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

I Background Information:

A 510(k) Number

K220193

B Applicant

Becton, Dickinson and Company

C Proprietary and Established Names

BD MAX Enteric Parasite Panel

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PCI	Class II	21 CFR 866.3990 - Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assay	MI - Microbiology
PCH	Class II	21 CFR 866.3990 - Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assay	MI - Microbiology
OOI	Class II	21 CFR 862.2570 - Instrumentation for clinical multiplex test systems	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determine for BD Max Enteric Parasite Panel (BD Max EPP) with stool specimens collected using the Copan FecalSwab Collection, Transport and

Preservation System (Copan FecalSwab) and BD FecalSwab Collection, Transport and Preservation System (BD FecalSwab). The Copan FecalSwab is also co-branded as the BD FecalSwab Collection, Transport and Preservation System (BD FecalSwab) and has been FDA-cleared under K142094 (Copan Italia SpA, legal manufacturer); the terms Copan FecalSwab, BD FecalSwab, and FecalSwab may be used interchangeably.

B Measurand:

Target DNA sequences with the BD MAX EPP detects nucleic acids from:

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

C Type of Test:

The BD MAX EPP is a Qualitative real-time polymerase chain reaction (PCR) assay for the amplification and detection of DNA from *Giardia lamblia, Cryptosporidium (C. hominis and C. parvum only), and Entamoeba histolytica.*

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The BD MAX EPP 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 EPP 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 or FecalSwab 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 ulcerative colitis, irritable bowel syndrome, or Crohn's disease.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

BD MAX EPP is performed on the BD MAX System

IV Device/System Characteristics:

A Device Description:

The BD MAX EPP assay together with the BD MAX System contain an instrument with associated hardware and accessories, disposable microfluidic cartridges, master mixes, unitized reagent strips, and extraction reagents. The instrument automates sample preparation including target lysis, DNA extraction and concentration, reagent rehydration, 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. For the BD MAX EPP, a test result may be called POS, NEG or UNR (Unresolved) based on the amplification status of the targets and of the Sample Processing control. IND (Indeterminate) or INC (Incomplete) results are due to BD MAX System failure.

B Principle of Operation:

The principles of the test remain unchanged from K143648, other than addition of stool specimens collected with the FecalSwab Collection, Transport, and Preservation System as an additional validated specimen type.

The BD MAX EPP is to be used with raw unpreserved stool specimens, 10% Formalin preserved stool specimens or stool in FecalSwab specimens. Unpreserved samples are placed in a BD MAX sample buffer tube (SBT) with a 10 µL transfer loop for analysis on the BD MAX System. Specimens preserved in 10% Formalin utilizes a plastic paddle (scoop) to place a stool sample into 15 ml of 10% Formalin media for transport before being placed into a SBT with a 10 µL transfer loop prior to analysis on the BD MAX System.

C Instrument Description Information:

1. Instrument Name:

***For the instrument**:*

BD MAX

For the assay:

BD MAX EPP

2. Specimen Identification:

   Unpreserved or 10% formalin-fixed stool or FecalSwab unpreserved collected stool specimens from symptomatic patients with suspected gastroenteritis, enteritis, or colitis.

3. Specimen Sampling and Handling:

   Once specimens (Unpreserved, 10% Formalin, or FecalSwab) are placed into a BD MAX SBT, the principles of the test are as described in K143648.

   The BD MAX EPP assay is designed for use on the BD MAX System (K111860), an automated sample-to-answer processor that enables on-board extraction of nucleic acids and PCR amplification to provide diagnostic results and remains unchanged from K143648. This submission is only for the addition of a new collection/transport device (FecalSwab) to the existing assay as performed on the BD MAX System. Since the previously cleared BD MAX System hardware was not modified to accommodate the new collection device, electrical safety evaluation is not required. Instrument software updates were reported regularly upon new assay submissions.

   No changes have been made to the BD MAX EPP assay contents, which are presented in Table 1. Table 2 provides the contents of the FecalSwab.

Table 1: BD MAX EPP Assay Contents

Contents	Quantity
BD MAX Enteric Parasite Panel Master Mix (A7) *Dried PCR Master Mix containing polymerase, nucleotides, and specific molecular probes (0.005% w/v) and primers (0.007% w/v) along with Sample Processing Control and PCR enzyme (1.8E -15% w/v).*	24 tests (2 x 12 tubes)
BD MAX Enteric Parasite Unitized Reagent Strips *Unitized Reagent Strip containing wash buffer with 0.1% v/v Tween 20 (0.7 mL), elution buffer (0.7 mL) and neutralization buffer with 0.02% v/v Tween 20 (0.7 mL) reagents and disposable pipette tips necessary for sample processing and DNA Extraction.*	24 tests
BD MAX Enteric Parasite Panel Extraction Tube (B2) *Oven-dried pellet containing DNA magnetic affinity beads (6.4% w/v), protease reagents (6.67% w/v) and Sample Processing Control.*	24 tests (2 x 12 tubes)
BD MAX Enteric Parasite Panel Sample Buffer Tubes *(with 1% v/v Triton X-100)*	24 tests (2 x 12 tubes)
Septum Caps	25

Table 2: FecalSwab Collection, Transport, and Preservation System Kit Content

Contents	Quantity
FecalSwab Transport and Preservation Medium *Screw cap tube with 2 ml of Copan's modified Cary-Blair Media*	1*
Flocked Swab *Plastic shaft swab with nylon flocked tip intended as transfer device for stool specimens*	1*

*****Note, 50 FecalSwab collection kits are contained in a shelf pack and 10 x 50 units are contained in a box.

4. Calibration:

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

5. Quality Control:

   External controls are not provided with the BD MAX EPP; however, recommendations for control preparation and testing are provide in the package inserts. Both panels include an individual Sample Processing Control (SPC) as part of every test that undergoes the extraction, concentration and amplification steps to monitor for potential inhibitory substances which may be present. Additionally, the SPC monitors for potential process inefficiency due to instrument or reagent failure. External quality control and SPC have not changed since the clearance of the BD MAX EPP K143648.

V Substantial Equivalence Information:

A Predicate Device Name(s):

BD MAX Enteric Parasite Panel, BD MAX Instrument

B Predicate 510(k) Number(s):

K143648

C Comparison with Predicate(s):

Device & Predicate Device(s):	Subject Device (K220193)	Predicate (K143648)
Device Trade Name	**BD MAX Enteric Parasite Panel**	**BD MAX Enteric Parasite Panel, BD MAX Instrument**
General Device Characteristic Similarities		
Intended Use/Indications for 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	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 or FecalSwab 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.	parasite 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 test 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 colitis, irritable bowel syndrome, or Crohn's disease.	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.
Organisms Detected	• *Giardia lamblia* • *Cryptosporidium (C. hominis and C. parvum only)* • *Entamoeba histolytica*	Same
Assay Format	Amplification: PCR Detection: Fluorogenic target-specific hybridization	Same
Mode of Detection	Presence of: • SSU rRNA gene specific for *Giardia lamblia* • Sequence contained within the Laxer locus specific for *Cryptosporidium (C. hominis and C. parvum only)* • SSU rRNA gene specific for *Entamoeba histolytica*	Same
Interpretation of Test Results	Automated (BD MAX System diagnostic software)	Same
Analysis Platform	BD MAX System	Same
PCR Sample Preparation	Automated by the BD MAX System	Same
Detection Probes	TaqMan Probe	Same
Assay Controls	Assay Controls Sample Processing Control (SPC)	Same
General Device Characteristic Differences		
Specimen Type	Unpreserved stool or 10% formalin-fixed or FecalSwab specimens	Unpreserved stool or 10% formalin-fixed

VI Standards/Guidance Documents Referenced:

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

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

   Within-lab precision and reproducibility studies were evaluated previously using unpreserved stool specimens. This represents the most challenging specimen type and therefore additional studies were not conducted. See K143648 for the BD MAX EPP.

2. Linearity:

   Not Applicable

3. Analytical Specificity/Interference:

   Analytical Specificity/Cross-Reactivity: Analytical specificity/cross-reactivity was evaluated previously in the presence of unpreserved stool which represents the most challenging specimen type. Please refer to K143648. No specificity issues were identified from additional studies conducted to support testing FecalSwab stool specimens.

   Interference Substances (exogenous/endogenous): Interfering substances were previously evaluated in the presence of unpreserved stool which contains the highest concentration of potential inhibitors. Please refer to K143648.

4. Assay Reportable Range:

   Not Applicable

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   Positive and Negative External Controls: External control materials are not provided by BD; however, Quality Control material was previously addressed in the original submission, please refer to K143648.

6. Detection Limit:

   Limit of Detection (LoD) Study: An LoD study was conducted to demonstrate the performance of the FecalSwab to preserve the target analytes for use with the BD Max EPP assay. The LoD study was performed for each of the three (3) targets of this assay (*Entamoeba histolytica, Cryptosporidium parvum*, *Giardia lamblia*) in FecalSwab specimens following a two-phase study design. The study was designed to ensure the FecalSwab does not introduce bias into the BD Max EPP assay. The LoD study prepared target analyte panels in unpreserved stool matrix and FecalSwab. The BD MAX EPP Sample Buffer Tubes were prepared for each specimen panel: unpreserved (UNP) stool specimens and FecalSwab (FS) specimens.

   In the first phase of the study, a preliminary LoD for each target was determined by testing multiple replicate specimens (n=12) of each target in negative clinical matrix at low concentrations until two critical concentrations were identified: a target concentration with 100% positive rate, and a lower concentration, within five-fold dilution, with a positive rate greater than zero and less than 100%. The preliminary LoD was defined in this study as the

lowest target concentration with a 100% positive rate. Target concentrations used for each spiked stool specimen for LoD determination is shown in Table 3**.**

Table 3: Target Concentrations Spiked into Stool Specimens for LoD Determination

Target Dilution Level	LoD unpreserved from BD MAX EPP PI (orgs/mL in stool)	*Cryptosporidium parvum* (orgs/mL in stool)	*Giardia lamblia* (orgs/mL in stool)	*Entamoeba histolytica* (orgs/mL in stool)
Dilution 1	5x	120,130	8,005	12,595
Dilution 2	1x	24,026	1,601	2,519
Dilution 3	0.2x	4,805	320	504
Dilution 4	0.04x	961	64	101
Dilution 5	0.008x	192	13	20

In the second phase of the study, the size of the test panels was increased to 20 replicates per concentration. The first concentration evaluated for each target was the preliminary LoD, as determined in the first phase of the study. If the positive rate for this test panel was exactly 95% (19/20), the preliminary LoD would be 'confirmed' for that target and specimen type. If the positive rate at the preliminary LoD was not 95%, additional testing would be conducted. The sample from the initial LoD was run in 20 replicates and achieved a 19/20 positives (95% positivity) rate and was considered the final LoD.

The LoD determination and confirmation positivity rates obtained for each *Entamoeba histolytica* level tested are reported in Table 4. The LoD determination required an additional dilution (4 orgs/mL stool) because the initial five dilutions obtained a positivity rate of 100%. The initial LoD concentration for *Entamoeba histolytica* was 20 orgs/mL stool for both FecalSwab and unpreserved specimens, corresponding to the lowest target level to achieve 100% positivity. Therefore, the confirmed LoD for *Entamoeba histolytica* target is 12 orgs/mL stool for both FecalSwab and unpreserved specimens, corresponding to the lowest target level to achieve ≥ 95% positivity.

Table 4: Positivity Rate Obtained for *Entamoeba Histolytica* Target for LoD Determination and Confirmation

		Unpreserved LoD from BD MAX EPP PI (x LoD)	Concentration orgs/mL Stool	FecalSwab positivity rate	Unpreserved positivity rate
Entameoba histolytica	**LoD Determination**	5x	12595	12/12 (100%)	12/12 (100%)
		1x	2519	12/12 (100%)	12/12 (100%)
		0.2x	504	12/12 (100%)	12/12 (100%)
		0.04x	101	12/12 (100%)	12/12 (100%)
		0.008x	**20**	**12/12 (100%)**	**12/12 (100%)**
		0.0016x	4	7/12 (58.3%)	9/12 (75.0%)

	LoD Confirmation	0.008x	20	20/20 (100%)	20/20 (100%)
		0.0048x	**12**	**20/20 (100%)**	**20/20 (100%)**
		0.0016x	4	16/20 (80.0%)	14/20 (70.0%)

The LoD determination and confirmation positivity rates obtained for each *Cryptosporidium parvum* level tested are reported in Table 5. The LoD determination step identified 961 orgs/mL stool as the initial *Cryptosporidium parvum* LoD for both FecalSwab and unpreserved specimens, which corresponded to the lowest target level to achieve 100% positivity. The initial LoD (961 orgs/mL stool) tested for the LoD confirmation step obtained a positivity rate below 95%. The second level tested for LoD confirmation (4805 orgs/mL stool) obtained a positivity rate of 100% for both FecalSwab and unpreserved specimens. Therefore, the confirmed LoD for *Cryptosporidium parvum* target is 4805 orgs/mL stool for both FecalSwab and unpreserved specimens, corresponding to the lowest target level to achieve ≥ 95% positivity.

Table 5: Positivity Rate Obtained for *Cryptosporidium Parvum* Target for LoD Determination and Confirmation

		Unpreserved LoD from BD MAX EPP PI (x LoD)	**Concentration orgs/mL stool**	**FecalSwab positivity rate**	**Unpreserved positivity rate**
Cryptosporidium parvum	**LoD Determination**	5x	120130	12/12 (100%)	12/12 (100%)
		1x	24026	12/12 (100%)	12/12 (100%)
		0.2x	4805	12/12 (100%)	12/12 (100%)
		0.04x	**961**	**12/12 (100%)**	**12/12 (100%)**
		0.008x	192	2/12 (16.7%)	5/12 (41.7%)
	LoD Confirmation	**0.2x**	**4805**	**20/20 (100%)**	**20/20 (100%)**
		0.04x	961	17/20 (85%)	15/20 (75%)

The LoD determination and confirmation of positivity rates obtained for each *Giardia lamblia* level tested are reported in Table 6. The LoD determination step identified 320 orgs/mL stool as the initial *Giardia lamblia* LoD for both FecalSwab and unpreserved specimens, corresponding to the lowest target level to achieve 100% positivity. The initial LoD (320 orgs/mL stool) tested for the LoD confirmation step obtained a positivity rate below 95%. The second level tested for LoD confirmation (1601 orgs/mL stool) obtained a positivity rate of 100% for both FecalSwab and unpreserved specimens. Therefore, the

confirmed LoD for *Giardia lamblia* is 1601 orgs/mL stool for both FecalSwab and unpreserved specimens, corresponding to the lowest target level to achieve ≥ 95%.

Table 6: Positivity Rate Obtained for *Giardia lamblia* Target for LoD Determination and Confirmation

		Unpreserved LoD from BD MAX EPP PI (x LoD)	**Concentration orgs/mL stool**	**FecalSwab positivity rate**	**Unpreserved positivity rate**
Giardia lamblia	**LoD Determination**	5x	8005	12/12 (100%)	12/12 (100%)
		1x	1601	12/12 (100%)	12/12 (100%)
		0.2x	**320**	**12/12 (100%)**	**12/12 (100%)**
		0.04x	64	6/12 (50%)	7/12 (58%)
		0.008x	13	2/12 (16.7%)	1/12 (8.3%)
	LoD Confirmation	**1x**	**1601**	**20/20 (100%)**	**20/20 (100%)**
		0.2x	320	18/20 (90%)	18/20 (90%)

In summary the study confirmed LoD for each of the three target organisms, *Entamoeba histolytica, Cryptosporidium parvum*, and *Giardia lamblia*, for the BD MAX EPP assay with FecalSwab specimens. The study demonstrated that there is a similar analytical sensitivity for detection of all targets when spiked into the FecalSwab device when compared to unpreserved specimen run in parallel. The LoD values for each target in the FecalSwab specimen was also similar to the LoD values from unpreserved stool specimen in the original submission K143648.

7. Assay Cut-Off:

Assay cut-offs remain unchanged from previously cleared versions of the BD MAX EPP (K14143648).

8. Accuracy (Instrument):

Not Applicable

9. Carry-Over:

Carry-over was established previously using unpreserved stool in EBP sample buffer which represents the worst-case scenario for carry-over contamination; therefore, additional studies were not necessary, please refer to K143648.

User Variability Studies

The User variability studies were carried out to determine if the preparation of the FecalSwab by different users introduces variability in the expected results for the BD MAX EPP assay. Six (6) different users prepared two (2) different FecalSwab samples from each of five (5) panel members made of one (1) negative panel member, three (3) panel members at 2x LoD, and one (1) panel member at 4x LoD of co-spiked targets. As *Giardia lamblia* and *Cryptosporidium parvum* are the most prevalent targets, they were used to evaluate user variability of the BD MAX EPP assay. Once the FecalSwab samples were prepared by various users, all subsequent steps, including the transfer to SBTs from each FecalSwab, were performed by a single user. Acceptance criteria were: 100% negative results for the twelve (12) negative samples, ≥95% positive results for the thirty-six (36) samples tested at 2x LoD, and 100% positive for the twelve (12) samples tested at 4x LoD. All samples yielded the expected assay results. The assay results obtained for both targets in this study, *Giardia lamblia* and *Cryptosporidium parvum*, at each level tested are displayed in Table 7 and all conditions met acceptance criteria.

Table 7: User Variability Results

Organism	Conc	Assay Results (POS/NEG/Total)	Pass/Fail Acceptance Criteria
Giardia lamblia	2x LoD	36 / 0 / 36	Pass
	4x LoD	12 / 0 / 12	
	NEG	0 / 12 / 12	
Cryptosporidium parvum	2x LoD	36 / 0 / 36	
	4x LoD	12 / 0 / 12	
	NEG	0 / 12 / 12	

B Comparison Studies:

1. Method Comparison with Predicate Device:

Specimen Stability Study

The purpose of the specimen stability study was to verify the preservation of amplifiable DNA from *Cryptosporidium parvum*, *Giardia lamblia*, and *Entamoeba histolytica* in both the FecalSwab collection device and SBT in specified storage conditions. The study was conducted through the storage of prepared positive and negative samples at specified temperatures and subsequent assay of the samples at selected stability test points using the BD MAX System and the BD MAX EPP assay. In order to be consistent with the storage conditions recommended in the current BD MAX EPP assay package insert for the two previously validated specimen types (unpreserved (UNP) and 10% Formalin), BD performed specimen stability testing to demonstrate that FecalSwab specimens were capable of being stored at 25°C ± 2°C for up to 48 hours or at 5°C ± 3°C for up to 120 hours (5 days) prior to testing. BD also confirmed that BD MAX EPP sample buffer tubes (SBTs) inoculated with FecalSwab specimens can be stored under these same conditions prior to or after the pre-warm step required for parasite testing as indicated in the labeling following a nested study protocol.

To confirm the stability of samples in FecalSwab media under the same conditions as current specimen claims, specimens were tested at baseline, after storage at each of the claimed conditions. Both positive and negative specimens were tested, with positive specimens being FecalSwab tubes containing pooled negative stool matrix spiked with a multiplex mixture containing target analytes at 3× the assay LoD. Four positive FecalSwab tubes and one negative FecalSwab tube from each of three lots were prepared for each time point, and two BD MAX Sample Buffer Tubes were prepared from each FecalSwab tube. Therefore, a minimum of 24 test results for positive specimens and six valid test results for negative specimens were required at each test point. The nested stability protocol was to establish stability of FecalSwab specimens after being transferred to the BD MAX EPP SBTs in two separate arms. The first part evaluated the stability of inoculated sample buffer tubes stored under the claimed stability conditions prior to the pre-warm processing step, while the second part evaluated stability of the inoculated SBTs stored under the claimed stability conditions after completion of the pre-warm step. All test points and storage conditions are shown in Table 8. Specimens were created by spiking BD MAX EPP targets (*Cryptosporidium parvum*, *Giardia lamblia*, and *Entamoeba histolytica*) into one clinical stool pool previously determined to be negative for all BD MAX EPP targets. The stock concentrations, unpreserved LoD concentration, and 3x LoD are shown in Table 9.

Table 8: Test Point and Storage Conditions

Test Point	FecalSwab		SBT - Prior to Pre-Warm		SBT - Post Pre-Warm	
1(Baseline)	N/A				N/A	
2	25 °C ± 2 °C	2 days (48 hrs.)	N/A			
3			25 °C ± 2 °C	2 days	N/A	
4				3 days	N/A	
5			N/A		25 °C ± 2 °C	2 days
6			N/A			3 days
7			5 °C ± 3 °C	5 days	N/A	
8				6 days	N/A	
9*			12 hrs. at 5 °C ± 3 °C*		5 °C ± 3 °C	5 days
10*			12 hrs. at 5 °C ± 3°C*			6 days
11		3 days (72 hrs.)	N/A			
12	5 °C ± 3 °C	5 days (120 hrs.)	N/A		N/A	
13			25 °C ± 2 °C	2 days	N/A	
14				3 days	N/A	
15			N/A		25 °C ± 2 °C	2 days
16			N/A			3 days
17			5 °C ± 3 °C	5 days	N/A	
18				6 days	N/A	
19*			12 hrs. at 5 °C ± 3 °C*			5 days

20*			12 hrs. at 5 °C ± 3 °C*	5 °C ± 3 °C	6 days
21		6 days (144 hrs.)	N/A		

*The SBTs from Test Points 9, 10, 19 and 20 were stored 12 hours at 5 °C ± 3 °C before starting the pre-Warm step, due to delayed availability of required equipment (pre-Warm stations). Since the additional storage time for conditions 9, 10, 19 and 20 represents a worst case compared to the original Test Points described in the protocol and is within the storage conditions included in BD MAX EPP package insert, there is no impact on the study.

Table 9: Organism Targets and Spike Levels

Organism	Lot Number	Stock Concentration (orgs/mL)	LoD Unpreserved (orgs/mL in stool)	3x LoD (orgs/mL)
Cryptosporidium parvum	200121	6.25E+06	4805	14416
Giardia lamblia	200121	6.25E+06	1601	4803
Entamoeba histolytica	323743	1.08E+05	12	36

Acceptance criteria for FecalSwab specimens tested at baseline included the following:

- 100% detection for all targets in spiked samples, and 0% detection for all targets (except the SPC internal control) in negative samples.

For all subsequent test points, the acceptance criteria were as follows:

- ≥95% detection for all targets in spiked samples, and 0% detection for all targets (except the SPC internal control) in negative samples

For each time point, it was required that there be a minimum of 24 valid results for positive and 6 valid results for negative samples. All results/test points passed the acceptance criteria and demonstrate that FecalSwab specimens were capable of being stored at 25°C ± 2°C for up to 48 hours or at 5°C ± 3°C for up to 120 hours (5 days) prior to testing. BD also confirmed that BD MAX EPP sample buffer tubes (SBTs) inoculated with FecalSwab specimens can be stored under these same conditions prior to or after the pre-warm step required for parasite testing as indicated in the labeling for the previously cleared assay, K143648.

2. Matrix Comparison:

Not Applicable

C Clinical Studies:

1. Clinical Sensitivity and Specificity:

The clinical studies were done to demonstrate clinical agreement between the FecalSwab specimen type and the unpreserved stool specimen type for the BD Max EPP targets by using

positive percent agreement (PPA) (sensitivity) and negative percent agreement (NPA) with an expected point estimate of at least 95% for each target compared to the original reference method algorithm for unpreserved stool in K143648. The main objective of this study was to estimate the rate of unresolved (UNR) results that occur due to sample processing control (SPC) failure and to estimate the rates of indeterminate (IND) results for FecalSwab stool. The non-reportable rate (UNR/IND) for FecalSwab stool samples should not be more than 5%.

In the study design, the prospective collection and testing protocols utilized fresh, unpreserved remnant stool specimens from patients suspected of acute gastroenteritis, enteritis, or colitis. The prospective collections were only used to estimate the rate of unresolved (UNR) results, as well as to estimate the rates of indeterminate (IND) and incomplete (INC) results for a FecalSwab stool sample.

The retrospective collection and testing protocols utilized previously characterized unpreserved retrospective stool specimens from subjects suspected of acute gastroenteritis, enteritis, or colitis, and these specimens were stored frozen or at 4 – 8 °C. The goal of the retrospective collection was to achieve 100 positive specimens on the BD MAX EPP assay across all targets by supplementing the enrollment with samples collected. Contrived samples were only used to demonstrate clinical agreement between the FecalSwab specimen type and the unpreserved stool specimen type for the BD MAX EPP targets as the primary objective. Specimen collection was performed at select external sites and the testing occurred at the BD internal R&D laboratory.

The prospective collection protocol, utilized fresh, unpreserved remnant stool specimens from patients suspected of acute gastroenteritis, enteritis, or colitis. Fresh, prospective collection was performed at six (6) external sites. Samples collected were utilized in the testing protocol for the exploratory objective. The prospective specimen collection site used a protocol using the following steps on each specimen enrolled in the study.

- The FecalSwab collection device was used to transfer a portion of the specimen into the FecalSwab tube, vortexed per user instructions, and then 150 µL of the FecalSwab tube contents were pipetted into a properly labeled BD MAX EPP Sample Buffer Tube.

- The BD MAX EPP user instructions were used to directly transfer a portion of the unpreserved stool specimen to a properly labeled BD MAX EPP Sample Buffer Tube.

- Both BD MAX EPP Sample Buffer Tubes were then shipped to BD for specimen testing under the study test protocol.

Upon receipt at the BD testing laboratory, the FecalSwab specimens in the BD MAX EPP Sample Buffer Tubes were tested as instructed. The Unpreserved Stool specimens were processed through the pre-warm step and then frozen for future testing. The results from evaluation of the prospectively collected specimens were used to calculate the non-reportable rates.

The retrospective collection protocol enrolled previously characterized unpreserved remnant stool specimens from subjects suspected of acute gastroenteritis, enteritis, or colitis, and

these specimens were stored frozen or at 4-8°C. There were two (2) external sites that utilized this retrospective protocol. Additional frozen retrospective samples from the previous clinical study already at the testing facility, and contrived samples were also enrolled, where applicable. Another protocol had enrolled fresh, unpreserved remnant stool specimens from patients suspected of acute gastroenteritis, enteritis, or colitis, and the remnant specimens obtained during that clinical study and have been stored frozen. Retrospective and contrived samples were evaluated under the testing protocol to evaluate the primary objective (to demonstrate clinical agreement between the FecalSwab specimen type and the unpreserved stool specimen type for the BD MAX EPP targets).

For testing of the retrospective samples, each frozen or refrigerated, unpreserved remnant specimen was brought to room temperature and tested using the BD MAX EPP assay per manufacturer's instructions by an internal BD R&D team. If the result from this test was concordant with the historical result provided by the vendor or the previous study result, then the unpreserved stool was provided to the BD Clinical Team. Per FDA guidance from previous communications, if the unpreserved BD MAX EPP assay result was discordant with the historical result, then the unpreserved specimen was tested 2 more times using the BD MAX EPP assay and a 2-out-of-3 algorithm applied to obtain truth. Once the "truth" of the unpreserved stool specimen had been established, it was provided to the BD Clinical team for FecalSwab preparation and testing. The unpreserved stool was transferred to a FecalSwab device, and that material was then pipetted into an EPP-labeled SBT and tested with the BD MAX EPP assay by the Clinical Team. The EPP FecalSwab result was compared to the final unpreserved specimen result. The same testing procedure was applied to retrospective specimens.

Since the workflow for all specimen types (Unpreserved or FecalSwab) is identical after the specimens are placed into the SBT, BD MAX analysis (testing of the SBT on the BD MAX system) was performed internally (BD R&D laboratory). Additionally, previous clinical studies for the BD MAX EPP (K143648) Assays have demonstrated that the variability between reagent lots, MAX instruments, and users do not impact the results of the assay. The BD MAX EPP assay was performed according to the clinical trial package insert, BD MAX. Instrument Manual and the Clinical Trial Training Manual. All runs were performed by trained employees who demonstrated proficiency prior to testing. The BD MAX / FecalSwab Study compliant external control rate is summarized in Table 10.

Table 10: BD MAX / FecalSwab Comparison Study Compliant External Control Rate (from Clinical Sample Runs Only)

		EC Pass Rate		**EC Fail Rate with Reportable Result**		**EC Fail Rate with Non-reportable Result**	
External Control	**Total Run**	**Percent**	**95% CI**	**Percent**	**95% CI**	**Percent**	**95% CI**
Positive	159	98.7% (157/159)	(95.5%, 99.7%)	0.0% (0/159)	(0.0%, 2.4%)	1.3% (2/159)	(0.3%, 4.5%)
Negative	159	97.5% (155/159)	(93.7%, 99.0%)	0.6% (1/159)	(0.1%, 3.5%)	1.9% (3/159)	(0.6%, 5.4%)
Overall	159	96.9% (154/159)	(92.9%, 98.6%)	0.6% (1/159)	(0.1%, 3.5%)	2.5% (4/159)	(1.0%, 6.3%)

Any sample included in a run in which an external control failed, or any sample for which an Unresolved, Indeterminate, or Incomplete result was obtained, was retested from the same Sample Buffer Tube along with valid external controls. If any targets of the panel have an unresolved or indeterminate result, then the result of the combined assay was defined as "non-reportable". BD Max non-reportable results categorizations are shown in Table 11.

Table 11: BD MAX Non-Reportable Result

Type	Description
Unresolved (UNR)	Indicative of an inhibitory specimen or reagent failure
Indeterminate (IND)	Indeterminate due to BD MAX System failure
Incomplete (INC)	Incomplete Run
External Control failure	All samples included in runs were considered non-reportable if at least one external control (positive or negative) does not give the expected result.

In addition to these specimens, contrived specimens were prepared for *Entamoeba histolytica*. 50% of the contrived specimens were to be prepared at 1.5x - 2x LoD in a unique (not pooled) specimen matrix consisting of a single negative stool specimen and collected using the FecalSwab. A total of 53 positive and 53 negative specimens were prepared using 53 unique negative specimen matrices that had been previously determined to be negative for all targeted analytes, per FDA request shown in Table 12.

Table 12: Contrived Samples Panel Target Level Description

xLoD	***Entamoeba***
1.99	27
4	10
7	10
10	6
Negative	53
Total	106

The overall PPA and NPA for the FecalSwab compared to the current unpreserved specimen collection is provided in Table 13. The NPA acceptance criteria were met for all target organisms in the retrospective testing. However, *Cryptosporidium (C. hominis and C. parvum only)* and *Entamoeba histolytica* did not meet the expected PPA performance, either with a PPA point estimate under 95% or with a 95% confidence interval around the PPA estimate extending below 90%. *Giardia lamblia* met the expected PPA point estimate but the 95% confidence interval around the PPA estimate extended below 90%.

In summary, the unexpected results arise from the low prevalence of positive specimens observed for the targets and target concentrations near or below the LoD.

Table 13: PPA and NPA Results Summary of Primary Endpoint (Retrospective Only)

Target	Pos N	PPA Result (95%CI)	Neg N	NPA Result (95%CI)
Entamoeba histolytica	5	80.0% (37.6%-96.4%)	741	100.0% (99.5%-100.0%)
Cryptosporidium (C. hominis and C. parvum only)	110	90.9% (84.1% – 95.0%)	636	99.8% (99.1%-100.0%)
Giardia lamblia	42	95.2% (84.2%-98.7%)	705	99.9% (99.2%-100.0%)

Individual organism's data comparisons are shown below. The data in Table 14 demonstrates that although the PPA lower bound for *Giardia lamblia* extends below 90%, the performance of the FecalSwab specimens is comparable with the performance of the unpreserved specimens. As all specimens with discrepant results have the target at or near the LoD based on Ct values, the degree of variability observed here is not surprising. In addition, the number of positive specimens in the study was less than the target number (~100 positive samples), which results in wider confidence intervals.

Table 14: Percent Agreement 2x2 Table for BD MAX EPP Assay FecalSwab Compared to Unpreserved Device Type for *Giardia lamblia* (by Overall)- Retrospective Specimen Only.

Giardia lamblia	**Unpreserved**		
FecalSwab	**Positive**	**Negative**	**Total**
Positive	40	1	41
Negative	2	704	706
Total	42	705	747
PPA: 95.2% (84.2%, 98.7%) NPA: 99.9% (99.2%, 100.0%)			

Cryptosporidium (C. hominis and C. parvum only) met the FDA's expected NPA performance goal (NPA: 99.8 % (95% CI: 99.1%, 100.0%)). *Cryptosporidium (C. hominis and C. parvum only)* had a 90.9% PPA with a 95% CI of 84.1% - 95.0% that does not meet the FDA's expected PPA performance goal (≥95% with the lower bound of the two-sided 95% confidence interval exceeding 90%). As described in Table 15**,** there were ten samples that were unpreserved Positive (POS) and FecalSwab Negative (NEG) and one sample that was unpreserved Negative (NEG) and FecalSwab Positive (POS). All discrepant samples were at or near the LoD. Stool samples with low target loads (at or near the LoD) have a higher probability of not being detected due to insufficient target DNA delivery to the PCR cartridge. Therefore, the likelihood of reproducible positive/negative results for samples near or at the LoD is variable. Additionally, three of the discrepant samples required 2-out-of-3 testing due to this variability at or near LoD.

Table 15: Percent Agreement 2x2 Table for BD MAX EPP Assay FecalSwab Compared to Unpreserved Device Type for *Cryptosporidium parvum* (by Overall) - Retrospective Specimen Only.

Cryptosporidium (C. hominis and C. parvum only)	**Unpreserved**		**Total**
FecalSwab	**Positive**	**Negative**	
Positive	100	1	101
Negative	10	635	645
Total	110	636	746
PPA: 90.9% (84.1%, 95.0%) NPA: 99.8% (99.1%, 100.0%)			

Entamoeba histolytica met the FDA's desirable NPA performance goal (NPA: 100.0% (95% CI: 99.5%, 100.0%)). *Entamoeba histolytica* had 80.0% PPA with a 95% CI of 37.6% - 96.4%, that does not meet the FDA's desirable PPA performance goal (≥95% with the lower bound of the two-sided 95% confidence interval exceeding 90%). As described in Table 16**,** there was one sample that was unpreserved Positive (POS) and FecalSwab Negative (NEG). The one discrepant sample was below the LoD. Stool samples with low target loads (at or near the LoD) have a higher probability of not being detected due to insufficient target DNA delivery to the PCR cartridge. Therefore, the likelihood of reproducible positive/negative results for samples near or at the LoD is variable. In addition, since the positive sample size was low, contrived testing was performed and met the FDA's desirable performance goal with no discrepant results (Table 17).

The data demonstrates that although the PPA is lower than the expected 95%-point estimate, and the 95% confidence interval around that estimate extends below 90% for *Entamoeba histolytica*, the performance of the FecalSwab specimens is comparable with the performance of the unpreserved specimens. As all specimens with discrepant results have the target at or near the LoD based on Ct values, the degree of variability observed here is not surprising. In addition, the number of positive specimens in the study was less than the target number (~100 positive samples), which results in wider confidence intervals.

Table 16: Percent Agreement 2x2 Table for BD MAX EPP Assay FecalSwab Compared to Unpreserved Device Type for *Entamoeba histolytica* (by Overall) - Retrospective Specimen Only.

Entamoeba histolytica	**Unpreserved**		**Total**
FecalSwab	**Positive**	**Negative**	
Positive	4	0	4
Negative	1	741	742
Total	5	741	746
PPA: 80.0% (37.6%, 96.4%) NPA: 100.0% (99.5%, 100.0%) OPA: 99.9% (99.2%, 100.0%)			

Table 17: Percent Agreement 2x2 Table for BD MAX EPP Assay FecalSwab Compared to Unpreserved Device Type for *Entamoeba histolytica* Contrived Specimens.

Entamoeba histolytica	**Unpreserved**		**Total**
FecalSwab	**Positive**	**Negative**	
Positive	53	0	53
Negative	0	53	53
Total	53	53	106
PPA: 100.0% (93.2%, 100.0%) NPA: 100.0% (93.2%, 100.0%) OPA: 100.0% (96.5%, 100.0%)			

In conclusion, the evidence provided herein indicates that the FecalSwab specimen type performs comparably to the currently market authorized unpreserved specimen type. The observation that some of the assay targets had a PPA point estimate or confidence interval around the PPA estimate that were lower than FDA's expected performance is due to either low prevalence of positive specimens or specimens containing target organisms at or below the LoD. For *Entamoeba histolytica* that had few positive specimens, contrived testing was performed and met the FDA's desirable performance goal (≥95% with the lower bound of the two-sided 95% confidence interval exceeding 90%), further supporting the claim that FecalSwab performance is substantially equivalent to performance of the current unpreserved specimen collection when tested on BD MAX EPP assay. All discrepant samples across all targets are consistent with low titer specimens at or near the limit of detection of the BD MAX EPP on the BD MAX System. Additionally, the exploratory objective of this study met the acceptance criteria with a nonreportable rate (NRR) of ≤ 5% (Table 18).

Table 18: Overall Non-Reportable Rate for Combined BD MAX EPP Target (Prospective FecalSwab Specimens Only)

Combined EPP Target	**Unresolved Rate**		**Indeterminate Rate**		**Incomplete Rate**		**Total UNR+IND Rate (Non-Reportable Rate)**	
Collection Device	**Initial [95% CI]**	**Final* [95% CI]**	**Initial [95% CI]**	**Final* [95% CI]**	**Initial [95% CI]**	**Final* (95% CI]**	**Initial [95% CI]**	**Final* [95% CI]**
FecalSwab	2.3% (8/344) [1.2%, 4.5%]	1.8% (6/329) [0.8%, 3.9%]	0.0% (0/344) [0.0%, 1.1%]	0.0% (0/329) [0.0%, 1.2%]	1.2% (4/344) [0.5%, 3.0%]	0.0% (0/329) [0.0%, 1.2%]	2.3% (8/344) [1.2%, 4.5%]	1.8% (6/329) [0.8%, 3.9%]
* The final rate is calculated with valid repeats only								

Collectively, the results of the clinical and analytical studies demonstrate that the FecalSwab specimen type is substantially equivalent to the current unpreserved specimen type for the BD MAX EPP Assay.

2. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not Applicable

D Clinical Cut-Off:

Not Applicable

E Expected Values/Reference Range:

The expected values/reference range for analytes on the BD MAX EP were established previously, see K143648.

F Other Supportive Instrument Performance Characteristics Data:

Not Applicable

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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