DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

August 27, 2015

BECTON, DICKINSON AND COMPANY
PAUL SWIFT
REGULATORY AFFAIRS MANAGER
7 LOVETON CIR.
SPARKS MD 21152

Re: K143648
Trade/Device Name: BD Max Enteric Parasite Panel, BD Max Instrument
Regulation Number: 21 CFR 866.3990
Regulation Name: Gastrointestinal microorganism multiplex nucleic acid based assay
Regulatory Class: II
Product Code: PCH, OOI
Dated: July 30, 2015
Received: July 31, 2015

Dear Mr. Swift:

This letter corrects our substantially equivalent letter of August 25, 2015.

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 regulation (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" (21CFR 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,

Ribhi Shawar -S

For Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health (OIR)
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)*
K143648

Device Name
BD MAX™ Enteric Parasite Panel

Indications for Use *(Describe)*
The BD MAX™ Enteric Parasite Panel performed on the BD MAX™ System is an automated in vitro diagnostic test for the direct qualitative detection of enteric parasitic pathogens. The BD MAX Enteric Parasite Panel detects nucleic acids from:

- Giardia lamblia
- Cryptosporidium (C. hominis and C. parvum only)
- Entamoeba histolytica

Testing is performed on unpreserved or 10% formalin-fixed stool specimens from symptomatic patients with suspected gastroenteritis, enteritis or colitis. The assay is intended to aid in the diagnosis of gastrointestinal infection when used in conjunction with clinical evaluation and other laboratory findings. The test is performed directly on the specimen, utilizing real-time polymerase chain reaction (PCR) for the amplification of specific targets. The test utilizes fluorogenic gene-specific hybridization probes for 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 Giardia lamblia, Cryptosporidium hominis and C. parvum, as well as Entamoeba histolytica infections. Results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decision. 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 and/or colitis 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.

This section applies only to requirements of the Paperwork Reduction Act of 1995.

DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.

The burden time for this collection of information is estimated to average 79 hours per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."

510(k) Summary

BD MAX Enteric Parasite Panel (EPP)

Summary Preparation Date:

August 18, 2015

Submitted by:

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

Contact:
Paul Swift, RAC
Regulatory Affairs Manager

Tel: 410-316-4905
Fax: 410-316-4188
Email: Paul_Swift@bd.com

Proprietary Names:

For the instrument:

BD MAX™

For the assay:

BD MAX™ Enteric Parasite Panel (EPP)

Common Names:

For the instrument:

Bench-top molecular diagnostics workstation

For the assay:

Enteric Parasite Nucleic Acid Test
Enteric Parasite 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):

PCH Gastrointestinal Pathogen Panel Multiplex Nucleic Acid-Based Assay System

Predicate Device

FilmArray Gastrointestinal (GI) Panel Kit [K140407 (May 2, 2014)], BioFire Diagnostics, LLC

Device Establishment

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

Registration Number: 3007420875

Performance standards

No performance standards have been developed under Section 514 of the Food, Drug and Cosmetic Act.

Intended Use

The BD MAX™ Enteric Parasite Panel performed on the BD MAX™ System is an automated *in vitro* diagnostic test for the direct qualitative detection of enteric parasitic pathogens. The BD MAX Enteric Parasite Panel detects nucleic acids from:

- *Giardia lamblia*
- *Cryptosporidium* (*C. hominis* and *C. parvum* only)
- *Entamoeba histolytica*

Testing is performed on unpreserved or 10% formalin-fixed stool specimens from symptomatic patients with suspected gastroenteritis, enteritis or colitis. The assay is intended to aid in the diagnosis of gastrointestinal infection when used in conjunction with clinical evaluation and other laboratory findings. The test is performed directly on the specimen, utilizing real-time polymerase chain reaction (PCR) for the amplification of specific targets. The test utilizes fluorogenic gene-specific hybridization probes for 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 *Giardia lamblia*, *Cryptosporidium hominis* and *C. parvum*, as well as *Entamoeba histolytica* infections. Results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decision. 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 and/or colitis 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™ System and the BD MAX™ Enteric Parasite Panel are comprised of an 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, NEG or UNR 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

Stool specimens are collected from patients and transported to the laboratory either unpreserved in a clean container or 10% formalin-fixed. The specimen is vortexed and a 10 µL loop is inserted to the depth of the loop into the specimen, and expressed using a swirling motion into a BD MAX Sample Buffer Tube (SBT). The SBT is closed with a septum cap and then heated on the BD Pre-Warm Heater to facilitate

lysis of the parasite organisms. The SBT is then vortexed and transferred to the BD MAX System. Once the work list is generated and the clinical sample is loaded on the BD MAX instrument with a EPP Unitized Reagent Strip (URS) and PCR Cartridge, the run is started and no further operator intervention is required. The BD MAX System automates sample preparation, including target organism lysis, DNA extraction and concentration, reagent rehydration, target nucleic acid sequence amplification and detection using real-time PCR. The interpretation of the signal is performed automatically by the BD MAX System. The assay also includes a Sample Processing Control (SPC) that is provided in the Extraction Tube and subjected to extraction, concentration and amplification steps. The SPC is incorporated into the lysis, extraction, concentration and amplification steps to monitor for the presence of potential inhibitory substances as well as system or reagent failures.

Following enzymatic cell lysis at an elevated temperature, the released nucleic acids are captured on magnetic affinity beads. The beads, with the bound nucleic acids, are washed and the nucleic acids are eluted by heat and high pH in Elution Buffer. Eluted DNA is pH neutralized and transferred to the Master Mix Tube to rehydrate the PCR reagents. After rehydration, the BD MAX System dispenses a fixed volume of DNA eluent 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 heat evaporation and 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 amplicons for enteric parasite targets and the Sample Processing Control in four different optical channels of the BD MAX System: *Giardia lamblia* target amplicons are detected in the FAM channel, *Cryptosporidium parvum/hominis* target amplicons are detected in the ROX channel, *Entamoeba histolytica* target amplicons are detected in the VIC channel and Sample Processing Control amplicons are detected in the Cy5.5 channel.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 DNA 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[1]

Table 1 shows the similarities and differences between the BD MAX Enteric Parasite Panel and the predicate device.

Table 1: Comparison to Predicate Device

ITEM	BD MAX Enteric Parasite Panel	FilmArray Gastrointestinal (GI) Panel Kit [K140407 (May 2, 2014)] BioFire Diagnostics, LLC
Specimen type	Unpreserved and 10% formalin-fixed stool.	Human stool samples collected in Cary Blair transport media.
Assay Format	Amplification: PCR Detection: fluorogenic target-specific hybridization.	Nested multiplex RT-PCR followed by high resolution melting analysis to confirm identity of amplified product.
Interpretation of Test Results	Automated (BD MAX System diagnostic software)	Automated test interpretation and report generation by the FilmArray™ Instrument.
Analysis Platform	BD MAX System	FilmArray™ Instrument
PCR Sample Preparation	Automated by the BD MAX System	Automated by the FilmArray™ Instrument
Detection Probes	TaqMan® Probe	Unknown – information not available publicly
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.

[1] 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.

Analytical Performance

Precision

Within-laboratory precision was evaluated for the BD MAX™ Enteric Bacterial Panel at one (1) site. The Precision panel consisted of 4 sample categories near the LoD. Each specimen contained negative stool matrix. Target strains were tested as follows:

- For moderate positives (MP): overall correct percentage of approximately 100% with 95% CI
- For low positives (LP): overall correct percentage of approximately 95% with 95% CI
- For true negatives (TN): overall correct percentage of approximately 100% with 95% CI
- For high negatives (HN): overall correct percentage between 20 and 80%

Testing was performed in triplicate, over 12 days, with 2 runs per day, by 2 different technologists. Precision study results are summarized below in Table 2.

Table 2: Within-laboratory Precision Testing

Panel Member Level	***Specimen/Target***	***Percentage Correct (%)***
High Negative (HN)	*G. lamblia*	48.6
	C. parvum	41.7
	E. histolytica	37.5
Low Positive (LP)	*G. lamblia*	98.6
	C. parvum	98.6
	E. histolytica	98.6
Moderate Positive (MP)	ALL	100
True Negative (TN)	ALL	100

Reproducibility

For the Site-to-Site reproducibility study, three (3) clinical sites were provided with a total of sixteen (16) panels, each consisting of 12 tubes. The panels used were the same as described under the Precision heading, above. Each site was asked to perform the study on eight (8) distinct days (consecutive or not), wherein each day, two (2) panels were tested, one (1) for each of two (2) technologists.

The overall Site-to-Site Reproducibility percent agreement was 100% for the TN and MP categories for all targets, and ranged from 38.2% to 48.6% and 97.2% to 98.6% for the HN and LP categories, respectively (**Table 3**). The qualitative and quantitative reproducibility across sites and by target is presented below in **Tables 4** through **9**. Ct.Score is an internal criterion used to determine final assay results and was selected as an additional means of assessing assay

reproducibility. Overall mean Ct.Score values with variance components (SD and %CV) are shown in **Tables 5**, **7** and **9**.

Table 3: Site-to-Site Reproducibility Study Results using one lot of the BD MAX Enteric Parasite Panel

Category	***Giardia lamblia***	***Cryptosporidium parvum***	***Entamoeba histolytica***
TN	100.0% (144/144) (97.4%, 100.0%)	100.0% (144/144) (97.4%, 100.0%)	100.0% (144/144) (97.4%, 100.0%)
HN	48.6% (70/144) (40.6%, 56.7%)	38.2% (55/144) (30.7%, 46.3%)	47.2% (68/144) (39.2%, 55.3%)
LP	97.2% (140/144) (93.1%, 98.9%)	97.2% (140/144) (93.1%, 98.9%)	98.6% (142/144) (95.1%, 99.6%)
MP	100.0% (144/144) (97.4%, 100.0%)	100.0% (144/144) (97.4%, 100.0%)	100.0% (144/144) (97.4%, 100.0%)

Table 4: *G. lamblia* Site-to-Site Qualitative Reproducibility Across Sites with Pooled Days, Runs and Replicates

Category	X LoD	SITE												Total			
		CIN				*CLE*				*BD NC*							
		Correct		*Incorrect*		*Correct*		*Incorrect*		*Correct*		*Incorrect*		*Correct*		*Incorrect*	
		n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
TN	0	48	100.0	0	0	48	100.0	0	0	48	100.0	0	0	144	100.0	0	0
HN	0.1-0.5	23	47.9	25	52.1	22	45.8	26	54.2	25	52.1	23	47.9	70	48.6	74	51.4
LP	0.8-1.2	46	95.8	2	4.2	47	97.9	1	2.1	47	97.9	1	2.1	140	97.2	4	2.8
MP	3-5	48	100.0	0	0	48	100.0	0	0	48	100.0	0	0	144	100.0	0	0

Table 5: *G. lamblia* Site-to-Site Quantitative Reproducibility Across Sites, Days, Runs and Within Run

Ct.score			Within Run		Between Run within Day		Between Day within Site		Between Site		Overall	
Category	*n*	*Mean*	*SD*	*%CV*	*SD*	*%CV*	*SD*	*%CV*	*SD*	*%CV*	*SD*	*%CV*
HN	74	31.2	1.17	3.7%	0.55	1.8%	0.56	1.8%	0.00	0.0%	1.41	4.5%
LP	140	28.7	0.78	2.7%	0.00	0.0%	0.00	0.0%	0.37	1.3%	0.86	3.0%
MP	144	26.8	0.44	1.6%	0.15	0.6%	0.00	0.0%	0.15	0.6%	0.49	1.8%

Table 6: *C. parvum* Site-to-Site Qualitative Reproducibility Across Sites with Pooled Days, Runs, and Replicates

Category	X LoD	SITE												Total			
		CIN				*CLE*				*BD NC*							
		Correct		*Incorrect*		*Correct*		*Incorrect*		*Correct*		*Incorrect*		*Correct*		*Incorrect*	
		n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
TN	0	48	100.0	0	0	48	100.0	0	0	48	100.0	0	0	144	100.0	0	0
HN	0.1-0.5	17	35.4	31	64.6	18	37.5	30	62.5	20	41.7	28	58.3	55	38.2	89	61.8
LP	0.8-1.2	46	95.8	2	4.2	47	97.9	1	2.1	47	97.9	1	2.1	140	97.2	4	2.8
MP	3-5	48	100.0	0	0	48	100.0	0	0	48	100.0	0	0	144	100.0	0	0

Table 7: *C. parvum* Site-to-Site Quantitative Reproducibility Across Sites, Days, Runs and Within Run

Ct.score			Within Run		Between Run within Day		Between Day within Site		Between Site		Overall	
Category	*n*	*Mean*	*SD*	*%CV*	*SD*	*%CV*	*SD*	*%CV*	*SD*	*%CV*	*SD*	*%CV*
HN	89	35.7	1.53	4.3%	0.54	1.5%	0.60	1.7%	0.43	1.2%	1.78	5.0%
LP	140	31.4	1.07	3.4%	0.41	1.3%	0.00	0.0%	0.11	0.3%	1.15	3.7%
MP	144	30.1	0.74	2.4%	0.44	1.5%	0.00	0.0%	0.17	0.6%	0.88	2.9%

Table 8: *E. histolytica* Site-to-Site Qualitative Reproducibility Across Sites with Pooled Days, Runs and Replicates

Category	X LoD	SITE												Total			
		CIN				*CLE*				*BD NC*							
		Correct		*Incorrect*		*Correct*		*Incorrect*		*Correct*		*Incorrect*		*Correct*		*Incorrect*	
		n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
TN	0	48	100.0	0	0	48	100.0	0	0	48	100.0	0	0	144	100.0	0	0
HN	0.1-0.5	22	45.8	26	54.2	26	54.2	22	45.8	20	41.7	28	58.3	68	47.2	76	52.8
LP	0.8-1.2	47	97.9	1	2.1	47	97.9	1	2.1	48	100.0	0	0	142	98.6	2	1.4
MP	3-5	48	100.0	0	0	48	100.0	0	0	48	100.0	0	0	144	100.0	0	0

Table 9: *E. histolytica* Site-to-Site Quantitative Reproducibility Across Sites, Days, Runs and Within Run

Ct.score			**Within Run**		**Between Run within Day**		**Between Day within Site**		**Between Site**		**Overall**	
Category	*n*	*Mean*	*SD*	*%CV*	*SD*	*%CV*	*SD*	*%CV*	*SD*	*%CV*	*SD*	*%CV*
HN	76	30.4	2.34	7.7%	1.35	4.4%	0.66	2.2%	0.32	1.0%	2.80	9.2%
LP	142	25.5	0.88	3.4%	0.26	1.0%	0.00	0.0%	0.00	0.0%	0.91	3.6%
MP	144	23.5	0.58	2.5%	0.37	1.6%	0.00	0.0%	0.09	0.4%	0.69	2.9%

For the Lot-to-Lot reproducibility study, two users each completed a single run of 12 panel members on a single instrument for each of two lots of reagents over a 5-day period. 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 agreement was 100% for the TN and MP categories for all targets, and ranged from 48.9% to 55.6% and 97.8% to 98.9% for the HN, and :LP categories, respectively (**Table 10**).

Table 10: Lot-to-Lot Reproducibility - Study Results using three lots of the BD MAX Enteric Parasite Panel

Target	Level	Correct	Total	% Correct	95% CI	
					LowerCI	*UpperCI*
G. lamblia	TN**	90	90	100.0%	95.9%	100.0%
	HN*	44	90	48.9%	38.8%	59.0%
	LP	88	90	97.8%	92.3%	99.4%
	MP	90	90	100.0%	95.9%	100.0%
C. parvum	TN	90	90	100.0%	95.9%	100.0%
	HN	50	90	55.6%	45.3%	65.4%
	LP	88	90	97.8%	92.3%	99.4%
	MP	90	90	100.0%	95.9%	100.0%
E. histolytica	TN	90	90	100.0%	95.9%	100.0%
	HN	45	90	50.0%	39.9%	60.1%
	LP	89	90	98.9%	94.0%	99.8%
	MP	90	90	100.0%	95.9%	100.0%

* HNs are dilutions of the LoD designed to produce results that are negative for 20% to 80% of replicates. As such, "% Correct" correlates to the percent of negative results.
** TNs were blanks, therefore, "% Correct" correlates to the percent of negative results.

Sample Storage

Collected specimens, either unpreserved or 10% formalin fixed stool, should be kept between 2 °C and 25 °C during transport. Protect against freezing or exposure to excessive heat.

Specimens, either unpreserved or 10% formalin fixed stool, can be stored for up to 120 hours (5 days) at 2-8 °C or for a maximum of 48 hours at 2-25 °C before testing.

Prior to or following pre-warm, inoculated Sample Buffer Tubes can be stored for testing (or retesting) for a total of up to:

Time	Temperature
120 hours (5 days)	2 - 8 °C
48 hours	2 - 25 °C

Controls

External Control materials are not provided by BD; 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:

- Commercially available positive control materials
- *Giardia intestinalis* (Lambl) (ATCC 3088D)
- *Cryptosporidium parvum* (ATCC PRA-67D)
- *Entamoeba histolytica* (ATCC 30459D)
- External negative control
- Use a non-inoculated BD MAX™ Enteric Parasite Panel SBT

The assay includes a Specimen 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.

Analytical Sensitivity

The analytical sensitivity (Limit of Detection or LoD) for the BD MAX Enteric Parasite Panel was determined for each assay target individually. Purified organisms were used to prepare targets that were inoculated into the Sample Buffer Tube along with pooled negative stool matrix (both unpreserved and 10% formalin fixed were evaluated, separately). The pooled negative stool matrix was created from stool specimens obtained from patients that were characterized by the BD MAX Enteric Parasite Panel. The organism concentrations were used to simulate positive samples with a wide range of organism loads. The LoD was determined for each organism tested with both unpreserved and 10% formalin fixed target-negative stool matrix. The results from the LoD study can be found below in **Table 11**.

Table 11: BD MAX Enteric Parasite Panel Target Limits of Detection

	Unpreserved [95% Confidence Interval]	**10% formalin fixed [95% Confidence Interval]**
Cryptosporidium parvum		
LoD (organism/mL in SBT) [95% confidence interval]	160.17 [96.93 – 264.22]	154.25 [100.46 – 236.57]
LoD (organism/mL in stool) [95% confidence interval]	24,026 [14,540 – 39,633]	92,550 [60,276 – 141,942]
Giardia lamblia		
LoD (organism/mL in SBT) [95% confidence interval]	10.67 [5.69 - 15.66]	10.04 [4.83 - 15.25]
LoD (organism/mL in stool) [95% confidence interval]	1,601 [854 - 2,349]	6,024 [2,898 - 9,150]
Entamoeba histolytica		
LoD (organism/mL in SBT) [95% confidence interval]	16.79 [11.97 – 23.42]	15.52 [10.99 – 21.76]
LoD (organism/mL in stool) [95% confidence interval]	2,519 [1,796 – 3,570]	9,300 [6,600 – 13,080]

Analytical Inclusivity

The objective of this study was to demonstrate that the BD MAX Enteric Parasite Panel is able to detect clinically relevant and geographically diverse serovars/strains/ subspecies for each of the BD MAX™ Enteric Parasite Panel targets found in various geographical origins. The study was designed to validate the functional performance of the BD MAX™ Enteric Parasite Panel by verifying the specificity of the assay's primers and probes for the targeted bacterial enteric analytes.

In total, eleven (11) distinct strains/isolates of whole organisms of *Giardia lamblia/intestinalis,* ten (10) distinct strains/isolates of whole organisms of *Entamoeba histolytica* and one (1) strain of *Cryptosporidium hominis* whole organism were screened with the BD MAX Enteric Parasite Panel at 2X the point estimate of the 95% LoD (**Table 12**). One genomic DNA preparation, representative of each BD MAX Enteric Parasite Panel target, was screened at 2X LoD (**Table 13**).

Due to the lack of availability of *Cryptosporidium parvum/hominis* organisms, commercially available genomic DNA libraries, prepared from nine (9) distinct *Cryptosporidium parvum* strains/isolates, were also evaluated as supplementary information.

An additional study was performed for *Cryptosporidium* due to the lack of commercially available organisms. DNA extracts from thirteen (13) clinical stool specimens with known *Cryptosporidium* spp. infection status, were obtained (**Table 14**). Because it is not possible to discriminate human or other DNA from parasite DNA by spectrophotometry, quantification of the DNA extracts was not performed. Instead, Sample Buffer Tubes were spiked with 10 µL of negative, pooled stool matrix and with 5 µL of the clinical specimen DNA extract, in duplicate.

Table 12: Inclusivity Organisms

Organism	ATCC ID	Organism	ATCC ID
Giardia lamblia	PRA-242	*Entamoeba histolytica*	PRA-358
Giardia lamblia	PRA-244	*Entamoeba histolytica*	50007
Giardia lamblia	PRA-247	*Entamoeba histolytica*	30890
Giardia lamblia	PRA-249	*Entamoeba histolytica*	30889
Giardia lamblia	30888	*Entamoeba histolytica*	30458
Giardia intestinalis	30957	*Entamoeba histolytica*	30459
Giardia intestinalis	50114	*Entamoeba histolytica*	PRA-357
Giardia intestinalis	50137	*Entamoeba histolytica*	50738
Giardia intestinalis	50581	*Entamoeba histolytica*	30190
Giardia intestinalis	50584	*Entamoeba histolytica*	50541
Giardia intestinalis	50585	*Cryptosporidium parvum*	502 (Tufts)

Table 13: Target Genomic DNA

Organism	Genomic DNA ATCC ID
Cryptosporidium parvum	PRA-67D
Giardia lamblia	30888D
Entamoeba histolytica	30459D

Table 14: Clinical Specimen DNA Extracts: *Cryptosporidium* spp. Positive

Organism	Subtype
Cryptosporidium hominis	IbA10G2
Cryptosporidium hominis	IbA10G2
Cryptosporidium hominis	Unknown
Cryptosporidium hominis	IbA10G2
Cryptosporidium hominis	IbA10G2
Cryptosporidium hominis	IbA10G2
Cryptosporidium hominis	Unknown

Organism	Subtype
Cryptosporidium parvum	Unknown
Cryptosporidium parvum	Unknown
Cryptosporidium parvum	Unknown
Cryptosporidium hominis	IeA11G3T3
Cryptosporidium hominis	IbA10G2
*Cryptosporidium ubiquitum**	Unknown

Analytical Specificity
The BD MAX Enteric Parasite Panel was performed on samples containing phylogenetically related species and other organisms (bacteria, viruses, parasites and yeast) likely to be found in stool specimens. Potentially cross-reacting organisms are summarized in **Tables 15** – **17**.

- Six (6) out of 6 *Entamoeba* spp. (*Entamoeba* species other than *E. histolytica*) produced negative results with the BD MAX Enteric Parasite Panel. The organisms were tested directly from stock at a 1:10 dilution to obtain the highest possible concentration in the Sample Buffer Tube, with concentrations ranging from $4.00x10^3$ organisms/mL to $2.90x10^5$ organisms/mL in the Sample Buffer Tube. *E. barretti* (ATCC 30996) was provided as non-titered stock.
- One (1) out of 1 *Cryptosporidium meleagridis* strain tested at a concentration ≥ $1x10^5$ cycts/mL in the Sample Buffer Tube, produced positive results with the BD MAX Enteric Parasite Panel. *Cryptosporidium meleagridis* has been documented in symptomatic human infection.
- One hundred thirteen (113) out of 113 bacterial strains, tested at a concentration ≥ $1x10^6$ CFU/mL in the Sample Buffer Tube, produced negative results with the BD MAX Enteric Parasite Panel.
- Fifteen (15) out of 15 viruses, produced negative results with the BD MAX Enteric Parasite Panel. Thirteen (13) were tested directly from stock at a 1:10 dilution to obtain the highest possible concentration in the Sample Buffer Tube, with concentrations ranging from $1.6x10^4$ $TCID_{50}$ - $8.9x10^7$ $TCID_{50}$. Human Papillomavirus was tested as plasmid in *Escherichia coli* and Rotavirus was tested as a high titer qualitative standard.
- Five (5) out of 5 phylogenetically unrelated parasites, tested at a concentration ≥ $1x10^5$ organisms/mL in the Sample Buffer Tube, produced negative results with the BD MAX Enteric Parasite Panel.
- Two (2) out of 2 *Candida* spp. tested at a concentration ≥ $1x10^6$ organisms/mL in the Sample Buffer Tube, produced negative results with the BD MAX Enteric Parasite Panel.
- Three (3) enteric organisms representing each target of the BD MAX Enteric Parasite Panel were tested, with results as follows:
  - One (1) of 1 *Cryptosporidium* spp.; *Cryptosporidium parvum* tested at a concentration ≥ $1x10^5$ cycts/mL in the Sample Buffer Tube, produced positive results for *Cryptosporidium* and negative results for all other targets with the BD MAX Enteric Parasite Panel.

- One (1) of 1 *Giardia* spp.; *Giardia lamblia* tested at a concentration $\geq 1x10^5$ cycts/mL in the Sample Buffer Tube, produced positive results for *Giardia* and negative results for all other targets with the BD MAX Enteric Parasite Panel.
- One (1) of 1 *Entamoeba* spp.; *Entamoeba histolytica* tested at a concentration $\geq 1x10^5$ cycts/mL in the Sample Buffer Tube, produced positive results for *Entamoeba* and negative results for all other targets with the BD MAX Enteric Parasite Panel.

Table 15: Potential Cross-Reactant Bacteria and Yeast

Genus	***Species***	***Strain***
Abiotrophia	*defectiva*	ATCC 49176
Acinetobacter	*baumannii*	ATCC 19606
	Iwoffii	ATCC 17925
Aeromonas	*hydrophila*	ATCC 49847
Alcaligenes	*faecalis* subsp. *faecalis*	ATCC 8750
Anaerococcus	*tetradius*	ATCC 35098
Arcobacter	*butzleri*	ATCC 49616
Arcobacter	*cryaerophilus*	ATCC 43157
Bacillus	*cereus*	ATCC 49064
Bacteroides	*caccae*	ATCC 43185
	merdae	ATCC 43184
	stercoris	ATCC 43183
Bifidobacterium	*adolescentis*	ATCC 15706
	longum	ATCC 15707
Camplylobacter	*coli*	ATCC 43134
	concisus	CCUG 17580
	curvus	CCUG 47528
	fetus subsp. *fetus*	ATCC 27374
	fetus subsp. *venerealis*	ATCC 19438
	gracilis	ATCC 33236
	hominis	ATCC BAA-381
	jejuni	ATCC 43429
	lari	ATCC 43675
	rectus	ATCC 33238
	upsaliensis	ATCC 49815
Candida	*albicans*	ATCC 24433
	catenulate	ATCC 18821
Cedecea	*davisae*	ATCC 33431
Chlamydia	*trachomatis*	ATCC VR-879
Citrobacter	*amalonaticus*	ATCC 25405
	fruendii [b]	ATCC 33128
	koseri	ATCC 27156
	sedlakii	ATCC 51115
Clostridium	*difficile*	ATCC 17858
		ATCC 43598
		CCUG 8864- ATCC 9689
		ATCC 43255
		ATCC BAA-1805
		ATCC 43593
	perfringens	ATCC 10543
Collinsella	*aerofaciens*	ATCC 35085
Corynebacterium	*genitalium*	ATCC 33030
Desulfovibrio	*piger*	ATCC 29098
Edwardsiella	*tarda*	ATCC 15947
Eggerthella	*lenta*	ATCC 25559
Enterobacter	*aerogenes*	ATCC 13048
	cloacae [b]	ATCC 35030
Enterococcus	*casseliflavus*	ATCC 49605
	cecorum	ATCC 43198
	dispar	ATCC 51266
	faecalis	ATCC 29212
	gallinarum	ATCC 49573
	hirae	ATCC 49612
	raffinosus	ATCC 49427
Escherichia	*coli*	ATCC 25922
	coli O157 stx 1	BD RD012313-01
	coli O157 stx 2	BD RD092612-01

Genus	***Species***	***Strain***
Escherichia	*coli*	ATCC 12014
		ATCC 8739
		ATCC 10536
		ATCC 33605
	fergusonii	ATCC 35469
	hermannii	ATCC 33650
	vulneris	ATCC 33821
Fusobacterium	*varium*	ATCC 27725
Gardnerella	*vaginalis*	ATCC 14019
Gemella	*morbillorum*	ATCC 27824
Hafnia	*alvei*	ATCC 11604
Helicobacter	*fennelliae*	ATCC 35683
	pylori	ATCC 43504
Klebsiella	*oxytoca*	ATCC 13182
	pneumoniae	ATCC 33495
Lactobacillus	*acidophilus*	ATCC 4355
	reuteri	ATCC 23272
Lactococcus	*lactis*	ATCC 15346
		ATCC 49032
Leminorella	*grimontii*	ATCC 33999
Listeria	*grayi*	ATCC 19120
	innocua	ATCC 33090
	monocytogenes	ATCC 19115
Morganella	*morganii*	ATCC 25830
Peptoniphilus	*asaccharolyticus*	ATCC 14963
Peptostreptococcus	*anaerobius*	ATCC 27337
Plesiomonas	*shigelloides*	ATCC 14029
Porphyromonas	*asaccharolytica*	ATCC 25260
Prevotella	*melaninogenica*	ATCC 25845
Proteus	*mirabilis*	ATCC 29906
	penneri	ATCC 35198
	vulgaris	ATCC 13315
Providencia	*alcalifaciens*	ATCC 27971
	rettgeri	ATCC 29944
	stuartii	ATCC 33672
Pseudomonas	*aeruginosa*	ATCC 27853
	fluorescens	ATCC 13525
Ruminococcus	*bromii*	ATCC 27255
Salmonella	*typhimurium*	ATCC 14028
	enteriditis	ATCC 13076
Serratia	*liquefaciens*	ATCC 35551
	marcescens	ATCC 13880
Shigella	*sonnei*	BD ENF 7142
	flexneri	ATCC 700930
Staphylococcus	*aureus*	ATCC 25923
	epidermidis	ATCC 12228
Stenotrophomonas	*maltophilia*	ATCC 13637
Streptococcus	*agalactiae*	ATCC 13813
	dysgalactiae	ATCC 43078
	intermedius	ATCC 27335
	uberis	ATCC 19436
Trabulsiella	*guamensis*	ATCC 49490
Veillonella	*parvula*	ATCC 10790
Vibrio	*cholerae*	BD ENF 13503
	parahaemolyticus	ATCC 17802
Yersinia	*bercovieri*	ATCC 43970
	enterocolitica	ATCC 9610
	rohdei	ATCC 43380

Table 16: Potential Cross-Reactant Viruses

Virus	Strain
Adenovirus type 2	ATCC VR-680
Adenovirus type 14	ATCC VR-15
Adenovirus type 40	ATCC VR-931
Adenovirus type 41	ATCC VR-930
Coxsackie A9	ATCC VR-186
Coxsackie B1	ATCC VR-687
HHV-5 Cytomegalovirus	ATCC VR-538
Enterovirus type 69	ATCC VR-785
Human Papillomavirus Type 16	ATCC 45113
Human Papillomavirus Type 18	ATCC 45152
Herpes Simplex Virus I	ATCC VR-539
Herpes Simplex Virus II	ATCC VR-734
Norovirus I	0810086CF
Norovirus II	0810087CF
Rotavirus	NATROTA

Table 171: Potential Cross-Reactant Parasites

Parasite	Strain
Blastocystis hominis	ATCC 50608
Encephalitozoon intestinalis	ATCC 50651
Encephalitozoon hellum	ATCC 50504
Encephalitozoon cuniculi	ATCC 50602
Pentatrichomonas hominis	ATCC 30098
Entamoeba barretti	ATCC 30996
Entamoeba dispar	ATCC PRA-260
Entamoeba gigivalis	ATCC 30927
Entamoeba invadens	ATCC 30994
Entamoeba moshkovskii	ATCC 30041
Entamobea ranarum	ATCC 50389
Cryptosporidium meleagridis	1867

Interfering Substances

Twenty-two (22) biological and chemical substances occasionally used or found in stool specimens were evaluated for potential interference with the BD MAX Enteric Parasite Panel near the LOD for each particular target. Included in this study was an Antibiotics Mixture, which consisted of a combination of 8 different antibiotics tested simultaneously, with each antibiotic at a concentration that may be excreted in a stool specimen. Three of the substances tested exhibited potential interference with the BD MAX Enteric Parasite Panel (refer to **Table 18**). Vagisil cream demonstrated potential interference at concentrations greater than 9% in stool. Whole human blood demonstrated potential interference at concentrations greater than 25% in stool. Additional testing with grossly bloody clinical stool specimens showed potential interference in 1 out of a total of 12 specimens tested. Substances that demonstrated interference may result in potential unresolved, indeterminate or false negative results.

Table 18: Endogenous and Commercial Exogenous Substances Tested with the BD MAX Enteric Parasite Panel

Brand Name or Description	Result	Brand Name or Description	Result
Fecal Fat	NI	Spermicidal Lubricant	NI
Human DNA	NI	Diaper Rash Cream	NI
Mucus	NI	Vagisil (>9%)	I
Whole Human Blood (>25%)	I	Laxatives	NI
Hydrocortisone Cream	NI	Anti-Diarrheal (liquid)	NI
Antiseptic Towelettes	NI	Anti-Diarrheal (pill)	NI
Enema	NI	Antibiotics Mixture	NI
Hemorrhoidal Gel	NI	Antacids	NI
Nystatin Cream	NI	Non-Steroidal Anti-Inflammatory (NSAID)	NI
10% Buffered Formalin	NI	Topical Antibiotic	NI
20% Buffered Formalin	NI	Grossly Bloody Stool	I

I: Interference with the BD MAX Enteric Parasite Panel.
NI: No reportable interference with the BD MAX Enteric Parasite Panel.

Carryover/Cross-Contamination

A study was conducted to investigate within-run carryover and between-run carryover while processing specimens with a high load of *Giardia lamblia, Cryptosporidium parvum and Entamoeba histolytica* in the BD MAX Enteric Parasite Panel. A panel made of one high positive member containing the three target organisms and one negative member was used to prepare numerous samples. Isolates of *Giardia lamblia, Entamoeba histolytica* and *Cryptosporidium parvum* were used for the high positive panel member (1×10^5 cysts/trophozoites per mL). 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. Two (2) operators performed a total of 15 runs with each run containing 24 samples.

Carryover contamination was assessed for each target in the BD MAX Enteric Parasite Panel. A total of 180 Sample Buffer Tubes, each containing the 3 BD MAX Enteric Parasite Panel targets, were assessed in the carryover contamination study. All of the 180 spiked Sample Buffer Tubes produced the expected positive results for all 3 target organisms. A total of 180 Sample Buffer Tubes, each negative for all 3 BD MAX Enteric Parasite Panel targets, were also assessed in the carryover contamination study. One hundred and seventy-eight (178) of the 180 spiked Sample Buffer Tubes produced the expected negative results for all 3 target organisms. One expected negative result was positive for *Giardia lamblia* and the second was positive for both *Giardia lamblia* and *Entamoeba histolytica* in a single Sample Buffer Tube.

Mixed Infection/Competitive Interference

The mixed infection/competitive interference study was designed to evaluate the ability of the BD MAX Enteric Parasite Panel to detect low positive results in the presence of other targets at high concentrations. Four (4) organisms (*Giardia lamblia, Cryptosporidium parvum* and two preparations of *Entamoeba histolytica*) were individually prepared at 2X their respective LOD to serve as a low target in the BD MAX Enteric Parasite Panel Sample Buffer Tube. A high target mix comprised of the organisms representative of the other two BD MAX Enteric Parasite Panel analytes at a concentration of $> 1x10^5$ organisms/mL in the Sample Buffer Tube was spiked into the Sample Buffer Tube along with 10 µL of unpreserved stool and tested to simulate mixed infections. The second target mix for *Entamoeba histolytica* also contained *Entamoeba dispar* prepared in the same manner as just described. All four low target organisms were successfully detected by the BD MAX Enteric Parasite Panel when combined with their respective simulated high target concentration mixed infection preparations.

Clinical Performance Studies

A clinical study was designed to assess the performance of the BD MAX Enteric Parasite Panel for the identification of *Giardia lamblia, Cryptosporidium parvum/hominis* and *Entamoeba histolytica*, from unpreserved or 10% formalin-fixed stool specimens from symptomatic patients with suspected gastroenteritis, enteritis and/or colitis. This multicenter study evaluated results obtained with the BD MAX Enteric Parasite Panel compared to those obtained with the reference method. Clinical centers were employed to collect and test patient specimens; whereas collection centers were employed to collect patient specimens, with the reference method and EPP testing being performed off-site.

The study involved a total of five (5) clinical sites where specimens were collected as part of routine patient care, enrolled into the trial, and tested on the BD MAX Enteric Parasite Panel as well as seven (7) collection sites. Only excess, de-identified patient specimens were used. Additionally, an internal site was involved as a clinical center to perform BD MAX Enteric Parasite Panel testing on specimens supplied by

collection centers. Samples tested at BD were obtained from all collection sites. Specimens collected at the sites consisted of a mix of 10% formalin-fixed and unpreserved specimens as well as a mix of prospective and retrospective specimens.

Clinical centers were selected for the clinical study based on a number of criteria, such as investigator and site personnel availability, number of specimens of interest tested for each target, prevalence, and familiarity with PCR methodology. The clinical centers were also selected according to the specimen types that they routinely collect.

A total of 2204 prospective specimens (1128 10% formalin-fixed, 1058 unpreserved and 18 non-compliant) and 411 retrospective specimens (148 10% formalin-fixed, 251 unpreserved and 12 non-compliant) were enrolled in the clinical evaluation. **Table 19** describes the number of compliant specimens enrolled by patient age and specimen type. A total of 128 retrospective specimens were not included in the performance calculations below as the historical results were not confirmed by an alternate PCR and bi-directional sequencing.

Table 19: Compliant clinical trial enrollment summary by age group and specimen type

Age Group	**Specimen Type**		
	Formalin 10%	**Unpreserved**	**Combined**
0-1 month	1	0	1
1 month to 2 years	111	51	162
2-12 years	218	76	294
13-18 years	121	77	198
19-21 years	37	34	71
Over 21 years	723	782	1505
Unknown	62	202	264
Total	**1273**	**1222**	**2495**

For the 10% formalin fixed specimen type, the BD MAX Enteric Parasite Panel identified 95.5% and 99.7% of the *Giardia lamblia* prospective positive and negative specimens, respectively, and 100% and 100% of the retrospective positive and negative specimens, respectively. For the unpreserved specimen type, the BD MAX Enteric Parasite Panel identified 94.4% and 100% of the *Giardia lamblia* prospective positive and negative specimens, respectively, and 98.6% and 94.9% of the retrospective positive and negative specimens, respectively (**Table 20**).

Table 20: *Giardia lamblia* – Clinical Performance

Specimen Type	Specimen Origin	BD MAX Enteric Parasite Panel	RM		Total
			P	N	
10% Formalin Fixed	Prospective	P	21	3[2]	*24*
		N	1[1]	996	*997*
		Total	*22*	*999*	*1021*
		SENSITIVITY (95% CI): 95.5% (78.2%, 99.2%) SPECIFICITY (95% CI): 99.7% (99.1%, 99.9%)			
	Retrospective	P	55	0	*55*
		N	0	71	*71*
		Total	*55*	*71*	*126*
		PPA (95% CI): 100.0% (93.5%, 100.0%) NPA (95% CI): 100.0% (94.9%, 100.0%)			
Unpreserved	Prospective	P	17	0	17
		N	1[3]	655	656
		Total	18	655	673
		SENSITIVITY (95% CI): 94.4% (74.2%, 99.0%) SPECIFICITY (95% CI): 100.0% (99.4%, 100.0%)			
	Retrospective	P	72	7[5, 6]	79
		N	1[4]	129	130
		Total	73	136	209
		PPA (95% CI): 98.6% (92.6%, 99.8%) NPA (95% CI): 94.9% (89.8%, 97.5%)			

1 The alternate PCR and bi-directional sequencing component of the reference method was negative for this specimen. The DFA component of the reference method was positive. Discrepant repeat testing with an alternate PCR and bi-directional sequencing was performed and gave a negative result. Discrepant repeat testing with DFA was done and gave a negative result. Discrepant repeat testing with the BD MAX™ Enteric Parasite Panel was performed in twelve (12) replicates and gave all negative results (0/12). The specimen was also tested using a *Giardia* antigen EIA as discrepant testing and gave a negative result.

2 One (1) specimen was tested using a *Giardia* antigen detecting EIA as discrepant testing and gave a negative result. Discrepant repeat testing with the BD MAX™ Enteric Parasite Panel was performed in six (6) replicates of this specimen and gave 1/6 positive result. No discrepant testing was performed for the other two (2) specimens.

3 The alternate PCR and bi-directional sequencing component of the reference method was negative for this specimen. The DFA segment of the reference method was positive. Discrepant repeat testing with the alternate PCR and bi-directional sequencing was performed and gave a negative result. Discrepant repeat testing with DFA was performed and gave a positive result. Discrepant repeat testing with the BD MAX™ Enteric Parasite Panel was performed in six (6) replicates and gave all negative results (0/6). This specimen was also tested using an antigen detecting EIA as discrepant testing and gave a negative result.

4 No discrepant testing was performed for this specimen.

5 One (1) specimen was tested using an antigen detecting EIA and a commercially-available molecular assay as discrepant testing and gave a positive result for both. Discrepant repeat testing with the BD MAX™ Enteric Parasite Panel was performed in six (6) replicates of this specimen and gave all positive results (6/6).

6 One (1) specimen was tested using an EIA and a commercially-available molecular assay as discrepant testing. The EIA gave a positive result and the molecular assay gave a negative result. Discrepant repeat testing with the BD MAX™ Enteric Parasite Panel was done in eleven (11) replicates of this specimen and gave 5 positive results (5/11). No discrepant testing was performed for the other five (5) specimens.

For the 10% formalin fixed specimen type, the BD MAX Enteric Parasite Panel identified 90.3% and 99.8% of the *Cryptosporidium parvum/hominis* prospective positive and negative specimens, respectively, and 93% and 100% of the retrospective positive and negative specimens, respectively. For the unpreserved specimen type, the BD MAX Enteric Parasite Panel identified 100% and 99.5% of the *Cryptosporidium parvum/hominis* prospective positive and negative specimens, respectively, and 97.7% and 98.4% of the retrospective positive and negative specimens, respectively (**Table 21**). Because DFA identifies *Cryptosporidium* to the genus level, DFA-positive specimens identified by bi-directional sequencing as other than *C. hominis* or *C. parvum* were considered reference method negative.

Table 21: *Cryptosporidium parvum/hominis* – Clinical Performance

Specimen Type	Specimen Origin	BD MAX™ Enteric Parasite Panel	RM		Total
			P	N	
10% Formalin Fixed	Prospective	P	56	2[3]	*58*
		N	6[1, 2]	951[4]	*957*
		Total	*62*	*953*	*1015*
		SENSITIVITY: 90.3% (80.5%, 95.5%) SPECIFICITY: 99.8% (99.2%, 99.9%)			
	Retrospective	P	40	0	*40*
		N	3[5]	78	*81*
		Total	*43*	*78*	*121*
		PPA: 93% (81.4%, 97.6%) NPA: 100% (95.3%, 100%)			
Unpreserved	Prospective	P	35	3[6]	38
		N	0	625	625
		Total	35	628	663
		SENSITIVITY: 100% (90.1%, 100%) SPECIFICITY: 99.5% (98.6%, 99.8%)			
	Retrospective	P	43	3[7]	46
		N	1	181	182
		Total	44	184	228
		PPA: 97.7% (88.2%, 99.6%) NPA: 98.4% (95.3%, 99.4%)			

1 All six (6) specimens were positive by the DFA component of the composite reference method. One (1) specimen sequenced as *C. parvum*, three (3) specimens were negative and two (2) were non-reportable by the alternate PCR and bi-directional sequencing components of the composite reference method.

2 Discrepant repeat testing with the alternate PCR and bi-directional sequencing was performed on all six (6) specimens. One (1) specimen sequenced as *Cryptosporidium parvum*, one (1) specimen sequenced as *Cryptosporidium felis* and the remaining four (4) were PCR negative by discrepant repeat testing. Discrepant testing was also performed using an antigen detecting EIA that does not distinguish between *Cryptosporidium* and *Giardia*. Two specimens were EIA negative and four specimens were EIA positive, of which two were positive for *Giardia* by other test methods.

3 One DFA-positive specimen was classified as reference method negative based on alternate PCR and bi-directional sequencing results that identified *Cryptosporidium meleagridis*.

4 Six DFA-positive specimens were classified as reference method negative based on alternate PCR and bi-directional sequencing results that identified (4) *Cryptosporidium canis*, (1) *C. meleagridis* and (1) *Cryptosporidium* spp. (undefined).

5 Discrepant repeat testing was performed with BD MAX Enteric Parasite Panel in twelve (12) replicates per specimen. One specimen was positive for five (5) of twelve (12) replicates and one specimen was positive for two (2) of twelve (12) replicates.

6 Discrepant repeat testing was performed with BD MAX Enteric Parasite Panel in six (6) replicates per specimen. One specimen was positive for five (5) of six (6) replicates and one specimen was positive for three (3) of six (6) replicates. A third specimen was negative in six (6) of six (6) replicates.

7 Discrepant repeat testing was performed with BD MAX Enteric Parasite Panel in six (6) replicates per specimen. Two specimens were negative for six (6) of six (6) replicates.

For the 10% formalin fixed specimen type, the BD MAX Enteric Parasite Panel identified 100% of the *Entamoeba histolytica* negative specimens for both the prospective and retrospective specimens. There were no prospective or retrospective 10% formalin fixed *Entamoeba histolytica* positive specimens found during the clinical evaluation. For the unpreserved specimen type, the BD MAX Enteric Parasite Panel identified 100% *Entamoeba histolytica* prospective negative specimens and 100% and 100% of the retrospective positive and negative specimens, respectively (**Table 22**). There were no prospective unpreserved *Entamoeba histolytica* positive specimens found during the clinical evaluation.

Table 22: *Entamoeba histolytica* – Clinical Performance

Specimen Type	Specimen Origin	BD MAX Enteric Parasite Panel	RM		Total
			P	N	
10% Formalin Fixed	Prospective	P	0	0	0
		N	0	827	827
		Total	0	827	827
		SENSITIVITY (95% CI): No data for calculation SPECIFICITY (95% CI): 100% (99.5%, 100%)			
	Retrospective	P	0	0	0
		N	0	54	54
		Total	0	54	54
		PPA (95% CI): No data for calculation NPA (95% CI): 100% (93.4%, 100%)			
Unpreserved	Prospective	P	0	0	0
		N	0	577	577
		Total	0	577	577
		SENSITIVITY (95% CI): No data for calculation SPECIFICITY (95% CI): 100% (99.3%, 100%)			
	Retrospective	P	11	0	11
		N	0	191	191
		Total	11	191	202
		PPA (95% CI): 100% (74.1%, 100%) NPA (95% CI): 100% (98.0%, 100%)			

As *Entamoeba histolytica* is a rare analyte, both prospective and retrospective testing efforts were unable to demonstrate the positive percent agreement of the BD MAX Enteric Parasite Panel. To supplement the prospective and retrospective data, an evaluation of contrived specimens was performed. Surrogate clinical specimens were prepared using residual specimens that had previously tested negative for all BD MAX Enteric Parasite Panel targets. Specimens were spiked at clinically relevant levels at various concentrations of the limit of detection for each specimen type. Users analyzing the specimens were blinded as to the specimen status.

For both the 10% formalin fixed and unpreserved specimen types, the BD MAX Enteric Parasite Panel correctly identified 100% of both the positive and negative specimens. The contrived study results obtained with the BD MAX Enteric Parasite Panel were compared to the expected results and are summarized in **Table 23**.

Table 23: *Entamoeba histolytica* – Contrived Specimen Performance

Specimen Type	BD MAX Enteric Parasite Panel	Expected Result		Total
		P	N	
Formalin 10%	P	50	0	50
	N	0	50	50
	Total	50	50	100
	PPA (95% CI): 100% (92.9%, 100%) NPA (95% CI): 100% (92.9%, 100%)			
Unpreserved	P	50	0	50
	N	0	50	50
	Total	50	50	100
	PPA (95% CI): 100% (92.9%, 100%) NPA (95% CI): 100% (92.9%, 100%)			

Performance of the BD MAX Enteric Parasite Panel by *Cryptosporidium hominis* and *Cryptosporidium parvum* species identification as observed during the clinical trial is presented below in **Table 24**. The species identification was obtained from the alternate PCR and bi-directional sequencing segment of the composite reference method. While the BD MAX Enteric Parasite Panel is designed to detect the species described below, the panel does not report results to the species level.

Table 24: *Cryptosporidium* PPA per species observed during the clinical trial

Cryptosporidium			PPA	
Specimen Type	Specimen Origin	Species	Estimate	95% CI
10% Formalin Fixed	Prospective	*hominis*	100.0% (17/17)	(81.6%, 100.0%)
		parvum	97.4% (37/38)	(86.5%, 99.5%)
	Retrospective	*hominis*	95.0% (19/20)	(76.4%, 99.1%)
		parvum	91.3% (21/23)	(73.2%, 97.6%)
Unpreserved	Prospective	*hominis*	100.0% (22/22)	(85.1%, 100.0%)
		parvum	100.0% (11/11)	(74.1%, 100.0%)
	Retrospective	*hominis*	96.2% (25/26)	(81.1%, 99.3%)
		parvum	100.0% (18/18)	(82.4%, 100.0%)

There were twenty-three (23) co-infections detected by the BD MAX Enteric Parasite Panel. **Table 25** below shows the co-infections detected by the BD MAX Enteric Parasite Panel during the clinical trial.

Table 25: Co-infections observed during the BD MAX Enteric Parasite Panel clinical trial

Co-Infections		Number of Co-Infections Observed	Number of Discrepant Co-Infections	Discrepant Target	Unavailable Reference Method Result for Comparison
Target 1	**Target 2**				
Cryptosporidium parvum/hominis	*Entamoeba histolytica*	3	1	*Cryptosporidium hominis/parvum*	1 *Cryptosporidium parvum/hominis*
Giardia lamblia	*Cryptosporidium parvum/hominis*	11	2	*Cryptosporidium parvum/hominis*	3 *Giardia lamblia* and 4 *Cryptosporidium parvum/hominis*
Giardia lamblia	*Entamoeba histolytica*	9	4[1]	*Giardia lamblia*	1 *Giardia lamblia* and 3 *Entamoeba histolytica*

[1] All four (4) are retrospective specimens with unconfirmed historical results.

Of the 2226 specimens initially evaluated with the BD MAX Enteric Parasite Panel, 1.5% of the 10% formalin fixed and 4.7% of the unpreserved specimens were initially reported as Unresolved. Following a valid repeat test, 0% of the 10% formalin fixed and 1.2% of the unpreserved specimens remained Unresolved. The total numbers provided in **Table 26** are based on compliant specimens and BD MAX Enteric Parasite Panel results.

Table 26: Unresolved Rates

		Initial Unresolved Rate with 95% CI		Final Unresolved Rate with Valid Repeat with 95% CI	
Specimen Type	**Specimen Origin**	**Percent**	**95% CI**	**Percent**	**95% CI**
Formalin 10%	Prospective	1.5% (16/1084)	(0.9%, 2.4%)	0.0% (0/1084)	(0.0%, 0.4%)
	Retrospective	1.4% (2/146)	(0.4%, 4.9%)	0.0% (0/146)	(0.0%, 2.6%)
Unpreserved	Prospective	5.6% (42/752)	(4.2%, 7.5%)	1.5% (11/747)	(0.8%, 2.6%)
	Retrospective	2.0% (5/244)	(0.9%, 4.7%)	0.4% (1/244)	(0.1%, 2.3%)

Of the 2226 specimens initially evaluated with the BD MAX Enteric Parasite Panel, 0.3% of the 10% formalin fixed and 0.1% of the unpreserved specimens were initially reported as Indeterminate. Following a valid repeat test, 0% of both the 10% formalin fixed and the unpreserved specimens remained Indeterminate. The total numbers provided in **Table 27** are based on compliant specimens and BD MAX Enteric Parasite Panel results.

Table 27: Indeterminate Rates

		Initial Indeterminate Rate with 95% CI		Final Indeterminate Rate with Valid Repeat with 95% CI	
Specimen Type	**Specimen Origin**	**Percent**	**95% CI**	**Percent**	**95% CI**
Formalin 10%	Prospective	0.4% (4/1084)	(0.1%, 0.9%)	0.0% (0/1084)	(0.0%, 0.4%)
	Retrospective	0.0% (0/146)	(0.0%, 2.6%)	0.0% (0/146)	(0.0%, 2.6%)
Unpreserved	Prospective	0.1% (1/752)	(0.0%, 0.7%)	0.0% (0/747)	(0.0%, 0.5%)
	Retrospective	0.0% (0/244)	(0.0%, 1.5%)	0.0% (0/244)	(0.0%, 1.5%)

Of the 2226 specimens initially evaluated with the BD MAX Enteric Parasite Panel, 0.6% of the 10% formalin fixed and 0.4% of the unpreserved specimens were initially reported as Incomplete. Following a valid repeat test, 0% of both the 10% formalin fixed and the unpreserved specimens remained Incomplete. The total numbers provided in **Table 28** are based on compliant specimens and BD MAX Enteric Parasite Panel results.

Table 28: Incomplete Rates

		Initial Incomplete Rate with 95% CI		Final Incomplete Rate with Valid Repeat with 95% CI	
Specimen Type	**Specimen Origin**	**Percent**	**95% CI**	**Percent**	**95% CI**
Formalin 10%	Prospective	0.6% (6/1084)	(0.3%, 1.2%)	0.0% (0/1084)	(0.0%, 0.4%)
	Retrospective	0.7% (1/146)	(0.1%, 3.8%)	0.0% (0/146)	(0.0%, 2.6%)
Unpreserved	Prospective	0.0% (0/752)	(0.0%, 0.5%)	0.0% (0/747)	(0.0%, 0.5%)
	Retrospective	1.6% (4/244)	(0.6%, 4.1%)	0.0% (0/244)	(0.0%, 1.5%)