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

I Background Information:

A 510(k) Number

K251721

B Applicant

Cepheid

C Proprietary and Established Names

Xpert GI Panel

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PCH	Class II	21 CFR 866.3990 - Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assay	Microbiology
OOI	Class II	21 CFR 862.2570 – Instrumentation for Clinical Multiplex Test Systems	CH- Chemistry

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for the Xpert GI Panel performed on GeneXpert Instrument systems.

B Measurand:

Targeted nucleic acid sequences of the following bacteria, parasites, and viruses:

Bacteria:
Campylobacter (*C. jejuni/C. coli*)
Shiga toxin-producing *Escherichia coli* (STEC) *stx1/stx2*
Salmonella spp.
Shigella/Enteroinvasive *Escherichia coli* (shigella EIEC)
Yersinia enterocolitica
Vibrio parahaemolyticus
Vibrio cholerae

Parasites:
Giardia (also known as *G. intestinalis*, *G. duodenalis* & *G. lamblia*)
Cryptosporidium

Virus:
Norovirus GI/GII

C Type of Test:

The Xpert GI panel is performed on the Cepheid GeneXpert Instrument systems equipped with 10-color modules and utilizes automated, qualitative real time polymerase chain reaction (PCR). The Xpert GI Panel simultaneously detects and identifies DNA and RNA from multiple bacteria or parasites as well as Norovirus using reverse transcription real-time polymerase chain reaction (PCR) with a nested PCR set-up. The first PCR involves qualitative reverse transcription followed by PCR, and the second PCR is a real-time PCR. The Xpert GI Panel test results are interpreted by GeneXpert Instrument Systems from measured fluorescent signals and embedded calculation algorithms and will be reported as positive or negative for each of the analytes.

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Xpert GI Panel, performed on the GeneXpert Instrument Systems, is a qualitative multiplexed *in vitro* diagnostic test that is capable of the simultaneous detection and identification of DNA and RNA from multiple bacteria, parasites and/or virus directly from stool samples in Cary Blair transport media obtained from individuals with signs and symptoms of gastrointestinal infection. The test utilizes automated, qualitative real time polymerase chain reaction (PCR). The following bacteria (including several diarrheagenic *E. coli/Shigella* pathotypes), parasites, and virus are identified using the Xpert GI Panel:

	Pathogens Detected	Pathogens Reported
Bacteria	*Campylobacter (C. jejuni/C. coli)*	*Campylobacter*
	Shiga toxin-producing *Escherichia coli* (STEC) *stx1/stx2*	STEC stx1
		STEC stx2
	Salmonella	*Salmonella*
	Shigella/Enteroinvasive *Escherichia coli*	*Shigella EIEC*
	Yersinia enterocolitica	*Yersinia*
	Vibrio parahaemolyticus	*V. parahaemolyticus*
	Vibrio cholerae	*V. cholerae*
Parasites	*Giardia* (also known as *G. intestinalis, G. duodenalis & G. lamblia)*	*Giardia*
	Cryptosporidium	*Cryptosporidium*
Virus	Norovirus GI/GII	Norovirus

Results are meant to be used in conjunction with other clinical, laboratory, and epidemiological data and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results do not rule out co-infection with pathogens not included in the Xpert GI Panel. The pathogen detected may not be the definite cause of the disease. Negative results in the setting of clinical illness compatible with gastroenteritis 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

D Special Instrument Requirements:

The Xpert GI panel is performed on Cepheid GeneXpert Instrument systems equipped with GeneXpert 10 color modules:

- GeneXpert Dx system (software version Dx 6.4 or higher)
- GeneXpert Infinity system (software version Xpertise 7.1 or higher)
- GeneXpert with Touchscreen (software version OS 2.1 or higher)

IV Device/System Characteristics:

A Device Description:

The Xpert Gastrointestinal (GI) Panel test, performed on Cepheid GeneXpert Instrument Systems equipped with 10-color modules, is an automated *in vitro* diagnostic test for qualitative detection and differentiation of eleven (11) pathogens in stool in Cary Blair specimens collected from individuals suspected of gastrointestinal infection. The results from the Xpert GI Panel test will be available in ≤75 mins.

B Principle of Operation:

The Xpert GI Panel is performed on the Cepheid GeneXpert Instrument Systems equipped with GeneXpert 10-color modules running software version 6.4 and higher (GeneXpert Dx), software version Xpertise 7.1 or higher (GeneXpert Infinity) or software version OS 2.1 or higher (GeneXpert with Touchscreen). The GeneXpert Instrument Systems automate and integrate sample purification, nucleic acid amplification, and detection of the target sequences from clinical specimens using reverse transcription (conversion of RNA template into DNA) followed by real-time polymerase chain reaction (real-time PCR) and melt curve analysis. The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. Each test requires the use of a single-use disposable GeneXpert cartridge that contains target-specific reagents and carries out the reverse transcription and PCR processes.

The Xpert GI Panel test includes reagents needed: (i) for sample preparation and (ii) to detect the different bacteria, parasites and virus. A Sample Processing Control (SPC), an Internal Control (IC) and a Probe Check Control (PCC) are also included in the cartridge. The PCC verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability. The IC is present to ensure adequate processing of RNA targets and monitor the presence of inhibitor(s) in the PCR reaction. The SPC is present to control for adequate extraction and processing of the target sequences in the PCR reaction. The SPC also acts as a control for functionality of melt curve analysis.

The Xpert GI Panel test results are interpreted by GeneXpert Instrument Systems from measured fluorescent signals and embedded calculation algorithms and will be reported as positive or negative for each of the targets.

The Xpert GI Panel test is designed for use with stool specimens collected and transferred to Cary Blair medium according to the Cary Blair manufacturer's instructions or institutional guidelines.

The stool sample in Cary Blair medium are mixed thoroughly. The user will then dip a transfer swab (provided) for 5 seconds into the sample and then transfer the inoculated swab into the sample chamber of the Xpert GI Panel cartridge. The user will break the swab by snapping the shaft against the notch in the sample chamber opening leaving the swab tip in the sample chamber and closing the cartridge lid. The Xpert GI Panel cartridge is then loaded onto the GeneXpert instrument, which performs hands-off automated sample processing and real-time PCR for detection of the target DNA/RNA. After the melt curve analysis, software automatically calculates the melt peak temperature and melt peak height of the pathogens detected using melt curve analysis.

C Instrument Description:

1. Instrument Name:

The Xpert GI Panel test is run on the GeneXpert Instrument Systems family which comprises GeneXpert Dx system, GeneXpert System with Touchscreen, and GeneXpert Infinity System. The most recent 510(k) product clearance for the GeneXpert Dx System with GeneXpert Dx software version 6.5 and the GeneXpert System with Touchscreen with Cepheid OS software version 2.1 was K250218. The last 510(k) product clearance for the GeneXpert Infinity System with GeneXpert Infinity Xpertise software version 7.1 was Xpert C. Difficile/EPI (K243730).

2. Specimen Identification:

Stool in Cary Blair Transport media collected from patients with suspected gastrointestinal infection.

3. Specimen Sampling and Handling:

The stool or rectal swab sample in Cary Blair medium will be mixed thoroughly. The user will then dip a transfer swab (provided) for 5 seconds into the sample and transfer the inoculated swab into the sample chamber of the Xpert GI Panel cartridge. The user will break the swab by snapping the shaft against the notch in the sample chamber opening leaving the swab tip in the sample chamber and closing the cartridge lid. The Xpert GI Panel cartridge is then loaded onto the GeneXpert instrument, which performs hands-off automated sample processing and real-time PCR for detection of the target DNA/RNA.

4. Calibration:

Please refer to K243730 Decision Summary for GeneXpert Infinity Systems Calibration information and K250218 Decision Summary for GeneXpert Dx System and GeneXpert System with Touchscreen calibration information.

5. Quality Control:

Each test includes a Sample Processing Control (SPC), an Internal Control (IC) and a Probe Check Control (PCC).

- **Sample Processing Control (SPC)—** The SPC is spores of *Bacillus globigii* included in each cartridge to ensure that the sample was processed correctly. The SPC verifies the lysis of hard to lyse pathogens (parasites and bacteria) if the pathogens are present and that sample processing is adequate. Additionally, this control ensures that the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that the PCR reagents are functional. The SPC also acts as a control for functionality of melt curve analysis. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria. The test result is INVALID if all targets are reported negative and the SPC does not meet the assigned acceptance criteria.
- **Internal Control (IC)** — The IC is an Armored RNA that is included in each cartridge to verify adequate processing of the sample. The IC verifies release of RNA from the sample and that the sample processing is adequate. Additionally, this control detects specimen-associated inhibition of the reverse transcription and PCR reactions. The IC should be positive in a negative sample and can be negative or positive in a positive sample. The IC passes if it meets the validated acceptance criteria. The test result is INVALID if all targets are reported negative and the IC does not meet the assigned acceptance criteria.
- **Probe Check Control (PCC)**— Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the assigned acceptance criteria.

- **External Controls**—Following good laboratory practice, external controls, not provided in the kit, should be used in accordance with the requirements of local and state accrediting organizations, as applicable.

V Substantial Equivalence Information:

A Predicate Device Name(s):

FilmArray Gastrointestinal (GI) Panel

B Predicate 510(k) Number(s):

K140407

C Comparison with Predicate(s):

Device & Predicate Device:	K251721	K140407
Device Trade Name	Xpert GI Panel	FilmArray Gastrointestinal (GI) Panel
General Device Characteristic Similarities		
Regulation	21 CFR 866.3990	Same
Product Code	PCH, OOI	Same
Intended Use/Indications For Use	The Xpert GI Panel, performed on the GeneXpert Instrument Systems, is a qualitative multiplexed in vitro diagnostic test that is capable of the simultaneous detection and identification of DNA and RNA from multiple bacteria, parasites and/or virus directly from stool samples in Cary Blair transport media obtained from individuals with signs and symptoms of gastrointestinal infection. The test utilizes automated, qualitative real time polymerase chain reaction (PCR). The following bacteria (including several	The FilmArray Gastrointestinal (GI) Panel is a qualitative multiplexed nucleic acid-based in vitro diagnostic test intended for use with the FilmArray Instrument. The FilmArray GI Panel is capable of the simultaneous detection and identification of nucleic acids from multiple bacteria, viruses, and parasites directly from stool samples in Cary Blair transport media obtained from individuals with signs and/or symptoms of gastrointestinal infection. The FilmArray GI Panel is indicated as an aid in the diagnosis of specific

Device & Predicate Device:	K251721	K140407
	diarrheagenic *E. coli/Shigella* pathotypes), parasites, and virus are identified using the Xpert GI Panel: Pathogens Detected: Bacteria: *Campylobacter* (*C. jejuni/C. coli*), Shiga toxin-producing *Escherichia coli* (STEC) *stx1/stx2*, *Salmonella*, Shigella/Enteroinvasive *Escherichia coli*, *Yersinia enterocolitica*, *Vibrio parahaemolyticus*, *Vibrio cholerae* Parasites: *Giardia* (also known as *G. intestinalis, G. duodenalis & G. lamblia*), *Cryptosporidium* Virus: Norovirus GI/GII Pathogens Reported: Bacteria: *Campylobacter*, STEC stx1, STEC stx2, *Salmonella, Shigella EIEC, Yersinia, V. parahaemolyticus, V. cholerae* Parasites: *Giardia, Cryptosporidium* Virus: Norovirus	agents of gastrointestinal illness and results are meant to be used in conjunction with other clinical, laboratory, and epidemiological data. The following bacteria (including several diarrheagenic E-coli/Shigella pathotypes), parasites, and viruses are identified using the FilmArray GI Panel: * Campylobacter (*C. jejuni/C. coli/C. upsaliensis*) * *Clostridium difficile* (*C. difficile*) toxin A/B * *Plesiomonas shigelloides* * *Salmonella* * Vibrio (*V. parahaemolyticus/V. vulnificus/V. cholerae*) including specific identification of *Vibrio cholerae* * *Yersinia enterocolitica* * Enteroaggregative *Escherichia coli* (EAEC) * Enteropathogenic *Escherichia coli* (EPEC) * Enterotoxigenic *Escherichia coli* (ETEC) *lt/st* * Shiga-like toxin-producing *Escherichia coli* (STEC) *stx1/stx2* (including specific identification of the *E. coli O157* serogroup within STEC)

Device & Predicate Device:	K251721	K140407
	Results are meant to be used in conjunction with other clinical, laboratory and epidemiological data and should not be used as the sole basis for diagnosis, treatment or other patient management decisions. Positive results do not rule out co-infection with pathogens not included in the Xpert GI Panel. The pathogen detected may not be the definite cause of the disease. Negative results in the setting of clinical illness compatible with gastroenteritis 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.	* Shigella/Enteroinvasive *Escherichia coli* (FIEC) * *Cryptosporidium* * *Cyclospora cayetanensis* * *Entamoeba histolytica* * *Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*) * Adenovirus F 40/41 * Astrovirus * Norovirus GI/GlI * Rotavirus A * Sapovirus (Genogroups I, II, IV, and V) Pathogens reported: *Campylobacter* *E. coli 0157* *Plesiomonas shigelloides* *Salmonella* *Vibrio* and *V. cholerae* *Yersinia enterocolitica* STEC (*stx1/2*) ETEC EPEC EIEC/*Shigella* EAEC Adenovirus F 40/41 Astrovirus Norovirus GI/GII Rotavirus A Sapovirus *Clostridium difficile* toxin A/B *Cryptosporidium* *Giardia lamblia* *Cyclospora cayetanensis* *Entamoeba histolytica* Positive results do not rule out co-infection with organisms not included in the FilmArray GI Panel.

Device & Predicate Device:	K251721	K140407
		The agent detected may not be the definite cause of the disease.
Specimen Type	Stool samples in Cary Blair transport media	Same
Test Format	Single Use	Same
Assay Results	Qualitative	Same
Time to Result	≤75 mins	≤75 mins
General Device Characteristic Differences		
Organisms Detected	*Campylobacter (C. jejuni/C. coli),* Shiga toxin-producing *Escherichia coli* (STEC) *stx1 and* STEC *stx2 (separate callouts), Salmonella, Shigella*/Enteroinvasive *Escherichia coli, Yersinia enterocolitica, Vibrio parahaemolyticus, Vibrio cholerae, Giardia*, *Cryptosporidium,* Norovirus GI/GII	Campylobacter species: *C. jejuni/C. coil/C. upsaliensis*. *Clostridioides* (Clostridium) *difficile* (toxin A/B) Salmonella *Yersinia enterocolitica Cryptosporidium Giardia lamblia Enterotoxigenic E. coli (ETEC) lt/st Shiga-like toxin-producing E. coli (STEC) stx1/stx2, E. coli O157* callout *Shigella/Enteroinvasive E. coli (EIEC) Norovirus Plesiomonas shigelloides*. Vibrio (*V. parahaemolyticus/V. vulnificus/V. cholerae*), *V. cholerae callout Yersinia enterocolitica* Adenovirus F40/41, Rotavirus A Astrovirus, Sapovirus (Genogroups I, II, IV, and V), *Cyclospora cayetanensis*, *Entamoeba histolytica*, Enteropathogenic *E. coli* (EPEC), and Enteroaggregative *E.*

Device & Predicate Device:	K251721	K140407
		coli (EAEC).
Technology/Detection	Nested multiplex real-time reverse transcription polymerase chain reaction (RT-qPCR) with detection by amplification and melt curve analysis.	Nested multiplex RT-PCR followed by high resolution melting analysis to confirm identity of amplified product
Internal Control	Sample Processing Control (SPC) Internal Control (IC) Probe Check Control (PCC)	Two controls (RNA Process Control and PCR2 Control) are included in each reagent pouch to control for sample processing and both stages of PCR and melt analysis.
Instrument Systems	Cepheid GeneXpert Instrument Systems	FilmArray Instrument
Software	GeneXpert Dx 6.4 or higher GeneXpert Infinity Xpertise 7.1 or higher Cepheid OS 2.1 or higher	BioFire FilmArray Software

VI Standards/Guidance Documents Referenced:

Guidance:

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

Standards:

Organization	Title	Type of Use
ASI AAMI ISO	14971:2019 Medical Devices – Applications of risk management to medical devices	Declaration of Conformity
CLSI	EP12-A2 – User Protocol for Evaluation of Qualitative test performance; Approved Guideline – Second Edition	General Use

Organization	Title	Type of Use
CLSI	EP14-A3 Evaluation of Commutability of Processed Samples; Approved Guideline -Third Edition	General Use
CLSI	EP15-A3- User verification of Precision and Estimation of Bias; Approved Guideline – Third Edition	General Use
CLSI	EP17-A2 – Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, Approved Guideline – Second Edition	General Use
CLSI	EP24-A2Assessment of Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves; Approved Guideline	General Use
CLSI	EP25-A – Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline	General Use
CLSI	EP05-A3 – Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition	General Use
CLSI	EP07 3rd edition – Interference Testing in clinical Chemistry	General Use
CLSI	EP09c 3rd Edition – Measurement Procedure Comparison and Bias Estimation Using Patient Samples	General Use
CLSI	MM13 2nd Edition – Collection Transport Preparation and Storage of Specimens for Molecular Methods	General Use
CLSI	LIS08-A – Standard Guide for Functional Requirements of Clinical Laboratory Information Management Systems	General Use
IEC	61326-1 Edition 3.0 2020-10 – Electrical equipment for measurement control and laboratory use – EMC requirements – Part 1: General Requirements	General Use
IEC	61326-2-6 Edition 3.0 2020-10 – Electrical Equipment for Measurement control and laboratory use – EMC requirements – Part 2-6: Particular Requirements – In vitro diagnostic (IVD) medical equipment	General Use
ANSI AAMI IEC	62304:2006/A1:2016 – Medical Devices software – Software life cycle processes	Declaration of conformity
ANSI AAMI IEC	60601-1-2:2014 – Medical electrical equipment – Part 1-2: General	General Use

Organization	Title	Type of Use
	requirements for basic safety and essential performance	

VII Performance Characteristics:

A Analytical Performance:

1. Precision/Reproducibility:

The reproducibility and precision of the Xpert GI Panel was evaluated in a multicenter blinded study across three sites utilizing a multi-factor nested design consisting of three contrived panels composed of low positive (~1x LoD), moderate positive (~3x LoD), and negative samples. The negative samples were pooled negative clinical stool matrix.

The positive samples were contrived by diluting target pathogen into pooled negative clinical stool matrix across three panels. Testing was conducted to assess reproducibility over days, lots of Xpert GI Panel cartridges, sites, and operators at each site. The percent agreement of the correct results compared to the expected results analyzed by each of the operators across site is shown in Table 1. Additionally, the combined agreement across sites for each sample (% total agreement) and the two-sided Wilson Score confidence intervals (CI) are presented in the last column.

Low and moderate *V. parahaemolyticus* positives were tested fresh within the 4-day window of specimen stability (2 - 8°C) as part of Panel 3. Panel 3 consisted of low and moderate positive V. parahaemolyticus and negative panel members. The results from the study are summarized in Table 1 below. There were no statistically significant differences in the Xpert GI Panel performance between study sites, lots and operators.

Table 1: Summary of Reproducibility Study Results - % Agreement

Panel	Sample	Site 1			Site 2			Site 3			% Total Agreement [95% CI]
		OP 1	OP2	Site	OP 1	OP 2	Site	OP 1	OP 2	Site	
Panel 1	*Campylobacter* Low Pos	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (144/144) [97.4-100.0]
		24/24	24/24	48/48	24/24	24/24	48/48	24/24	24/24	48/48	
Panel 1	*Campylobacter* Mod Pos	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (144/144) [97.4-100.0]
		24/24	24/24	48/48	24/24	24/24	48/48	24/24	24/24	48/48	
Panel 1	STEC *stx2* Low Pos	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (144/144) [97.4-100.0]
		24/24	24/24	48/48	24/24	24/24	48/48	24/24	24/24	48/48	
Panel 1	STEC *stx2* Mod Pos	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (144/144) [97.4-100.0]
		24/24	24/24	48/48	24/24	24/24	48/48	24/24	24/24	48/48	
Panel 1	Negative	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (144/144) [97.4-100.0]
		24/24	24/24	48/48	24/24	24/24	48/48	24/24	24/24	48/48	
Panel 2	*Salmonella* Low Pos	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (144/144) [97.4-100.0]
		24/24	24/24	48/48	24/24	24/24	48/48	24/24	24/24	48/48	
Panel 2	*Salmonella* Mod Pos	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (143/143) [97.4-100.0]
		24/24	24/24	48/48	23/23[a]	24/24	47/47	24/24	24/24	48/48	
Panel 2	*Yersinia* Low Pos	100%	100%	100%	100%	96%	98%	100%	100%	100%	99.3% (143/144) [96.2-99.9]
		24/24	24/24	48/48	24/24	23/24	47/48	24/24	24/24	48/48	
Panel 2	*Yersinia* Mod Pos	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (143/143) [97.4-100.0]
		24/24	24/24	48/48	23/23[a]	24/24	47/47	24/24	24/24	48/48	
Panel 2	*Cryptosporidium* Low Pos	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (144/144) [97.4-100.0]
		24/24	24/24	48/48	24/24	24/24	48/48	24/24	24/24	48/48	
Panel 2	*Cryptosporidium* Mod Pos	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (143/143) [97.4-100.0]
		24/24	24/24	48/48	23/23[a]	24/24	47/47	24/24	24/24	48/48	
Panel 2	*Giardia* Low Pos	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (144/144) [97.4-100.0]
		24/24	24/24	48/48	24/24	24/24	48/48	24/24	24/24	48/48	
Panel 2	*Giardia* Mod Pos	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (144/144) [97.4-100.0]
		24/24	24/24	48/48	24/24	24/24	48/48	24/24	24/24	48/48	
Panel 2	*Shigella* EIEC Low Pos	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (144/144) [97.4-100.0]
		24/24	24/24	48/48	24/24	24/24	48/48	24/24	24/24	48/48	
Panel 2	*Shigella* EIEC Mod Pos	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (144/144) [97.4-100.0]
		24/24	24/24	48/48	24/24	24/24	48/48	24/24	24/24	48/48	
Panel 2	Norovirus Low Pos	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (144/144) [97.4-100.0]
		24/24	24/24	48/48	24/24	24/24	48/48	24/24	24/24	48/48	
Panel 2	Norovirus Mod Pos	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (144/144) [97.4-100.0]
		24/24	24/24	48/48	24/24	24/24	48/48	24/24	24/24	48/48	
Panel 2	Negative	100%	96%	98%	100%	96%	98%	100%	100%	100%	98.6% (142/144) [95.1-99.6]
		24/24	23/24	47/48	24/24	23/24	47/48	24/24	24/24	48/48	
Panel 3	*V. parahaemolyticus* Low Pos	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (108/108) [96.6-100.0]
		18/18	18/18	36/36	18/18	18/18	36/36	18/18	18/18	36/36	
Panel 3	*V. parahaemolyticus* Mod Pos	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (108/108) [96.6-100.0]
		18/18	18/18	36/36	18/18[b]	18/18	36/36	18/18	18/18	36/36	
Panel 3	Negative	100%	100%	100%	100%	100%	100%	100%	100%	100%	100% (108/108) [96.6-100.0]
		18/18	18/18	36/36	18/18	18/18	36/36	18/18	18/18	36/36	

[a] One sample was non-determinate on both initial and retest and was excluded from the analyses.
[b] One moderate positive sample tested positive for *V. parahaemolyticus* and *V. cholerae*. This sample was considered concordant for *V. parahaemolyticus*.

The evaluation of reproducibility and within-laboratory precision of the underlying analyte response (Ct, melt peak (MP), or melt valley (MV) values) for the Xpert GI Panel was analyzed using nested Analysis of Variance (ANOVA). The mean response (Ct, MP or MV), standard deviation (SD), and coefficient of variation (CV) between-sites, between-operators, between-lots, between-days, between-runs and within-run for each panel member are presented in Table 2.

Table 2: Summary of Nested ANOVA by Coefficient of Variation

Sample	Response	N	Mean Ct, MP, or MV	Variance Source													
				Site		Operator		Lot		Day		Run		Within-Run		Total	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Campylobacter Low Pos	MP	144	15.6	0.6	3.6	0.0	0.0	1.2	7.6	0.7	4.4	0.0	0.0	1.9	12.2	2.4	15.4
	MV	144	-13	0.4	3.3	0.0	0.0	0.9	7.2	0.6	4.3	0.0	0.0	1.5	11.6	1.9	14.7
Campylobacter Mod Pos	MP	144	16.4	0.6	3.5	0.0	0.0	0.6	3.8	0.6	3.7	0.0	0.0	1.6	9.9	1.9	11.8
	MV	144	-13.7	0.6	4.3	0.0	0.0	0.5	3.8	0.5	3.5	0.0	0.0	1.3	9.5	1.6	11.6
STEC *stx2* Low Pos	MP	144	12.9	0.9	6.6	0.0	0.0	0.7	5.7	0.0	0.0	0.1	0.9	1.7	13.0	2.0	15.7
	MV	144	-12.1	0.7	6.0	0.0	0.0	0.7	5.7	0.3	2.2	0.0	0.0	1.7	13.9	2.0	16.3
STEC *stx2* Mod Pos	MP	144	14.1	1.1	8.0	0.0	0.0	0.5	3.2	0.8	5.7	0.4	3.2	1.8	12.7	2.3	16.7
	MV	144	-13.4	1.0	7.4	0.0	0.0	0.4	2.9	0.7	5.3	0.5	3.9	1.8	13.5	2.3	17.0
Salmonella Low Pos	Ct	144	34.6	0.4	1.0	0.2	0.4	0.0	0.0	0.3	0.8	0.0	0.0	0.7	1.9	0.8	2.3
Salmonella Mod Pos	Ct	143[a]	33.7	0.2	0.7	0.2	0.5	0.3	0.8	0.0	0.0	0.2	0.7	0.6	1.8	0.8	2.2
Yersinia Low Pos	MP	143[b]	14.2	0.4	2.8	0.0	0.0	0.0	0.0	0.0	0.0	0.7	5.0	1.4	10.0	1.6	11.5
	MV	143	-11.7	0.4	3.6	0.0	0.0	0.0	0.0	0.0	0.0	0.6	4.8	1.2	9.9	1.4	11.6
Yersinia Mod Pos	MP	143[a]	14.7	0.6	3.7	0.0	0.0	0.5	3.2	0.0	0.0	0.0	0.0	1.8	12.0	1.9	12.9
	MV	143[a]	-12.3	0.5	4.0	0.0	0.0	0.4	3.0	0.0	0.0	0.2	1.4	1.4	11.1	1.5	12.2
Cryptosporidium Low Pos	Ct	144	23.3	0.6	2.6	0.0	0.0	0.0	0.0	0.5	2.1	0.0	0.0	1.6	6.8	1.8	7.5
Cryptosporidium Mod Pos	Ct	143[a]	22.3	0.5	2.0	0.0	0.0	0.0	0.0	0.4	1.6	0.5	2.2	1.0	4.4	1.2	5.5
Giardia Low Pos	Ct	144	27.4	1.3	4.6	0.0	0.0	1.2	4.4	0.7	2.4	1.2	4.3	1.6	5.7	2.7	9.9
Giardia Mod Pos	Ct	144	26.6	1.2	4.5	0.0	0.0	1.4	5.4	0.4	1.6	1.1	4.2	1.3	4.9	2.6	9.7
Shigella EIEC Low Pos	Ct	144	32.8	0.4	1.1	0.1	0.3	0.2	0.5	0.1	0.4	0.0	0.0	0.8	2.3	0.9	2.7
Shigella EIEC Mod Pos	Ct	144	31.9	0.1	0.4	0.2	0.6	0.0	0.0	0.0	0.1	0.0	0.0	0.7	2.3	0.8	2.5
Norovirus Low Pos	Ct	144	33.2	0.3	0.9	0.0	0.0	0.2	0.6	0.3	0.8	0.3	0.8	0.6	2.0	0.8	2.5
Norovirus Mod Pos	Ct	144	32	0.3	0.9	0.0	0.1	0.1	0.4	0.1	0.4	0.1	0.3	0.6	1.8	0.7	2.1
V.	MP	108	15.5	1.3	8.4	0	0	0.9[c]	5.8[c]	NA[c]	NA[c]	0.8	5.1	2	12.9	2.7	17.2

Sample	Response	N	Mean Ct, MP, or MV	Variance Source													
				Site		Operator		Lot		Day		Run		Within-Run		Total	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
parahaemolyticus Low Pos	MV	108	-13.5	1.1	8.1	0	0	0.8[c]	5.7[c]	NA[c]	NA[c]	0.8	6.2	1.8	13.5	2.4	17.9
V. parahaemolyticus Mod Pos	MP	108	16.2	1.1	6.7	0	0	0.8[c]	5.1[c]	NA[c]	NA[c]	0.2	1.5	2.2	13.4	2.6	15.9
	MV	108	-14.1	0.9	6.2	0	0	0.8[c]	5.6[c]	NA[c]	NA[c]	0.1	0.8	2	14.1	2.3	16.4

[a]One (1) sample excluded due to a non-determinate result
[b]One (1) sample excluded due to negative *Yersinia* result with missing melt peak and melt valley values
[c]The variation of lot and day are confounded and cannot be separated.

2. Linearity:

Not applicable as this is a qualitative assay.

3. Analytical Specificity/Interference:

a. Analytical Reactivity:

The analytical reactivity (inclusivity) of the Xpert GI Panel test was evaluated by utilizing multiple clinically relevant strains for each Xpert GI Panel target pathogen, representative of genotypic differences from various geographical regions. All strains were tested at ≤3x LoD with a minimum of five replicates using the Xpert GI Panel test, except for one strain which was assessed using *in silico* analysis against the Xpert GI Panel primer and probe sequences. If a target pathogen strain reported negative results for one replicate or more at ≤3x LoD, the strain was subsequently tested at a higher concentration. A strain was considered detected when all replicates at a test level were reported positive. Table 3 to Table 13 present the evaluated strains, test levels and the results for detection.

Table 3: Inclusivity Results for *Campylobacter*

Species/Subtype	Strain ID	Test Level (x LoD)	Test Concentration (CFU/mL)	Reported Results
Campylobacter coli	CCUG 10960	3x LoD	549	Positive
	CCUG 53138	3x LoD	549	Positive
	ATCC 43478	3x LoD	549	Positive
	CCUG 36766	3x LoD	549	Positive
	CCUG 59141	3x LoD	549	Positive
Campylobacter jejuni	CCUG 10259	3x LoD	549	Positive
	Zeptometrix 0801650	3x LoD	549	Positive
Campylobacter jejuni subsp. doylei	CCUG 24567T	3x LoD	549	Positive
	CCUG 11284T	3x LoD	549	Positive
	CCUG 14541	3x LoD	549	Positive
Campylobacter jejuni subsp. jejuni	CCUG 33057	3x LoD	549	Positive
	CCUG 6824	3x LoD	549	Positive
	ATCC 33560	3x LoD	549	Positive
Campylobacter fetus	CCUG 71557	3.9x LoD	720	Negative
		8.2x LoD	1,500	Negative

Species/Subtype	Strain ID	Test Level (x LoD)	Test Concentration (CFU/mL)	Reported Results
		131x LoD	24,000	Negative
Campylobacter lari	CCUG 15031	3.9x LoD	720	Negative
		131x LoD	24,000	Negative
Campylobacter upsaliensis	CCUG 14913T	3.9x LoD	720	Negative
		8.2x LoD	1,500	Negative
		131x LoD	24,000	Negative
	CCUG 24191	3.9x LoD	720	Negative
		8.2x LoD	1,500	Negative
		131x LoD	24,000	Negative

Table 4: Inclusivity Results for *Cryptosporidium*

Target Pathogen Strain	Strain ID	Test Concentration (oocysts/mL or cp/mL for Synthetic DNA)	Multiple of LoD	Reported Results
Cryptosporidium parvum	IDT Synthetic DNA, L25642	3,690	3x LoD	Positive
Cryptosporidium	IDT Synthetic DNA, EF179381	3,690	3x LoD	Positive
Cryptosporidium canis	IDT Synthetic DNA, AF112576	3,690	3x LoD	Positive
Cryptosporidium ubiquitum	IDT Synthetic DNA,	3,690	3x LoD	Positive
Cryptosporidium hominis	IDT Synthetic DNA,	3,690	3x LoD	Positive
Cryptosporidium muris	Waterborne Inc. P104, RN66	480,000	390x LoD	Negative [(a)]
	IDT Synthetic DNA, AB089284	100,000	81xLoD	Positive [(b)]

[(a)] Two out of five replicates and one out of five replicates were detected at 134xLoD and 390x LoD respectively.
[(b)] Three out of five replicates, eight out of ten replicates and nine out of ten replicates were detected at 8.1x LoD, 33x LoD and 57x LoD respectively.

Table 5: Inclusivity Results for STEC

Target Pathogen Strain	Strain ID	Test Concentration (CFU/mL)	Multiple of LoD	Reported Results
STEC *stx1* (O103)	NCTC 13782	1,872	3x LoD	Positive
STEC *stx1* (O45)	Microbiologics 01098P (CDC 00-)	1,872	3x LoD	Positive
STEC *stx2* (O121)	Statens Serum Institute D6088	9,030	3x LoD	Positive
STEC *stx2* (O145)	NCTC 13797	9,030	3x LoD	Positive
STEC *stx2* (O113)	Statens Serum Institute D5586	9,030	3x LoD	Positive
STEC *stx2* (O104)	NCTC 13796	9,030	3x LoD	Positive
STEC *stx1/stx2* (O26)	NCTC 13733	1,872	3x LoD (stx1)	Positive
			0.6x LoD (stx2)	Positive
STEC *stx1/stx2* (O111)	NCTC 13794	1,872	3x LoD (stx1)	Positive
			0.6x LoD (stx2)	Positive
STEC *stx1/stx2* (O157)	Microbiologics 0617P (ATCC 35150)	1,872	3x LoD (stx1)	Positive
			0.6x LoD (stx2)	Positive

Table 6: Inclusivity Results for Norovirus

Target Pathogen Strain	Strain ID	Test Concentration (cp/mL)	Multiple of LoD	Reported Results
Norovirus GII.4	Clinical Specimen, DLS0113053, CerbaXpert	60	0.2x LoD	Positive [a]
Norovirus	Clinical Specimen, 460878, Precision for	298	1x LoD	Positive
Norovirus GI.6	Clinical Specimen 13CA514199, Karolinska Hospital Sweden	298	1x LoD	Positive
		3	0.01x LoD	
Norovirus GII.3[P12]	Clinical Specimen 435625, Precision for Medicine U.S.	894	3x LoD	Positive
		9	0.03x LoD	
Norovirus	Clinical Specimen 487208, Precision for Medicine	894	3x LoD	Positive
Norovirus	Clinical Specimen 461526, Precision for Medicine	894	3x LoD	Positive
Norovirus	Clinical Specimen 487198, Precision for Medicine	894	3x LoD	Positive
Norovirus GI	Clinical Specimen GI 1, Karolinska Hospital	Unknown [b]	NA	Positive
	Clinical Specimen GI E, Karolinska Hospital	Unknown [b]	NA	Positive
Norovirus GII	Clinical Specimen GII 1, Karolinska Hospital	Unknown [b]	NA	Positive
	Clinical Specimen GII 2, Karolinska Hospital	Unknown [b]	NA	Positive
	Clinical Specimen GII 3, Karolinska Hospital	Unknown [b]	NA	Positive
	Clinical Specimen, GII 4 Karolinska Hospital	Unknown [b]	NA	Positive
	Clinical Specimen GII 5, Karolinska Hospital	Unknown [b]	NA	Positive
	Clinical Specimen GII A, Karolinska Hospital	Unknown [b]	NA	Positive
	Clinical Specimen GII B, Karolinska Hospital	Unknown [b]	NA	Positive
	Clinical Specimen GII C, Karolinska Hospital	Unknown [b]	NA	Positive
	Clinical Specimen GII D, Karolinska Hospital	Unknown [b]	NA	Positive
Norovirus GI.1	IDT Synthetic RNA, NC-001959	12,600	4x LoD	Positive
Norovirus GI.2	IDT Synthetic RNA, NMZ223426	25,200	8x LoD	Positive
Norovirus GI.4	IDT Synthetic RNA, MH393671	12,600	4x LoD	Positive
Norovirus GI.5	IDT Synthetic RNA, MT908122	9,390	3x LoD	Positive
Norovirus GI.7	IDT Synthetic RNA, MT357994	25,200	8x LoD	Positive
Norovirus GII.2	IDT Synthetic RNA, KJ407074,	9,390	3x LoD	Positive
Norovirus GII.4	IDT Synthetic RNA, X86557	9,390	3x LoD	Positive
Norovirus GII.10	IDT Synthetic RNA, MT501863	9,390	3x LoD	Positive
Norovirus GII.12	IDT Synthetic RNA, HQ449728	18,800	6x LoD	Positive
Norovirus GII.15	IDT Synthetic RNA, OK247589	18,800	6x LoD	Positive
Norovirus GII.17	IDT Synthetic RNA, KT190704	9,390	3x LoD	Positive

[a] The strain is considered detected since 19 from 20 replicates were reported positive at <1 LoD.
[b] Clinical specimens with unknown titers and one replicate tested per specimen.

Table 7: Inclusivity Results for *Salmonella*

Target Pathogen Strain	Strain ID	Test Concentration (CFU/mL)	Multiple of LoD	Reported Results
Salmonella bongori	CCUG 63587	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *salamae*	CCUG 30039T	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *arizonae*	CCUG 6322T	2,400	1.9x LoD	Positive
	CCUG 63588	2,400	1.9x LoD	Positive
	CCUG 63589	2,400	1.9x LoD	Positive

Target Pathogen Strain	Strain ID	Test Concentration (CFU/mL)	Multiple of LoD	Reported Results
Salmonella enterica subsp. *diarizonae*	CCUG 30040T	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *houtenae*	CCUG 30041T	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *indica*	CCUG 30038T	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Agona	CCUG 21287	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Anatum	CCUG 21243	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Bareilly	CCUG 12616	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Berta	CCUG 27106	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Blockley	CCUG 21263	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Branderup	CCUG 50923	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Derby	CCUG 21276	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Enteritidis	CCUG 34136T	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Hadar	CCUG 21271	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Heidelberg	CCUG 21289	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* serotype Typhimurium 4,5,12: i: 1,2	CCUG 18375	2,400	1.9x LoD	Positive
	ATCC 14028	2,400	1.9x LoD	Positive
	CCUG 42060T	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Infantis	CCUG 12615	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Javiana	CCUG 21235	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Litchfield	NCTC 6028	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Mbandaka	CCUG 21272	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Mississippi	Clinical Specimen S027019, Public Health Agency of Sweden	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Montevideo	CCUG 21239	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Muenchen	CCUG 21254	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Newport	CCUG 21283	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Oranienburg	CCUG 12649	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Panama	CCUG 21275	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Paratyphi A	NCTC 5702	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Poona	CCUG 39842	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Reading	NCTC 5720	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Saintpaul	CCUG 21282	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Sandiego	NCTC 6024	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica*	CCUG 21280	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Senftenberg	CCUG 37886	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Stanley	CCUG 26623	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Thompson	CCUG 12652	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Typhi	Clinical Specimen 22-00912, Public Health Agency of Sweden	2,400	1.9x LoD	Positive
Salmonella enterica subsp. *enterica* Typhimurium	CCUG 35118	2,400	1.9x LoD	Positive

Table 8: Inclusivity Results for *Shigella*

Target Pathogen Strain	Strain ID	Test Concentration (CFU/mL)	Multiple of LoD	Reported Results
Shigella boydii (Subgroup C, serotype 8)	CCUG 37892	612	3x LoD	Positive
Shigella boydii (Subgroup C, serotype 10)	CCUG 9564	612	3x LoD	Positive
Shigella boydii (Subgroup C, serotype 11)	ATCC 12031	612	3x LoD	Positive
Shigella dysenteriae (Subgroup A, serotype 1)	NCTC 4837 [(a)]	204	1x LoD	Positive
	NCTC 8217 [(a)]	612	3x LoD	Positive
	NCTC 8571 [(a)]	612	3x LoD	Positive
Shigella dysenteriae (Subgroup A,	NCTC 9345	612	3x LoD	Positive
Shigella dysenteriae (Subgroup A,	NCTC 9348	612	3x LoD	Positive
Shigella flexneri (Subgroup B, serotype	CCUG 56439T	1,224	6x LoD	Positive
Shigella flexneri (Subgroup B, serotype 3)	CCUG 21251	612	3x LoD	Positive
Shigella flexneri (Subgroup B, serotype	CCUG 37906	612	3x LoD	Positive
Shigella flexneri (Subgroup B, serotype 6)	CCUG 39080	612	3x LoD	Positive
	ATCC 15391	612	3x LoD	Positive
Shigella sonnei (Subgroup D)	CCUG 9567	612	3x LoD	Positive
Shigella dysenteriae (Subgroup A, serotype 9)	ATCC 49547	*In silico* analysis	NA	100% match with primer and probe

[(a)] Shigella strains carrying STEC *stx1* gene.

Table 9: Inclusivity Results for EIEC

Target Pathogen Strain	Strain ID	Test Concentration (CFU/mL)	Multiple of LoD	Reported Results
Enteroinvasive *Escherichia coli* (EIEC)	CCUG 38092	204	1x LoD	Positive
	CCUG 38094	612	3x LoD	Positive
	NCTC 9013	70,000	343x LoD	Negative [(a)]

[(a)] Secondary PCR assay could confirm the absence of EIEC target gene ipaH in strain NCTC 9013, i.e., target gene loss confirmed.

Table 10: Inclusivity Results for *Vibrio cholerae*

Target Pathogen Strain	Strain ID	Test Concentration (CFU/mL)	Multiple of LoD	Reported Results
Vibrio cholerae O:139	CCUG 34707	1,200	2.6x LoD	Positive
Vibrio cholerae O:1	CCUG 9118T	1,200	2.6x LoD	Positive

Table 11: Inclusivity Results for *V. parahaemolyticus*

Target Pathogen Strain	Strain ID	Test Concentration (CFU/mL)	Multiple of LoD	Reported Results
Vibrio parahaemolyticus	CCUG 19113	1,467	3x LoD	Positive
	CCUG 15657T	1,467	3x LoD	Positive
	CCUG 43362	1,467	3x LoD	Positive

Table 12: Inclusivity Results for *Yersinia*

Target Pathogen Strain	Strain ID	Test Concentration (CFU/mL)	Multiple of LoD	Reported Results
Yersinia enterocolitica biotype 1 (O:8)	CCUG 33055	1,044	3x LoD	Positive
Yersinia enterocolitica biotype 2 (O:9)	CCUG 8239A	1,044	3x LoD	Positive
Yersinia enterocolitica biotype 2 (O:5, 2)	NCTC 10463	1,044	3x LoD	Positive
Yersinia enterocolitica biotype 4 (O:3)	CCUG 34604	1,044	3x LoD	Positive

Table 13: Inclusivity Results for *Giardia*

Target Pathogen Strain	Strain ID	Test Concentration (cysts/mL)	Multiple of LoD	Reported Results
Giardia intestinalis	ATCC 30888	1	3x LoD	Positive
	ATCC 50114	1	3x LoD	Positive

b. Analytical Specificity (Exclusivity) and Microbial Interference:

The analytical specificity (exclusivity), microbial interference and in-assay cross reactivity of the Xpert GI Panel test were evaluated using microorganisms commonly found in stool and rectal flora. A total of 136 microorganisms were either tested with the Xpert GI Panel test (131 microorganisms) or assessed using *in silico* analysis directed against the Xpert GI Panel probe and primer sequences (five microorganisms). The non-target microorganisms evaluated using the Xpert GI Panel test were diluted into clinical stool matrix at high concentrations and tested in absence of target pathogens using three replicates, and in presence of target pathogens diluted to ≤ 3x LoD using six replicates. Non-target bacteria were tested at 1E6 CFU/mL and non-target viruses and parasites/yeast were tested at ≥ 1E4 units/mL. The in-assay cross reactivity was evaluated using a subset of target pathogen strains, individually tested at high concentrations using six replicates, to determine potential cross-reactivity with the primers and probes included in the Xpert GI Panel test. Target pathogen strains evaluated for in-assay cross reactivity were tested at 1E6 units/mL, except for *Cryptosporidium parvum* which was tested at 9.94E4 oocysts/mL.

No cross-reactivity or microbial interference was observed for any of the tested non-target microorganisms using the Xpert GI Panel test. The risk for cross reactivity or interference with the Xpert GI Panel test for other select pathogens was determined to be low using *in silico* analysis for five of the non-target microorganisms. No in-assay cross reactivity was observed for any of the Xpert GI Panel target pathogens tested at high concentrations. Table 14**,** Table 15**,** and Table 16 present the bacteria, viruses, parasites/yeast evaluated in the Xpert GI Panel analytical (exclusivity) and microbial interference study respectively. Table 17 presents the target pathogen strains evaluated for in-assay cross reactivity of the Xpert GI Panel test.

Table 14: Non-Target Bacteria included in Analytical Specificity and Microbial Interference Studies

Non-target Microorganism	Strain ID	Concentration Tested
Abiotrophia defectiva	CCUG 27639	1E6 CFU/mL
Acinetobacter baumannii	CCUG 19096T	1E6 CFU/mL

Non-target Microorganism	Strain ID	Concentration Tested
Acinetobacter lwoffii	ZeptoMetrix 0801909	1E6 CFU/mL
Aeromonas caviae	CCUG 25939	1E6 CFU/mL
Aeromonas salmonicida (Aeromonas	ATCC 7965	1E6 CFU/mL
Aeromonas schubertii	CCUG 27820	1E6 CFU/mL
Aeromonas sobria	CCUG 14830	1E6 CFU/mL
Aeromonas veronii	CCUG 27821T	1E6 CFU/mL
Alcaligenes faecalis subsp. *faecalis*	CCUG 1814T	1E6 CFU/mL
Anaerococcus tetradius	CCUG 46590T	1E6 CFU/mL
Arcobacter butzleri	CCUG 30485	1E6 CFU/mL
Arcobacter cryaerophilus	CCUG 17801	1E6 CFU/mL
Bacillus cereus	ZeptoMetrix 0801823	1E6 CFU/mL
Bacteroides caccae	ATCC 43185	1E6 CFU/mL
Bacteroides fragilis	ZeptoMetrix 0801583	1E6 CFU/mL
Bacteroides stercoris	ATCC 43183	1E6 CFU/mL
Bacteroides thetaiotaomicron	CCUG 10774	1E6 CFU/mL
Phocaeicola vulgatus (Bacteroides vulgatus)	ATCC 8482	1E6 CFU/mL
Bifidobacterium adolescentis	CCUG 18363T	1E6 CFU/mL
Bifidobacterium bifidum	CCUG 45217	1E6 CFU/mL
Bifidobacterium longum subsp. *longum*	ATCC 15707	1E6 CFU/mL
Brevundimonas diminuta	CCUG 2031	1E6 CFU/mL
Cedecea davisae	CCUG 12370	1E6 CFU/mL
Chlamydia trachomatis	ZeptoMetrix 0801775	1E6 CFU/mL
Citrobacter amalonaticus	CCUG 4860A	1E6 CFU/mL
Citrobacter freundii	ZeptoMetrix 0801563	1E6 CFU/mL
Citrobacter koseri	CCUG 4859	1E6 CFU/mL
Citrobacter sedlakii	CCUG 30794	1E6 CFU/mL
Clostridium difficile	ZeptoMetrix 0801619	1E6 CFU/mL
Hathewaya histolytica (Clostridium histolyticum)	ATCC 19401	1E6 CFU/mL
Clostridium novyi	ATCC 17861	1E6 CFU/mL
Clostridium perfringens	ATCC 13124	1E6 CFU/mL
Thoamsclavelia ramosa (Clostridium ramosum)	CCUG 24038	1E6 CFU/mL
Clostridium septicum	ATCC 12464	1E6 CFU/mL
Paeniclostridium sordellii (Clostridium sordellii)	DSMZ 2141	1E6 CFU/mL
Clostridium tetani	ATCC 19406	1E6 CFU/mL
Collinsella aerofaciens	CCUG 28087	1E6 CFU/mL
Corynebacterium genitalium	CCUG 65575	1E6 CFU/mL
Corynebacterium lipophiloflavum	CCUG 37336	1E6 CFU/mL
Desulfovibrio piger	NA	*In silico* analysis
Edwardsiella tarda	CCUG 1638	1E6 CFU/mL
Eggerthella lenta	ATCC 43055	1E6 CFU/mL
Klebsiella aerogenes (Enterobacter aerogenes)	ZeptoMetrix 0801518	1E6 CFU/mL
Enterobacter cancerogenus	ATCC 35316	1E6 CFU/mL
Enterobacter cloacae subsp. *cloacae*	ZeptoMetrix 0801830	1E6 CFU/mL
Enterococcus faecalis	ZeptoMetrix 0801637	1E6 CFU/mL

Non-target Microorganism	Strain ID	Concentration Tested
Enterococcus faecium	ZeptoMetrix 0804210	1E6 CFU/mL
Enteroaggregative *E. coli* EAEC	ZeptoMetrix 0801919	1E6 CFU/mL
Enteropathogenic *E. coli* EPEC	ZeptoMetrix 0801938	1E6 CFU/mL
Enterotoxigenic *E. coli* ETEC	ZeptoMetrix 0801624	1E6 CFU/mL
Escherichia fergusonii	CCUG 18766	1E6 CFU/mL
Escherichia hermannii	CCUG 15714	1E6 CFU/mL
Pseudescherichia vulneris (Escherichia vulneris)	CCUG 15715	1E6 CFU/mL
Fusobacterium varium	ATCC 8501	1E6 CFU/mL
Gardnerella vaginalis	ZeptoMetrix 0801894	1E6 CFU/mL
Gemella morbillorum	CCUG 18164	1E6 CFU/mL
Hafnia alvei	CCUG 41547T	1E6 CFU/mL
Helicobacter fennelliae	CCUG18820	1E6 CFU/mL
Helicobacter pylori	ZeptoMetrix 0804383	1E6 CFU/mL
Haemophilus influenzae	ZeptoMetrix 0801679	1E6 CFU/mL
Klebsiella oxytoca	ZeptoMetrix 0801881	1E6 CFU/mL
Klebsiella pneumoniae subsp. *pneumoniae*	CCUG 225T	1E6 CFU/mL
Lactobacillus acidophilus	ATCC 314	1E6 CFU/mL
Limosilactobacillus reuteri	CCUG 33624	1E6 CFU/mL
Lactococcus lactis subsp. lactis	CCUG 32211	1E6 CFU/mL
Leminorella grimontii	CCUG 20909B	1E6 CFU/mL
Listeria monocytogenes	ZeptoMetrix 0801534	1E6 CFU/mL
Megamonas hypermegale	CCUG 5856	1E6 CFU/mL
Megasphaeara elsdenii	ATCC 25940	1E6 CFU/mL
Morganella morganii subsp. *morganii*	ZeptoMetrix 0804010	1E6 CFU/mL
Neisseria gonorrhoeae	ZeptoMetrix 0801482	1E6 CFU/mL
Parabacteroides merdae	CCUG 38734	1E6 CFU/mL
Peptoniphilus asaccharolyticus	ATCC 14963	1E6 CFU/mL
Peptostreptococcus anaerobius	CCUG 7835	1E6 CFU/mL
Photobacterium damselae subsp. *damselae*	CCUG 13626	1E6 CFU/mL
Pleisomonas shigelloides	CCUG 410T	1E6 CFU/mL
Porphyromonas asaccharolytica	CCUG 7834T	1E6 CFU/mL
Prevotella melaninogenica	ATCC 25845	1E6 CFU/mL
Proteus mirabilis	ZeptoMetrix 0801544	1E6 CFU/mL
Proteus penneri	CCUG 15722	1E6 CFU/mL
Proteus vulgaris	ZeptoMetrix 0801898	1E6 CFU/mL
Providencia alcalifaciens	ZeptoMetrix 0801906	1E6 CFU/mL
Pseudomonas aeruginosa	CCUG 551T	1E6 CFU/mL
Ruminococcus bromii	ATCC 27255	1E6 CFU/mL
Serratia fonticola	CCUG 14186	1E6 CFU/mL
Serratia liquefaciens	CCUG 9285T	1E6 CFU/mL
Serratia marcescens subsp. *marcescens*	ZeptoMetrix 0801723	1E6 CFU/mL
Shewanella algae	CCUG 39064	1E6 CFU/mL
Shimwellia blattae	CCUG 14803BT	1E6 CFU/mL
Staphylococcus aureus subsp. *aureus*	ATCC 25923	1E6 CFU/mL
Staphylococcus epidermidis	ZeptoMetrix 0801651	1E6 CFU/mL

Non-target Microorganism	Strain ID	Concentration Tested
Stenotrophomonas maltophilia	ZeptoMetrix 0801569	1E6 CFU/mL
Streptococcus agalactiae	CCUG 4208	1E6 CFU/mL
Streptococcus intermedius	ZeptoMetrix 0801895	1E6 CFU/mL
Streptococcus pyogenes	CCUG 4207	1E6 CFU/mL
Streptococcus salivarius subsp. *salivarius*	ZeptoMetrix 0801896	1E6 CFU/mL
Streptococcus suis	CCUG 7984	1E6 CFU/mL
Trabulsiella guamensis	ATCC 49492	1E6 CFU/mL
Veillonella parvula	ATCC 10790	1E6 CFU/mL
Vibrio vulnificus	CCUG 48492	1E6 CFU/mL
Yersinia bercovieri	CCUG 26329T	1E6 CFU/mL
Yersinia frederiksenii	CCUG 11293	1E6 CFU/mL
Yersinia intermedia	CCUG 11292T	1E6 CFU/mL
Yersinia kristensenii	CCUG 11294	1E6 CFU/mL
Yersinia mollaretii	CCUG 26331	1E6 CFU/mL
Yersinia rohdei	CCUG 38833	1E6 CFU/mL

Table 15: Non-Target Viruses included in Analytical Specificity and Microbial Interference Studies

Non-target Microorganism	Strain ID	Concentration Tested
Adenovirus Type 1	ZeptoMetrix 0810050CF	1E6 $TCID_{50}$/mL
Adenovirus Type 3	ZeptoMetrix 0810062CF	5E5 $TCID_{50}$/mL[(a)] 1E4 $TCID_{50}$/mL[(b)]
Adenovirus Type 4	ZeptoMetrix 0810070CF	5E4 $TCID_{50}$/mL[(a)]
Adenovirus Type 5	ZeptoMetrix 0810020CF	1E6 $TCID_{50}$/mL
Adenovirus Type 8	ZeptoMetrix 0810069CF	2E4 $TCID_{50}$/mL[(a)] 1E4 $TCID_{50}$/mL[(b)]
Adenovirus Type 14	ZeptoMetrix 0810108CF	1E5 $TCID_{50}$/mL
Adenovirus Type 18	NA	*In silico* analysis
Adenovirus Type 31	ZeptoMetrix 0810073CF	1E5 $TCID_{50}$/mL
Adenovirus Type 40	ZeptoMetrix 0810084CF	1E5 $TCID_{50}$/mL
Adenovirus Type 41	ZeptoMetrix 0810085CF	1E5 $TCID_{50}$/mL
Astrovirus	ATCC VR-1936	1E6 $TCID_{50}$/mL
Parvovirus	ZeptoMetrix 0810064C	1E6 IU/mL
Cytomegalovirus	ATCC VR-538	5E5 $TCID_{50}$/mL
Enterovirus	ATCC VR-836	5E5 $TCID_{50}$/mL
Hepatitis A virus	ATCC VR-1541	5E5 $TCID_{50}$/mL
Herpes Simplex Virus Type 2	ZeptoMetrix 0810006CF	5E5 $TCID_{50}$/mL
Human coxsackievirus	ZeptoMetrix 0810074CF	5E5 $TCID_{50}$/mL
Rhinovirus	ZeptoMetrix 0810012CFN	2E4 $TCID_{50}$/mL[(a)] 1E5 $TCID_{50}$/mL[(b)]
Rotavirus	ATCC VR-2551	1E6 $TCID_{50}$/mL
Sapovirus	ATCC VR-3237SD	1E7 genome copies/mL[(a)] 1E6 genome copies/mL[(b)]

[(a)] Concentration of the non-target microorganism tested with *Salmonella enterica, Giardia lamblia, Vibrio cholerae* and negative sample.
[(b)] Concentration of the non-target microorganism tested with *Campylobacter jejuni,* STEC stx1 and stx2, EIEC*, Yersinia enterocolitica, Vibrio parahaemolyticus, Cryptosporidium parvum* and Norovirus GI and GII.

Table 16: Non-Target Parasites/Yeast included in Analytical Specificity and Microbial Interference Studies

Non-target Microorganism	Strain ID	Concentration Tested
Blastocystis hominis	NA	*In silico* analysis
Candida albicans	ZeptoMetrix 0801504	1E6 CFU/mL
Cyclospora cayetanensis	ATCC PRA-3000SD	1E7 copies/mL[(a)] 1E6 copies/mL[(b)]
Encephalitozoon cuniculi	NA	*In silico* analysis
Encephalitozoon hellum	NA	*In silico* analysis
Entamoeba dispar	ATCC PRA-368	N/A[(c)]
Entamoeba histolytica	ATCC 30459	2E4 cells/mL[(a)] 1E4 cells/mL[(b)]
Entamoeba invadens	ATCC 30994	5E4 cells/mL
Pentatrichomonas hominis	ATCC 30000	1E6 cells/mL
Trichomonas vaginalis	ZeptoMetrix 0801805	1E5 trophozoites/mL
Entamoeba histolytica	ATCC 30459	2E4 cells/mL[(a)] 1E4 cells/mL[(b)]

[(a)] Concentration of the non-target microorganism tested with *Salmonella enterica, Giardia lamblia, Vibrio cholerae* and negative sample.
[(b)] Concentration of the non-target microorganism tested with *Campylobacter jejuni,* STEC *stx1* and *stx2*, EIEC*, Yersinia enterocolitica, Vibrio parahaemolyticus, Cryptosporidium parvum* and Norovirus GI and GII.
[(c)] For *Entamoeba dispar*, ATCC PRA-368, no titer or cell count was available according to vendor ATCC. According to the Certificate of Analysis, the microorganism was released based on visual observation methods.

Table 17: Target Pathogen Strains evaluated in Cross-Reactivity Study

Target Pathogen	Strain ID	Concentration Tested
Campylobacter coli	CCUG 53138	1E6 CFU/mL
	ATCC 43478	1E6 CFU/mL
	CCUG 36766	1E6 CFU/mL
Shigella boydii (Subgroup C, serotype 8)	CCUG 37892	1E6 CFU/mL
Shigella boydii (Subgroup C, serotype 10)	CCUG 9564	1E6 CFU/mL
Shigella flexneri (Subgroup B, serotype 2a)	CCUG 56439T	1E6 CFU/mL
Shigella flexneri (Subgroup B, serotype 4a)	CCUG 37906	1E6 CFU/mL
Enteroinvasive *Escherichia coli* (EIEC)	CCUG 38094	1E6 CFU/mL
Escherichia coli (STEC), O111	NCTC 13794	1E6 CFU/mL
Escherichia coli (STEC), O113	D5586	1E6 CFU/mL
Escherichia coli (STEC), O104	NCTC 13796	1E6 CFU/mL
Salmonella bongori	CCUG 63587	1E6 CFU/mL
Salmonella enterica subsp. *indica*	CCUG 30038T	1E6 CFU/mL
Salmonella enterica subsp. enterica	NCTC 5702	1E6 CFU/mL
Vibrio cholerae, O:139 (non-O:1)	CCUG 34707	1E6 CFU/mL
Vibrio cholerae, O:1	CCUG 9118T	1E6 CFU/mL
Vibrio parahaemolyticus	CCUG 43362	1E6 CFU/mL
	CCUG 19113	1E6 CFU/mL
	CCUG 15657T	1E6 CFU/mL
Norovirus GI.3 [P3]	Clinical Specimen, 460878, Precision for Medicine U.S.	1E6 cp/mL
Norovirus GII.7	Clinical Specimen, 461526, Precision for Medicine U.S.	1E6 cp/mL

Target Pathogen	Strain ID	Concentration Tested
Yersinia entercolitica, subsp. *enterocolitica*, biotype 1 (serotype O:8)	CCUG 33055	1E6 CFU/mL
Yersinia entercolitica, subsp. *enterocolitica*, biotype 2 (serotype O:5,27)	NCTC 10463	1E6 CFU/mL
Yersinia entercolitica, subsp. *enterocolitica*, biotype 4 (serotype O:3)	CCUG 34604	1E6 CFU/mL
Cryptosporidium parvum	Waterborne Inc. P102C Iowa	9.94E4 oocysts/mL
Cryptosporidium hominis	Waterborne Inc. TU502	1E6 oocysts/mL
Giardia intestinalis	ATCC 30888	1E6 cysts/mL
	ATCC 50114	1E6 cysts/mL

c. Competitive Inhibition:

Competitive inhibition of the Xpert GI Panel test, caused by clinically relevant co-infections, was evaluated by testing 12 target pathogen combinations using a total of seven target pathogens, i.e., *Campylobacter jejuni*, *Salmonella enterica*, *Giardia lamblia*, *Yersinia enterocolitica*, STEC *stx1*, STEC *stx2* and Norovirus GI. The target pathogens were tested at low concentrations, i.e., ≤3x LoD in presence of one or more additional target pathogen(s) at a high concentration in negative clinical stool matrix. The high-test concentrations were 1E6 CFU/mL for bacterial pathogens, 1E5 cysts/mL for *Giardia lamblia*, and 1E5 cp/mL for Norovirus GI. All target pathogen combinations were diluted in clinical stool matrix and tested with six replicates. The study results showed no competitive inhibition for common gastrointestinal co-infections with the Xpert GI Panel test. Table 18 presents the target pathogen combinations and the reported results.

Table 18: Summary of Competitive Inhibition Study Results

High Titer Target Pathogen	Low Titer Target Pathogen	Low Titer Testing Concentration (≤3x LoD)	Reported Result for Low Titer Target Pathogen
STEC stx1 (1E6 CFU/mL) STEC stx2 (1E6 CFU/mL)	*Campylobacter jejuni*	549 CFU/mL	Positive
	Salmonella enterica	1,863 CFU/mL	Positive
Yersinia enterocolitica (1E6 CFU/mL)	*Campylobacter jejuni*	549 CFU/mL	Positive
	Salmonella enterica	1,863 CFU/mL	Positive
Norovirus GI (1E5 cp/mL)	*Campylobacter jejuni*	549 CFU/mL	Positive
	Salmonella enterica	1,863 CFU/mL	Positive
Campylobacter jejuni (1E6 CFU/mL) *Salmonella enterica* (1E6 CFU/mL)	STEC stx1	1,872 CFU/mL	Positive
	STEC stx2	9,030 CFU/mL	Positive
Yersinia enterocolitica (1E6 CFU/mL)	STEC stx1	1,872 CFU/mL	Positive
	STEC stx2	9,030 CFU/mL	Positive
Norovirus GI (1E5 cp/mL)	STEC stx1	1,872 CFU/mL	Positive
	STEC stx2	9,030 CFU/mL	Positive
Campylobacter jejuni (1E6 CFU/mL) *Salmonella enterica* (1E6 CFU/mL)	*Yersinia enterocolitica*	1,044 CFU/mL	Positive

High Titer Target Pathogen	Low Titer Target Pathogen	Low Titer Testing Concentration (≤3x LoD)	Reported Result for Low Titer Target Pathogen
STEC stx1 (1E6 CFU/mL) STEC stx2 (1E6 CFU/mL)	*Yersinia enterocolitica*	1,044 CFU/mL	Positive
Campylobacter jejuni (1E6 CFU/mL) *Salmonella enterica* (1E6 CFU/mL)	Norovirus GI	894 cp/mL	Positive
Giardia lamblia (1E5 cysts/mL) *Yersinia enterocolitica* (1E6 CFU/mL)	Norovirus GI	894 cp/mL	Positive
STEC *stx1* (1E6 CFU/mL) STEC *stx2* (1E6 CFU/mL)	Norovirus GI	894 cp/mL	Positive
Norovirus GI (1E5 cp/mL)	*Giardia lamblia*	738 cysts/ml	Positive
	Yersinia enterocolitica	1,044 CFU/mL	Positive

d. Potentially Interfering Substances Study:

A total of seven endogenous and twenty-nine exogenous substances, that may be encountered in clinical stool specimens, as well as four method-specific substances for collecting and storing clinical specimens, were evaluated for potential interference with the Xpert GI Panel test performance. Table 19 presents the evaluated substances and the corresponding test concentration in raw stool. Each potentially interfering substance was tested individually in clinical negative stool matrix in the absence and presence of representative Xpert GI Panel target pathogens respectively at a concentration of 3x LoD using six replicates. The representative target pathogens included EIEC, *Yersinia enterocolitica*, *Cryptosporidium parvum* and Norovirus and they were selected to include at least one of each pathogen type (bacteria, parasite, virus), covering both detection methods (amplification and melt analysis).

None of the tested substances showed interference with the Xpert GI Panel test performance, except for overfilling of stool sample above the Cary Blair medium transport vial max fill line. The stool overfill condition resulted in five pressure errors, two false negative results for *Cryptosporidium parvum* and delayed Ct values for the amplification targets (EIEC, *Cryptosporidium parvum* and Norovirus). Thus, overfilling stool in the Cary Blair medium vial may be a potential risk for non-determinate results or false negative results at low target pathogen levels. These results highlight the importance of performing the stool sample collection correctly and according to the Cary Blair manufacturer´s instructions.

Table 19: Potential Interfering Substances Study Results

Type of Substance	Substance	Test Concentration in Raw Stool
Endogenous Substances	Human whole blood	10% v/v
	Mucin	5% w/v
	Fecal fat – triglycerides	5% v/v
	Fecal fat – cholesterol	5% w/v
	Human stool (overfill of Cary Blair vial)	Filled above vial max fill line
	Bile Salts	9 mg/g
	Human urine	50% v/v
	Amoxicillin	5% w/v

Type of Substance	Substance	Test Concentration in Raw Stool
Exogenous Substances	Ampicillin	5% w/v
	Aspartame	5% w/v
	Azithromycin	1% w/v
	Bacitracin	50% w/v
	Ceftriaxone	16 mg/mL
	Ciprofloxacin	5% w/v
	Doxycycline	1% w/v
	Fluvastatin	1% w/v
	Glycerin	50% v/v
	Nystatin	50% w/v
	Metronidazole	60.8 mg/mL
	Vancomycin	12.5 mg/mL
	Naproxen sodium	10% w/v
	Bisacodyl	5% w/v
	Bismuth subsalicylate	1% w/v
	Calcium carbonate	5% w/v
	Docusate sodium	50% w/v
	Hydrocortisone	50% w/v
	Loperamide hydrochloride	5% w/v
	Magnesium hydroxide	10% w/v
	Phenylephrine hydrochloride	30% w/v
	Sodium phosphate	5% w/v
	Nonoxynol-9	50% v/v
	Steric acid	5% w/v
	Palmitic acid	5% w/v
	Bleach 10%	50% v/v
	Ethanol	0.2% v/v
	Mineral Oil	50% v/v
Method Specific Substance (Cary Blair Medium)	Copan Fecal Swab	N/A
	Remel Cary Blair	N/A
	Para-Pak C&S	N/A
	MCC C&S Medium Transport	N/A

4. Assay Reportable Range:

Not applicable as this is a qualitative assay.

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

a. Specimen Stability:

A specimen stability study was conducted using positive and negative samples in fresh clinical stool matrix to determine the stability of the Xpert GI Panel target pathogens in clinical stool matrix. The samples were tested at 2°C and 8°C for up to five days and at 15°C and 25°C for up to either 72 hours or 4 days. A minimum of eight replicates for each positive and negative sample were tested for each time point and temperature condition using one Xpert GI Panel reagent lot. All the Xpert GI Panel target pathogens were tested either as target mixes or as single

targets in fresh clinical stool matrix at ≤3x LoD. The negative sample consisted of fresh negative clinical stool without any Xpert GI Panel target pathogens.

The data from specimen stability support a specimen storage claim in clinical stool matrix (stool in Cary Blair) at 2°C - 8°C for up to 4 days and at 15°C - 25°C for up to 24 hours.

6. Detection Limit:

The analytical limit of detection (LoD) of the Xpert GI Panel test was determined by using two strains per Xpert GI Panel target pathogen. Each Xpert GI Panel target pathogen strain was serially diluted in clinical stool matrix and tested using two reagent lots across three testing days. The highest observed LoD, as determined by Probit regression analysis (95th Percentile), for each target pathogen strain from the two reagent lots was selected for LoD verification. Verification and confirmation of the estimated LoD for each Xpert GI Panel target pathogen strain was performed using one reagent lot across three testing days with a minimum of 20 replicates. The titer with a positive reported result greater than or equal to 95% was determined as the verified LoD. The verified LoD was then confirmed by levels tested below and above with reported results of <95% and 100% respectively. The verified and confirmed LoD values for each Xpert GI Panel target pathogen strain are presented in Table 20.

Table 20: Limit of Detection of the Xpert GI Panel Analytes

Target Pathogen	Strain	Strain ID	Confirmed LoD
Campylobacter	*Campylobacter coli*	CCUG 11283T	46 CFU/mL
	Campylobacter jejuni	CCUG 41359	183 CFU/mL
Shigella/EIEC	*Shigella sonnei*	CCUG 68726T	82 CFU/mL
	Enteroinvasive *Escherichia coli*	CCUG 46406	204 CFU/mL
	Salmonella bongori	CCUG 30042T	261 CFU/mL
Salmonella	*Salmonella enterica*	NCTC 13171	1,242 CFU/mL
STEC *stx1/stx2*	STEC *stx1*	Statens Serum Institut MHI813	624 CFU/mL
	STEC *stx2*	Statens Serum Institut 31	3,010 CFU/mL
	STEC *stx1_2*	Statens Serum Institut EDL933	565 (stx1) CFU/mL
	STEC *stx1_2*	Statens Serum Institut EDL933	683 (stx2) CFU/mL
Vibrio cholerae	*Vibrio cholerae*	NCTC 8457	136 CFU/mL
	Vibrio cholerae	CCUG 67718	459 CFU/mL
Vibrio parahaemolyticus	*Vibrio parahaemolyticus*	CCUG 14474T	127 CFU/mL
	Vibrio parahaemolyticus	CCUG 67711	489 CFU/mL
Yersinia enterocolitica	*Yersinia enterocolitica*	CCUG 52867T	348 CFU/mL
	Yersinia enterocolitica	CCUG 12369T	106 CFU/mL
Cryptosporidium	*Cryptosporidium hominis*	Waterborne Inc. TU502	72 oocysts/mL
	Cryptosporidium parvum	Waterborne Inc. P102C, Iowa	246 oocysts/mL
Giardia	*Giardia lamblia*	Waterborne Inc. P101, H3	246 cysts/mL
	Giardia intestinalis	ATCC 30957	0.36 cysts/mL
Norovirus	Norovirus GI	Clinical sample GI.3[P3]	298 cp/mL
	Norovirus GII	Clinical sample GII.4 Sydney	27 cp/mL

7. Assay Cut-Off:

The Xpert GI Panel test detects 11 target pathogens, either from amplification analysis or melt curve analysis. The Xpert GI Panel test also detects two internal controls, one internal control

(IC) detected by amplification analysis and one sample process control (SPC) detected by both amplification and melt analysis. The amplification signal is defined as the cycle threshold (Ct) when the amplification curve reaches the set threshold. The melt curve signal is defined as the temperature at which half of the DNA strands are in the random coil or single-stranded (ssDNA) state. Each target pathogen and internal control, within the cycle threshold or melt threshold values specifications are reported as POSITIVE. Each target pathogen and internal control, outside the cycle threshold or melt threshold values specifications are reported as NEGATIVE. If the SPC and/or the IC report a negative result, a test with a reported positive result for a target pathogen is reported as POSITIVE. If the SPC and/or IC report a negative result, a test with a reported negative result for all target pathogens is reported as INVALID. The cycle threshold and melt threshold specifications are included as automatic calculations in the assay definition file (ADF) of the Xpert GI Panel test.

8. Accuracy (Instrument):

Not applicable.

9. Carry-Over:

A study was conducted to demonstrate that the single-use, self-contained Xpert GI Panel cartridge exhibits no carry-over contamination. The carry-over contamination evaluation was conducted by testing a negative sample immediately after testing a positive sample at high concentration in the same GeneXpert module. This procedure was repeated until 10 high positive and 11 negative replicates had been alternately tested for two GeneXpert modules respectively. The positive sample consisted of representative target pathogens at high concentrations in clinical stool matrix, i.e., EIEC at 1E6 CFU/mL, *Giardia lamblia* at 1E5 cysts/mL and Norovirus at 1E5 cp/mL. The target pathogens were selected to include at least one of each pathogen type (bacteria, parasite, virus). The negative sample consisted of negative clinical stool matrix without any target pathogens. All 20 replicates of the positive sample (10 replicates for each GeneXpert module) were correctly reported as positive for the target pathogens included. All 22 replicates of the negative sample (11 replicates for each GeneXpert module) were correctly reported as Negative. Thus, no carry-over contamination in the GeneXpert modules were observed.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Not applicable

2. Matrix Comparison:

Not applicable

C Clinical Studies:

1. Clinical Performance:

The clinical performance of the Xpert GI Panel was evaluated in a multicenter study at nineteen geographically diverse clinical sites within (13) and outside (6) of the United States. Clinical specimens were prospectively collected between July 2023 to December 2023. The clinical study utilized leftover, de-identified stool specimens in Cary Blair media collected from individuals suspected of GI infection. A total of 1658 prospectively collected fresh stool specimens in Cary Blair media were initially enrolled in the study of which only 1592 eligible specimens were

included in the study. All clinical specimen testing using the Xpert GI Panel were performed by trained operators at 14 clinical testing sites.

Due to the low prevalence observed for specific analytes in the prospective study cohort, the sample size for this study was supplemented with pre-selected archived specimens sourced from sites within the United States. To minimize bias, pre-selected specimens were randomized and tested in a blinded manner at 5 of the 19 clinical sites. Pre-selected specimens were identified by standard of care results and confirmed using comparator test results prior to testing with the Xpert GI Panel. A total of 103 pre-selected specimens were included in the clinical performance analysis. In addition, if sufficient prospective and pre-selected archived specimens were not obtained for specific analytes, the sample size was supplemented with contrived samples for those analytes.

Overall initial and final non-determinant rates for Xpert GI panel clinical studies were 3.4% (74/2150) and 0.2% (4/2150), respectively.

Prospective Specimens

Demographic information (sex, age, and healthcare setting) of the eligible prospective specimens is presented in Table 21.

Table 21: Demographics of Eligible Prospective Specimens

Prospectively collected Fresh specimens (N=1592)	Number of specimens (%)
Sex	
Female	907 (57.0%)
Male	685 (43.0%)
Age (years)	
<18	187 (11.7%)
18-21	29 (1.8%)
22-49	398 (25.0%)
50-64	344 (21.6%)
>=65	634 (39.8%)
Healthcare Setting	
ER Patient	133 (8.4%)
Inpatient/Hospitalized	338 (21.2%)
Outpatient	569 (35.7%)
Unknown	552 (34.7%)

The clinical performance of each Xpert GI Panel analyte was compared to those of an FDA cleared molecular assay (Nucleic Acid Amplification Test) for most analytes. A composite of three FDA-cleared molecular assays was used for *Campylobacter* and *Yersinia.* Specimens were considered positive if at least two of the three comparator assays had positive results, and the specimens were considered negative if at least two of three comparator assays had negative results. A composite of 2 PCR assays followed by bi-directional sequencing was used for Norovirus. For STEC *stx1*, STEC *stx2*, and *V. parahaemolyticus* analytes, if the FDA cleared molecular assay was positive, a second FDA cleared molecular assay was performed to provide species differentiation. Specimens with discrepant results were investigated on an independent

FDA cleared molecular assay. For each analyte in the Xpert GI Panel, the performance (Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), and the 95% confidence interval (CI)) of the Xpert GI Panel as compared to the comparator method in prospective specimens is presented in Table 22. The number of specimens included in the performance calculations for each analyte were based on availability of valid results for Xpert GI Panel and the comparator method for the analyte and are presented in Table 22.

Table 22: Clinical Performance of Xpert Panel GI Test in Prospectively Collected Specimens

Analyte	Total	Positive Percent Agreement				Negative Percent Agreement			
		TP	FN	PPA (%)	95%CI	TN	FP	NPA (%)	95%CI
Campylobacter	604	62	4[a]	93.9	85.4 - 97.6	533	5[a]	99.1	97.8 - 99.6
Salmonella	1568	49	5[b]	90.7	80.1 - 96.0	1512	2	99.9	99.5 - 100.0
V. parahaemolyticus	1505	0	0	N/A	N/A	1505	0	100.0	99.7 - 100.0
V. cholerae	1568	0	1[c]	0	0.0 - 79.3	1566	1[c]	99.9	99.6 - 100.0
Yersinia	603	15	0	100	79.6 – 100.0	578	10[d]	98.3	96.9 - 99.1
***Shigella* EIEC**	1568	34	3[e]	91.9	78.7 - 97.2	1527	4[e]	99.7	99.3 - 99.9
STEC *stx1*	1497	6	0	100.0	61.0 - 100.0	1491	0	100.0	99.7 - 100.0
STEC *stx2*	1497	2	0	100.0	34.2 - 100.0	1494	1	99.9	99.6 - 100.0
Cryptosporidium	1568	32	0	100.0	89.3 - 100.0	1534	2[f]	99.9	99.5 - 100.0
Giardia	1568	20	1[g]	95.2	77.3 - 99.2	1543	4[g]	99.7	99.3 - 99.9
Norovirus GI/GII	1521	46	1	97.9	88.9 - 99.6	1448	26	98.2	97.4 - 98.8

Abbreviations: CI, confidence interval; FN, false negative; FP, false positive; N/A, not available; NPA, negative percent agreement; PPA, positive percent agreement; TN, true negative; TP, true positive

[a] Of 4 specimens with FN *Campylobacter* results, 4 were positive by all 3 comparator tests. Of 5 specimens with FP *Campylobacter* results, 2 were negative by all 3 comparator tests and 3 were positive by only 1 of the 3 comparator tests. The sample size for NPA is smaller for *Campylobacter* because only a portion of the samples with negative results by Xpert GI Panel and FDA cleared molecular assay was tested with the complete composite comparator.

[b] Of 5 specimens with FN *Salmonella* results, 3 were also negative and 2 were not evaluable by discrepant analysis.

[c] One specimen with FN *V. cholerae* was also negative by discrepant analysis. One specimen with FP *V. cholerae* was negative by discrepant analysis.

[d] Of 10 specimens with FP *Yersinia* results, 6 were negative by all 3 comparator tests and 4 were positive by only 1 of the 3 comparator tests. The sample size for NPA is smaller for *Yersinia* because only a portion of the samples with negative results by Xpert GI Panel and FDA cleared molecular assay was tested with the complete composite comparator.

[e] Of 3 specimens with FN *Shigella* EIEC results, 1 was also negative, 1 was positive, and 1 was not evaluable by discrepant analysis. Of 4 FP *Shigella* EIEC results, 1 was also positive, 2 were negative, and 1 was not evaluable by discrepant analysis.

[f] Of 2 specimens with FP *Cryptosporidium* results, both specimens were not evaluable by discrepant analysis.

[g] One specimen with FN *Giardia* result was also negative by the discrepant analysis. Of 4 specimens with FP *Giardia* results, 3 were negative and 1 was not evaluable by discrepant analysis.

Multianalyte Detection - Mixed Infections

The number of specimens with multi-analyte detection by Xpert GI Panel are presented in Table 23. Each combination is listed, along with the total number of occurrences observed, and the number of instances where Xpert results for a given analyte were discrepant relative to the comparator.

Table 23: Multi-analyte Combinations Detected by Xpert GI Panel

Analyte 1	Analyte 2	Analyte 3	N of co-infections	N of discrepant co-infections	Discrepant analyte(s)
Cryptosporidium	Norovirus	N/A	1	1	Norovirus
Cryptosporidium	*Giardia*	N/A	1	0	N/A
Yersinia	Norovirus	N/A	2	2	Specimen 1: *Yersinia,* Norovirus; Specimen 2: *Yersinia*, Norovirus, *Shigella* EIEC[a]
Shigella EIEC	Norovirus	N/A	2	1	*Shigella* EIEC, Norovirus
Shigella EIEC	*Giardia*	N/A	1	1	*Shigella* EIEC
STEC *stx1*	*Cryptosporidium*	N/A	1	0	N/A
STEC *stx1*	*Shigella* EIEC	N/A	1	0	N/A
STEC *stx1*	STEC *stx2*	N/A	1	0	N/A
Salmonella	Norovirus	N/A	2	0	N/A
Salmonella	*Giardia*	Norovirus	1	1	Norovirus
Salmonella	*Yersinia*	N/A	1	0	N/A
Salmonella	STEC *stx1*	STEC *stx2*	1	0	N/A
Campylobacter	Norovirus	N/A	2	1	Norovirus
Campylobacter	*Cryptosporidium*	N/A	1	0	N/A
Campylobacter	*Yersinia*	N/A	2	0	N/A
Campylobacter	*Shigella* EIEC	N/A	3	1	*Campylobacter*
Campylobacter	*Shigella* EIEC	Norovirus	1	1	*Campylobacter,* Norovirus
Campylobacter	*Salmonella*	N/A	1	0	N/A
Total co-infections			25	9	N/A
Co-detection with 2 Analytes			22	7	
Co-detection with 3 Analytes			3	2	

[a]For specimen 2, *Yersinia* and Norovirus were not detected by the comparator method and *Shigella* EIEC was detected by the comparator method.

Of the 25 specimens with multi-analyte detections by Xpert GI Panel, 16 (64%; 16/25) agreed with the comparator. A total of 9 specimens (36%; 9/25) contained one or more analytes that were not concordant with the comparator test method.

Pre-selected Archived Specimens

Demographic information (sex, age, and healthcare setting) of the eligible pre-selected archived specimens is presented in Table 24.

Table 24: Demographics of Eligible Pre-selected Archived Specimens

Frozen Archived Specimens (N=103)	Number of Specimens (%)
Sex	
Female	51 (49.5%)
Male	52 (50.5%)
Age (years)	
<18	3 (2.9%)
18-21	2 (1.9%)

Frozen Archived Specimens (N=103)	Number of Specimens (%)
22-49	39 (37.9%)
50-64	21 (20.4%)
>=65	38 (36.9%)
Healthcare Setting where specimen was collected	
ER patient	21 (20.4%)
Inpatient/Hospitalized	14 (13.6%)
Outpatient	25 (24.3%)
Unknown	43 (41.7%)

Archived specimens (n=103) were pre-selected for *Salmonella*, *Shigella* EIEC, *Cryptosporidium*, *Giardia*, and Norovirus and deemed eligible for inclusion in the study. The clinical performance of each Xpert GI Panel analyte was compared to that of an FDA cleared molecular assay and/or a composite of 2 PCR assays followed by bi-directional sequencing. Specimens with discrepant results were investigated on an independent FDA cleared molecular assay. For each analyte in the Xpert GI Panel, the performance (Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), and the 95% confidence interval (CI)) of the Xpert GI Panel as compared to the comparator method in pre-selected archived specimens is presented in Table 25.

Table 25: Clinical Performance of Xpert GI Panel in Pre-selected Archived Specimens

Analyte	Total	Positive Percent Agreement				Negative Percent Agreement			
		TP	FN	PPA (%)	95%CI	TN	FP	NPA (%)	95%CI
Salmonella	68	6	1[a]	85.7	48.7 - 97.4	61	0	100.0	94.1 - 100.0
Shigella EIEC	68	15	0	100.0	79.6 - 100.0	52	1[b]	98.1	90.1 - 99.7
Cryptosporidium	68	3	1[c]	75	30.1 - 95.4	64	0	100.0	94.3 - 100.0
Giardia	68	13	0	100.0	77.2 - 100.0	55	0	100.0	93.5 - 100.0
Norovirus GI/GII	35	17	0	100.0	81.6 - 100.0	18	0	100.0	82.4 - 100.0

Abbreviations: CI, confidence interval; FN, false negative; FP, false positive; N/A, not available; NPA, negative percent agreement; PPA, positive percent agreement; TN, true negative; TP, true positive

[a]One specimen with FN *Salmonella* result was also negative by discrepant analysis.

[b]One specimen with FP *Shigella* EIEC was negative by discrepant analysis.

[c]One specimen with FN *Cryptosporidium* result was also negative by discrepant analysis.

Contrived Samples

A total of 468 contrived samples were included in the study to supplement the sample size due to the low observed prevalence for *V. parahaemolyticus*, *V. cholerae*, *Yersinia*, *Shigella* EIEC, STEC *stx1*, STEC *stx2*, *Cryptosporidium*, and *Giardia* in prospectively collected clinical specimens. The contrived samples were prepared by spiking representative strains (multiple strains per pathogen) at concentrations ranging from <3x the analytical limit of detection (LoD) to >800x LoD into unique negative clinical stool matrix that were confirmed negative by the Xpert GI Panel prior to preparation. A total of 65 *V. parahaemolyticus*, 65 *V. cholerae*, 45 *Yersinia*, 15 *Shigella* EIEC, 65 STEC *stx1*, 65 STEC *stx2*, 32 *Cryptosporidium*, and 35 *Giardia* positive samples were contrived and tested with 81 negative samples in a blinded fashion at a minimum of 3 sites.

The performance of the Xpert GI Panel in contrived samples was calculated relative to the expected result and presented in Table 26.

Table 26: Clinical Performance of Xpert GI Panel using Eligible Contrived Specimens

Analyte	Total	Positive Percent Agreement				Negative Percent Agreement			
		TP	FN	PPA (%)	95%CI	TN	FP	NPA (%)	95%CI
V. parahaemolyticus	146	63	2[a]	96.9	89.5 - 99.2	81	0	100.0	95.5 - 100.0
V. cholerae	146	65	0	100.0	94.4 - 100.0	81	0	100.0	95.5 - 100.0
Yersinia	126	45	0	100.0	92.1 - 100.0	81	0	100.0	95.5 - 100.0
Shigella EIEC	96	15	0	100.0	79.6 - 100.0	81	0	100.0	95.5 - 100.0
STEC stx1	146	65	0	100.0	94.4 - 100.0	81	0	100.0	95.5 - 100.0
STEC stx2	146	65	0	100.0	94.4 - 100.0	81	0	100.0	95.5 - 100.0
Cryptosporidium	113	32	0	100.0	89.3 - 100.0	81	0	100.0	95.5 - 100.0
Giardia	116	35	0	100.0	90.1 - 100.0	81	0	100.0	95.5 - 100.0

Abbreviations: CI, confidence interval; FN, false negative; FP, false positive; NPA, negative percent agreement; PPA, positive percent agreement; TN, true negative; TP, true positive

[a]Of 2 specimens with FN *V. parahaemolyticus* results, both were contrived samples spiked with the same strain that were not detected. It is possible that variability in test performance was due to variability of the clinical stool matrix composition.

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

Expected values for each analyte as determined by Xpert GI Panel in prospectively collected fresh specimens from individuals suspected of gastrointestinal infection stratified by age group (years) are presented in Table 27.

Table 27: Expected Values per analyte by Xpert GI Panel

Pathogen	Overall[a]	<18	18-21	22-49	50-64	≥65
Campylobacter	4.3% (67/1568)	5.9% (11/185)	17.2% (5/29)	4.6% (18/394)	3.9% (13/336)	3.2% (20/624)
Salmonella	3.3% (51/1568)	6.5% (12/185)	6.9% (2/29)	4.1% (16/394)	3.3% (11/336)	1.6% (10/624)
V. parahaemolyticus	0.0% (0/1505)	0.0% (0/179)	0.0% (0/29)	0.0% (0/377)	0.0% (0/321)	0.0% (0/599)
V. cholerae	0.1% (1/1568)	0.0% (0/185)	0.0% (0/29)	0.3% (1/394)	0.0% (0/336)	0.0% (0/624)
Yersinia	1.6% (25/1568)	2.2% (4/185)	0.0% (0/29)	1.0% (4/394)	1.5% (5/336)	1.9% (12/624)
***Shigella* EIEC**	2.4% (38/1568)	3.2% (6/185)	0.0% (0/29)	4.3% (17/394)	2.4% (8/336)	1.1% (7/624)
STEC *stx1*	0.4% (6/1497)	0.6% (1/173)	0.0% (0/25)	0.3% (1/374)	0.6% (2/320)	0.3% (2/605)
STEC *stx2*	0.2% (3/1497)	0.0% (0/173)	0.0% (0/25)	0.0% (0/374)	0.3% (1/320)	0.3% (2/605)
Cryptosporidium	2.2% (34/1568)	5.4% (10/185)	0.0% (0/29)	3.8% (15/394)	2.1% (7/336)	0.3% (2/624)
Giardia	1.5% (24/1568)	1.6% (3/185)	0.0% (0/29)	3.8% (15/394)	0.9% (3/336)	0.5% (3/624)
Norovirus	4.7% (72/1521)	7.2% (13/181)	0.0% (0/29)	5.0% (19/381)	3.4% (11/327)	4.8% (29/603)

[a] Including prospectively collected clinical specimens with valid results for both the Xpert GI Panel and the comparator method. For *Campylobacter* and *Yersinia*, specimens with valid results for Xpert GI Panel and the FDA cleared molecular comparator were included.

The prevalence of multi-analyte combinations detected by the Xpert GI Panel is presented in Table 28**.** The Xpert GI Panel detected a total of 25 specimens with co-detections among the 1429 prospectively collected specimens with valid test results for all 11 pathogens by both Xpert GI Panel and the comparator method. This represents 1.7% of all prospectively collected specimens with valid test results for all 11 pathogens.

Table 28: Expected Values for Analytes in Multi-analyte Detections by Xpert GI Panel

Multianalyte Detection Combinations[a]	Number of Specimens with Mixed Infections (n/N)	Prevalence of Mixed infections (%)
Cryptosporidium and Norovirus	1/25	4.0%
Cryptosporidium and *Giardia*	1/25	4.0%
Yersinia and Norovirus	2/25	8.0%
Shigella EIEC and Norovirus	2/25	8.0%
Shigella EIEC and *Giardia*	1/25	4.0%
STEC *stx1* and *Cryptosporidium*	1/25	4.0%
STEC *stx1* and *Shigella* EIEC	1/25	4.0%
STEC *stx1* and STEC *stx2*	1/25	4.0%
Salmonella and Norovirus	2/25	8.0%
Salmonella, *Giardia*, and Norovirus	1/25	4.0%
Salmonella and *Yersinia*	1/25	4.0%
Salmonella, STEC *stx1*, and STEC *stx2*	1/25	4.0%
Campylobacter and Norovirus	2/25	8.0%
Campylobacter and *Cryptosporidium*	1/25	4.0%
Campylobacter and *Yersinia*	2/25	8.0%
Campylobacter and *Shigella* EIEC	3/25	12.0%
Campylobacter, *Shigella* EIEC, and Norovirus	1/25	4.0%
Campylobacter and *Salmonella*	1/25	4.0%

[a] Including 1429 specimens with valid test results for all 11 target pathogens by both Xpert GI Panel and comparator method. For analytes where the comparator method was a composite of 3 FDA-cleared molecular assays, specimens with valid results for Xpert GI Panel and the FDA cleared molecular comparator were included.

F Other Supportive Instrument Performance Characteristics Data:

Not applicable

VIII Proposed Labeling:

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

IX Conclusion:

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