™ Enteric Bacterial Panel (BD MAX EBP), performed on the BD MAX™ System. The BD MAX xEBP is performed with the Master Mix contained in the BD MAX xEBP reagent kit and the EBP reagents, including the Master Mix contained in the BD MAX™ EBP reagent kit. The BD MAX xEBP assay and accompanying Assay Definition File (ADF) were developed to allow inclusion of both BD MAX EBP and BD MAX xEBP master mix reagents in one test run.

The BD MAX™ System and the BD MAX™ Extended Enteric Bacterial Panel is run with the instrument with associated hardware and accessories, disposable microfluidic cartridges, master mixes, unitized reagent strips, extraction reagents, and sample buffer tubes. 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. A test result may be called as POS (Positive), NEG (Negative), or UNR (Unresolved) for each of the assay's targets, based on the amplification status of the target and of the Sample Processing Control. IND (Indeterminate) or INC (Incomplete) results are due to BD MAX™ System failure.

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.

Substantial Equivalence[i]

Table 1 shows the similarities and **Table 2** shows the differences between the BD MAX™ Extended Enteric Bacterial Panel and the predicate device.

Table 1: Similarities Comparison to Predicate Device

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 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 co-infection 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.	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 - 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

[i] The term "substantial equivalence" as used in this 510(k) notification is limited to the definition of substantial equivalence as found in the Federal Food, Drug and Cosmetic Act, as amended and as applied under 21 CFR 807, Subpart E under which a device can be marketed without pre-market approval or reclassification. A determination of substantial equivalency under this notification is not intended to have any bearing whatsoever on the resolution of patent infringement suits or any other patent matters. No statements related to, or in support of substantial equivalence herein shall be construed as an admission against interest under the US Patent Laws or their application by the courts.

Similarities		
Item	*BD MAX™ xEBP*	*FilmArray GI Panel (K160459)*
		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
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.

Table 2: Differences Comparison to Predicate Device

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)

Analytical Performance

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 in **Table 3.**

Table 3: 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.

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 4.** The quantitative reproducibility results across sites by target are presented in **Table 5** and **Table 6.** 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 5** and **Table 6.**

Table 4: 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 5: Quantitative Site-to-Site Reproducibility for all Targets and Sample Processing Control

Target	*PCR Metric*	*Category*	*N*	*Mean*	*Within Run*		*Between Run Within Day*		*Between Day Within Site*		*Between Site*		*Total*	
					SD	*%CV*	*SD*	*%CV*	*SD*	*%CV*	*SD*	*%CV*	*SD*	*%CV*
Vibrio	Ct. Score	HN	46	36.9	1.11	3.0	0.00	0.0	0.00	0.0	0.40	1.1	1.18	3.2
		LP	90	32.1	0.43	1.3	0.00	0.0	0.00	0.0	0.13	0.4	0.45	1.4
		MP	90	31.7	0.55	1.7	0.00	0.0	0.00	0.0	0.13	0.4	0.56	1.8
	Cycle End Point	HN	46	805.1	334.64	41.6	0.00	0.0	0.00	0.0	103.89	12.9	350.40	43.5
		LP	90	2725.7	398.88	14.6	303.13	11.1	250.16	9.2	588.42	21.6	812.29	29.8
		MP	90	3076.6	459.04	14.9	127.59	4.1	92.07	3.0	0.00	0.0	485.26	15.8
Plesiomonas shigelloides	Ct. Score	HN	63	36.0	1.44	4.0	0.05	0.1	0.00	0.0	0.43	1.2	1.50	4.2
		LP	89	31.5	0.73	2.3	0.00	0.0	0.02	0.1	0.52	1.7	0.90	2.9
		MP	89	30.7	0.73	2.4	0.00	0.0	0.00	0.0	0.16	0.5	0.74	2.4
	Cycle End Point	HN	63	596.5	284.02	47.6	0.00	0.0	0.00	0.0	0.00	0.0	284.02	47.6
		LP	89	2349.7	332.60	14.2	0.00	0.0	0.00	0.0	250.45	10.7	416.36	17.7
		MP	89	2320.9	342.77	14.8	313.96	13.5	86.03	3.7	416.63	18.0	630.12	27.1
Yersinia enterocolitica	Ct. Score	HN	58	36.3	0.99	2.7	0.35	1.0	0.13	0.4	0.00	0.0	1.06	2.9
		LP	90	32.9	0.69	2.1	0.00	0.0	0.00	0.0	0.37	1.1	0.78	2.4
		MP	89	32.7	0.58	1.8	0.14	0.4	0.06	0.2	0.23	0.7	0.64	2.0
	End Point	HN	58	599.6	232.01	38.7	139.57	23.3	0.00	0.0	121.25	20.2	296.66	49.5
		LP	90	1191.1	272.51	22.9	107.96	9.1	0.00	0.0	121.52	10.2	317.31	26.6
		MP	89	1264.2	363.00	28.7	0.00	0.0	0.00	0.0	139.76	11.1	388.98	30.8
ETEC	Ct. Score	HN	48	36.8	1.37	3.7	0.00	0.0	0.00	0.0	0.00	0.0	1.37	3.7
		LP	88	33.7	0.74	2.2	0.00	0.0	0.09	0.3	0.29	0.9	0.80	2.4
		MP	90	32.4	0.67	2.1	0.00	0.0	0.00	0.0	0.26	0.8	0.72	2.2
	Cycle End Point	HN	48	976.0	577.46	59.2	0.00	0.0	0.00	0.0	0.00	0.0	577.46	59.2
		LP	88	2049.6	511.41	25.0	61.48	3.0	0.00	0.0	121.96	6.0	529.33	25.8
		MP	90	2640.0	491.69	18.6	217.12	8.2	0.00	0.0	263.58	10.0	598.65	22.7
SPC	Ct. Score	TN	90	27.2	0.35	1.3	0.00	0.0	0.00	0.0	0.18	0.7	0.40	1.5
	Cycle End Point	TN	90	6020.7	672.52	11.2	0.00	0.0	0.00	0.0	148.61	2.5	688.74	11.4

Table 6: 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 7.** The quantitative results across lots and by target are presented in **Table 8** and **Table 9.** Ct. 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 8** and **Table 9.**

Table 7: 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 8: Quantitative Lot-to-lot for all Targets and SPC

Target	***PCR Metric***	***Category***	***N***	***Mean***	***Within Run***		***Between Run Within Day***		***Between Day Within Site***		***Between Site***		***Total***	
					SD	*%CV*	*SD*	*%CV*	*SD*	*%CV*	*SD*	*%CV*	*SD*	*%CV*
Vibrio	Ct. Score	HN	53	36.6	1.18	3.2	0.00	0.0	0.00	0.0	0.00	0.0	1.18	3.2
		LP	90	32.2	0.42	1.3	0.18	0.6	0.00	0.0	0.23	0.7	0.51	1.6
		MP	90	31.9	0.61	1.9	0.00	0.0	0.00	0.0	0.20	0.6	0.64	2.0
	Cycle End Point	HN	53	931.1	369.81	39.7	0.00	0.0	0.00	0.0	0.00	0.0	369.81	39.7
		LP	90	3124.7	189.01	6.0	0.00	0.0	70.08	2.2	135.26	4.3	242.75	7.8
		MP	90	3033.5	207.01	6.8	0.00	0.0	53.48	1.8	101.18	3.3	236.53	7.8
Plesiomonas shigelloides	Ct. Score	HN	64	35.8	1.41	3.9	0.20	0.6	0.00	0.0	0.00	0.0	1.42	4.0
		LP	90	31.3	0.52	1.7	0.05	0.2	0.00	0.0	0.31	1.0	0.61	2.0
		MP	90	30.7	0.68	2.2	0.29	0.9	0.00	0.0	0.52	1.7	0.90	2.9
	Cycle End Point	HN	64	686.9	320.86	46.7	0.00	0.0	0.00	0.0	104.23	15.2	337.36	49.1
		LP	90	2444.1	411.42	16.8	0.00	0.0	0.00	0.0	269.42	11.0	491.79	20.1
		MP	90	2428.7	170.72	7.0	140.04	5.8	0.00	0.0	353.06	14.5	416.43	17.1
Yersinia enterocolitica	Ct. Score	HN	69	36.1	1.08	3.0	0.70	1.9	0.00	0.0	0.00	0.0	1.29	3.6
		LP	90	32.4	0.73	2.3	0.03	0.1	0.00	0.0	0.58	1.8	0.94	2.9
		MP	89	32.3	0.87	2.7	0.18	0.6	0.00	0.0	0.25	0.8	0.93	2.9
	End Point	HN	69	731.3	252.85	34.6	154.25	21.1	0.00	0.0	12.15	1.7	296.44	40.5
		LP	90	1339.7	140.87	10.5	0.00	0.0	0.00	0.0	72.45	5.4	158.41	11.8
		MP	89	1370.3	162.58	11.9	25.73	1.9	0.00	0.0	19.29	1.4	165.73	12.1
ETEC	Ct. Score	HN	53	37.0	1.06	2.9	0.13	0.3	0.00	0.0	0.00	0.0	1.06	2.9
		LP	88	33.6	0.65	1.9	0.00	0.0	0.00	0.0	0.43	1.3	0.78	2.3
		MP	90	32.4	0.74	2.3	0.00	0.0	0.00	0.0	0.46	1.4	0.87	2.7
	Cycle End Point	HN	53	838.3	308.54	36.8	105.56	12.6	0.00	0.0	64.26	7.7	332.37	39.6
		LP	88	2056.8	267.41	13.0	0.00	0.0	0.00	0.0	323.86	15.7	419.99	20.4
		MP	90	2244.2	315.85	14.1	0.00	0.0	0.00	0.0	332.48	14.8	458.59	20.4
SPC	Ct. Score	TN	90	27.2	0.30	1.1	0.00	0.0	0.00	0.0	0.06	0.2	0.30	1.1
	Cycle End Point	TN	90	5544.7	567.21	10.2	0.00	0.0	0.00	0.0	282.58	5.1	633.70	11.4

Table 9: 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

Storage and Stability

- Collected specimens, either unpreserved stool or stool stored in 15 mL Cary-Blair transport media should be kept between 2 °C and 25 °C during transport. Protect against exposure to excessive heat.
- Specimen can be stored for up to 120 hours (5 days) at 2–8 °C or for up to 24 hours at 2-25 °C before testing.
- BD MAX Extended Enteric Bacterial Panel Master Mix is stable at 2–25 °C through the stated expiration date. Do not use expired components.
- BD MAX Extended Enteric Bacterial Master Mix Tubes are provided in sealed pouches. To protect product from humidity, immediately re-seal after opening. Master Mix tubes are stable for up to 14 days at 2–25 °C after initial opening and re-sealing of the pouch.
- Unreconstituted Master Mix tubes are stable for up to 24 hours at 2–25 °C after being removed from their protective pouch.

Controls

External Control materials are not provided by BD. External Positive and Negative Controls are not used by the BD MAX System software for the purpose of sample test result interpretation. External Controls are treated as if they were patient samples. However, Quality Control strains and procedures are included in the package insert. Various types of External Controls are recommended to allow the user to select the most appropriate for their laboratory quality control program:

a. External Negative Control: Commercially available control material or a previously characterized sample known to be negative. BD recommends that the External negative Control be prepared prior to the External Positive Control in order to reduce the potential for contamination as a result of control preparation.

b. External Positive Control: Commercially available control materials, such as the ATCC strains listed below, or previously characterized samples known to be positive:
    - *Yersinia enterocolitica* (ATCC 9610)
    - *Vibrio cholerae* (ATCC 14033)
    - *Vibrio parahaemolyticus* (ATCC 17802)
    - ETEC (ATCC 35401)
    - *Plesiomonas shigelloides* (ATCC 14029)

The assay includes a Specimen Processing Control (SPC) that is present in the Extraction Tube. The Sample Processing Control monitors the efficiency of DNA capture, washing and elution during the sample processing steps, as well as the efficiency of DNA target amplification and detection during PCR analysis.

Analytical Sensitivity

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 539 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 (**Table 10**).

Table 10: 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[a] (CFU/SBT)*	*LoD[a] (CFU/mL in stool)*	*LoD[a] (CFU/SBT)*	*LoD[a] (CFU/mL in stool)*	*LoD[a] (CFU/SBT)*	*LoD[a] (CFU/mL in stool)*
Plesiomonas shigelloides ATCC 14029[b]; ATCC 14030[c]	458	45,752	155	15,481	257	25,712
Yersinia enterocolitica ATCC 9610[b]; CCUG 4588[c]	209	20,900	311	31,099	227	22,723
ETEC ST/LT ATCC 35401[b]; CCUG 47194[c]	34	3,434	539	53,852	137	13,706
Vibrio cholerae ATCC 14033[b]; ENF 9786[c]	149	14,942	43	4,344	252	25,238
Vibrio parahaemolyticus ATCC17802[b]; ENF 5887[c]	207	20,708	80	8,031	124	12,424
Vibrio vulnificus ATCC 27562[b] 2; ENF 10727[c]	131	13,093	80	7,959	79	7,860

[a]LoD concentrations are expressed in CFU/SBT and CFU/mL, except for *Vibrio*, which is expressed in cells/SBT and cells/mL.
[b]1st strain
[c]2nd strain

Analytical 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.

Analytical Specificity (Cross-Reactivity and Exclusivity)

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.

- Most 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 $\leq 1.0 \times 10^7$ cells/mL in stool with these 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, *Vibrio* HENC.

Interfering Substances

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 (**Table 11**).

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 (> 2 x 10^6 CFU/mL of stool). Results demonstrated no reportable interference with any microorganism tested (**Table 12**).

Table 11: 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.

Table 12: Microorganisms Tested for Interference with the BD MAX Extended Enteric Bacterial 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.

Carryover/Cross-Contamination

A study was conducted to investigate within-run carryover and between-run carryover while processing samples with high bacterial load of analytes in the BD MAX™ Extended Enteric Bacterial Panel. A panel made of one high positive member containing one target organism and one negative member was used to prepare numerous samples. The enterotoxigenic *Escherichia coli* strain was used to represent the high positive panel member (~1 x 10^6 CFU/mL of SBT). The negative member did not contain any target analyte. 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. Of the 108 negative samples tested in this study, one (1) sample produced a positive ETEC ST/LT result.

Mixed Infection/Competitive Interference

The mixed infection/competitive interference study was designed 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. A mix of three (3) out of four (4) organisms (*Plesiomonas shigelloides, Yersinia enterocolitica,* ETEC ST/LT, and *Vibrio cholerae*) were prepared at < 2x their respective LoD to serve as

low targets in the BD MAX Extended Enteric Bacterial Panel Sample Buffer Tube. The fourth BD MAX Enteric Bacterial Panel analyte (absent from the low targets mix) was spiked into the Sample Buffer Tube at a concentration ≥ 1 x 10^6 CFU/mL (representing a high load target) along with 10 µL of unpreserved stool and tested to simulate mixed infections. 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 three (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 three (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.

Clinical Performance Studies

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 both prospective fresh and prospective frozen specimens, was a combination of bacterial culture followed by alternate PCR assay and bi-directional sequencing on presumptive positive isolates for *Yersinia enterocolitica*, *Vibrio* and *Plesiomonas shigelloides*. For ETEC, the comparator method was two alternate PCR assays and bi-directional sequencing performed from the stool specimens. For retrospective specimens, the historical results were recorded at the collection site. The historical results were confirmed using an alternate 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 13** describes the number of compliant specimens enrolled by patient age and specimen type with a total of 2403 compliant specimens overall. **Table 14** through **Table 22** describe the performance characteristics of the BD MAX Extended Enteric Bacterial Panel that were observed during the clinical trial.

Table 13: Compliant Clinical Trial Enrollment Summary 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

***Vibrio* Performance Results**

For the Cary-Blair preserved specimen type, the BD MAX Extended Enteric Bacterial Panel identified 100% and 99.6% of the *Vibrio* prospective positive and negative specimens, respectively, and 100% of the retrospective positive and negative specimens. For the unpreserved specimen type, the BD MAX Extended Enteric Bacterial Panel identified 99.8% of the *Vibrio* negative specimens. No unpreserved prospective positive was found for *Vibrio*, therefore no performance can be estimated. 100% and 97.8% of the retrospective positive and negative specimens respectively was identified (**Table 14**).

As *Vibrio* prevalence is low, an evaluation of contrived samples was performed to supplement data collected in the study. These were prepared by spiking four (4) different strains for each species of *Vibrio* detected by BD MAX Extended Enteric Bacterial Panel in negative stool matrix. Strains were spiked at various clinically relevant loads and randomly distributed among three (3) clinical sites for BD MAX Extended Enteric Bacterial Panel testing. A positive agreement of 100% was obtained across the tested loads. Results are shown in **Table 15.**

Table 14: *Vibrio* – Overall Performance

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

While the BD MAX Extended Enteric Bacterial Panel is designed to detect *V. paraheamoliticus, V. cholerae* and *V. vulnificus*, the panel does not report results to the species level. The *Vibrio* species identification was obtained by sequencing of the alternate PCR performed from the reference method presumptive positive isolate. During the study, equal number of *V. cholerae* and *V. parahaemoliticus* were obtained and no *V. vulnificus* was obtained. Three (3) *V. parahaemoliticus* and three (3) *V. cholerae* were obtained.

Table 15: *Vibrio* Contrived Samples Results per Specimen Type

Specimen Type	***BD MAX***	***Expected Result***			***PPA (%)***	***NPA (%)***
		Positive	*Negative*	***Total***		
Cary-Blair Preserved	Positive	48	0	48	100 (92.6, 100)	100 (97.4, 100)
	Negative	0	144	144		
	Total	48	144	192		
Unpreserved	Positive	48	0	48	100 (92.6, 100)	100 (97.4, 100)
	Negative	0	144	144		
	Total	48	144	192		

***Plesiomonas shigelloides* Performance Results**

For the Cary-Blair preserved specimen type, the BD MAX Extended Enteric Bacterial Panel identified 99.9% of the *Plesiomonas shigelloides* prospective negative specimens, and 100% of the retrospective positive and negative specimens. No Cary-Blair prospective positive *P. shigelloides* were identified; therefore no performance can be estimated. For the unpreserved specimen type, the BD MAX Extended Enteric Bacterial Panel identified 99.9% of the *P. shigelloides* negative specimens. No prospective positive *P. shigelloides* were identified; therefore no performance can be estimated. 100% and 97.9% of the retrospective positive and negative specimens respectively was identified (**Table 16**).

As *P. shigelloides* prevalence is low, an evaluation of contrived samples was performed to supplement data collected in the study. These were prepared by spiking 12 different strains of

P. shigelloides in negative stool matrix. Strains were spiked at various clinically relevant loads and randomly distributed among three (3) clinical sites for BD MAX Extended Enteric Bacterial Panel testing. A positive agreement of 100% was obtained across the tested loads. Results are shown in **Table 17.**

Table 16: *Plesiomonas shigelloides* – Overall Performance

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

Table 17: Plesiomonas shigelloides Contrived Samples Results per Specimen Type

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

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

Yersinia enterocolitica Performance Results

For the Cary-Blair preserved specimen type, the BD MAX Extended Enteric Bacterial Panel identified 99.9% of the *Yersinia enterocolitica* prospective negative specimens and 100% of the retrospective negative specimens. No Cary-Blair prospective and retrospective positive *Y. enterocolitica* were identified, therefore no performance can be estimated. For the unpreserved specimen type, the BD MAX Extended Enteric Bacterial Panel identified 100% of the *Y. enterocolitica* negative prospective specimens. No prospective positive *Y. enterocolitica* were identified, therefore no performance can be estimated. 100% of the retrospective positive and negative specimens was identified (**Table 18**).

As *Y. enterocolitica* prevalence is low, an evaluation of contrived samples was performed to supplement data collected in the study. These were prepared by spiking twelve (12) different strains of *Y. enterocolitica* in negative stool matrix. Strains were spiked at various clinically relevant loads and randomly distributed among three (3) clinical sites for BD MAX Extended Enteric Bacterial Panel testing. A positive agreement of 100% was obtained across the tested loads, except for one Cary-Blair preserved sample type at clinical load which the agreement obtained is 97.9% (Results are shown in **Table 19**).

Table 18: *Yersinia enterocolitica* – Overall Performance

Specimen Type	***Specimen Origin***	***BD MAX***	***Reference Method***			***PPA (%)***	***NPA (%)***
			Positive	*Negative*	***Total***		
Cary-Blair preserved	Prospective	Positive	0	1	1	No Data for Calculation	99.9 (99.6, 100)
		Negative	0	1341	1341		
		Total	0	1342	1342		
	Retrospective	Positive	0	0	0	No Data for Calculation	100 (89.3, 100)
		Negative	0	32	32		
		Total	0	32	32		
Unpreserved	Prospective	Positive	0	0	0	No Data for Calculation	100 (99.6, 100)
		Negative	0	863	863		
		Total	0	863	863		
	Retrospective	Positive	9	0	9	100% (70.1%, 100%)	100 (92.4, 100)
		Negative	0	47	47		
		Total	9	47	56		

Table 19: *Yersinia enterocolitica* Contrived Samples Results per Specimen Type

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

[b] 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) Performance Results

For the Cary-Blair preserved specimen type, the BD MAX Extended Enteric Bacterial Panel identified 100% and 99.8% of the ETEC prospective positive and negative specimens, respectively, and 100% of the retrospective positive and negative specimens. For the unpreserved specimen type, the BD MAX Extended Enteric Bacterial Panel identified 100% and 99.9% of the ETEC prospective positive and negative specimens, respectively, and 90% and 96.3% of the retrospective positive and negative specimens respectively was identified (**Table 20**).

Table 20: Enterotoxigenic *E. coli* (ETEC LT/ST) – Overall Performance

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

The ETEC toxin identification was obtained by sequencing of the alternate PCR performed from stool specimens (**Table 21**). While the BD MAX Extended Enteric Bacterial Panel is designed to detect the toxin types described below, the panel does not report results to the species or toxin level.

Table 21: ETEC Performance per Toxin Observed During the Clinical Trial

ETEC		***LT***	***ST***			***Unknown[c]***
			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

[c] ETEC toxin was detected but the specific species not identified.

Discrepant Results

There were nineteen (19) discrepant results in the clinical trial. Eighteen (18) false-positive (FP) and one (1) false-negative (FN) result. Three (3) retrospective specimens, one (1) ETEC FN, one (1) ETEC FP and one (1) *Vibrio* FP, were not tested in the discrepant analysis, due to limited specimen volume. Of the sixteen (16) FP discrepant specimens tested, there were seven (7) FP *Vibrio*, four (4) *P. shigelloides*, one (1) *Y. enterocolitica* and four (4) ETEC. Five (5) out of the seven (7) *Vibrio* discrepant specimens were negative for both BD MAX Extended Enteric Bacterial Panel and the comparator method. The two (2) remaining *Vibrio* FP specimens, obtained a positive status in the BD MAX Extended Enteric Bacterial Panel coupled with a negative status in the comparator method testing in the discordance study.

Out of the four (4) *P. shigelloides* discrepant results, one (1) was negative for both BD MAX Extended Enteric Bacterial Panel and the comparator method. Two (2) specimens obtained a positive status on the BD MAX Extended Enteric Bacterial Panel coupled with a negative status in the comparator method testing in the discordance study. They were also both positive for another target on the BD MAX Extended Enteric Bacterial Panel, ETEC and *Y. enterocolitica*, respectively. One (1) specimen, a Retrospective specimen, was *Vibrio* true positive in the study and *P. shigelloides* FP. In discrepant analysis this specimen was *P. shigelloides* negative for the comparator method and positive for *Vibrio* and *P. shigelloides* (1/3). The *Y. enterocolitica* FP specimen was negative for both BD MAX Extended Enteric Bacterial Panel and the comparator method in discrepant analysis. One (1) of the six (6) ETEC FP was negative for both BD MAX Extended Enteric Bacterial Panel and the comparator method. Two (2) ETEC specimens obtained a positive status on the BD MAX Extended Enteric Bacterial Panel coupled with an ETEC positive status in the RM testing in the discordance study. One (1) ETEC specimen obtained a positive status on the BD MAX Extended Enteric Bacterial Panel coupled with a negative status in the comparator method testing in the discordance study.

Non-Reportable

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 incompletes were reported during this study (**Table 22**).

Table 22: Non-reportable Rates

Specimen Type	***Unresolved Rate***			***Indeterminate Rate***		***Incomplete 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)*
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 0/1430 (0.0, 0.3)
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. 0/958 (0.0, 0.4)	0 0/958 (0.0, 0.4)