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

I Background Information:

A 510(k) Number

K232672

B Applicant

Genetic Signatures Limited

C Proprietary and Established Names

EasyScreen Gastrointestinal Parasite Detection Kit

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
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:

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Genetic Signatures EasyScreen Gastrointestinal Parasite Detection Kit is a rapid *in vitro* nucleic acid amplification assay for the qualitative detection of pathogenic gastrointestinal parasite nucleic acid from the stool of patients with signs and/or symptoms of gastroenteritis. The test, based on real-time PCR, detects the nucleic acid of the following organisms:

- *Cryptosporidium* spp.
- *Giardia intestinalis*
- *Dientamoeba fragilis*
- *Entamoeba histolytica*
- *Blastocystis hominis*
- *Enterocytozoon bieneusi*

- *Encephalitozoon intestinalis*
- *Cyclospora cayetanensis*

The kit is compatible with stool specimens that are unpreserved or frozen or in transport media including Cary Blair or C&S media from symptomatic patients with suspected gastroenteritis. It is required that the stool is first processed using the EasyScreen Sample Processing Kit. Nucleic acid extraction and real-time PCR set up are performed on the automated Genetic Signatures GS1 platform.

The EasyScreen Gastrointestinal Parasite Detection Kit includes all reagents required to detect the specific protozoan gene sequences using real-time PCR amplification of the extracted nucleic acids and fluorogenic target-specific hybridization probes for the detection of the amplified nucleic acid. The EasyScreen Gastrointestinal Parasite Detection kit also incorporates an Extraction Control (EC) and an Internal Positive Control (IPC) to ensure the reliability of the extracted nucleic acid and to detect the presence of any inhibitors, respectively.

This device is an *in vitro* diagnostic (IVD) intended to be used by trained personnel in clinical, pathology or hospital laboratories as an aid in the diagnosis of gastrointestinal illness. This test is intended for use, in conjunction with clinical presentation, laboratory findings, and epidemiological information, as an aid in the differential diagnosis of infections by *Dientamoeba fragilis*, *Blastocystis hominis*, *Enterocytozoon bieneusi*, *Cyclospora cayetanensis*, *Entamoeba histolytica*, *Encephalitozoon intestinalis*, *Cryptosporidium* spp. (including *C. hominis* and *C. parvum*), and *Giardia intestinalis*. 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 indicate 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.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

Following limitations apply: (not an exhaustive list)

i. Whole blood at greater than 0.63% and Mucin at greater than 0.75 mg/mL demonstrated interference with the *EasyScreen* Gastrointestinal Parasite Detection Kit. Caution must be exercised in interpretation of the results of this assay when testing stool samples with blood and/or mucin potentially present at or above these concentrations.

ii. This test has been validated for use with only QuantStudio Dx and Applied Biosystems 7500 Fast Dx real-time PCR systems. Other real-time PCR analyzers from different manufacturers may perform differently and should not be used with this this test.

iii. The performance of this test has been validated with stool specimen that has been collected from patients with signs and symptoms of gastroenteritis and is either unpreserved fresh or diluted in Cary Blair or C&S transport medium per manufacturer's instructions. It has not been validated for other specimen types, rectal swabs or samples stored in other transport media or fixatives.

iv. This kit is only compatible with nucleic acid from stool samples prepared using an *EasyScreen* Sample Processing Kit (SP008B).

v. Samples subjected to more than one thaw from frozen storage have not been validated for use with the *EasyScreen* Gastrointestinal Parasite Detection Kit (EP005) and should not be used for the test.

vi. Performance of this assay has been validated only for the target organisms indicated in the Intended Use statement. It has not been validated for any other non-target organisms. The assay is qualitative and does not provide a quantitative value for target organisms present in the stool specimens.

vii. During competitive interference testing, the detection of low positive *D. fragilis* and *B. hominis* was attenuated by presence of high amounts of co-targets *C. parvum* and *E. histolytica*, respectively. In regions of high prevalence or endemicity of *Cryptosporidium* spp and *Entamoeba histolytica*, negative EasyScreen Gastrointestinal Parasite Detection Kit assay results should be interpreted cautiously. Perform additional tests if gastrointestinal infections/co-infections by *D. fragilis* and *B. hominis*, respectively, are clinically suspected.

D Special Instrument Requirements:

- Genetic Signatures GS1
- QuantStudio Dx Real-Time PCR Instrument with QuantStudio Dx software
- Applied Biosystems 7500 Fast Dx Real-Time PCR instrument with SDS Software

IV Device/System Characteristics:

A Device Description:

EasyScreen Gastrointestinal Parasite Detection Kit consists of four (4) components:

1. The *EasyScreen* Sample Processing Kit (SP008B), which is a set of reagents for specimen processing, extraction, and purification of nucleic acids. (Required but not provided.)

2. Genetic Signatures GS1, which is an automated platform for processing of human stool samples for the extraction of purified nucleic acids (using SP008B reagents) and setting up of real time PCR runs (using EP005 or EP005-HT reagents). (Required but not provided.)

3. The *EasyScreen* Gastrointestinal Parasite Detection Kit (EP005, for 100 reactions; EP005-HT, for 500 reactions), which is a set of PCR components, reaction mastermixes, and controls designed for multiplexed detection and identification of eight (8) potentially pathogenic gastrointestinal parasites via real-time PCR.

   Amplified targets are detected by fluorophore-labeled probes. The real-time PCR is an 8-plex assay, detecting the following protozoan targets in each fluorescent channel:

Detection Channel	Available Fluorophores	Reaction Mix (Panel) A	Reaction Mix (Panel) B	Reaction Mix (Panel) C
Channel 1	FAM, etc.	*Dientamoeba fragilis*	*Blastocystis hominis*	*Enterocytozoon bieneusi*
Channel 2	VIC, HEX, TET, etc.	Extraction Control (EC)	Internal Process Control (IPC)	Extraction Control (EC)
Channel 3	ROX, Texas Red, etc.	*Cyclospora cayetanensis*	*Entamoeba histolytica*	*Encephalitozoon intestinalis*
Channel 4	Cy5, LightCycler Red640, etc.	*Cryptosporidium* spp.	*Giardia intestinalis*	Not used

4. Real-Time PCR Instruments:
   a) *QuantStudio Dx* Real-Time PCR Instrument with QuantStudio Dx software (**K123955**).
   b) Applied Biosystems *7500 Fast Dx* Real-Time PCR instrument with SDS Software (**K141220**).

Additional components indicated as "Required but Not Supplied" are specified in the package insert and include items and laboratory equipment for sample processing and PCR set up.

B Principle of Operation:

1. *EasyScreen* Gastrointestinal Parasite Detection Kit is a multiplexed real time PCR assay, designed to simultaneously detect and identify up to eight (8) potentially pathogenic gastrointestinal parasites from human stool samples.

2. The device workflow is compatible with only nucleic acids prepared using the *EasyScreen* Sample Processing kit (SP008B) in the GS1 automated nucleic acid extraction platform.

3. Kit SP008B contains Reagent 1 and 2 which are combined to make a "Conversion Reagent." Individual stool samples are mixed and heated with this conversion reagent which lyses microbial cells and converts all Cytosine bases to Uracil (detected as Thymine) to create "3base nucleic acids."

4. Kit SP008B also contains Nucleic Acid extraction reagents, which are used on the stool lysate for further processing to elute the nucleic acids on the GS1 automated platform. The purified eluate proceeds to the PCR set-up step in the GS1 platform.

5. *EasyScreen* Gastrointestinal Parasite Detection Kit (EP005 or EP005-HT) contains all the reagents to be used for real-time PCR amplification and detection of the target gastrointestinal parasites. These reagents are appropriately dispensed to uniquely designated sample wells in the GS1 platform.

6. In the GS1 platform, aliquots of the purified nucleic acids eluted from the processed stool sample are then added to EP005 PCR reagents in designated sample wells, which selectively amplify the genetic targets of the target parasites. Each stool eluate is tested across three (3) Reaction Mixes or Panels (i.e., Panel A, B, and C), each of which include an internal control. Panel A and C detect an endogenous gene contained in bacteria present in the stool sample via the Extraction Controls (EC), which act as sampling and extraction controls. Panel B features an incorporated Internal Positive Control (IPC) sequence which acts to detect the presence of any inhibitors after extraction from the primary sample. The IPC also confirms the integrity of the PCR reagents.

7. The amplification reactions in presence of the EP005 PCR Reagents are carried out by the Life Technologies QuantStudio Dx or Applied Biosystems 7500 Fast Dx thermocycler systems. The real-time PCR software displays all collected data and provides the raw threshold cycle (Ct) values for automated analysis using software tools developed by Genetic Signatures.

C Instrument Description Information:

1. Instrument Name:
   Genetic Signatures GS1

2. Specimen Identification:
   Each specimen barcode is scanned into the system by the user when loading the sample tube into the pedestal. Specimen barcodes are tracked by software tools developed by Genetic Signatures through nucleic acid extraction, PCR setup, run, and result analysis, as well as

through result reporting into Laboratory Information Systems at the Customer / End User establishment.

3. Specimen Sampling and Handling:
Specimens are aliquoted into a reagent tube and heated for 3base conversion. After the heating step, the inoculated sample tubes are scanned and loaded onto the GS1 instrument. During extraction, the instrument pipettes samples into the processing plate and, at the end of the process, into the final elution plate. These samples are then pipetted by GS1 from the extraction elution plate into the PCR plate. Sample placements in the elution and PCR plates are tracked via the software.

4. Calibration:
At each semi-annual preventative maintenance, the instrument is calibrated using a manufacturer calibration block and software. If an instrument is found to be out of calibration, alignment is checked and adjusted as needed, then calibration is repeated.

5. Quality Control:
Upon installation, qualification procedures for installation (IQ), operation (OQ), and performance (PQ) are completed by Genetic Signatures to verify performance of the instrument and assay. After each preventative maintenance, OQ is performed. A daily maintenance and weekly maintenance are required before operating the instrument.

V Substantial Equivalence Information:

A Predicate Device Name(s):
BD MAX Enteric Parasite Panel, BD MAX System

B Predicate 510(k) Number(s):
K143648

C Comparison with Predicate(s):

Device & Predicate Device(s):	Device: K232672	Predicate: K143648
Device Trade Name	EasyScreen Gastrointestinal Parasite Detection Kit	BD MAX Enteric Parasite Panel
General Device Characteristic Similarities		
Intended Use/Indications For Use	The Genetic Signatures EasyScreen Gastrointestinal Parasite Detection Kit is a rapid *in vitro* nucleic acid amplification assay for the qualitative detection of pathogenic gastrointestinal parasite nucleic acid from the stool of patients with signs and/or symptoms of gastroenteritis. The test, based on real-time PCR, detects the nucleic acid of the following organisms: • *Cryptosporidium spp.* • *Giardia intestinalis* • *Dientamoeba fragilis* • *Entamoeba histolytica*	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*

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	• *Blastocystis hominis* • *Enterocytozoon bieneusi* • *Encephalitozoon intestinalis* • *Cyclospora cayetanensis* The kit is compatible with stool specimens that are unpreserved or frozen or in transport media including Cary Blair or C&S media from symptomatic patients with suspected gastroenteritis. It is required that the stool is first processed using the EasyScreen Sample Processing Kit. Nucleic acid extraction and real-time PCR set up are performed on the automated Genetic Signatures GS1 platform. The EasyScreen Gastrointestinal Parasite Detection Kit includes all reagents required to detect the specific protozoan gene sequences using real-time PCR amplification of the extracted nucleic acids and fluorogenic target-specific hybridization probes for the detection of the amplified nucleic acid. The EasyScreen Gastrointestinal Parasite Detection kit also incorporates an Extraction Control (EC) and an Internal Positive Control (IPC) to ensure the reliability of the extracted nucleic acid and to detect the presence of any inhibitors, respectively. This device is an *in vitro* diagnostic (IVD) intended to be used by trained personnel in clinical, pathology or hospital laboratories as an aid in the diagnosis of gastrointestinal illness. This test is intended for use, in conjunction with clinical presentation, laboratory findings, and epidemiological information, as an aid in the differential diagnosis of infections by *Dientamoeba fragilis*, *Blastocystis hominis*, *Enterocytozoon bieneusi*, *Cyclospora cayetanensis*, *Entamoeba histolytica*, *Encephalitozoon intestinalis*, *Cryptosporidium* spp. (including *C. hominis* and *C. parvum*), and *Giardia*	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

Device & Predicate Device(s):	Device: K232672	Predicate: K143648
	intestinalis. 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 indicate 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.	bowel syndrome, or Crohn's disease.
Technology	Multiplex nucleic acid amplification and detection	Same
Specimen types	• Unpreserved (fresh) stool • Stool specimen in preservative (see below for differences in the preservatives used)	Same
General Device Characteristic Differences		
Organisms detected	• *Dientamoeba fragilis* • *Blastocystis hominis* • *Enterocytozoon bieneusi* • *Encephalitozoon intestinalis* • *Cyclospora cayetanensis* • *Giardia lamblia* • *Cryptosporidium* spp • *Entamoeba histolytica*	• *Giardia lamblia* • *Cryptosporidium hominis* • *C. parvum* • *Entamoeba histolytica*
Specimen preservative types and collection conditions	Stool specimens in Cary Blair and C&S transport media	10% formalin-fixed stool specimens
Sample processing and PCR analysis platform	• Genetic Signatures GS-1 • Thermo Scientific QuantStudio Dx • Applied Biosystems 7500 Dx	BD Max System

VI Standards/Guidance Documents Referenced:

Standard	Document title	FDA-Recognition	Use
Class II Special Controls Guideline (2015)	Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assays for Detection and Identification of Microorganisms and Toxin Genes from Human Stool Specimens	FDA guidelines	Clinical and analytical studies
CLSI EP37, 1st Ed. (2018)	Supplemental Tables for Interference Testing in Clinical Chemistry	Complete	Interference study
CLSI EP39, 1st Ed (2021)	A Hierarchical Approach to Selecting Surrogate Samples for the Evaluation of In Vitro Medical Laboratory Tests	Complete	Carry over / cross contamination study

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Site-to-site reproducibility of the *EasyScreen* Gastrointestinal Parasite Detection Kit was evaluated in two (2) separate studies—the first study with four (4) targets, namely, *D. fragilis*, *C. parvum*, *E. bieneusi*, and *E. intestinalis*; and a second study with remaining four (4) targets, namely, *C. cayetanensis*, *B. hominis*, *E. histolytica*, and *G. intestinalis*—as described below.

A. First Study (*D. fragilis*, *C. parvum*, *E. bieneusi*, and *E. intestinalis*)

For the indicated targets, site-to-site reproducibility of the *EasyScreen* Gastrointestinal Parasite Detection Kit was evaluated at three (3) clinical sites, two (2) in the US and one (1) outside the US (OUS), with two (2) operators at each site for ten (10) days.

For each of the indicated target analyte, a sample panel was contrived by spiking an unpreserved negative stool matrix with varying analyte concentrations relative to the target's pre-determined LoD, e.g., a Low Positive (LP) at 2X LoD and a Moderate Positive (MP) at 4X LoD. Each panel also featured a True Negative (TN) sample, a negative clinical stool sample with no (or an undetectable amount of the) target analyte.

Each positive panel member was tested in triplicate for each of five (5) extraction runs resulting in a total of 90 (i.e., 3 x 2 x 5 x 3) data points per panel member. The negative samples were tested at the three (3) sites by two (2) operators at each site for a total of ten (10) extraction runs in triplicate resulting in a total of 180 (i.e., 3 x 2 x 10 x 3) data points.

B. Second Study (*C. cayetanensis*, *B. hominis*, *E. histolytica*, and *G. intestinalis*)

For the indicated targets, site-to-site reproducibility of the *EasyScreen* Gastrointestinal Parasite Detection Kit was evaluated at one (1) external clinical site in the US and one (1) in-house site outside the US with three (3) operators at each site for five (5) to seven (7) days.

Panel members *B. hominis*, *E. histolytica*, and *G. intestinalis* were tested in triplicate for each of five (5) or six (6) extraction runs resulting in a total of 99 (i.e., 3 x 5 x 3 + 3 x 6 x 3) data points per panel member, whereas *C. cayetanensis* was tested in-house in triplicate for each run resulting in a total of 54 (i.e., 3 x 6 x 3) data points at 2X LoD (LP) and a total of 51 (i.e., 3 x 5 x 3 + 2 x 1 x 3) data points at 4X LoD (MP) concentration. The negative samples (TN) were tested in triplicate at the two (2) sites by three (3) operators at each site, resulting in a total of 165 (i.e., 3 x 12 x 3 + 2 x 2 x 3 + 3 x 5 x 3) data points.

Due to operational difficulties with *C. cayetanensis* organisms (i.e., unreliable sourcing, isolates not culturable, transit degradation, time constraints, etc.), testing at adequate numbers could not be completed at the US site and additional testing to fulfil the replicate number requirements was carried out at the OUS site with two (2) operators, testing the target analyte in twelve (12) replicates per run and two (2) runs per operator, resulting in an additional total of 48 (i.e., 2 x 2 x 12) data points.

C. Results of site-to-site reproducibility studies.

Analyte-specific site-to-site qualitative reproducibility testing results from both studies are presented in **Tables 1 and 2** below as percent agreement (Correct/Total) of tested valid samples

with expected results (i.e., proportion of correctly estimated sample over total samples), along with the 95% confidence intervals. Samples testing invalid were removed from analysis.

Table 1: qualitative reproducibility (First study)

Target and Sample		Site 1		Site 2		Site 3		Total		95% CI	
		%	Correct /N	%	Correct /N	%	Correct /N	%	Correct /N	LL	UL
D. fragilis	TN	100	(59/59)	100	(59/59)	100	(59/59)	100	(177/177)	97.94	100
	LP	100	(30/30)	100	(30/30)	100	(30/30)	100	(90/90)	95.98	100
	MP	100	(30/30)	100	(30/30)	100	(30/30)	100	(90/90)	95.98	100
E. bieneusi	TN	100	(59/59)	100	(59/59)	100	(59/59)	100	(177/177)	97.94	100
	LP	100	(30/30)	100	(30/30)	100	(29/29)	100	(89/89)	95.94	100
	MP	100	(30/30)	100	(30/30)	100	(30/30)	100	(90/90)	95.98	100
C. parvum	TN	100	(59/59)	100	(59/59)	100	(59/59)	100	(177/177)	97.94	100
	LP	100	(30/30)	100	(28/28)	100	(30/30)	100	(88/88)	95.90	100
	MP	96.4	(27/28)	100	(29/29)	100	(30/30)	98.9	(87/88)	93.83	99.97
E. intestinalis	TN	100	(59/59)	100	(59/59)	100	(59/59)	100	(177/177)	97.94	100
	LP	100	(30/30)	100	(30/30)	100	(28/28)	100	(88/88)	95.90	100
	MP	100	(30/30)	100	(29/29)	100	(30/30)	100	(89/89)	95.94	100

Table 2: qualitative reproducibility (Second study)

Target and Sample		Site 1		Site 2		Total		95% CI	
		%	Correct /N	%	Correct /N	%	Correct /N	LL	UL
B. hominis	TN	100	(120/120)	100	(45/45)	100	(165/165)	97.79	100
	LP	100	(54/54)	100	(45/45)	100	(99/99)	96.34	100
	MP	100	(54/54)	100	(45/45)	100	(99/99)	96.34	100
C. cayetanensis §	TN	100	(120/120)	N/A		100	(120/120)	96.97	100
	LP	97.1	(99/102)	N/A		97.1	(99/102)	91.64	99.39
	MP	100	(99/99)	N/A		100	(99/99)	96.34	100
G. intestinalis	TN	100	(120/120)	100	(45/45)	100	(165/165)	97.79	100
	LP	100	(54/54)	100	(45/45)	100	(99/99)	96.34	100
	MP	100	(54/54)	100	(45/45)	100	(99/99)	96.34	100
E. histolytica	TN	100	(120/120)	100	(45/45)	100	(165/165)	97.79	100
	LP	100	(54/54)	100	(45/45)	100	(99/99)	96.34	100
	MP	100	(54/54)	100	(45/45)	100	(99/99)	96.34	100

§ *C. cayetanensis* results are excluded from the site-to-site reproducibility considerations.

For all targets evaluated at >1 sites (which excludes *C. cayetanensis*), the overall site-to-site qualitative reproducibility percent agreement was 100% for all targets at the Low Positive (2X LoD) analyte level, as well as for all targets except *C. parvum* at the Medium Positive (4X LoD) analyte level. Overall detection of *C. parvum* at 4X LoD across all testing sites was at 98.9% (95% CI: 93.8–99.9). *C. cayetanensis* was not assessed for site-to-site reproducibility. Within-site reproducibility for *C. cayetanensis* (tested twice at a single site) was 97.1% (95% CI: 91.64–99.39) for LP samples and 100% (95% CI: 96.3–100) for MP samples. All True Negative (TN) samples (100%) were correctly identified in these tests.

Therefore, the analysis of site-to-site qualitative reproducibility of the *EasyScreen* Gastrointestinal Parasite Detection Kit showed acceptably consistent performance of the EP005 workflow across all test sites.

2. Analytical Reactivity/Inclusivity:

Analytical reactivity (inclusivity) of the *EasyScreen* Gastrointestinal Parasite Detection Kit was investigated by testing eighty-two (82) isolates representing the eight (8) target parasites (with 4–16 isolates per target, as available) (as shown in **Table 3**). Isolates were selected to represent various temporal, geographic, and phylogenetic diversity for each analyte. Isolates tested represent clinically relevant subspecies or serotypes and are biased toward more common species and known human pathogens. For clinically relevant organisms, genotypes were established with sequence-based analysis using genotyping assays and review of published literature.

For inclusivity testing, organisms were initially diluted to 1X–3X LoD for the corresponding target in unpreserved negative stool or transport media with preservative (*C. cayetanensis* only) and tested with the *EasyScreen* Gastrointestinal Parasite Detection Kit, which detected all isolates at all tested concentrations.

Table 3. Isolates for inclusivity testing with* EasyScreen *Gastrointestinal Parasite Detection Kit.

Target organism	Strain/ Designation	Product ID / Clinical ID	Geographic Origin
Dientamoeba fragilis	Genotype 1	Culture "B"	Sydney, AUS
	Genotype 2	Clinical #598	Sydney, AUS
	Genotype 1	Clinical #465	Sydney, AUS
	Genotype 1	Clinical #351	Sydney, AUS
	inconclusive	Clinical #409	Sydney, AUS
	Genotype 1	Clinical #862	Sydney, AUS
	Genotype 1	Clinical #054	NY, US
Cyclospora cayetanensis	N/A	Clinical #17	NY, US
	N/A	Clinical #18	NY, US
	N/A	Clinical #1	NY, US
	N/A	Clinical #4	NY, US
	N/A	Clinical #5	NY, US
	N/A	Clinical #7	NY, US
	N/A	Clinical #12	NY, US
	N/A	Clinical #14	NY, US
	N/A	Clinical #15	NY, US
	N/A	Clinical #20	NY, US
Cryptosporidium hominis	IbA10G2	Clinical#249	Sydney, AUS
	IbA10G2	Clinical#246	Sydney, AUS
	IbA10G2	Clinical#257	Sydney, AUS
	IbA10G2	Clinical#444	Sydney, AUS
	Id	Clinical#205	Sydney, AUS
	IfA14G1	Clinical#058	NY, US
Cryptosporidium parvum	IIaA18G3R1	Clinical#223	Sydney, AUS
	IIaA18G3R1	Clinical#243	Sydney, AUS
	IIa	Clinical#204	Sydney, AUS
	IIa	Clinical#244	Sydney, AUS
	IIaA	Clinical#234	Sydney, AUS

Target organism	Strain/ Designation	Product ID / Clinical ID	Geographic Origin
	IIaA	Clinical#240	Sydney, AUS
	IIaA	Clinical#248	Sydney, AUS
	IIaA17G2R1	P102C	Iowa, US
	IIaA20G3R1	Clinical#017	NY, US
	IIaA20G3R1	Clinical#011	NY, US
Blastocystis hominis	DL (ST- 3)	ATCC 50626 [a]	unknown
	NTY (ST- 1)	ATCC 50610 [a]	unknown
	NandII (ST- 1)	ATCC 50177	Maryland, US
	BT1 (ST- 4)	ATCC 50608	US
	ST- 3	Clinical#851	Sydney, AUS
	ST- 3	Clinical#910	Sydney, AUS
	ST- 3	Clinical#690	Sydney, AUS
	ST- 3	Clinical#835	Sydney, AUS
	ST- 4	Clinical#198	Sydney, AUS
	ST- 8[#]	Clinical#155	Sydney, AUS
	ST- 2[#]	Clinical#123	Sydney, AUS
	ST- 1	Clinical#009	NY, US
	ST- 1	Clinical#014	NY, US
	ST- 1	Clinical#022	NY, US
	ST- 2	Clinical#023	NY, US
	ST- 3	Clinical#053	NY, US
Entamoeba histolytica	HU-21:AMC	ATCC 30457	Arkansas, US
	200:NIH	ATCC 30458	unknown
	H-458: CDC	ATCC 30889	Asia
	HK-9 clone 6	ATCC 50544	Korea
	HB-301: NIH CL-1-3	ATCC 50547	Burma
	unknown	Clinical#1	Sydney, AUS
	unknown	Clinical#3	Sydney, AUS
	unknown	Clinical#4	Sydney, AUS
	unknown	Clinical#6	Sydney, AUS
	unknown	Clinical#7	Sydney, AUS
	unknown	Clinical#047	NY, US
	unknown	Clinical#050	NY, US
Giardia lamblia (a.k.a. G. intestinalis)	CM	ATCC PRA-242	unknown
	G1M	ATCC PRA-251	unknown
	Mario	ATCC PRA-244	US
	DAN	ATCC PRA-247	US
	BE-1	ATCC PRA-249	Canada
	WB clone C6 (AI)	ATCC 50803	Alaska, US
	Portland-1 (AI)	ATCC 30888	Oregon, US
	PR-15	ATCC PRA-42	Brazil
	JH (AII)	ATCC 50584	Alaska, US
	GS clone H7 (B)	ATCC 50581	Virginia, US
Enterocytozoon bieneusi	Genotype A	Clinical #7	Sydney, AUS
	Genotype D	Clinical #8	Sydney, AUS
	Genotype A	Clinical #5	Sydney, AUS
	Genotype K	Clinical #587	Sydney, AUS

Target organism	Strain/ Designation	Product ID / Clinical ID	Geographic Origin
	Genotype D	Clinical #10	Sydney, AUS
	Genotype D	Clinical #11	Sydney, AUS
	Genotype K	Clinical #12	Sydney, AUS
Encephalitozoon intestinalis	Alveolar isolate	ATCC 50506	NY, US
	CDC: V297	ATCC 50651	CA, US
	CDC: V307	ATCC 50603	Georgia, US
	Nasal isolate	ATCC 50507	NY, US

3. Analytical Specificity/Cross reactivity:

A. Whole organism/genome wet testing:

Analytical Specificity or cross-reactivity of the *EasyScreen* Gastrointestinal Parasite Detection Kit was established by testing a total of 94 different organisms (including viruses, bacteria, yeasts, and protozoa), as well as some commonly used microbiological media, in the *EasyScreen* Gastrointestinal Parasite Detection assay. In the whole organism/genome wet testing study, isolates from cultures, where available, or purified nucleic acids were diluted in negative stool matrix to a range of 10^6–10^9 CFU or copies/mL, with the exception of *Entamoeba dispar* which could not be procured at a sufficiently high concentration, or in a culturable form, to achieve the desired concentration and was tested at 1.5 x 10^3 organisms/mL. The potential cross reactants were tested in triplicate, with acceptance criteria for no cross-reactivity set at no positive replicate for a given target in Panels A–C of the *EasyScreen* Gastrointestinal Parasite Detection assay.

The study results showed no cross-reactivity the viral, fungal, bacterial, and protozoal microorganisms tested at the concentrations indicated in **Table 4**. None of the microbiological media used for organism propagation were cross-reactive either. However, the whole organism/genome wet testing study demonstrated three (3) cross-reacting protozoa—all of which were predicted from *in silico* alignments with their congeneric protozoa in the Panels A and C of the *EasyScreen* Gastrointestinal Parasite Detection assay:

i. *Cryptosporidium muris* (Strain Waterborne P104, tested at 6.25 x 10^5 organisms/mL), positive signal in Panel A.
ii. *Encephalitozoon cuniculi* (ATCC 50789, tested at 10^6 organisms/mL), positive signal in Panel C, and
iii. *Encephalitozoon hellem* (ATCC 50604, tested at 5 x 10^5 organisms/mL), positive signal in Panel C.

Table 4. Organisms with NO cross-reactivity observed in the EasyScreen Gastrointestinal Parasite Detection Kit Analytical Specificity Study.

Organism; strain	ID details
Viruses (tested at 10^6–10^9 copies/mL)	
Coxsackie virus B5	Vircell MBC062-R
Cytomegalovirus	Vircell MBC016
Sapovirus	ATCC VR3237SD
Adenovirus Type 41	ATCC VR-930DQ
Astrovirus	ATCC VR-3238SD
Bocavirus	ATCC VR-3251SD
Enterovirus 71	ATCC VR-1775DQ
Norovirus G1	ATCC VR-3234SD
Norovirus G2	ATCC VR-3235SD
Rotavirus A	ATCC VR-2018DQ

Organism; strain	ID details
Adenovirus Type 5; Adenoid 75	ATCC VR-5D
Yeasts (tested at 0.5–1 x 10^7 CFU/mL)	
Saccharomyces cerevisiae	ATCC MYA-796
Candida albicans	ATCC MYA-2876D-5
Bacterial strains (tested at 10^6–10^9 CFU/mL)	
Abiotrophia defectiva; Strain SC10	ATCC 49176
Acinetobacter baumannii	ATCC 19606D-5
Actinomyces naeslundii	ATCC 12104D5
Aeromonas hydrophila; CDC 359-60	ATCC 7965D
Akkermansia muciniphila; Strain Muc	ATCC BAA-835D-5
Alcaligenes faecalis; subsp. *faecalis*	ATCC 8750D-5
Anaerococcus tetradius	ATCC 35098
Arcobacter butzleri	ATCC 49616
Atopobium vaginae	ATCC BAA55
Bacillus cereus; Strain 971	ATCC 14579D
Bacteroides fragilis	ATCC 25285D-5
Bifidobacterium adolescentis	ATCC 15703D-5
Bifidobacterium bifidum	ATCC 29521
Campylobacter hominis	ATCC BAA-381D-5
Campylobacter jejuni	ATCC 33560D-5
Campylobacter lari	ATCC BAA-1060D-5
Capnocytophaga gingivalis	ATCC 33624D-5
Cedecea davisae	ATCC 33431
Chlamydia trachomatis; Serovar D	ATCC VR-885D
Chryseobacterium gleum	ATCC 35910
Citrobacter freundii	ATCC 8090D
Clostridioides difficile	ATCC BAA-1870DQ
Clostridium perfringens	ATCC 13124DQ
Corynebacterium glutamicum; Strain 534	ATCC 13032D-5
Cronobacter sakazakii	ATCC BAA-894D-5
Desulfovibrio piger; Strain VPI C3-23	ATCC 29098
Edwardsiella tarda; Strain CDC 1483-59	ATCC 15947
Eggerthella lenta; 1899B	ATCC 25559D-5
Enterococcus faecalis	ATCC 700802DQ
Enterococcus faecium	ATCC BAA-472D-5
Escherichia coli; CFT073	ATCC 700298D-5
Escherichia coli; Strain CDC EDL 1284	ATCC 43893
Eubacterium rectale	ATCC 33656
Faecalibacterium prausnitzii; Strain: VPI C13-51	ATCC 27768
Fusobacterium varium	ATCC 27725
Gardnerella vaginalis	ATCC 49145D-5
Gemella morbillorum	ATCC 27824
Hafnia alvei; HER 1272	ATCC 51873D-5
Helicobacter pylori; J99	ATCC 700824D-5
Klebsiella oxytoca	ATCC 700324D
Lactobacillus acidophilus	ATCC 4357D-5
Lactococcus lactis; subsp. lactis	ATCC 19435D-5
Leminorella grimontii	ATCC 33999

Organism; strain	ID details
Listeria monocytogenes	ATCC 19115D-5
Mycobacterium abscessus	ATCC 19977D-5
Mycobacterium avium; Strain K-10	ATCC BAA-968D-5
Mycobacterium tuberculosis	ATCC 25177D-5
Mycoplasma hominis Strain PG21	ATCC 23114D
Mycoplasma salivarium	ATCC 23064D
Neisseria flava	ATCC 14221D
Peptoniphilus asaccharolyticus	ATCC 14963
Peptostreptococcus anaerobius	ATCC 49031D-5
Plesiomonas shigelloides	ATCC 51903D
Porphyromonas asaccharolytica	ATCC 25260
Porphyromonas levii	ATCC 29147
Prevotella melaninogenica; Strain VPI 2381	ATCC 25845D-5
Proteus mirabilis	ATCC 12453DQ
Proteus vulgaris	ATCC 29905DQ
Providencia stuartii	ATCC 33672D
Pseudomonas aeruginosa	ATCC 47085DQ
Ruminococcus bromii; Strain VPI 6883	ATCC 27255
Salmonella enterica; serovar *Typhimurium*	ATCC 700720DQ
Serratia marcescens; CDC 3100-71	ATCC 27137D-5
Shigella flexneri type 2 Strain 24570	ATCC 29903D-5
Shigella sonnei	ATCC 29930
Staphylococcus aureus; subsp. *aureus*	ATCC 25923D-5
Staphylococcus haemolyticus	ATCC 29970D-5
Stenotrophomonas maltophila	ATCC 13637D-5
Streptococcus mitis NS 51	ATCC 49456D-5
Streptococcus pyogenes	ATCC 12344D-5
Streptococcus sanguinis	ATCC 10556D-5
Streptococcus thermophilus	ATCC BAA-250D-5
Trichomonas vaginalis	ATCC PRA-98D
Veillonella parvula	ATCC 10790D-5
Vibrio cholerae	Vircell MBC118-R
Vibrio parahaemolyticus	ATCC 17802D-5
Yersinia pseudotuberculosis	ATCC 6902D-5
Protozoa (tested at 1.5 x 10^3 organisms/mL)	
Entamoeba dispar SAW 760*****	ATCC PRA-260

***** *Entamoeba dispar* was not available for shipment for testing at a higher concentration or culturable form. *E. dispar* was thus subsequently investigated as a synthetic RNA target at a higher concentration.

B. In silico *analysis:*

Sequence alignments were performed to identify organisms of high similarity using the BLAST tool to interrogate sequences in GenBank. Sequences with identity of 90–100% to the target sequence were analyzed *in silico* for cross reactivity potential where the organisms were not available for wet testing, with a focus on sequence identity under the EP005 primer and probe regions. When assessing the likelihood of cross reactivity, the number of mismatches and location of the mismatches were considered. The location of a mismatch was defined as "significant" if

located within the first 5 nucleotides at the 3' end of the primer. ("Significant location" is not applicable to location of mismatches within probes.)

Organisms were assigned into three (3) categories indicating their potential to cross-react based on the GenBank sequence match to the primers and probes (in the context of the "3base" sequences), namely,

(i) High level of sequence match with up to 5 mismatches with non-significant locations (cross reactive potential: High).
(ii) Moderate level of sequence match with 4-6 mismatches that have non-significant locations (cross reactive potential: Moderate).
(iii) Low level of sequence match with 7 or more mismatches including in significant locations (cross reactive potential: Low).

For potentially cross-reactive organisms, literature searches were also conducted to identify whether any such organisms were known to infect humans. The results of *in silico* analyses are summarized in **Table 5** (potential cross reactants with no clinical relevance) and **6** (potential cross reactants with clinical relevance as assessed from published literature) below.

Table 5. **EasyScreen** ***Gastrointestinal Parasite Detection Kit target*** **in silico** ***analysis: potential cross reactants of no clinical relevance (known animal pathogens).***

Organism / representative GenBank accession	**Cognate EP005 target**	**Forward primer match ***	**Reverse primer match ***	**Probe match ***
B. cycluri AY590116	*Blastocystis hominis*	100%	100%	100%
B. lapemi AY266471		100%	100%	90.3% (1 mismatch, 2 deletions)
B. pythoni AY266472		100%	100%	90.3% (2 mismatches, 1 deletion)
B. ratti AY590114		100%	100%	100%
C. cercopitheci AF111185	*Cyclospora cayetanensis*	95.7% (1 mismatch)	100%	100%
C. colobi AF111186		95.7% (1 mismatch)	100%	100%
C. papionis AF111187		91.3% (2 mis-matches)	100%	91.3% (2 mismatches)
C. bovis EF514234	*Cryptosporidium* spp.	100%	100%	96.7% (1 insertion)
C. wrairi U11440 #		100%	100%	100%
Ecytonucleospora hepatopenaei OR168078	*Enterocytozoon bieneusi*	100%	100%	96% (1 mismatch)
Enterospora nucleophila KF135641		100%	100%	96% (1 mismatch)
Obruspora papernae HG005137		100%	100%	96% (1 mismatch)
E. nuttalli LC042219 #	*Entamoeba histolytica*	100%	100%	100%
G. microti AF006676	*Giardia intestinalis*	100%	100%	100%

Organism / representative GenBank accession	Cognate EP005 target	Forward primer match *	Reverse primer match *	Probe match *
Eimeria hermani KJ000078	*Cyclospora cayetanensis*	100%	100%	78.3% (3 mismatches, 2 deletions)
C. baileyi KT151546 #	*Cryptosporidium* spp.	100%	100%	90% (2 mismatches, 1 insertion)
Histomonas meleagridis AJ920323	*Dientamoeba fragilis*	100%	100%	81.5 % (5 mismatches)
Pseudotrichomonas keilini HM581663		100%	100%	85.2% (4 mismatches)
Trichomitus sp. 1 (ex Geochelone sulcata) JX515400		100%	100%	88.9% (2 deletions, 1 mismatch)
Nucleospora salmonis HQ418210	*Enterocytozoon bieneusi*	100%	100%	88% (3 mismatches)
E. lacerate AF067144	*Encephalitozoon intestinalis*	88% (3 mis-matches)	92% (2 mis-matches)	95.7% (1 mismatch)
E. pogonae KR998311		88% (3 mis-matches)	96% (1 mismatch)	95.7% (1 mismatch)
G. ardeae Z17210	*Giardia intestinalis*	95.7% (1 mismatch)	100%	81.8% (4 mismatches)
Trochochilodon flavus JN867018	*Blastocystis hominis*	96% (1 mismatch)	96% (1 mismatch)	80.6% (5 mismatches, 1 deletion)
Colpodella tetrahymenae AF330214	*Cryptosporidium* spp.	100%	96% (1 mismatch)	70% (4 mismatches, 4 insertions, 1 deletion)
C. fragile EU162754		100%	100%	76.7% (5 mismatches, 1 insertion, 1 deletion)
C. serpentis AF093499		100%	100%	83.3% (2 mismatches, 3 insertions)
C. struthionis AJ697751		100%	100%	66.7% (7 mismatches, 3 insertions)
Giardia muris X65063	*Giardia. intestinalis*	91.3% (2 mis-matches)	100%	45.5% (7 mismatches, 5 insertions)

***** For the purpose of calculating % match, any nucleotide inserted or deleted underneath a primer or probe (relative to the GenBank entry for the species in question) was considered one (1) mismatch. Thus, a primer or probe with four (4) mismatches, four (4) insertions and one (1) deletion would be considered to have nine (9) mismatches.

<3 cases of human infection reported worldwide.

Table 6. **EasyScreen *Gastrointestinal Parasite Detection Kit target* in silico *analysis: potential cross reactants of clinical relevance (known human pathogens).***

Organism / representative GenBank accession	Cognate EP005 target	Forward primer match *	Reverse primer match *	Probe match *	Predicted cross reactive potential
C. canis AB210854	*Cryptosporidium* spp.	100%	100%	93.3% (2 mismatches)	High
C. felis AF112575		100%	100%	93.3% (2 mismatches)	
C. meleagridis EF179381		100%	100%	100%	
C. tyzzeri OQ826430		100%	100%	100%	
C. muris L19069	*Cryptosporidium* spp.	100%	100%	80% (2 mismatches, 4 insertions)	Moderate
Chilomastix mesnili KC960586	*Giardia intestinalis*	95.7% (1 mismatch)	100%	81.8% (4 mismatches)	
E. dispar KP722600 **§**	*Entamoeba histolytica*	87.5% (2 mismatches, 1 insertion)	100%	100%	
Isospora belli TDQ060661	*Cyclospora cayetanensis*	82.6% (4 mismatches)	72% (7 mismatches)	56.5% (3 mismatches, 3 insertions, 4 deletions)	Low
C. andersoni AB513869	*Cryptosporidium* spp.	100%	100%	80% (3 mismatches, 3 insertions)	
E. bangladeshi KR025412	*Entamoeba histolytica*	62.5% (8 mismatches, 1 insertion)	100%	100%	
E. ecuadoriensis DQ286373		62.5% (9 mismatches)	100%	100%	
E. moshkovskii MN536500 **§**		70.8% (6 mismatches, 1 insertion)	81.5% (5 mismatches)	100%	

***** For the purpose of calculating % match, any nucleotide inserted or deleted underneath a primer or probe (relative to the GenBank entry for the species in question) was considered one (1) mismatch. Thus, a primer or probe with four (4) mismatches, four (4) insertions and one (1) deletion would be considered to have nine (9) mismatches.

§ *E. dispar* and *E. moshkovskii* infect humans but are non-pathogenic.

In silico analysis identified several targets for further investigation as potential cross- reacting organisms. However, only cases with three (3) or more reported human infections world-wide were further analyzed and investigated using synthetic RNA targets as described below.

C. Confirmatory Wet testing of synthetic RNA targets from clinically relevant protozoa.

Table 6 lists seven (7) potentially cross-reacting organisms (*in silico* predicted cross-reactivity with the *EasyScreen* Gastrointestinal Parasite Detection Kit: High or Moderate) that are also clinically relevant (as assessed from literature evidence for human infection). However, these

organisms were generally not available in either a culturable form or as clinical samples. For their further testing, synthetic double-stranded DNA targets—incorporating the T7 promoter at the 5' end of the sequence—were commercially designed ('gBlock' from IDT) from the Accession numbers in **Table 6**. Alternatively, where available, whole genome sequences (approximately 800–1000bp in length) were commercially obtained. These sequences were used to make synthetic *in vitro* transcribed (IVT) RNA. After quantification, the IVT RNA was extracted using the SP008B kit at between 10^8 and 10^9 copies/mL (see below) and tested with the *EasyScreen* Gastrointestinal Parasite Detection Kit. Results are presented in **Table 7**. With the exception of the *Chilomastix mesnili*. and *Entamoeba dispar*, all *in silico* targets of clinical relevance that were predicted to cross-react in the cognate *EasyScreen* assay tested positive in that assay.

Table 7. Wet testing of synthetic IVT RNA targets (P = positive; N = negative):

Organism	EP005 target to which similarity exists	gBlock Lot number	Cop-ies/ mL	Panel		
				A	B	C
Cryptosporidium meleagridis	*Cryptosporidium* spp.	109271385	10^8	**P**	N	N
Cryptosporidium tyzzeri		109271386	10^8	**P**	N	N
Cryptosporidium canis		109271381	8×10^8	**P**	N	N
Cryptosporidium felis		109271383	8×10^8	P	N	N
Entamoeba dispar ^	*Entamoeba histolytica*	103550577	10^9	N	N	N
Chilomastix mesnili	*Giardia intestinalis*	109609223	10^9	N	N	N

^ Wet-testing of *E. dispar* whole organism was negative at low copy number (Table 4), but it was selected for wet-testing with synthetic targets at a high copy number (10^9 copies/mL).

Therefore, wet testing of whole organisms or whole genomes or of synthetic RNA informed by *in silico* analysis identified the following seven (7) human pathogenic protozoa that are congeneric to two (2) target protozoal parasites in the *EasyScreen* Gastrointestinal Parasite Detection Kit and cross-react with the EP005 assays: *Cryptosporidium meleagridis*, *C. tyzzeri*, *C. canis*, *C. felis*, *C. muris, Encephalitozoon cuniculi*, and *E. hellem*.

4. Analytical Specificity/Interference:

Twenty-three (23) biological and chemical substances that may be present in clinical stool specimens were evaluated for potential interference with the *EasyScreen* Gastrointestinal Parasite Detection Kit with three (3) target analytes—one (1) representative chosen per panel, namely, *Dientamoeba fragilis* (Panel A), *Giardia intestinalis* / *lamblia* (Panel B), and *Enterocytozoon bieneusi* (Panel C)—tested at 2X LoD. Interferent concentrations chosen for evaluation were determined from the recommendations of FDA-recognized Consensus Standard CLSI EP37 along with a review of analytical studies from prior FDA-cleared GI panel devices. In ten (10) replicates tested, Interference (**I**) was defined as <100% target positivity (≤9/10) achieved OR a change of >15% in the average Ct values in test (i.e., with interferent) samples relative to baseline (i.e., no interferent). Of all substances evaluated (as shown in **Table 8**), two (2) of the tested substances (i.e., Whole Blood and Mucin) exhibited potential interference at different concentrations tested with the *EasyScreen* Gastrointestinal Parasite Detection Kit assay.

Table 8. Endogenous and exogenous substances tested with the EasyScreen Gastrointestinal Parasite Detection Kit as potential interferents. (I = Interference noted.)

Potential Interferent / Active agent (use)	Interferent conc.	*D. fragilis* (panel A)	*G. intestinalis* (panel B)	*E. bieneusi* (panel C)
Barium sulfate	10 % w/v	No interference observed in any Panel		
Calcium carbonate	2.5 % w/v			
Canesten (Clotrimazole 200 mg; 1% v/v) (antifungal)	30 % w/v			
Diaper rash cream (Zinc oxide)	30 % w/v			
Doxycycline (antibiotic)	10 mg/mL			
Dulcolax (Bisacodyl 5mg) (laxative)	20 % w/v			
Fatty acids (Stearic acid, Palmitic acid)	5 % w/v			
Fecal fat (Triglycerides, Cholesterol)	5 % w/v			
Gaviscon 10 mL (Sodium Alginate 500mg, Sodium Bicarbonate 213mg, Calcium Carbonate 325mg) (antacid)	10 % w/v			
Hydrozole (Hydrocortisone 1% v/v, Clotrimazole 1% v/v) (antifungal)	30 % w/v			
Imodium (Loperamide hydrochloride) (Anti-diarrheal)	10 % w/v			
KY gel (Chlorhexidine gluconate; Methyl benzoate) (lubricant)	30 % w/v			
Metronidazole (antibiotic)	10 % w/v			
Mineral oil	50 % w/v			
Naproxen sodium 275 mg (pain reliever)	10 % w/v			
Nystatin suspension (antifungal)	25 % w/v			
Pepto-Bismol Max Strength (Bismuth subsalicylate) (Anti-diarrheal)	10 % w/v			
Rectinol (Zinc oxide 200 mg, Cinchocaine hydrochloride 5mg) (hemorrhoid cream)	30 % w/v			
Vagisil (Benzocaine 50mg/g, Resorcinol 20mg/g) (feminine itching cream medication)	30 % w/v			
Vaseline (white petroleum jelly)	30 % w/v			
Wet Ones (Benzalkonium Chloride, Ethanol) (Antibacterial Hand Wipes)	30 % v/v			
Purified Mucin protein	3 mg/mL	No inter-ference	No inter-ference	**I**
	1.5 mg/mL	Not tested	Not tested	**I**
	0.75 mg/mL	Not tested	Not tested	No inter-ference
Whole Blood	5 % v/v	**I**	No inter-ference	**I**
	2.50%	**I**	Not tested	**I**
	1.25%	**I**	Not tested	No inter-ference
	0.63%	No inter-ference	Not tested	Not tested

Whole blood and Mucin demonstrated potential interference with the *EasyScreen* Gastrointestinal Parasite Detection Kit at concentrations greater than 0.63% and 0.75 mg/mL, respectively. This is indicated as a limitation in labeling.

5. Analytical Specificity/Mixed infections:

A. Microbial Interference:

The microbial interference study was designed to evaluate the ability of the *EasyScreen* Gastrointestinal Parasite Detection Kit to detect low positive target analytes in presence of high concentrations of extraneous non-protozoal micro-organisms that may be present in high concentrations in clinical stool specimens. All target analytes in Panel A–C of the *EasyScreen* Gastrointestinal Parasite Detection Kit were tested at 2X LoD in a negative stool matrix in presence or absence of the following eight (8) selected bacterial or fungal isolates at 10^6 CFU/mL: *Pseudomonas aeruginosa* (ATCC 47085DQ), *Enterococcus faecalis* (ATCC 700802DQ), *Candida albicans* (ATCC MYA-2876D-5), *Bacteroides fragilis* (ATCC 25285D-5), *Clostridioides perfringens* (ATCC 13124DQ), *Klebsiella pneumoniae* (ATCC 13883DQ), non-pathogenic *Escherichia coli* (ATCC 25922DQ), and *Saccharomyces cerevisiae* (ATCC MYA-796).

In the ten (10) target replicates tested, relative to baseline (i.e., with no interferent), microbial interference (**I**) was defined as <100% target positivity (≤9/10) achieved OR a change of >15% in the average Ct values in test (i.e., with interferent) samples; moderate microbial interference (**MI**) was defined as 100% (10/10) target positivity but showing 11–15% change in test Ct values; whereas no reportable interference (**NI**) was defined at 100% (10/10) target positivity with test Ct changes at or below 10%.

In the presence of potential microbial interferents in the *EasyScreen* Gastrointestinal Parasite Detection assay, all targets showed <10% change in average Ct values relative to baseline, with 100% (10/10) target positivity amongst replicates. Therefore, no microbial interference was observed when testing target organisms with the *EasyScreen* Gastrointestinal Parasite Detection Kit.

B. Competitive Interference:

The competitive interference study was designed to evaluate the ability of the *EasyScreen* Gastrointestinal Parasite Detection Kit to detect low positive target analytes in presence of high concentrations of potential competitor co-target protozoan analyte(s) that may be present in high concentrations in clinical stool specimens. All target analytes in Panel A–C of the *EasyScreen* Gastrointestinal Parasite Detection Kit were tested at 2X LoD in a negative stool matrix in presence or absence of other targets as shown in **Table 8**. Targets used as potential competitors were tested at 10^5 org/mL (whole organisms) except for *C. cayetanensis*, for which a synthetic *in vitro* transcribed RNA target was used at 10^8 copies/mL to serve as a high concentration competitor.

As before, in the ten (10) target replicates tested, relative to baseline (i.e., with no competitor), competitive interference (**I**) was defined as <100% target positivity (≤9/10) achieved OR a change of >15% in the average Ct values in test (i.e., with competitor) samples; moderate competitive interference (**MI**) was defined as 100% (10/10) target positivity but showing 11–15% change in test Ct values; whereas no reportable interference (**NI**) was defined at 100% (10/10) target positivity with test Ct changes at or below 10%.

In the course of these studies, moderate competitive interference was observed when testing low positive (2X LoD) *D. fragilis* and *G. intestinalis* targets with *C. cayetanensis* and *E. histolytica* competitors, respectively, with average Ct change of 12% and 11% (relative to baseline). Further, competitive interference (at 20% positivity) was seen with low positive *D. fragilis* and *B. hominis* targets with *C. parvum* and *E. histolytica* competitors, respectively. These were resolved upon reducing the competitor concentrations to 5 x 10^4 org/mL and 10^4 org/mL, respectively. However, a limitation is included in labeling to recommend testing with other methods upon a negative assay if *D. fragilis* or *B. hominis* is clinically suspected. All other low positive targets were successfully detected by the *EasyScreen* Gastrointestinal Parasite Detection Kit when combined with other competing targets at a high concentration, as shown in **Table 9** below.

Table 9: Target analytes and potential microbial competitors tested in the* EasyScreen *Gastrointestinal Parasite Detection Kit assay.

Panel	Target analyte at 2X LoD	Competitor analyte	Competitor concentration (org/mL, *except *C. cayetanensis*, copies/mL)	Competitive Interference observed
A	*D. fragilis*	*C. cayetanensis*	10^8 *	Moderate
	D. fragilis	*C. parvum*	10^5	**Interference**
	D. fragilis	*C. parvum*	*5 x 10^4 §*	No reportable interference
	C. cayetanensis	*D. fragilis*	10^5	
	C. cayetanensis	*C. parvum*	10^5	
	C. parvum	*D. fragilis*	10^5	
	C. parvum	*C. cayetanensis*	10^8 *	
B	*B. hominis*	*E. histolytica*	10^5	**Interference**
	B. hominis	*E. histolytica*	*10^4 §*	No reportable interference
	B. hominis	*G. intestinalis*	10^5	
	E. histolytica	*B. hominis*	10^5	
	E. histolytica	*G. intestinalis*	10^5	
	G. intestinalis	*B. hominis*	10^5	
	G. intestinalis	*E. histolytica*	10^5	Moderate
C	*E. bieneusi*	*E. intestinalis*	10^5	No reportable interference
	E. intestinalis	*E. bieneusi*	10^5	

§ Tests repeated at a lower concentration of competing targets, which resolved the interference.

6. Specimen Stability:

To provide evidence in support of the stability of specimens stored for testing with the *EasyScreen* Gastrointestinal Parasite Detection Kit, a range of storage conditions were evaluated using the methodology described below.

A. Specimen stability at 2–8°C.

From the EP005 Panels A–C, individual target analytes (i.e., whole organisms for targets except *C. cayetanensis*, which employed a synthetic RNA target) were spiked at 3X LoD into unpreserved negative stool matrix or Cary-Blair medium. Failure of testing in negative stool matrix at 3X LoD for *C. cayetanensis* and *B. hominis* necessitated re-testing at 10X LoD for these two (2) targets in the negative stool matrix.

Baseline (time zero) test results were established by testing the contrived samples with *EasyScreen* Gastrointestinal Parasite Detection Kit on the day of preparation. Aliquots prepared for each

analyte in each matrix were stored at 2–8ºC for at least (3) weeks with weekly testing and acceptance criteria set at 100% positivity (10/10 replicates). Based on the test observations, all target analytes are stable for three (3) weeks in unpreserved negative stool matrix. For analytes in Cary Blair matrix, except *C. cayetanensis* and E. *histolytica*, all targets are stable for three (3) weeks, whereas *C. cayetanensis* and E. *histolytica* are stable for two (2) weeks in the Cary Blair matrix.

B. Fresh vs. Frozen specimen stability:

A fresh versus frozen study was conducted to support the use of frozen samples in the analytical studies and to provide a scientific rationale for the acceptable use of frozen prospective (Category II) and retrospective (Category III) samples in the clinical studies with the *EasyScreen* Gastrointestinal Parasite Detection Kit. Individual sample dilutions were prepared for each analyte in the Panels A–C in unpreserved negative stool matrix, including Negative (no target, 10 replicates), Low Positive (2X LoD, 20 replicates) and Moderately Positive (4X LoD, 10 replicates) samples. Baseline ("fresh") test results were established by testing the contrived samples on the day of sample preparation. Aliquots of each analyte at each concentration were stored frozen at -20 ± 5°C and thawed for examination at four (4) weeks (28–30 days). Acceptance criteria for replicate results of each analyte were set at: 4X LoD, 100% (10/10 replicates) positive; 2X LoD, ≥95% (≥19/20) positive; and Negative, 0% (0/10) positive.

All eight (8) target parasites showed 100% detection at 4X LoD and ≥95% detection at 2X LoD at the 4-week timepoint after being frozen at -20 ± 5°C, except for *B. hominis*, which was tested at earlier at 3 weeks due to time constraints. Average Ct values at the 4-week timepoint (3-week timepoint for *B. hominis*) were within ±10 % of the baseline average Ct values for all targets and concentrations assessed. The results supported a frozen storage stability claim of three (3) weeks for all targets other than *B. hominis* (frozen storage stability of two (2) weeks for *B. hominis* was acceptable) when tested with the *EasyScreen* Gastrointestinal Parasite Detection Kit following manufacturer's instructions.

7. Detection Limit:

The analytical sensitivity (Limit of Detection / LoD) of the *EasyScreen* Gastrointestinal Parasite Detection Kit was established for all targets in a matrix of either unpreserved confirmed negative stool or Cary Blair media. All samples were evaluated following the device's Instructions for Use. LoD, evaluated from two (2) isolates per target, is expressed in organisms (org/mL) or genome copy number (copies/mL) per mL of sample, where one organism is defined as one (1) haploid genome, determined by digital PCR or quantitative PCR for either the 18S rRNA gene, or a single-copy gene target, and where necessary, divided by the published 18S rRNA gene copy number for that organism.

Following a preliminary range-finding study, LoDs were established with a minimum of twenty (20) extraction replicates using two (2) different isolates (or two (2) strains/genotype, where available) for each target. For LoD confirmation, the acceptance criteria were set at ≥95% detection of the specified target AND <95% detection at 0.5X LoD. When the acceptance criteria were not met in the first instance, targets were tested at 2-times lower or higher concentration as required until the criteria were met. These studies employed both AB 7500DX and QSDX analyzers. The results of the LoD studies showed comparable performance with minimal variability observed between LoD values obtained across different isolates, PCR analyzers and EP005 reagent batches with all targets showing an LoD within a ±2-fold dilution across all variables. The final LoD for each organism was defined for each sample matrix as the lowest concentration tested meeting the LoD criteria when results were combined for all instruments and kit lots tested (see **Table 10**).

Table 10: Analytical Sensitivity (LoD) results for EasyScreen Gastrointestinal Parasite Detection Kit target parasite in different matrices:

Reagent Panel	Target	LoD (analyzers: QSDX and 7500DX)	
		unpreserved stool matrix (org/mL)	Cary Blair matrix (org/mL)
A	*Dientamoeba fragilis*	62.5	320
	Cyclospora cayetanensis	1.56	5
	Cryptosporidium parvum	2060	7723
B	*Blastocystis hominis*	6.25	12.5
	Entamoeba histolytica	45	112.5
	Giardia intestinalis	1425	981
C	*Enterocytozoon bieneusi*	4	4
	Encephalitozoon intestinalis	5000	5000

8. Assay Cut-Off:

For all target parasites, cut-off values for threshold Cycle (Ct) were determined in analytical studies on both QSDX and AB7500 DX thermocyclers and verified using the multi-site clinical study data. Target-specific cut-offs (defining a negative) were determined during analytical studies using Ct values recorded for LoD or near LoD samples. For each targeted analyte, sample Ct value metrics from the clinical studies were directly compared to the Reference method (i.e., alternative NAAT followed by bidirectional sequencing, as indicated in FDA's 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*) and used a mathematical validation approach that avoided false negatives and shifted false positive calls to negative calls while retaining the near LoD sample Ct values to positive calls. The accepted and locked in cut-off Ct values for each target (see **Table 11** below) were applied to the results of the candidate assay. Apart from target-specific Ct cut-off values, cut-offs for extraction control (EC) and internal positive control (IPC) were established as Ct ≤37 and ≤40 respectively through prior use with other stool-based *EasyScreen* assays from Genetic Signatures.

Table 11: Cut-offs for EP005 Panel

Panel	Target	Cutoff Ct
A	*D. fragilis*	<41
	C. cayetanensis	<45
	Cryptosporidium spp. (including *C. parvum*)	<42
B	*B. hominis*	<43
	E. histolytica	<45
	G. lamblia/G. intestinalis	<40
C	*E. bieneusi*	<44
	E. intestinalis	<41

9. Carry-Over / Cross Contamination:

A carry-over study was conducted to investigate the potential for cross contamination or carry over between wells when using the workflow of the *EasyScreen* Gastrointestinal Parasite Detection Kit. Pooled high positive samples were prepared containing one representative member of each panel (*Cyclospora cayetanensis*, *Giardia lamblia* and *Enterocytozoon bieneusi*) consisting of at least 1 x 10^6 copies/mL of each target *in vitro* transcript (IVT) in negative stool matrix, following the recommendations provided in FDA-recognized voluntary consensus standard CLSI EP39. IVTs

were used in this study as most of the analytical targets available were not able to be extracted at the high copy numbers required. A negative sample was prepared containing negative stool matrix containing no target analyte and tested in one stage.

Sixteen (16) replicates of pooled high positive sample and fourteen (14) replicates of negative sample were extracted in each run in an alternating negative and positive sample pattern. In this sample pattern, there were 6 and 8 negative wells surrounded by, respectively, 4 and 3 positive wells around them. A total of five (5) extractions and PCR setups were performed with each run containing one negative processing control and thirty (30) samples, which were then seeded into 96 wells of a PCR plate by the GS1 platform. Since each panel gets one set of reagents, one representative organism per panel is acceptable. Target detection in PCR was assessed for all samples, with pre-defined acceptance criteria met at 100% (240/240) detection for pooled high positive samples and 0% detection for negative samples (0/210) and the negative processing control (0/15), demonstrating that in the *EasyScreen* Gastrointestinal Parasite Detection Kit workflow, there was no reportable carry over/cross contamination between the wells during sample preparation or PCR set up.

B Comparison Studies:

1. Method Comparison with Predicate Device:

N/A

2. Matrix Equivalency between Cary-Blair and C&S:

A matrix equivalency study was conducted in support of the use of C&S media (e.g., Meridian Parapak #900612) as an alternative to Cary Blair media (e.g., Thermo Scientific Remel #R21610) when storing and/or transporting human clinical stool specimens for later processing with the *EasyScreen* Gastrointestinal Parasite Detection Kit. For each analyte in the Panels A–C, individual sample dilutions were prepared in C&S matrix to include Negative (no analyte), Low Positive (1X–2X LoD), and High Positive (5X LoD) samples. Performance characteristics of the samples contrived in C&S media were ascertained at the selected analyte levels using five (5) extraction replicates of High Positives, twenty-five (25) replicates of Low Positives, and ten (10) replicates of Negative samples. Acceptance criteria for replicate results of each analyte were set at: 5X LoD, 100% (5/5 replicates) positive; 1–2X LoD, ≥95% (≥24/25) positive; and Negative, 0% (0/10) positive. For each target, the initial LoD values previously obtained with Cary-Blair matrix during Analytical Sensitivity studies served as the baseline for this study. As shown in **Table 12**, for all targets and analyte concentrations tested, the targets diluted in C&S matrix met the acceptance criteria based on LoD in Cary Blair matrix, demonstrating equivalence for use in the *EasyScreen* Gastrointestinal Parasite Detection Kit.

Table 12. C&S Matrix equivalency for targets with various analyte concentrations tested in EasyScreen Gastrointestinal Parasite Detection Kit assay.

Target analyte	Tested analyte level in C&S matrix relative to LoD in Cary-Blair matrix	Detection (%)	Avg Ct
D. fragilis	1X LoD	24/25 (96%)	33.17
	5X LoD	5/5 (100%)	30.9
C. cayetanensis	2X LoD	25/25 (100%)	33.33
	5X LoD	5/5 (100%)	33.45
C. parvum	1X LoD	25/25 (100%)	29.69

Target analyte	Tested analyte level in C&S matrix relative to LoD in Cary-Blair matrix	Detection (%)	Avg Ct
	5X LoD	5/5 (100%)	26.94
B. hominis	1X LoD	25/25 (100%)	31.60
	5X LoD	5/5 (100%)	29.36
E. histolytica	1X LoD	25/25 (100%)	34.80
	5X LoD	5/5 (100%)	32.73
G. intestinalis	1X LoD	25/25 (100%)	34.55
	5X LoD	5/5 (100%)	31.34
E. bieneusi	1X LoD	25/25 (100%)	34.87
	5X LoD	5/5 (100%)	32.69
E. intestinalis	1X LoD	25/25 (100%)	33.66
	5X LoD	5/5 (100%)	29.97

C Clinical Studies:

Clinical Study details:

A multicenter clinical study was conducted to assess the performance of the *EasyScreen* Gastrointestinal Parasite Detection kit for the identification of *Cryptosporidium spp., Cyclospora cayetanensis, Giardia intestinalis, Dientamoeba fragilis, Entamoeba histolytica, Blastocystis hominis, Enterocytozoon bieneusi*, and *Encephalitozoon intestinalis* using stool specimens from symptomatic patients with suspected gastroenteritis. The study evaluated results obtained with the *EasyScreen* Gastrointestinal Parasite Detection kit, in comparison to those obtained with a reference method. Following FDA's Class II Special Controls Guidelines *"Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assays for Detection and Identification of Microorganisms and Toxin Genes from Human Stool Specimens"* the reference method for the clinical studies was chosen to be two (2) well-characterized and validated Nucleic Acid Amplification Tests (NAAT) followed by bi-directional sequencing (referred to as "alternative NAAT").

For the clinical study, sites were selected based on several criteria, including investigator and study staff availability, number of specimens of interest, target prevalence and familiarity with PCR methodology. The three (3) participating US sites for prospective sample collection and testing were geographically diverse by location, and an additional US site performed retrospective sample procurement along with testing.

The clinical study was designed in an All-Comers mode to prospectively collect stool samples from symptomatic subjects to be tested fresh (Category I samples) or after frozen storage (Category II samples). Stool specimens were collected from patients of any age (ranging <5 to ≥60 years), who presented with signs and/or symptoms of gastroenteritis and were referred for testing. However, given the low prevalence of some of the target parasites in specific geographic areas as well as problems in enrollment during COVID-19 pandemic-adjacent times, the enrollment of retrospective, positive-identified (Category III) samples was considered acceptable along with the inclusion of randomly distributed negative samples, all to be tested in a masked/blinded manner. Finally, for two (2) low-prevalence target parasites, namely, *E. bieneusi* and *E. intestinalis*, for which retrospective samples were not available despite best efforts, contrived (Category IV) samples in a negative stool matrix were prepared and enrolled at an outside the US (OUS) internal site for testing at the US sites.

All specimens were processed with the *EasyScreen* Sample Processing Kit using the automated Genetic Signatures GS1 platform and tested with the *EasyScreen* Gastrointestinal Parasite Detection kit (hereafter collectively referred to as "EP005") device following manufacturer's instructions.

True analyte status ("positive" or "negative") of each sample was established for each target parasite by comparison with the reference method of alternative NAATs conducted at the OUS site. For each target analyte, the alternative NAATs consisted of two (2) separate single-plex, PCR amplification tests which targeted regions not covered by or adjacent to the EP005 assay reagents. Amplicons from all PCR-positive reactions sequenced with bi-directional Sanger sequencing at the internal site. Samples producing sequence data that met the acceptability criteria listed in Section VII(D)(1) of the Class II Special Controls guidelines were reported as *positive* by alternative NAAT.

Results from the EP005 device were also compared against an FDA-cleared assay following its device labeling. In this comparison, only those three (3) protozoan targets were evaluated that are detected by the comparator—namely, *Giardia lamblia* (a.k.a. *G. intestinalis*), *Cryptosporidium* species *hominis* and *parvum*, as well as *Entamoeba histolytica.* Category III samples enrolled in the EP005 clinical study were not tested with the comparator that is not validated for use with that specimen type.

A total of 2,806 specimens (Categories I–IV) were collected with 880 samples excluded due to invalid and/or missing data, storage and volume limitations, improperly contrived samples with incorrect targets, which left 1,926 analyzable specimens for performance evaluation (see **Table 13**).

Table 13: Sample Details (N = 1,926)

Prospective and retrospective	US1 N = 204		US2 N=483		US3 N=966		US4 N=252		OUS1 N=21		Total N=1926	
	N	%	N	%	N	%	N	%	N	%	N	%
Stool type												
Transport Media (incl. Cary Blair / C&S)	157	76.96	219	45.34	242	25.05	252	100	0	0	870	45.17
Fresh	47	23.04	164	33.95	674	69.77	0	0	0	0	885	45.95
Frozen	0	0.00	100	20.70	50	5.18	0	0	21	100	171	8.88
SEX												
F	133	65.2	280	57.97	579	59.94	101	40.08	0	0	1,093	56.75
M	71	34.8	202	41.82	386	39.96	151	59.92	0	0	810	42.06
unknown	0	0.00	1	0.21	1	0.1	0	0.00	21	100	23	1.19
AGE (years)												
≤5	8	3.92	27	5.59	27	2.80	29	11.51	2	9.52	93	4.83
6–21	28	13.73	69	14.29	89	9.21	32	12.7	6	28.57	224	11.63
22–59	87	42.65	224	46.38	406	42.03	136	53.97	8	38.1	861	44.7
60≥	81	39.71	163	33.75	444	45.96	53	21.03	2	9.52	743	38.58
Unknown	0	0.00	0	0.00	0	0.00	2	0.79	3	14.29	5	0.26

Of these 1,926 samples with demographic data, 200 samples requiring a repeat test were invalid on retest, making for a 10.4% (200/1926) invalid rate. Therefore, 1,726 prospective (n = 1461) and

retrospective (n = 265) clinical samples were available for analysis with both EP005 and corresponding alternative NAAT reference method. Addition of Category IV samples (n = 165) with *E. bieneusi* and *E. intestinalis* brought the total analyzable study sample count up to 1,891.

Based on evaluation of clinical study data provided from 1,891 stool specimens, the performance of the *EasyScreen* Gastrointestinal Pathogen Detection Kit is acceptable with PPA for individual target parasites ranging between 91–99% with lower limit of 95% CI at ≥80% and NPA ≥99% when compared to the reference method.

A. Performance Estimates of EP005 relative to the Reference Method:

This section summarizes the target analyte-specific comparison of *EasyScreen* Gastrointestinal Parasite Detection kit (EP005) results to the reference method for the 1,461 prospective clinical samples, 265 retrospective clinical samples, and the 165 contrived samples with valid results on both tests. For results from clinical studies presented in **Tables 14–22**, the following abbreviations are used: all relative to the reference method, True Positive, TP; True Negative, TN; False Positive, FP; False negative, FN; Positive Percent Agreement (sensitivity), PPA; Negative Percent Agreement (specificity), NPA; lower (LL), upper limit (UL) of the 95% Confidence Interval (CI).

Table 14. Performance metrics of *D. fragilis* by Analysis groups

Group	N	TP	TN	FP	FN	PPA (%)	95% CI		NPA (%)	95% CI	
							LL	UL		LL	UL
Prospective	1461	13	1441	5	2 [a]	86.67	62	96	99.65	99	100
Retrospective	265	37	225	1	2 [b]	94.87	83	99	99.56	98	100

a) Two (2) FN prospective samples were not available for duplicate, investigational EP005 retests.
b) Two (2) FN retrospective samples were again found negative in duplicate EP005 retests and were originally reported negative for *D. fragilis* by a US site's laboratory-developed test.

Table 15. Performance metrics of *C. cayetanensis* by Analysis groups

Group	N	TP	TN	FP	FN	PPA (%)	95% CI		NPA (%)	95% CI	
							LL	UL		LL	UL
Prospective	1461	1	1454	6	0	100	21	100	99.59	99	100
Retrospective	265	43	216	5	1 [a]	97.73	88	100	97.74	95	99

a) One (1) FN retrospective sample was found positive for *C. cayetanensis* in duplicate, investigational EP005 retests.

Table 16. Performance metrics of *Cryptosporidium* spp. by Analysis groups

Group	N	TP	TN	FP	FN	PPA (%)	95% CI		NPA (%)	95% CI	
							LL	UL		LL	UL
Prospective	1461	3	1453	5	0	100	44	100	99.66	99	100
Retrospective	265	39	216	6	4 [a]	90.7	78	96	97.3	94	99

a) Three of the four (3/4) FN samples were found positive for *Cryptosporidium* in duplicate, investigational EP005 retests. The fourth (1/4) sample was found negative for *Cryptosporidium* in duplicate.

Table 17. Performance metrics of *B. hominis* by Analysis groups

Group	N	TP	TN	FP	FN	PPA (%)	95% CI		NPA (%)	95% CI	
							LL	UL		LL	UL
Prospective	1461	47	1401	11	2 [a]	95.92	86	99	99.22	99	100
Retrospective	265	76	186	2	1 [b]	98.7	93	100	98.94	96	100

a) Two (2) FN prospective samples were not available for duplicate, investigational EP005 retests.
b) One (1) FN retrospective sample was found positive for *B. hominis* in duplicate, investigational EP005 retests.

Table 18. Performance metrics of *E. histolytica* by Analysis groups

Group	N	TP	TN	FP	FN	PPA (%)	95% CI		NPA (%)	95% CI	
							LL	UL		LL	UL
Prospective	1461	0	1457	4	0				99.73	99	100
Retrospective	265	31	231	2	1 [a]	96.88	84	99	99.14	97	100

a) One (1) FN sample was co-infected with *B. hominis* and was found positive for both targets in duplicate, investigational EP005 retests.

Table 19. Performance metrics of *G. intestinalis* by Analysis groups

Group	N	TP	TN	FP	FN	PPA (%)	95% CI		NPA (%)	95% CI	
							LL	UL		LL	UL
Prospective	1461	2	1452	7	0	100	34	100	99.52	99	100
Retrospective	265	33	219	12	1 [a]	97.06	85	99	94.81	91	97

a) One (1) FN sample was co-infected with *B. hominis* and was found positive for both targets in duplicate, investigational EP005 retests.

Table 20. Performance metrics of *E. bieneusi* by Analysis groups

Group	N	TP	TN	FP	FN	PPA (%)	95% CI		NPA (%)	95% CI	
							LL	UL		LL	UL
Prospective	1461	6	1448	7	0	100	61	100	99.52	99	100
Retrospective	265	1	262	2	0	100	21	100	99.24	97	100
Contrived	165	75	89	0	1 [a]	98.68	92.92	99.77	100	95.86	100

a) One (1) FN sample was a contrived sample spiked with *E. bieneusi* at near-LoD.

Table 21. Performance metrics of *E. intestinalis* by Analysis groups

Group	N	TP	TN	FP	FN	PPA (%)	95% CI		NPA (%)	95% CI	
							LL	UL		LL	UL
Prospective	1461	0	1454	7	0				99.52	99	100
Retrospective	265	2	263	0	0	100	34	100	100	99	100
Contrived	165	73	86	0	6 [a]	92.41	84.4	96.47	100	95.72	100

a) All six (6) FN samples were contrived samples spiked with *E. intestinalis* at near-LoD.

B. Performance Estimates of EP005 relative to the Comparator:

This section summarizes the target analyte-specific comparison of *EasyScreen* Gastrointestinal Parasite Detection kit EP005 results to results obtained from the predicate for 760 samples with valid results on both tests (as shown in **Table 22**). This performance metrics estimation excluded all samples (n = 126) that tested invalid (on EP005) or UNR (i.e., unresolved, on the comparator).

Table 22: 2x2 Performance Comparison Tables for EP005 vs. comparator.

Target: *G. intestinalis*		**Comparator Test**		
		Positive	**Negative**	**Total**
EP005	**Positive**	1	2	3
	Negative	1	756	757
	Total	2	758	760
Target: *Cryptosporidium* spp.		**Comparator Test**		
		Positive	**Negative**	**Total**
EP005	**Positive**	1	0	1
	Negative	2	757	759
	Total	3	757	760
Target: *Entamoeba histolytica*		**Comparator Test**		
		Positive	**Negative**	**Total**
EP005	**Positive**	0	3	3
	Negative	0	757	757
	Total	0	760	760

Table 23: Performance estimates of EP005 relative to the comparator.

Target	**N**	**PPA (%)**	**95% CI**		**NPA (%)**	**95% CI**	
			LL	**UL**		**LL**	**UL**
G. intestinalis	760	50	9.5	90.5	99.7	99	100
Cryptosporidium spp.	760	33.3	6.1	79.2	100	99.5	100
E. histolytica [a]	760				99.6	98.9	100

a) *EasyScreen* Gastrointestinal Parasite Detection kit identified no true-positive and three false-positive *Entamoeba histolytica* samples.

C. Co-infections detected by EP005 in clinical study samples with validation by the reference method.

This section summarizes the number of multi-parasite (n = 68) Category I–IV samples detected by the *EasyScreen* Gastrointestinal Parasite Detection Kit (EP005) as presented in **Table 24**. Column N represents unique number of samples with targets validated by the reference method. Samples for any analytes with discrepant results between EP005 and reference method were not considered for this summary.

Table 24: Tabulation of Co-Infections as detected by both EP005 and the reference method.

Co-Infections	**N** [a]
B. hominis, C. cayetanensis	3
B. hominis, Cryptosporidium spp.	2
B. hominis, Cryptosporidium spp., *G. intestinalis*	2
B. hominis, D. fragilis	23 [b]
B. hominis, D. fragilis, C. cayetanensis	1
B. hominis, D. fragilis, G. intestinalis	1
B. hominis, E. bieneusi	2
B. hominis, E. histolytica	9 [c]
B. hominis, G. intestinalis	10
B. hominis, E. bieneusi, G. intestinalis	2
C. cayetanensis, D. fragilis	1
C. cayetanensis, G. intestinalis	1
Cryptosporidium spp., *G. intestinalis*	2

Co-Infections	**N** [a]
Cryptosporidium spp.*, E. bieneusi*	1
Cryptosporidium spp.*, D. fragilis*	1
D. fragilis, G. intestinalis	2 [d]
E. bieneusi, G. intestinalis	1
Totals	73

a) Not counted in this enumeration are five (5) retrospective samples which were discrepant for a second target that was detected upon EP005 retests: #201-1161 (*D. fragilis* TP + *Cryptosporidium* spp. FN→TP), #201-1187 (*G. intestinalis* TP + *B. hominis* FN→TP), #201-1198 (*B. hominis* TP + *G. intestinalis* FN→TP), #201-1211 (*B. hominis* TP + *E. histolytica* FN→TP), and #201-1243 (*G. intestinalis* TP + *Cryptosporidium* spp. FN→TP).

b) Not counted in this category was one (1) discrepant prospective sample (#103-0050) that was additionally positive for *D. fragilis* by the reference method but FN by EP005 and enough sample was not available for EP005 retest.

c) In this category, one (1) retrospective sample (#201-1066) was additionally positive for *D. fragilis* by the reference method but persistently FN by EP005. This was not enumerated under *D. fragilis*.

d) Not counted in this category was one (1) discrepant retrospective sample (#201-1223) that was additionally positive for *D. fragilis* by the reference method but persistently FN by EP005.

D Clinical Cut-Off:
N/A

E Expected Values/Reference Range:
N/A

F Other Supportive Instrument Performance Characteristics Data:
N/A

VIII Proposed Labeling:

Proposed 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.