510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:

K143648

B. Purpose for Submission:

To obtain a substantial equivalence determination for a new device

C. Measurand:

Target nucleic acids from:

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

D. Type of Test:

Qualitative real-time polymerase chain reaction (PCR) assay

E. Applicant:

BD Diagnostics Systems
Becton, Dickinson and Company

F. Proprietary and Established Names:

BD MAX™ Enteric Parasite Panel (EPP)
BD MAX™ System

G. Regulatory Information:

1. Regulation section:

   866. 3990 - Gastrointestinal microorganism multiplex nucleic acid-based assay

2. Classification:

   II

3. Product code:

   PCH, OOI

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   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.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   BD MAX™ System

I. 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 instrument includes a pre-warm heater as part of the sample preparation. 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 NEG (negative), POS (positive) or UNR (unresolved) 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.

Reagents provided with the BD MAX Enteric Parasite Panel

- BD MAX Enteric Parasite Master Mix: Oven-dried PCR Master Mix containing TaqMan® specific molecular probe and primers along with Sample Processing Control-specific Taqman probe and primers.

- BD MAX Enteric Parasite Reagent Strip: Unitized reagent strip containing all liquid reagents and disposable pipette tips necessary for specimen processing and DNA extraction.

- BD MAX Enteric Parasite Extraction Tube: Oven-dried DNA magnetic affinity beads, protease reagents, and Sample Processing Control

- BD MAX Enteric Bacterial Panel Sample Buffer Tube (with septum caps)

Equipment and materials required but not provided

- BD Pre-warm Heater
- BD MAX PCR Cartridges
- VWR Multi-Tube Vortex Mixer or equivalent
- Vortex Genie 2 or equivalent
- Nalgene® Cryogenic Vial Holder or equivalent
- Disposable gloves, powderless
- 10 µL loops (BD Catalog no. 220216)

For unpreserved stool specimen types:

- Dry, clean containers for the collection of stool specimens

For 10% formalin-fixed stool specimen types:

- 10% formalin transport (15 mL)

J. Substantial Equivalence Information:

1. Predicate device name(s):
   FilmArray Gastrointestinal (GI) Panel Kit, BioFire Diagnostics, LLC

2. Predicate 510(k) number(s):
   K140407

3. Comparison with predicate:

Similarities		
Item	Device:	Predicate:
	BD MAX Enteric Parasite Panel	FilmArray Gastrointestinal Panel Kit
Intended Use	Detects nucleic acids of enteric parasitic pathogens from the stool samples of patients with symptoms of gastrointestinal infection as an aid in the diagnosis of gastrointestinal illness.	Same (See below for target organism differences)
Target organism DNA detected	*Giardia lamblia, Cryptosporidium (C. hominis* and *C. parvum only)*, *Entamoeba histolytica*	Same (See below for noted differences)
Test Interpretation	Automated	Same
Analyte	Nucleic acids	Same
Technology	Multiplex nucleic acid amplification and detection	Same (See below for noted differences)

Differences		
Item	Device	Predicate
	BD MAX Enteric Parasite Panel	FilmArray Gastrointestinal Panel Kit
Instrumentation	BD MAX Instrument	FilmArray Instrument
Specimen Types	Unpreserved or 10% formalin-fixed stool specimens	Cary Blair stool specimens
Organisms Detected	*Giardia lamblia, Cryptosporidium (C.*	*Campylobacter* (*C. jejuni/C. coli/C.*

Differences		
Item	Device	Predicate
	hominis and *C. parvum only)*, *Entamoeba histolytica*	*upsaliensis*), *Clostridium difficile* (*C. difficile*) toxin A/B, *Plesiomonas shigelloides, Salmonella, Vibrio* (*V. parahaemolyticus/V. vulnificus/ V. cholerae*), including specific identification of *Vibrio cholera, 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), *Cyclospora cayetanensis,· Cryptosporidium* (genus claim), Adenovirus F 40/41, Astrovirus,·Norovirus GI/GII, Rotavirus A, Sapovirus (Genogroups I, II, IV, and V)
Technology	Amplification: RT-PCR Detection: fluogenic target specific hybridization	Amplification: Nested multiplex PCR Detection: high resolution melting analysis

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

Not applicable

L. Test Principle:

Stool specimens are collected from patients and transported to the laboratory in a clean container, unpreserved 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. The Sample Buffer Tube is closed with a septum cap and then heated on the BD Pre-warm Heater to facilitate lysis of the parasite organisms. The Sample Buffer Tube is then vortexed and transferred to the BD MAX System. Once the work list is generated and the sample is loaded on the BD MAX instrument with a BD MAX Enteric Parasite Panel Unitized Reagent Strip 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 that is provided in the Extraction Tube and subjected to extraction, concentration and amplification steps. The Sample Processing Control 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 neutralized and transferred to the Master Mix Tube to rehydrate the PCR reagents. After rehydration, the BD MAX System dispenses a fixed volume of PCR-ready solution 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 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Site-to-site reproducibility

   Site-to-site reproducibility was evaluated for the BD MAX Enteric Parasite Panel at three clinical sites by two operators at each site for eight days. Each panel member was tested in triplicate for each run resulting in a total of 144 data points per panel member. The panel members were prepared as organism mixes of *G. lamblia cysts, C. parvum* oocysts, and *E. histolytica* trophozoites spiked at varying concentrations per target in negative unpreserved stool matrix:

   Moderate Positive (MP): 2 to 5x LOD
   Low Positive (LP): 0.6 to 1.2x LOD
   High Negative (HN): 0.1 to 0.5x LOD
   True Negative (TN): No Target

   Three replicates of each positive mix and true negative sample were included in each run. Panel members were prepared by spiking SBTs with organism mixes. Testing panels were shipped to testing sites and prior to testing, the user expressed a 10µl loop of pooled stool matrix (previously determined to be negative for all analytes by the BD MAX Enteric Bacterial Panel) into each sample.

   Site-to-site reproducibility results are presented as percent agreement with expected result (%), (ratio of count with expected result/total count), and [95% confidence interval] in Table 1.

   Table 1. Site-to-Site Reproducibility Overall Results

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%]

   The qualitative and quantitative reproducibility across sites and by target is presented below in Tables 2 through 7. Ct.Score is an internal criterion used to determine final assay results and was selected as an additional means of assessing assay reproducibility.

Table 2. *Giardia lamblia* Site-to-Site Qualitative Reproducibility

Category	SITE												Total			
	1				2				3							
	Correct		Incorrect		Correct		Incorrect		Correct		Incorrect		Correct		Incorrect	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
TN	48	100	0	0	48	100	0	0	48	100	0	0	144	100	0	0
HN	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	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	48	100	0	0	48	100	0	0	48	100	0	0	144	100	0	0

Table 3. *Giardia lamblia* Site-to-Site Quantitative Reproducibility

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 4. *C. parvum* Site-to-Site Qualitative Reproducibility

Category	SITE												Total			
	1				2				3							
	Correct		Incorrect		Correct		Incorrect		Correct		Incorrect		Correct		Incorrect	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
TN	48	100	0	0	48	100	0	0	48	100	0	0	144	100	0	0
HN	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	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	48	100	0	0	48	100	0	0	48	100	0	0	144	100	0	0

Table 5. *C. parvum* Site-to-Site Quantitative Reproducibility

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 6. *E. histolytica* Site-to-Site Qualitative Reproducibility

Category	SITE												Total			
	1				2				3							
	Correct		Incorrect		Correct		Incorrect		Correct		Incorrect		Correct		Incorrect	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
TN	48	100	0	0	48	100	0	0	48	100	0	0	144	100	0	0
HN	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	47	97.9	1	2.1	47	97.9	1	2.1	48	100	0	0	142	98.6	2	1.4
MP	48	100	0	0	48	100	0	0	48	100	0	0	144	100	0	0

Table 7. *E. histolytica* Site-to-Site Quantitative Reproducibility

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%

Lot to Lot Reproducibility

Lot-to-Lot reproducibility was evaluated with three replicates of each panel member and three production lots at a single site, with two user/runs per day performed over five days. The panels used were the same as described for Site-to-Site reproducibility. Results from five days of the Site-to-Site 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. Lot-to-Lot reproducibility is presented in Tables 8 and 9 below.

Table 8. Lot-to-Lot Qualitative Reproducibility

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%

* TNs contained no organisms. "% Correct" correlates to the percent of negative results.

** HNs are dilutions of the LoD designed to produce results that are negative for 20% to 80% of replicates. "% Correct" correlates to the percent of negative results.

Table 9. Lot-to-Lot Quantitative Reproducibility

Target	Ct.score			Within Run		Between Run within Day		Between Day within Lot		Between Lot		Overall	
	Category	n	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
G. lamblia	HN	46	31.3	0.96	3.1%	0.50	1.6%	0.35	1.1%	0.54	1.7%	1.25	4.0%
	LP	88	29.4	0.90	3.1%	0.15	0.5%	0.25	0.9%	0.57	1.9%	1.11	3.8%
	MP	90	27.1	0.41	1.5%	0.37	1.4%	0.00	0.0%	0.30	1.1%	0.63	2.3%
C. parvum	HN	40	36.8	1.59	4.3%	1.16	3.1%	0.00	0.0%	1.09	3.0%	2.25	6.1%
	LP	88	33.2	1.72	5.2%	0.74	2.2%	0.07	0.2%	1.35	4.1%	2.31	7.0%
	MP	90	32.1	1.25	3.9%	1.09	3.4%	0.00	0.0%	1.90	5.9%	2.52	7.9%
E. histolytica	HN	45	30.7	2.70	8.8%	0.00	0.0%	0.83	2.7%	0.18	0.6%	2.83	9.2%
	LP	89	26.0	0.94	3.6%	0.41	1.6%	0.00	0.0%	0.61	2.4%	1.20	4.6%
	MP	90	24.5	0.87	3.5%	0.52	2.1%	0.21	0.9%	0.89	3.6%	1.37	5.6%

Precision

Within-laboratory precision was evaluated at one site over 12 days with two runs per day (one each by two technologists) using the same panel as described for the site-to-site reproducibility study. Within-laboratory precision results are presented in Tables 10 and 11 below.

Table 10. Precision Study Qualitative Results

Category	*G. lamblia* %, (n), [95% CI]	*C. parvum* %, (n), [95% CI]	*E. histolytica* %, (n), [95% CI]
TN*	100.0% (72/72) [94.9%, 100.0%]	100.0% (72/72) [94.9%, 100.0%]	100.0% (72/72) [94.9%, 100.0%]
HN*	48.6% (35/72) [37.4%, 59.9%]	41.7% (30/72) [31.0%, 53.2%]	37.5% (27/72) [27.2%, 49.0%]
LP	98.6% (71/72) [92.5%, 99.8%]	98.6% (71/72) [92.5%, 99.8%]	98.6% (71/72) [92.5%, 99.8%]
MP	100.0% (72/72) [94.9%, 100.0%]	100.0% (72/72) [94.9%, 100.0%]	100.0% (72/72) [94.9%, 100.0%]

*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 11. Precision Study Quantitative Results

Target	Ct.score			Within Run		Between Run within Day		Between Day		Overall	
	Category	n	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV
G. lamblia	HN	37	31.5	1.40	4.5%	0.64	2.0%	0.33	1.0%	1.58	5.0%
	LP	71	29.0	0.86	3.0%	0.00	0.0%	0.00	0.0%	0.86	3.0%
	MP	72	26.9	0.43	1.6%	0.23	0.8%	0.00	0.0%	0.48	1.8%
C. parvum	HN	42	36.3	1.74	4.8%	0.47	1.3%	0.84	2.3%	1.98	5.5%
	LP	71	31.6	1.12	3.5%	0.25	0.8%	0.00	0.0%	1.14	3.6%
	MP	72	30.3	0.56	1.9%	0.35	1.1%	0.00	0.0%	0.66	2.2%
E. histolytica	HN	45	29.9	2.56	8.6%	0.00	0.0%	0.19	0.6%	2.57	8.6%
	LP	71	25.5	0.67	2.6%	0.40	1.6%	0.00	0.0%	0.78	3.1%
	MP	72	23.6	0.64	2.7%	0.39	1.6%	0.13	0.5%	0.76	3.2%

b. *Linearity/assay reportable range:*

Not applicable

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

Positive and Negative External Controls:

External control materials are not provided by BD; however, Quality Control strains and procedures are recommended in the package insert. External positive controls are intended to monitor for substantial reagent failure, and External negative controls are used to detect reagent or environmental contamination (or carry-over) by target nucleic acids. 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 patient samples. Various types of external controls are recommended to allow the user to select the most appropriate for their laboratory quality control program. Due to lack of commercial availability of organism oocysts/cysts for all targets and burdensome culture methods for the trophozoite form of the target organisms, BD recommends genomic DNA preparations according to the listing below:

Commercially available external positive control materials
- *Giardia intestinalis* (Lambl) (ATCC 3088D) (2,475 pg/mL gDNA)
- *Cryptosporidium parvum* (ATCC PRA-67D) (11,025 pg/mL gDNA)
- *Entamoeba histolytica* (ATCC 30459D) (150 pg/mL gDNA)

Recommended external negative control
- A non-inoculated BD MAX™ Enteric Parasite Panel SBT

During the clinical study, the above mentioned external positive and negative controls were included in each run. A failed control invalidated the entire run. In the case of a failed control, the testing was repeated for all samples in the run from the appropriately stored SBTs, along with a new set of controls. A total of 253 external positive controls and 253 external negative controls were tested resulting in valid expected results for 242 (95.6%) and 240 (94.9%) of positive controls and negative controls, respectively. Three runs were invalidated due to Incomplete results (1.2%), and one run was invalidated due to an Indeterminate result (7.4%). Positive control testing resulted in five valid but incorrect results (2.0%) and two Unresolved results (0.8%). Negative control testing resulted in three Unresolved results (1.2%).

Internal Sample Processing Control:

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

reported as Unresolved.

Sample Processing Control Effectiveness Study

A Sample Processing control effectiveness study was performed to evaluate the effectiveness of the SPC to monitor for substantial reagent or process failure. This study was designed to test for failure in the extraction process, failure in PCR amplification and/or failure due to the presence of PCR inhibitors.

Twenty-four replicates were tested for each of the following conditions:
- Failure in PCR amplification was tested by replacing the BD MAX Enteric Bacterial Panel Master Mix tube with an empty Snap Tube (expected result = IND).
- Failure in the Extraction process was tested by replacing the BD MAX Enteric Bacterial Panel Extraction tube with an empty Snap Tube (expected result = UNR).
- Failure due to the presence of PCR inhibitors was tested by placing 100 mM EDTA solution into an empty Snap Tube of the BD MAX System test rack prior to beginning a run (expected result = UNR).
- Valid SPC control (expected result = positive SPC)

Each test condition produced the expected SPC detection or failures for all replicates.

Specimen Stability Study:

Stability studies were performed to demonstrate that target DNA is stable in both unpreserved and 10% formalin-fixed stool specimens prior to testing with the BD MAX EPP. The claimed storage conditions for either unpreserved or 10% formalin-fixed stool are at 2 – 25 °C for a maximum of 48 hours and at 2 – 8 °C for up to 120 hours (five days). The claimed storage conditions for samples inoculated into Sample Buffer Tubes are at 2 – 25 °C for a maximum of 48 hours and at 2 – 8 °C for up to 120 hours.

C. parvum, G. lamblia, and *E. histolytica* target organisms were spiked into negative unpreserved and fixed stool matrices at a final target concentration of 2X LoD. Contrived specimens were also inoculated into SBTs and stability was evaluated either before or after the pre-warm step of the BD MAX EPP procedure. Testing included 24 replicates for each test condition at baseline, 24, and 48 hours for storage claims at 25°C ± 2°C, and at 48 hours and 120 hours for storage claims at 5 °C ± 3°C.

The specimen stability studies support the claimed storage times and conditions for target DNA in both unpreserved and 10% formalin fixed stool. The SBT stability studies support the claimed storage times and conditions for target DNA in SBT either before or after the pre-warm step.

d. Detection limit:

Limit of Detection (LoD) was determined in an analytical study: Stock organisms were purified and quantified for 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 (unpreserved or 10% formalin-fixed) that was pre-determined to be negative for all the targets detected by the BD MAX Enteric Parasite Panel. Each organism was tested with a minimum of 30 replicates per specimen type (unpreserved or 10% formalin-fixed), by a single operator, using three different production lots of the BD MAX Enteric Parasite Panel kits. Each LOD, defined as the lowest concentration at which greater than 95% of all replicates are expected to test positive, ranged from 10.67 to 160.17 organisms per milliliter (in SBT) and 1,601 to 24,026 organisms per milliliter (in stool) for unpreserved specimens, and 10.04 to 154.25 organisms per milliliter (in SBT) and 6,024 to 92,550 (in stool) for 10% formalin-fixed specimens.

Results are shown in the following table.

Table 12. BD MAX Enteric Parasite Panel Limit of Detection

	Unpreserved	**10% formalin fixed**
Cryptosporidium parvum		
LoD (organism/mL in SBT[a]) [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]

[a] SBT: Sample Buffer Tube

Inclusivity/reactivity

Inclusivity was assessed through testing clinically relevant and geographically diverse organism isolates for each of the assay target organisms. Twenty-two isolates representing the three assay targets were tested (11 isolates of *Giardia lamblia* and *Giardia intestinalis*, 10 isolates of *Entamoeba histolytica*, and one isolate of *Cryptosporidium hominis*). The isolates were tested at 2X the LOD in unpreserved

stool matrix either as target pools containing two assay targets each or individually (Table 13). The BD MAX EPP detected all isolates.

Genomic DNA extracts from 13 individual human clinical stool specimens (nine *C. hominis*, three *C. parvum,* and one *C. ubiquitum*) collected during routine clinical testing or from a Cryptosporidiosis outbreak in 2005 were also tested (Table 14). The assay identified all 13 genomic DNA extracts as being positive for *C. hominis*/*parvum*.

Additionally, genomic DNA and prepared genomic DNA libraries were also evaluated. One genomic DNA representative of each BD MAX Enteric Parasite Panel target was tested at 2X the LOD for the assay (Table 15). Due to the lack of availability of *Cryptosporidium parvum* and *Cryptosporidium hominis* organisms, commercially available genomic DNA libraries prepared from nine distinct *Cryptosporidium parvum* isolates were also evaluated as supplementary information. The BD MAX EPP detected all samples at the concentrations tested.

Table 13. Isolates Evaluated for Inclusivity

Organism	ATCC ID	Strain/Designation	Geographic Origin
Giardia lamblia	PRA-242	CM	Florida
Giardia lamblia	PRA-244	Mario	U.S.
Giardia lamblia	PRA-247	DAN	U.S.
Giardia lamblia	PRA-249	BE-1	Canada
Giardia lamblia	30888	Portland-1	Oregon
Giardia intestinalis	30957	WB	Maryland
Giardia intestinalis	50114	KS	Pennsylvania
Giardia intestinalis	50137	New Orleans-1	Louisiana
Giardia intestinalis	50581	GS clone H7	Alaska
Giardia intestinalis	50584	JH	West Virginia
Giardia intestinalis	50585	AB	Peru
Entamoeba histolytica	30190	HB-301:NIH	Burma
Entamoeba histolytica	50541	HK-9 Clone 1	Korea
Entamoeba histolytica	PRA-358	F22	U.S.
Entamoeba histolytica	50007	DKB	London
Entamoeba histolytica	30890	HM-3:IMSS	Mexico
Entamoeba histolytica	30889	H-458:CDC	Asia
Entamoeba histolytica	30458	200:NIH	U.S.
Entamoeba histolytica	30459	HM-1:IMSS	Mexico
Entamoeba histolytica	PRA-357	IP:1182:2	Honduras
Entamoeba histolytica	50738	Rahman	U.K.
Cryptosporidium parvum	502 (Tufts)	TU	U.S.

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

Organism	Subtype	Geographic Origin (Year)
Cryptosporidium hominis	IbA10G2	Spray Park (2005)
Cryptosporidium hominis	IbA10G2	Spray Park (2005)
Cryptosporidium hominis	Unknown	Spray Park (2005)
Cryptosporidium hominis	IbA10G2	Spray Park (2005)
Cryptosporidium hominis	IbA10G2	Spray Park (2005)
Cryptosporidium hominis	IbA10G2	Spray Park (2005)
Cryptosporidium hominis	Unknown	Routine Clinical (2006)
Cryptosporidium parvum	Unknown	Routine Clinical (2010)
Cryptosporidium parvum	Unknown	Routine Clinical (2011)
Cryptosporidium parvum	Unknown	Routine Clinical (2012)
Cryptosporidium hominis	IeA11G3T3	Spray Park (2005)
Cryptosporidium hominis	IbA10G2	Spray Park (2005)
*Cryptosporidium ubiquitum**	Unknown	Spray Park (2005)

* *C. ubiquitum* has been previously identified in the literature as *C. parvum* genotype cervine and in 2010 was reclassified as *C. ubiquitum* (Fayer, et al., 2010; Ong, et al., 2002).

Table 15. Genomic DNA Evaluated for Inclusivity

Organism	ATCC ID	Isolate/Strain
Cryptosporidium parvum	PRA-67D	Iowa
Giardia lamblia	30888D	Oregon
Entamoeba histolytica	30459D	Mexico

e. *Analytical specificity:*

Cross reactivity:

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 listed in Tables 16 - 18. Results are summarized below:

- 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 $\geq 1x10^5$ cysts/mL in the Sample Buffer Tube, produced positive results with the BD MAX Enteric Parasite Panel. *C. meleagridis* has been documented in

symptomatic human infection. (Table 18 *****). *C. meleagridis* will be listed as a potential cross reactant with the *C. parvum/C. hominis* assay target.

- 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 ≥ $1x10^5$ cysts/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 ≥ $1x10^5$ cysts/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 ≥ $1x10^5$ cysts/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 16. Potential Cross-reactant Bacteria and Yeast

Genus	Species	ID
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

Genus	Species	ID
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

Enterococcus	*casseliflavus*	ATCC 49605	*Streptococcus*	*agalactiae*	ATCC 13813
	cecorum	ATCC 43198		*dysgalactiae*	ATCC 43078
	dispar	ATCC 51266		*intermedius*	ATCC 27335
	faecalis	ATCC 29212		*uberis*	ATCC 19436
	gallinarum	ATCC 49573	*Trabulsiella*	*guamensis*	ATCC 49490
	hirae	ATCC 49612	*Veillonella*	*parvula*	ATCC 10790
	raffinosus	ATCC 49427	*Vibrio*	*cholerae*	BD ENF 13503
Escherichia	*coli*	ATCC 25922		*parahaemolyticus*	ATCC 17802
	coli O157 stx 1	BD RD012313-01	*Yersinia*	*bercovieri*	ATCC 43970
	coli O157 stx 2	BD RD092612-01		*enterocolitica*	ATCC 9610
				rohdei	ATCC 43380

Table 17. Potential Cross-reactant Viruses

Virus	ID
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 18. Potential Cross-reactant Parasites

Parasite	ID
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

Interference:

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. Antibiotics were included as 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 exhibited potential interference with the BD MAX Enteric Parasite Panel (refer to Table 19). 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 one out of a total of 12 specimens tested. Substances that demonstrated interference may result in unresolved (UNR), indeterminate (IND), or false negative assay results.

Table 19. Endogenous and Commercial Exogenous Substances Tested

Potential Interferent	Result	Potential Interferent	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.

Fresh versus Frozen Study

A fresh versus frozen study was performed to support the use of frozen unpreserved stool specimens in the clinical study; the test is not intended for use on frozen samples. Seven individual negative clinical unpreserved stool specimens were used to prepare a panel of sixty-six positive samples spiked with each of the three target organisms at various concentrations. The panel included low positive (2X LoD; 24 replicates), moderate positive (4X LoD; 24 replicates), as well as high positive samples (10X, 50X, and 100X LoD; 6 replicates each). Panels were tested with the BD MAX EPP at baseline, stored at -20 °C, and then exposed to a total of 10 freeze/thaw cycles. The samples were tested again after every other freeze/thaw cycle. One *C. parvum* replicate and one *E. histolytica* replicate produced Unresolved results at baseline testing and were excluded from analysis. All replicates with positive baseline results were positive with the BD MAX EPP for up to 10 freeze/thaw cycles. The results indicate that up to 10 freeze/thaw cycles with unpreserved stool should not significantly affect the performance of the BD MAX EPP as compared to fresh specimen testing.

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 was prepared with one high positive member containing all three target organisms and one negative member. Isolates of *Giardia lamblia*, *Entamoeba histolytica* and *Cryptosporidium parvum* were spiked at 1 x 10^5 cysts/trophozoites per mL to prepare the high positive panel member. 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 three 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 three target organisms. A total of 180 Sample Buffer Tubes, each negative for all three 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. 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. The overall carryover/cross-contamination rate was 1.1%.

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 concentration target in the BD MAX Enteric Parasite Panel Sample Buffer Tube. A high concentration target mix of the other two BD MAX Enteric Parasite Panel analytes was spiked into the Sample Buffer Tube at a final concentration of > 1x10^5 organisms/mL 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 as just described for the other high concentration targets. All four low concentration target organisms were successfully detected by the BD MAX Enteric Parasite Panel when combined with other organisms at high concentrations to simulate mixed infections.

f. Assay cut-off:

Assay cut-offs for the BD MAX Enteric Parasite Panel were determined in analytical verification experiments and then subsequently validated using data from the multi-site clinical study. PCR metrics from the clinical study were graphically and statistically

analyzed as compared to results from the reference method for each targeted analyte. ROC curve analysis was performed separately for each PCR metric to confirm the optimal cutoffs for all analytes as compared to the reference method.

2. Comparison studies:

   a. *Method comparison with predicate device:*

      Not applicable

   b. *Matrix comparison:*

      Not applicable

3. Clinical studies:

   A multicenter clinical study was conducted 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 suspected of gastroenteritis, enteritis and/or colitis. The study evaluated results obtained with the BD MAX Enteric Parasite Panel compared to those obtained with the reference method.

   The study involved a total of five (5) clinical sites where specimens were collected and tested on the BD MAX Enteric Parasite Panel, as well as seven (7) sites which served as collection sites. Clinical centers were selected for the study based on a number of criteria, such as investigator and site personnel availability, number of specimens of interest, target prevalence, routinely collected specimen types, and familiarity with PCR methodology. Additionally, an internal site was involved as a clinical center to perform BD MAX Enteric Parasite Panel testing on specimens supplied by collection sites. Samples tested at BD were obtained from all collection sites. Specimens consisted of a mix of 10% formalin-fixed and unpreserved specimens as well as a mix of prospective and retrospective specimens. Only excess, de-identified patient specimens were used.

   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. Retrospective unpreserved specimens were stored at -20 °C or colder. Out of 978 compliant prospective unpreserved specimens, 287 were stored at -20 °C or colder. A total of 128 retrospective specimens were not included in the performance calculations as the historical results were not confirmed by an alternate PCR and bi-directional sequencing.

Table 20. 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 21).

Table 21. *Giardia lamblia* - Clinical Performance

Specimen Type	Specimen Origin	BD MAX EPP	Reference Method		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[4, 5]	79
		N	1	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, and the DFA component was positive. Discrepant repeat testing with alternate PCR/ sequencing and DFA were performed and both produced negative results. Discrepant repeat testing with the BD MAX™ Enteric Parasite Panel was performed in twelve (12) replicates of this specimen and gave all negative results (0/12). Discrepant testing with a *Giardia* antigen EIA also gave a negative result.

2 Discrepant testing with a *Giardia* antigen EIA gave a negative result for one (1) specimen, and discrepant repeat testing with the BD MAX™ Enteric Parasite Panel in six (6) replicates of this specimen and gave a positive result for one replicate (1/6). 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, and the DFA component was positive. Discrepant repeat testing with the alternate PCR/sequencing gave a negative result, and discrepant repeat testing with DFA gave a positive result. Discrepant repeat testing with the BD MAX™ Enteric Parasite Panel was performed in six (6) replicates of this specimen and gave all negative results (0/6). Discrepant testing with a *Giardia* antigen EIA also gave a negative result.

4 Discrepant testing on one (1) specimen with a *Giardia* antigen EIA and a commercially-available molecular assay gave positive results for both. Discrepant repeat testing with the BD MAX™ Enteric Parasite Panel performed in six (6) replicates of this specimen gave all positive results (6/6).

5 Discrepant testing on one (1) specimen with a *Giardia* antigen EIA gave a positive result, and discrepant testing with a commercially-available 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 22). 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 22. *Cryptosporidium hominis/parvum* - Clinical Performance

Specimen Type	Specimen Origin	BD MAX EPP	Reference Method		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 specimens were positive by the DFA component of the reference method. One 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) replicates (5/12) and one specimen was positive for two (2) replicates (2/12).

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) specimens. 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 23. There were no prospective unpreserved *Entamoeba histolytica* positive specimens found during the clinical evaluation.

Table 23. *Entamoeba histolytica* - Clinical Performance

Specimen Type	Specimen Origin	BD MAX EPP	Reference Method		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 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 24.

Table 24. *Entamoeba histolytica* – Contrived Specimen Performance

Specimen Type	BD MAX EPP	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 25. 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 25. *Cryptosporidium* PPA per species observed during the clinical trial

Cryptosporidium			**PPA**	
Specimen Type	**Specimen Origin**	**Species**	**%Point Estimate (n/N)**	**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 26 below shows the co-infections detected by the BD MAX Enteric Parasite Panel during the clinical trial.

Table 26. 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**				
C. parvum/ hominis	*E. histolytica*	3	1	*C. parvum/ hominis*	1 *C. parvum/ hominis*
G. lamblia	*C. parvum/ hominis*	11	2	*C. parvum/ hominis*	3 *G. lamblia* and 4 *C. parvum/ hominis*
G. lamblia	*E. histolytica*	9	4[1]	*G. lamblia*	1 *G. lamblia* and 3 *E. 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 27 are based on compliant specimens and BD MAX Enteric Parasite Panel results.

Table 27. 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 28 are based on compliant specimens and BD MAX Enteric Parasite Panel results.

Table 28. 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 29 are based on compliant specimens and BD MAX Enteric Parasite Panel results.

Table 29. 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%)

In the event of an unsuccessful pre-warm step with the BD MAX Pre-Warm heater, the BD MAX System will display an error message. There were a total of 211 pre-warm runs during the BD MAX EPP clinical study. A single BD MAX Pre-Warm failure was observed for a failure rate of 0.5% (1/211).

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

In the BD MAX Enteric Parasite Panel clinical study, reportable results from compliant specimens were obtained from clinical sites and compared to a composite reference method. The study population was grouped based on specimen type and age. The number and percentage of positive cases by target, as determined by the BD MAX Enteric Parasite Panel during the prospective segment of the clinical trial, are presented below in Table 30. Table 30 includes data from all clinical trial sites, including a state reference laboratory that enrolled specimens that were presumptively positive for *Cryptosporidium* as well as specimens collected outside of the US.

Table 30. BD MAX Enteric Parasite Panel Expected Values

		BD MAX Enteric Parasite Panel Positive Rate					
		Giardia lamblia		*Cryptosporidium parvum/hominis*		*Entamoeba histolytica*	
Specimen Type	**Age Group**	**Number of Specimens**	**Expected Value**	**Number of Specimens**	**Expected Value**	**Number of Specimens**	**Expected Value**
Formalin 10%	1 month to 2 years	103	3.9% (4/103)	103	4.9% (5/103)	103	0.0% (0/103)
	2-12	185	5.4% (10/185)	185	8.6% (16/185)	185	0.0% (0/185)
	13-18	105	0.0% (0/105)	105	6.7% (7/105)	105	0.0% (0/105)
	19-21	32	3.1% (1/32)	32	9.4% (3/32)	32	0.0% (0/32)
	Over 21	658	1.7% (11/658)	658	4.1% (27/658)	658	0.0% (0/658)
	Unknown	1	0.0% (0/1)	1	0.0% (0/1)	1	0.0% (0/1)
	Overall study	1084	2.4% (26/1084)	1084	5.4% (58/1084)	1084	0.0% (0/1084)
Unpreserved	1 month to 2 years	2	0.0% (0/2)	2	0.0% (0/2)	2	0.0% (0/2)
	2-12	41	2.4% (1/41)	41	0.0% (0/41)	41	0.0% (0/41)
	13-18	59	0.0% (0/59)	59	0.0% (0/59)	59	0.0% (0/59)
	19-21	19	0.0% (0/19)	19	0.0% (0/19)	19	0.0% (0/19)
	Over 21	499	1.0% (5/499)	498	0.2% (1/498)	498	0.0% (0/498)
	Unknown	116	11.2% (13/116)	116	34.5% (40/116)	116	0.0% (0/116)
	Overall study	736	2.6% (19/736)	735	5.6% (41/735)	735	0.0% (0/735)
Combined	1 month to 2 years	105	3.8% (4/105)	105	4.8% (5/105)	105	0.0% (0/105)
	2-12	226	4.9% (11/226)	226	7.1% (16/226)	226	0.0% (0/226)
	13-18	164	0.0% (0/164)	164	4.3% (7/164)	164	0.0% (0/164)
	19-21	51	2.0% (1/51)	51	5.9% (3/51)	51	0.0% (0/51)
	Over 21	1157	1.4% (16/1157)	1156	2.4% (28/1156)	1156	0.0% (0/1156)
	Unknown	117	11.1% (13/117)	117	34.2% (40/117)	117	0.0% (0/117)
	Overall study	1820	2.5% (45/1820)	1819	5.4% (99/1819)	1819	0.0% (0/1819)

N. Instrument Name:

BD MAX System

O. System Descriptions:

1. Modes of Operation:

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

Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

Yes ____X___ or No _____

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

Yes _____ or No ___X____

2. Software:

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

   Yes ___X___ or No _____

3. Specimen Identification:

   Specimens are labeled with a barcode.

4. Specimen Sampling and Handling:

   A disposable inoculating loop is used to place 10 µL of the unpreserved or 10% formalin stool specimen into a SBT which is then vortexed and placed onto the system.

5. Calibration:

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

6. Quality Control:

   See section M.1c above.

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

Not applicable

Q. Proposed Labeling:

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

R. Conclusion:

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