May 29, 2024

Genetic Signatures Limited
Neralie Coulston
Global Head, Regulatory Affairs
7 Eliza Street
Newtown, NSW 2042
Australia

Re: K232672
Trade/Device Name: EasyScreen Gastrointestinal Parasite Detection Kit
Regulation Number: 21 CFR 866.3990
Regulation Name: Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assay
Regulatory Class: Class II
Product Code: PCH, OOI
Dated: April 28, 2024
Received: April 29, 2024

Dear Neralie Coulston:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

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

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled “Deciding When to Submit a 510(k) for a Change to an Existing Device” (https://www.fda.gov/media/99812/download) and “Deciding When to Submit a 510(k) for a Software Change to an Existing Device” (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Ribhi Shawar -S

Ribhi Shawar, Ph.D. (ABMM)
Chief
General Bacteriology and Antimicrobial Susceptibility Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K232672

Device Name
EasyScreen™ Gastrointestinal Parasite Detection Kit

Indications for Use *(Describe)*
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.

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

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

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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

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

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

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

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

510(k) SUMMARY
K232672

1 GENERAL INFORMATION

Submitted by: Genetic Signatures Limited, 7 Eliza Street Newtown, NSW 2042, Australia

Contact Person: Neralie Coulston
Phone: +61 2 9870 7580
Email: neralie.coulston@geneticsignatures.com

Date Prepared May 27th, 2024

Proposed Device

Trade and Common Name:	*EasyScreen*™ PCR Gastrointestinal Parasite Detection Kit
Product Code(s):	PCH (Class 2) – Gastrointestinal Pathogen Panel Multiplex Nucleic Acid-Based Assay
C.F.R. Section:	866.3990
Classification Panel/Medical Specialty:	Microbiology
Device Class:	Class II

Predicate Device

Predicate Device:	BD MAX™ Enteric Parasite Panel (EPP)
Predicate 510(k):	K143648
Predicate Product Code(s):	PCH (Class 2) – Gastrointestinal Pathogen Panel Multiplex Nucleic Acid-Based Assay
Predicate C.F.R. Section:	866.3990
Predicate Classification Panel/Medical Specialty:	Microbiology
Predicate Device Class:	Class II

2 DEVICE DESCRIPTION

2.1 Device Details

The ***EasyScreen™*** **Gastrointestinal Parasite Detection Kit (EP005)** is designed to simultaneously identify 8 potential pathogens of the gastrointestinal tract, from human stool samples. The device is only compatible with nucleic acids prepared using an ***EasyScreen™*** **Sample Processing Kit (SP008B**).

A stool sample from a patient suspected of having gastroenteritis (usually liquid or soft stool) is collected and transported to the testing laboratory. A portion of the stool material is taken using a swab or pipette and processed with the *EasyScreen*™ Sample Processing Kit (SP008B), which lyses cells and converts the nucleic acid to a 3base™ form.

An aliquot of purified eluate is then added to the PCR reagents supplied in the EP005 kit, which selectively amplify the genetic targets of *Cryptosporidium* spp., *Giardia intestinalis*, *Entamoeba histolytica, Dientamoeba fragilis, Blastocystis hominis*, *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis* and *Cyclospora cayetanensis*. The reaction mix is manufactured to detect an Extraction Control (EC) and features an incorporated Internal Positive Control (IPC) to determine the reliability of the extracted nucleic acid and to detect the presence of any inhibitors after extraction from the primary sample.

Amplified targets are detected with probes labeled with fluorophores as detected by the real-time PCR platform. The PCR amplification takes approximately 150 minutes, depending on the PCR platform used. A positive control is included to ascertain that the detection reagents and analyzer are functioning correctly.

The detection channels for the ***EasyScreen™*** **Gastrointestinal Parasite Detection Kit** are shown in Table 1 below.

Table 1. Protozoan Targets Detected in Each Fluorescent Channel. Note: spp. = species.

Detection Channel	Reaction Mix A	Reaction Mix B	Reaction Mix C
Channel 1	*Dientamoeba fragilis*	*Blastocystis hominis*	*Enterocytozoon bieneusi*
Channel 2	Extraction Control	Internal Positive Control	Extraction Control
Channel 3	*Cyclospora cayetanensis*	*Entamoeba histolytica*	*Encephalitozoon intestinalis*
Channel 4	*Cryptosporidium* spp.	*Giardia intestinalis*	Not used

The amplified nucleic acid targets are detected by probes labeled with fluorophores, as detected by the real-time PCR platform. If no amplification occurs for a given target, then there will not be any significant increase in fluorescence. Each probe fluoresces at a given wavelength and the signals are measured and distinguished from each other by the real-time PCR platform. The real-time PCR software interprets all data collection and provides the information for automated or manual result analysis. The assay is semi-automated.

2.2 Reagents

The EP005 kit contains 10 tubes of each panel (including 5 PCR components and 5 PCR mastermix), 3 tubes of Reverse Transcription Reagent and 5 tubes of Gastrointestinal Parasite Positive Control. The device should be stored at -15 to -25 ºC.

2.3 Intended 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.

2.4 Substantial Equivalence Device Comparison

The device with substantial equivalence to EP005 that was selected is the BD MAX™ Enteric Parasite Panel, 510(k) K143648, manufactured by Becton Dickenson, Franklin Lakes, NJ. Both

the proposed device and the predicate device have similar intended uses and technological characteristics. The proposed EP005 assay is compared to the predicate assay in Table 2 below.

Table 2. Comparison of EP005 vs BD Max EPP

Feature	Predicate	Device
	BD MAX™ Enteric Parasite Panel	***EasyScreen*™ Gastrointestinal Parasite Detection Kit**
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 parasitic pathogens. The BD MAX Enteric Parasite Panel detects nucleic acids from: • *Giardia lamblia* • *Cryptosporidium* (*C. hominis* and *C. parvum* only) • *Entamoeba histolytica* Testing is performed on unpreserved or 10% formalin-fixed stool specimens from symptomatic patients with suspected gastroenteritis, enteritis or colitis. The assay is intended to aid in the diagnosis of gastrointestinal infection when used in conjunction with clinical evaluation and other laboratory findings. The test is performed directly on the specimen, utilizing real-time polymerase chain reaction (PCR) for the amplification of specific targets. The test utilizes fluorogenic gene-specific hybridization probes for detection of the amplified DNA. This test is intended for use, in conjunction with clinical presentation, laboratory findings, and epidemiological information, as an aid in the differential diagnosis of *Giardia lamblia*, *Cryptosporidium hominis* and *C. parvum*, as well as *Entamoeba histolytica* infections. Results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decision. Positive results do not rule out co-infection with other organisms that are not detected by this test, and may not be the sole or definitive cause of patient illness.	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

Feature	Predicate	Device
	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.	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.
SIMILARITIES		

Feature	Predicate	Device
Described intended Purpose of device	Detects nucleic acids of enteric parasitic pathogens from the stool samples of patients with symptoms of gastrointestinal infection as an aid in the diagnosis of gastrointestinal illness.	Same
Analyte	Nucleic acids	Same
Technology	Multiplex nucleic acid amplification and detection	Same
Target organism nucleic acid detected	• *Giardia lamblia* • *Cryptosporidium hominis* and *C. parvum* • *Entamoeba histolytica*	Same
Interpretation of test results	Automated (BD MAX™ System diagnostic software)	Automated via a validated macro-enabled Excel sheet.
Specimen Types	Unpreserved and preserved stool (see below for differences in the preservatives used)	Same
DIFFERENCES		
Feature	**Predicate**	**Device**
Analysis* platform	BD MAX System	Life Technologies QuantStudio™ Dx or Applied Biosystems® 7500 Fast Dx
Additional Targets Detected	none	• *Dientamoeba fragilis* • *Blastocystis hominis*. • *Enterocytozoon bieneusi* • *Encephalitozoon intestinalis* • *Cyclospora cayetanensis*
Specimen Types	• Fresh stool • 10% formalin-fixed stool specimens	• Fresh and frozen stool • Stool in Cary Blair/C&S

*The analysis platforms are manufactured by different suppliers with the predicate device using the BD MAX™ system and the *EasyScreen*™ Gastrointestinal parasite kit using the Life Technologies QuantStudio™ Dx or Applied Biosystems® 7500 Fast Dx, these perform the same function in terms of detecting an increase in fluorescence during amplification.

The differences noted above add extra sample types and target organisms, thereby increasing the breadth of the test's utility, while operating under the same Intended Use.

3 SUMMARY OF PERFORMANCE TESTING

3.1 Overview of Analytical Performance Studies

Analytical studies were performed to establish functional parameters for the ***EasyScreen*™ Gastrointestinal Parasite Detection Kit** in accordance with the 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 (issued November 2, 2015). The following studies were performed, and reports were submitted along with study protocol, line data, and summary of analysis.

(a) Analytical Sensitivity

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. For each target, LoD was established with a minimum of twenty (20) extraction replicates using two (2) different target isolates (or two (2) strains/genotype, where available) and is expressed as 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.

LoD is defined as the lowest concentration at which ≥95% of all replicates are expected to test positive. For any given target, the LoD acceptance criteria were set at ≥95% detection of the specified target AND <95% detection at 0.5X LoD. LoD studies showed comparable performance with minimal variability observed between LoD values obtained across different isolates, PCR analyzers (AB 7500DX or QSDX) 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 3**).

Table 3. Analytical Sensitivity (LoD) results for EasyScreen™ Gastrointestinal Parasite Detection Kit target analytes 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

(b) Multisite Reproducibility

The precision of performance of the *EasyScreen*™ Gastrointestinal Parasite Detection Kit amongst laboratories was evaluated with a site-to-site qualitative reproducibility study incorporating Study #1 at two US clinical sites and one in-house site outside the US (OUS) and Study #2 at another US clinical site and the OUS site. Study #1 evaluated four of the assay target organisms—namely, *D. fragilis*, *C. parvum*, *E. bieneusi*, and *E. intestinalis*—with two operators at each site for ten days. Study #2 evaluated remaining four of the assay targets—namely, *C. cayetanensis*, *B. hominis*, *E. histolytica*, and *G. intestinalis*— with three operators at each site for 5–7 days. For each indicated target organism, a sample panel was contrived by spiking an unpreserved negative stool matrix—a True Negative (TN) sample with no or undetectable target—with varying organism 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.

In Study #1, the negative samples were tested in triplicate by two operators at each of three sites for ten extraction runs, resulting in a total of 180 (i.e., 3 x 2 x 10 x 3) data points, whereas each positive panel member was tested in triplicate for each of five extraction runs, resulting in a total of 90 (i.e., 3 x 2 x 5 x 3) data points per panel member. In Study #2, for panel members *B. hominis*, *E. histolytica*, and *G. intestinalis*, the negative samples were tested in triplicate by three operators at each of the two sites, 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, whereas positive samples were tested in triplicate for each run resulting in a total of 99 (i.e., 3 x 5 x 3 + 3 x 6 x 3) data points per panel member. For *C. cayetanensis*, operational difficulties with sites resulted in a total of 102 (i.e., 3 x 6 x 3 + 2 x 12 x 2) data points for member LP and 99 (i.e., 3 x 5 x 3 + 2 x 1 x 3 + 2 x 12 x 2) data points for member MP.

Analyte-specific site-to-site qualitative reproducibility testing results from both studies are presented in **Tables 4 and 5** 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 excluded from analysis.

Table 4. 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 5. 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). 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.

(c) Analytical Specificity

Analytical Specificity or cross-reactivity of the ***EasyScreen*™ Gastrointestinal Parasite Detection Kit** was established in a stepwise design.

A. Whole organism/genome wet testing:

A total of 94 organisms—represented by culture isolates where available or purified nucleic acids as substitutes—along with seven different microbiological media were tested for cross-reactivity with the ***EasyScreen*™ Gastrointestinal Parasite Detection Kit**. The isolates/nucleic acids were diluted in negative stool matrix to a range of 10^6–10^9 CFU or copies/mL (except of *Entamoeba dispar* which could not be procured at a sufficiently high concentration, or in a culturable form, to achieve the desired input concentration). The potential cross-reactants were tested in triplicate with acceptance criteria set at no detection to signify no cross-reactivity for any given target in Panels A–C of the ***EasyScreen*™ Gastrointestinal Parasite Detection Kit**. In the organism or genome wet testing, no cross-reactivity was observed with the viral, fungal, bacterial, and protozoal microorganisms tested at the concentrations indicated in **Table 6**, except for three cross-reacting protozoa—all of which were predicted from the *in silico* alignments with their congeneric protozoa in Panels of the ***EasyScreen*™ Gastrointestinal Parasite Detection Kit**:

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 6. 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
Adenovirus Type 5; Adenoid 75	ATCC VR-5D
Microbiological Media	
Cooked Meat Media	ATCC 724
Diamond vitamin Solution	ATCC MD-2692

Organism; strain	ID details
Keister's Modified TYI-S-33 Giardia Medium	ATCC PRA-2695
Modified Reinforced Clostridial Medium	ATCC 2107
Soybean-Casein Digest Medium	ATCC 18
Supplemented Tryptic Soy Broth	ATCC 2722
Tryptic Soy Medium with 5% Defibrinated Sheep Blood	ATCC 260
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
Listeria monocytogenes	ATCC 19115D-5
Mycobacterium abscessus	ATCC 19977D-5

Organism; strain	ID details
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 assay 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 are summarized in **Table 7** below. In silico analysis identified several targets for further investigation as potential cross-reacting organisms. However, only cases with three or more reported human infections world-wide were further analyzed and investigated as synthetic RNA targets as described below.

Table 7. **EasyScreen™** ***Gastrointestinal Parasite Detection Kit*** **in silico** ***analysis summary.***

Organism / representative GenBank accession	**Cognate EP005 target**	**Evidence for human infection?**
In silico **predicted cross reactive potential: High**		
B. cycluri AY590116	*Blastocystis hominis*	No
B. lapemi AY266471		No
B. pythoni AY266472		No
B. ratti AY590114		No
C. cercopitheci AF111185	*Cyclospora cayetanensis*	No
C. colobi AF111186		No
C. papionis AF111187		No
C. bovis EF514234	*Cryptosporidium* spp.	No
C. canis AB210854		**YES**
C. felis AF112575		**YES**
C. meleagridis EF179381		**YES**
C. tyzzeri OQ826430		**YES**
C. wrairi U11440		No #
Ecytonucleospora hepatopenaei OR168078	*Enterocytozoon bieneusi*	No
Enterospora nucleophila KF135641		No
Obruspora papernae HG005137		No
E. nuttalli LC042219	*Entamoeba histolytica*	No #
G. microti AF006676	*Giardia intestinalis*	No
In silico **predicted cross reactive potential: Moderate**		
Eimeria hermani KJ000078	*Cyclospora cayetanensis*	No
C. baileyi KT151546	*Cryptosporidium* spp.	No #
C. muris L19069		**YES**
Histomonas meleagridis AJ920323	*Dientamoeba fragilis*	No
Pseudotrichomonas keilini HM581663		No
Trichomitus sp. 1 (ex Geochelone sulcata) JX515400		No
Nucleospora salmonis HQ418210	*Enterocytozoon bieneusi*	No
E. lacerate AF067144	*Encephalitozoon intestinalis*	No
E. pogonae KR998311		No

Organism / representative GenBank accession	Cognate EP005 target	Evidence for human infection?
Chilomastix mesnili KC960586	*Giardia intestinalis*	**YES**
G. ardeae Z17210		No
E. dispar KP722600	*Entamoeba histolytica*	**YES §**
***In silico* predicted cross reactive potential: Low**		
Trochochilodon flavus JN867018	*Blastocystis hominis*	No
Isospora belli TDQ060661	*Cyclospora cayetanensis*	**YES**
Colpodella tetrahymenae AF330214	*Cryptosporidium* spp.	No
C. andersoni AB513869		**YES**
C. fragile EU162754		No
C. serpentis AF093499		No
C. struthionis AJ697751		No
E. bangladeshi KR025412	*Entamoeba histolytica*	**YES**
E. ecuadoriensis DQ286373		**YES**
E. moshkovskii MN536500		**YES §**
Giardia muris X65063	*Giardia. intestinalis*	No

^ Wet-testing of *E. dispar* whole organism was negative at low copy number (Table 6), but it was selected for wet-testing with synthetic targets so that a high copy number (10^9 copies/mL) could be tested.
§ *E. dispar* and *E. moshkovskii* infect humans but are non-pathogenic.
# Only one or two cases of human infection reported worldwide.

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

Table 7 lists six potentially cross-reacting organisms (predicted cross-reactivity with the ***EasyScreen*™ Gastrointestinal Parasite Detection Kit**: High or Moderate) that are also clinically relevant ("YES" to 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 obtained as a 'gBlock' from IDT using the Accession numbers in **Table 7** and whole genome sequence where available. These sequences were used to make synthetic in vitro transcribed (IVT) RNA (800-1000 nucleotides in length). After quantitation, 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 8. 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 8. Wet testing of synthetic IVT RNA targets (P = positive; N = negative):

Organism	EP005 target to which similarity exists	gBlock Lot number	Copies/ mL	Panel		
				A	B	C
Cryptosporidium meleagridis	*Cryptosporidium* spp.	109271385	10^8	P	N	N
Cryptosporidium tyzzeri	*Cryptosporidium* spp.	109271386	10^8	P	N	N
Cryptosporidium canis	*Cryptosporidium* spp.	109271381	8×10^8	P	N	N
Cryptosporidium felis	*Cryptosporidium* spp.	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

Therefore, wet testing of whole organisms or whole genomes or of synthetic RNA informed by *in silico* analysis identified the following seven human pathogenic protozoa that are congeneric to two 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*. According to literature, these cross-reacting organisms occur primarily in animals, are rare in humans, and do not show any difference in disease severity compared to the more commonly encountered pathogenic species.

(d) Analytical Reactivity

Analytical reactivity or inclusivity of the *EasyScreen*™ Gastrointestinal Parasite Detection Kit was investigated by testing eighty-two isolates representing the eight target parasites (with 4–16 isolates per target, as available) (as shown in **Table 9**). 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, target 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 9. 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

Target organism	Strain/ Designation	Product ID / Clinical ID	Geographic Origin
	IIaA	Clinical#234	Sydney, AUS
	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	unknown
	NTY (ST- 1)	ATCC 50610	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
	Genotype D	Clinical #10	Sydney, AUS
	Genotype D	Clinical #11	Sydney, AUS
	Genotype K	Clinical #12	Sydney, AUS
	Alveolar isolate	ATCC 50506	NY, US

Target organism	Strain/ Designation	Product ID / Clinical ID	Geographic Origin
Encephalitozoon intestinalis	CDC: V297	ATCC 50651	CA, US
	CDC: V307	ATCC 50603	Georgia, US
	Nasal isolate	ATCC 50507	NY, US

(e) Interfering substances

Twenty-three 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 target analytes—one 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 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 10**), two 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 10. 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			

Potential Interferent / Active agent (use)	Interferent conc.	*D. fragilis* (panel A)	*G. intestinalis* (panel B)	*E. bieneusi* (panel C)
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 interference	No interference	**I**
	1.5 mg/mL	Not tested	Not tested	**I**
	0.75 mg/mL	Not tested	Not tested	No interference
Whole Blood	5 % v/v	**I**	No interference	**I**
	2.50%	**I**	Not tested	**I**
	1.25%	**I**	Not tested	No interference
	0.63%	No interference	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.

(f) 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 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 for the ***EasyScreen*™ Gastrointestinal Parasite Detection Kit**, 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**.

(g) Competitive Inhibition

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 11**. 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. Upon reducing the competitor concentrations to 5 x 10^4 org/mL and 10^4 org/mL, respectively, competitive interference was no longer observed. 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 11** below.

Table 11. 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	

Panel	Target analyte at 2X LoD	Competitor analyte	Competitor concentration (org/mL, *except *C. cayetanensis*, copies/mL)	Competitive Interference observed
	E. intestinalis	*E. bieneusi*	10^5	No reportable interference

§ Tests repeated at a lower concentration of competing targets, which showed no reportable interference.

(h) Cross-Contamination (Carry Over)

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 replicates of pooled high positive sample and fourteen 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 extractions and PCR setups were performed with each run containing one negative processing control and thirty 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.

(i) Specimen Stability

To provide evidence in support of stability of specimens to be tested with the ***EasyScreen*™ Gastrointestinal Parasite Detection Kit**, a range of transport and 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 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 2–8°C for at least three 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 weeks in unpreserved negative stool matrix. For analytes in Cary Blair matrix, except *C. cayetanensis* and E. *histolytica*, all targets are stable for three

weeks, whereas *C. cayetanensis* and E. *histolytica* are stable for two 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 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 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 support a frozen storage stability claim of three weeks for all targets other than *B. hominis* (frozen storage stability of two weeks for *B. hominis* is acceptable) when tested with the ***EasyScreen*™ Gastrointestinal Parasite Detection Kit**.

(j) Reagent Stability/Shelf-Life

With the use of Panel A–C target analytes diluted to 2X LoD in an unpreserved negative stool matrix, a reagent stability (shelf life) study was conducted to evaluate the shelf life of the reagents in the ***EasyScreen*™ Gastrointestinal Parasite Detection Kit** workflow—i.e., the EasyScreen™ Sample Processing Kit (SP008B) and the PCR amplification reagents and controls (EP005)—following the recommendations in FDA-recognized Consensus Standard CLSI EP25-A and other guidelines.

Since there is no direct data output available for intermediate reagents in the Kit SP008B, stability of SP008B was assessed by using the ***EasyScreen*™ Gastrointestinal Parasite Detection Kit** in the stability studies. Three batches of the SP008B kit were stored at the standard storage temperature for the product (i.e., 15–25ºC) for 38–48 months and then used with one lot of EP005 for testing the contrived samples. Conversely, four batches of the EP005 kit were stored at the standard storage temperature for the product (i.e., frozen at -25ºC to -15ºC) for 19, 25, 30, and 32 months, and then used to test the contrived samples.

All samples (individual whole organisms at 2X LoD concentration) were extracted in sets of five extraction replicates using the GS1 instrument according to the *EasyScreen*™ Sample Processing Kit SP008B User Guide and real-time PCR was performed on the QSDX analyzer according to the ***EasyScreen*™ Gastrointestinal Parasite Detection Kit** User Guide. For both sets of studies, the acceptance criteria were set at 100% positivity of all targets in the EP005 assay at 2X LoD for all replicates at the timepoint(s) tested.

For all the batches of SP008B kit (aged 38–48 months) tested, all EP005 target analytes were detected at 5/5 replicates (i.e., 100% detection) at 2X LoD, thereby meeting the performance criteria. Similarly, for all the batches of EP005 reagents (aged 19–32 months), all analytical targets

at 2X LoD were detected at 5/5 replicates (i.e., 100% detection). This study provided confirmation for a stable shelf-life of 24 months for the ***EasyScreen*™ Sample Processing Kit SP008B** when stored at room temperature (15–25°C) and for the ***EasyScreen*™ Gastrointestinal Parasite Detection Kit** when stored frozen (-25ºC to -15ºC).

Additionally, a similar study was conducted to verify the in-use (freeze thaw) stability of reagents of the ***EasyScreen*™ Gastrointestinal Parasite Detection Kit** i.e., the PCR mastermix and PCR components—when these reagents are mixed and pooled on first thaw (the reaction mix) and then stored at -25°C to -15°C with up to five freeze-thaw cycles permitted per tube to simulate in-use conditions. Two separate tubes of mixed EP005 reagents that underwent five freeze-thaw cycles over days were used as before in testing Panel A–C target analytes at 2X LoD. As with the other stability studies, the acceptance criteria were set at 100% of analytical targets (5/5 extraction replicates) testing positive at a concentration of 2X LoD.

At the 5th freeze-thaw, all PCR replicates at 2X LoD achieved 5/5 positivity (i.e., 100% detection for all analytical targets), thus meeting the performance criteria. This study provided confirmation for the in-use (freeze thaw) stability of the ***EasyScreen*™ Gastrointestinal Parasite Detection Kit** reagents for up to four (4) freeze thaws.

(k) C&S Matrix Equivalency

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 extraction replicates of High Positives, twenty-five replicates of Low Positives, and ten 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
	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

Target analyte	Tested analyte level in C&S matrix relative to LoD in Cary-Blair matrix	Detection (%)	Avg Ct
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

3.2 Overview of Clinical Performance Studies

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 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 tested with the candidate ***EasyScreen*™ Gastrointestinal Parasite Detection Kit** (hereafter referred to as "EP005") device, and the 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 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 were sequenced with bi-directional Sanger sequencing at the OUS 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 those of the predicate device where the predicate was available for use following its device labeling. In this comparison, only those three (3) protozoan targets that are detected by the comparator were evaluated —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 as it is not validated for use with those specimen types.

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 14–21**, 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.

Based on evaluation of clinical study data provided from 1,891 stool specimens, the performance of the ***EasyScreen*™ Gastrointestinal Pathogen Detection Kit (EP005)** is acceptable with PPA for target parasites ranging between 91–98% with lower limit of 95% CI at ≥80% and NPA ≥99% when compared to the reference method for combined groups.

B. Performance Estimates of EP005 relative to the Predicate:

This section summarizes the target analyte-specific comparison of ***EasyScreen*™ Gastrointestinal Parasite Detection Kit** results to those obtained from the predicate for 760 samples with valid results on both tests. This performance metrics analysis 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 = 64) Category I–IV samples detected by the ***EasyScreen*™ Gastrointestinal Parasite Detection Kit** 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
Cryptosporidium spp., *E. bieneusi*	1
Cryptosporidium spp., *D. fragilis*	1
D. fragilis, G. intestinalis	2 [d]
E. bieneusi, G. intestinalis	1

Co-Infections	N [a]
Totals	64

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.