

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY**

**A. 510(k) Number:**

K142501

**B. Purpose for Submission:**

Clearance of new device

**C. Measurand:**

Target RNA sequences from norovirus genogroup I and genogroup II

**D. Type of Test:**

An *in vitro* molecular diagnostic test for the direct, qualitative detection and differentiation of norovirus genogroup I and genogroup II RNA from stool specimens

**E. Applicant:**

Cepheid

**F. Proprietary and Established Names:**

Xpert<sup>®</sup> Norovirus

**G. Regulatory Information:**

1. Regulation section: 21 CFR 866.3990
2. Classification: Class II
3. Product code: PIQ, OOI
4. Panel: Microbiology (83)

**H. Intended Use:**

1. Intended use(s):

The Cepheid Xpert Norovirus Assay, performed on the GeneXpert<sup>®</sup> Instrument Systems, is a qualitative *in vitro* diagnostic test for the identification and differentiation of

norovirus genogroup I and genogroup II RNA from raw or unpreserved unformed stool specimens collected from individuals with symptoms of acute gastroenteritis. The test utilizes automated real-time reverse transcriptase polymerase chain reaction (RT-PCR) to detect norovirus RNA. The Xpert Norovirus Assay is intended to aid in the diagnosis of norovirus infections when used in conjunction with clinical evaluation, laboratory findings, and epidemiological information. The assay also aids in the detection and identification of norovirus infections in the context of outbreaks.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

GeneXpert<sup>®</sup> Instrument Systems (Software version 4.3 or higher)

**I. Device Description:**

The Xpert Norovirus Assay is a, automated *in vitro* diagnostic test for detection and differentiation of nucleic acid sequences for norovirus genogroup I and genogroup II from human stool specimens collected from individuals with symptoms of acute gastroenteritis. The test utilizes automated, multiplex real-time, reverse transcriptase polymerase chain reaction (RT-PCR) to detect specific viral gene sequences associated with norovirus genogroup I and genogroup II.

The Xpert Norovirus Assay is performed on the Cepheid GeneXpert Instrument Systems (GeneXpert Dx, GeneXpert Infinity-48, GeneXpert Infinity-48s, and GeneXpert Infinity-80 systems). The GeneXpert Instrument System platform automates sample preparation, amplification and real-time detection. The GeneXpert Instrument Systems require the use of single-use, disposable cartridges (the Xpert Norovirus Cartridges) that hold the PCR reagents and host the PCR process.

The Xpert Norovirus Assay includes reagents for the detection and differentiation of nucleic acid sequences for norovirus genogroup I and genogroup II human stool specimens collected from patients with symptoms of acute gastroenteritis. All reagents except the Sample Reagent are contained pre-loaded in the cartridge. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge. The SPC is present to control for adequate processing of the target viruses and to monitor the presence of inhibitors in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity and dye stability.

The single-use, multi-chambered fluidic cartridges are designed to complete sample

preparation and real-time RT-PCR for detection and differentiation of norovirus genogroup I and genogroup II viral RNA in 90 minutes or less. The GeneXpert Instrument Systems, comprised of the GeneXpert Dx Systems and the GeneXpert Infinity Systems, have 1 to 80 randomly accessible modules, depending upon the instrument, that are each capable of performing separate sample preparation and real-time PCR and RT-PCR tests. Each module contains a syringe drive for dispensing fluids (i.e., the syringe drive activates the plunger that works in concert with the rotary valve in the cartridge to move fluids between chambers), an ultrasonic horn for lysing cells or spores, and a proprietary I-CORE<sup>®</sup> thermocycler for performing real-time PCR and RT-PCR and detection.

Specimens are collected following the user's institution standard procedures for collecting stool specimens for norovirus testing and sent to the GeneXpert<sup>®</sup> testing area for processing. The specimen may be stored at 2–8 °C for up to two days prior to processing. When ready to process the specimen, a single-use disposable dry swab is used for transfer of the stool specimen to the Sample Reagent bottle that is provided with the Xpert Norovirus Assay kit. The user vortexes the capped Sample Reagent bottle for 10 seconds and transfers the entire contents to the sample chamber in the top of the disposable fluidic cartridge with a transfer pipette. The user initiates a test from the system user interface and places the cartridge into the GeneXpert instrument platform, which performs hands-off real-time, multiplex polymerase chain reaction (PCR) for detection of RNA. The results are automatically generated at the end of the process in a report that can be viewed and printed.

The Xpert Norovirus Assay kit contains sufficient reagents to process 10 specimens or quality control samples.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

Luminex xTAG<sup>®</sup> Gastrointestinal Pathogen Panel (xTAG GPP)

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

K121894

3. Comparison with predicate:

Similarities		
	Device	Predicate Device
Item	Xpert Norovirus (K142501)	Luminex xTAG GPP (K121894)
General Intended Use	<p>The Cepheid Xpert Norovirus Assay, performed on the GeneXpert<sup>®</sup> Instrument Systems, is a qualitative <i>in vitro</i> diagnostic test for the identification and differentiation of norovirus genogroup I and genogroup II RNA from raw or unpreserved unformed stool specimens collected from individuals with symptoms of acute gastroenteritis. The test utilizes automated real-time reverse transcriptase polymerase chain reaction (RT-PCR) to detect norovirus RNA. The Xpert Norovirus Assay is intended to aid in the diagnosis of norovirus infections when used in conjunction with clinical evaluation, laboratory findings, and epidemiological information. The assay also aids in the detection and identification of norovirus infections in the context of outbreaks.</p>	<p>The xTAG<sup>®</sup> Gastrointestinal Pathogen Panel (GPP) is a multiplexed nucleic acid test intended for the simultaneous qualitative detection and identification of multiple viral, parasitic, and bacterial nucleic acids in human stool specimens from individuals with signs and symptoms of infectious colitis or gastroenteritis. The following pathogen types, subtypes and toxin genes are identified using the xTAG<sup>®</sup> GPP:</p> <ul style="list-style-type: none"> <li>● <i>Campylobacter</i> (<i>C. jejuni</i>, <i>C. coli</i> and <i>C. lari</i> only)</li> <li>● <i>Clostridium difficile</i> (<i>C. difficile</i>) toxin A/B</li> <li>● <i>Cryptosporidium</i> (<i>C. parvum</i> and <i>C. hominis</i> only)</li> <li>● <i>Escherichia coli</i> (<i>E. coli</i>) O157</li> <li>● Enterotoxigenic <i>Escherichia coli</i> (ETEC) LT/ST</li> <li>● <i>Giardia</i> (<i>G. lamblia</i> only - also known as <i>G. intestinalis</i> and <i>G. duodenalis</i>)</li> <li>● Norovirus GI/GII</li> <li>● Rotavirus A</li> <li>● <i>Salmonella</i></li> <li>● Shiga-like Toxin producing <i>E. coli</i> (STEC) stx 1/stx 2</li> <li>● <i>Shigella</i> (<i>S. boydii</i>, <i>S. sonnei</i>, <i>S. flexneri</i> and <i>S. dysenteriae</i>)</li> </ul> <p>The detection and identification of specific gastrointestinal microbial nucleic acid from individuals exhibiting signs and symptoms of gastrointestinal infection aids in the diagnosis of gastrointestinal infection when used in conjunction with clinical evaluation, laboratory findings and epidemiological information. A gastrointestinal microorganism multiplex nucleic acid-based assay also</p>

Similarities		
	Device	Predicate Device
Item	Xpert Norovirus (K142501)	Luminex xTAG GPP (K121894)
		<p>aids in the detection and identification of acute gastroenteritis in the context of outbreaks.</p> <p><b>xTAG<sup>®</sup> GPP positive results are presumptive and must be confirmed by FDA-cleared tests or other acceptable reference methods.</b></p> <p>The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Confirmed positive results do not rule out co-infection with other organisms that are not detected by this test, and may not be the sole or definitive cause of patient illness. Negative xTAG Gastrointestinal Pathogen Panel 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.</p> <p><b>xTAG<sup>®</sup> GPP is not intended to monitor or guide treatment for <i>C. difficile</i> infections.</b></p> <p>The xTAG<sup>®</sup> GPP is indicated for use with the Luminex<sup>®</sup> MAGPIX<sup>®</sup> instrument.</p>
Specimen Types	Human stool	Human stool
Test Principle	Real-time reverse transcriptase polymerase chain reaction (RT-PCR)	Real-time reverse transcriptase polymerase chain reaction (RT-PCR)

<b>Differences</b>		
	<b>New Device</b>	<b>Predicate Device</b>
<b>Item</b>	<b>Xpert Norovirus (K142501)</b>	<b>Luminex xTAG GPP (K121894)</b>
Product Code	PIQ	PCH
Analyte Detected	Detects and differentiates between Norovirus GI/GII.	Detects norovirus but does not differentiate between Norovirus GI/GII.
Technology Principles of Operation	Amplification: multiplex real-time RT-PCR Detection: fluorogenic target-specific hybridization	Multiplex RT-PCR and multiplex target specific primer extension followed by fluorescence-activated sorting of labeled beads coupled to streptavidin-conjugated biotinylated products.
Sample Pre-treatment	Place swab dipped in specimen into provided tube of sample reagent. Vortex 10 seconds.	30-45 minutes of manual sample pre-treatment preparation.
Nucleic Acid Isolation and purification	Self-contained and automated in the GeneXpert Cartridge and GeneXpert Instrument Systems. No reagent preparation - all reagents are contained in the cartridge.	Multi-step manual reagent preparation for use with NucliSENS <sup>®</sup> EasyMAG extraction Kit (BioMerieux).
Instrument Systems	Cepheid GeneXpert Dx Systems and GeneXpert Infinity Systems	Nucleic Acid Purification System PCR Thermocycler Luminex <sup>®</sup> 100/200 <sup>®</sup> or MAGPIX <sup>®</sup> instruments
Internal Controls	Sample processing control (SPC) and probe check control (PCC) integrated in assay/instrument system.  External controls available but not provided.	Internal control added to each sample. External control processed with each batch of samples.
Time to obtain test results	Test results: 90 minutes or less for sample preparation and RT-PCR.	Test results in <5 hours, not including 30-45 minute sample pre-treatment.

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

Not Applicable

## L. Test Principle:

The Xpert Norovirus Assay is automated and utilizes reverse real-time RT-PCR to detect specific viral gene sequences associated with norovirus genogroup I and genogroup II. The primers and probes in the Xpert Norovirus Assay are designed to amplify and detect unique gene sequences within the conserved region of the Norovirus genome. The stool specimens are collected from individuals with symptoms of acute gastroenteritis and transported to the laboratory in a clean container. A swab is inserted into the stool specimen and then placed in a tube containing sample reagent. Following a brief vortexing, the eluted sample is transferred into the sample chamber of the disposable fluidic cartridge (the GeneXpert cartridge). The GeneXpert cartridge is loaded onto the GeneXpert<sup>®</sup> Instrument System platform, which performs hands-off automated sample processing and real-time RT-PCR for identification and differentiation of norovirus genogroup I and genogroup II. Test results are obtained in 90 minutes.

## M. Performance Characteristics (if/when applicable):

### 1. Analytical performance:

#### a. *Precision/Reproducibility:*

A reproducibility study was performed using a panel of 7 specimens with varying concentrations of norovirus GI and norovirus GII; the panel was tested two times on five different days by two different operators, at each of three sites (7 samples x 2 times/day x 5 days x 2 operators x 3 sites). One lot of Xpert Norovirus Assay cartridges was used at each of the 3 testing sites. Results are presented in the table below.

#### Summary of Reproducibility Results

Sample ID	Site 1	Site 2	Site 3	% Total Agreement
Negative	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
GI - High Negative	30.0% (6/20)	15.0% (3/20)	30.0% (6/20)	25.0% (15/60)
GI - Low Positive	100% (20/20)	85.0% (17/20)	95.0% (19/20)	93.3% (56/60)
GI - Mod Positive	100% (19/19)	100% (20/20)	100% (20/20)	100% (59/59) <sup>a</sup>
GII - High Negative	25.0% (5/20)	30.0% (6/20)	35.0% (7/20)	30.0% (18/60)
GII - Low Positive	100% (20/20)	95.0% (19/20)	90.0% (18/20)	95.0% (57/60)
GII - Mod Positive	95.0% (19/20)	100% (20/20)	100% (20/20)	98.3% (59/60)

<sup>a</sup>One sample 2x indeterminate.

The reproducibility of the Xpert Norovirus Assay was also evaluated in terms of the fluorescence signal expressed in Ct values for each target detected. The mean,

standard deviation (SD), and coefficient of variation (CV) between-sites, between-days, and between-operators for each panel member are presented in the table below.

### Summary of Reproducibility Data

Sample	N <sup>a</sup>	Mean Ct	Between-Site		Between-Day		Between-Operator		Within-Assay		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Negative	60	31.9	0.17	0.5	0.06	0.2	0.06	0.2	0.26	0.8	0.32	1.0
GI - High Negative	60	39.4	0	0	0.46	1.2	0	0	1.80	4.6	1.86	4.7
GI - Low Positive	59	37.9	0.29	0.8	0	0	0.36	1.0	1.03	2.7	1.13	3.0
GI - Mod Positive <sup>b</sup>	57	34.7	0.09	0.2	0.07	0.2	0	0	0.41	1.2	1.01	1.2
GII - High Negative	54	38.9	0	0	0	0	0.77	2.0	1.77	4.5	1.93	5.0
GII - Low Positive	60	37.3	0	0	0	0	0.58	1.6	1.33	3.6	1.45	3.9
GII - Mod Positive <sup>b</sup>	59	34.3	0.22	0.6	0	0	0	0	0.45	1.3	0.50	1.5

<sup>a</sup> Results with non-zero Ct values out of 60.

<sup>b</sup> 3 sample outliers (2 GI Mod Pos and 1 GII Mod Pos) that were more than 5 standard deviations from the mean were considered outliers and were removed from the analysis.

**Instrument System Precision:** An in-house precision study was conducted to compare the performance of the GeneXpert Dx and the GeneXpert Infinity instrument systems. A panel of 7 samples with varying concentrations of norovirus GI and norovirus GII was tested on 12 different days by two operators. Each operator conducted four runs of each panel member per day on each of the two instrument systems (7 samples x 4 times/day x 12 days x 2 operators x 2 instrument systems). Three lots of Xpert Norovirus Assay cartridges were used for the study. The Xpert Norovirus Assay was performed according to the Xpert Norovirus Assay procedure. Results are presented in the table below.

### Summary of Instrument System Precision Results (Dx vs. Infinity)

Sample	GeneXpert Dx			Infinity			% Total Agreement by Sample
	Op 1	Op 2	Inst	Op 1	Op 2	Inst	
Negative	100% (48/48)	100% (48/48)	100% (96/96)	100% (48/48)	100% (48/48)	100% (96/96)	100% (192/192)
GI - High Negative	14.6% (7/48)	10.4% (5/48)	12.5% (12/96)	14.6% (7/48)	25.0% (12/48)	19.8% (19/96)	16.2% (31/192)
GI - Low Positive	100% (48/48)	97.9% (47/48)	99.0% (95/96)	97.9% (47/48)	97.9% (47/48)	97.9% (94/96)	98.4% (189/192)
GI - Mod Positive	100% <sup>a</sup> (47/47)	100% (48/48)	100% (95/95)	100% (48/48)	100% (48/48)	100% (96/96)	100% (191/191)
GII - High Negative	25.0% (12/48)	29.2% (14/48)	27.1% (26/96)	29.2% (14/48)	31.3% (15/48)	30.2% (29/96)	28.7% (55/192)
GII - Low Positive	89.6% (43/48)	89.6% (43/48)	89.6% (86/96)	83.3% (40/48)	95.7% (44/46)	87.5% (84/96)	88.5% (170/192)
GII - Mod Positive	100% (48/48)	100% (48/48)	100% (96/96)	100% (48/48)	100% <sup>b</sup> (47/47)	100% (95/95)	100% (191/191)

<sup>a</sup> One GI Mod Pos sample not tested.

<sup>b</sup> One GII Mod Pos sample indeterminate and not retested.

The precision of the Xpert Norovirus Assay was also evaluated in terms of the fluorescence signal expressed in Ct values for each target detected. The mean, standard deviation (SD), and coefficient of variation (CV) between-instruments, between-lots, between-days, between-operators, and within-assays for each panel member are presented in the table below.

### Summary of Precision Data

Sample	N <sup>a</sup>	Mean Ct	Between-Instrument		Between-Lot		Between-Day		Between-Operator		Within-Assay		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Negative	192	31.8	0	0	0.44	1.4	0	0	0.08	0.2	0.39	1.2	0.59	1.9
GI - High Negative	188	38.6	0.19	0.5	0.25	0.7	0.18	0.5	0	0	1.40	3.6	1.45	3.8
GI - Low Positive	192	37.1	0.39	1.1	0.26	0.7	0.19	0.5	0	0	0.95	2.6	1.08	2.9
GI - Mod Positive	191	34.0	0	0	0.36	1.1	0.04	0.1	0.08	0.2	0.38	1.1	0.53	1.6
GII - High Negative	178	38.7	0.16	0.4	0	0	0.29	0.7	0	0	2.03	5.3	2.06	5.3
GII - Low Positive	187	37.6	0.10	0.2	0	0	0	0	0.45	1.2	1.65	4.4	1.71	4.6
GII - Mod Positive	191	34.3	0	0	0.09	0.2	0	0	0.17	0.5	0.42	1.2	0.46	1.3

<sup>a</sup> Results with non-zero Ct values out of 192.

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

The Xpert Norovirus Assay includes a Sample Processing Control (SPC) and a Probe Check Control (PCC). The success rate was >95% on the first attempt and was acceptable.

Recommended external quality control organisms were also tested at the sites. External controls should be used in accordance with accrediting institutions and government regulations. External controls are commercially available and not provided in the test kit; however, the catalog numbers for a source of external controls are provided in the “Materials Available but Not Provided” section of the Xpert Norovirus Assay Package Insert.

*c. Detection limit:*

The limit of detection (LoD) study was performed to evaluate the analytical sensitivity of the Xpert Norovirus Assay using norovirus GI.3 and norovirus GII.4 diluted into a negative stool matrix. The LoD of the Xpert Norovirus Assay is defined as the lowest concentration (copies/mL) per sample that can be reproducibly distinguished from negative samples with 95% confidence. The LoD was determined using seven (7) dilutions per norovirus genotype with a minimum of 23 replicates per dilution using two reagent lots across 3 testing days. Dilutions were prepared with a pooled negative clinical stool matrix to achieve final concentrations ranging from  $3.32 \times 10^4$

copies/mL to  $3.98 \times 10^6$  copies/mL for the norovirus GI.3 genotype and concentrations ranging from  $1.03 \times 10^4$  copies/mL to  $4.97 \times 10^5$  copies/mL for the norovirus GII.4 genotype.

The LoD was estimated for each reagent lot using probit regression and the highest LoD observed per genotype from both lots was further verified by testing 20 replicates (verification of LoD).

The LoDs of the Xpert Norovirus Assay for the two genotypes are presented in the table below.

**Limit of Detection of the Xpert Norovirus Assay**

<b>Norovirus</b>	<b>Limit of Detection (95% CI)</b>
GI.3	$5.7 \times 10^5$ (copies/mL) ( $4.64 \times 10^5 - 6.67 \times 10^5$ )
GII.4	$3.0 \times 10^5$ (copies/mL) ( $1.25 \times 10^5 - 1.78 \times 10^5$ )

*d. Analytical Reactivity (Inclusivity)*

The analytical reactivity of the Xpert Norovirus Assay was evaluated against thirty-one genotypes representing norovirus genogroups GI and GII. The studies were carried out with positive clinical stool specimens or RNA transcripts (when clinical specimens were not readily available) representing multiple GI and GII genotypes at levels near the LoD. Positive clinical stool specimens were tested for six (6) GI genotypes and fourteen (14) GII genotypes including the recently circulating Norovirus GII.4 Sydney strain and the previously dominant Norovirus GII.4 New Orleans strain with the Xpert Norovirus Assay. Eleven (11) RNA transcripts were tested for three (3) GI genotypes and eight (8) GII genotypes with the Xpert Norovirus Assay. The results of the analytical reactivity (inclusivity) study demonstrated that the Xpert Norovirus Assay appropriately identified all the temporally and geographically diverse samples selected to represent the range of norovirus genotypes. The table below shows the data for the analytical studies.

**Analytical Reactivity Results of the Xpert Norovirus Assay**

<b>Norovirus Strain</b>	<b>Estimated Concentration (copies/mL)<sup>a</sup></b>	<b>Result</b>	
		<b>GI</b>	<b>GII</b>
GI.1	$9.0 \times 10^6$	POS	NEG
GI.2	$3.7 \times 10^8$	POS	NEG

Norovirus Strain	Estimated Concentration (copies/mL) <sup>a</sup>	Result	
		GI	GII
GI.3	1.4 x 10 <sup>6</sup>	POS	NEG
GI.4	1.0 x 10 <sup>5</sup>	POS	NEG
GI.5 <sup>b</sup>	2.5 x 10 <sup>5</sup>	POS	NEG
GI.6 <sup>b</sup>	2.5 x 10 <sup>5</sup>	POS	NEG
GI.7 <sup>b</sup>	2.5 x 10 <sup>5</sup>	POS	NEG
GI.8	3.7 x 10 <sup>5</sup>	POS	NEG
GI.14	3.0 x 10 <sup>6</sup>	POS	NEG
GII.1	3.6 x 10 <sup>6</sup>	NEG	POS
GII.2	1.1 x 10 <sup>5</sup>	NEG	POS
GII.3 <sup>b</sup>	1.3 x 10 <sup>3</sup>	NEG	POS
GII.4 (2006a)	1.2 x 10 <sup>5</sup>	NEG	POS
GII.4 (2006b)	2.4 x 10 <sup>5</sup>	NEG	POS
GII.4 (2008)	4.3 x 10 <sup>5</sup>	NEG	POS
GII.4 (2009) New Orleans	1.7 x 10 <sup>5</sup>	NEG	POS
GII.4 (2010)	9.6 x 10 <sup>4</sup>	NEG	POS
GII.4 (2012) Sydney	1.2 x 10 <sup>5</sup>	NEG	POS
GII.5 <sup>b</sup>	1.3 x 10 <sup>3</sup>	NEG	POS
GII.6 <sup>b</sup>	1.3x 10 <sup>3</sup>	NEG	POS
GII.7	8.0 x 10 <sup>4</sup>	NEG	POS
GII.8 <sup>b</sup>	1.3 x 10 <sup>3</sup>	NEG	POS
GII.9 <sup>b</sup>	1.3 x 10 <sup>3</sup>	NEG	POS
GII.10 <sup>b</sup>	1.3 x 10 <sup>3</sup>	NEG	POS
GII.11	2.6 x 10 <sup>5</sup>	NEG	POS
GII.12	5.7 x 10 <sup>5</sup>	NEG	POS
GII.13	6.9 x 10 <sup>5</sup>	NEG	POS
GII.14	1.5 x 10 <sup>5</sup>	NEG	POS
GII.15	1.7 x 10 <sup>5</sup>	NEG	POS
GII.16 <sup>b</sup>	1.3x 10 <sup>3</sup>	NEG	POS
GII.17 <sup>b</sup>	1.3x 10 <sup>3</sup>	NEG	POS

<sup>a</sup> An estimated concentration or titer was provided based on a Ct value; because of the difficulty in culturing norovirus particles, an exact concentration cannot be provided. The Ct value for each clinical specimen in the inclusivity study was extrapolated to the titer obtained from the LoD study for well-characterized GI and GII samples using a standard curve at CDC

<sup>b</sup> Naked RNA transcripts were used for these strains because clinical samples were not available at the time of testing.

e. Analytical specificity (Cross Reactivity):

The analytical specificity of the Xpert Norovirus Assay was evaluated by testing a panel of 68 organisms, consisting of 54 bacteria, 1 fungus, 9 viruses, and 4 parasites, representing common gastroenteritis pathogens or those potentially encountered in stool. All organisms were tested with a minimum of three replicates. The bacteria and fungus were tested at concentrations  $\geq 10^6$  CFU/mL. The viruses were tested at concentrations  $\geq 10^5$  TCID<sub>50</sub>/mL, with the exception of two viruses obtained from clinical samples with unknown concentrations. The parasites were tested at concentrations  $\geq 10^6$  copies/mL. All organisms tested were correctly reported as Norovirus GI not DETECTED; Noro GII not DETECTED by the Xpert Norovirus Assay. Results are shown in the table below.

**Analytical Specificity of the Xpert Norovirus Assay**

Organism	Strain ID	Concentration
<i>Acinetobacter baumannii</i>	CCUG 3477	$>3.0 \times 10^8$ CFU/mL
<i>Anaerococcus prevotii</i> <sup>a</sup>	ATCC 9321	$6.7 \times 10^8$ CFU/mL
<i>Bacterioides fragilis</i> <sup>a</sup>	ATCC 25285	$1.4 \times 10^9$ CFU/mL
<i>Campylobacter coli</i>	ATCC 43478	$1.8 \times 10^8$ CFU/mL
<i>Campylobacter jejuni</i>	ATCC 33560	$1.3 \times 10^8$ CFU/mL
<i>Campylobacter lari</i>	ATCC 35221	$3.4 \times 10^7$ CFU/mL
<i>Citrobacter freundii</i>	ATCC 33128	$1.5 \times 10^9$ CFU/mL
<i>Clostridium difficile</i> <sup>a</sup>	ATCC 9689	$2.2 \times 10^8$ CFU/mL
<i>Clostridium sordelli</i> <sup>a</sup>	DSMZ 2141	$2.0 \times 10^8$ CFU/mL
<i>Eggerthella lenta</i>	ATCC 43055	$>3.0 \times 10^7$ CFU/mL
<i>Enterobacter cloacae</i>	ATCC 70021	$1.0 \times 10^9$ CFU/mL
<i>Enterococcus casseliflavus</i>	ATCC 25788	$1.0 \times 10^9$ CFU/mL
<i>Enterococcus faecalis</i>	ATCC 29212	$5.4 \times 10^8$ CFU/mL
<i>Enterococcus faecium</i>	ATCC 9756	$8.2 \times 10^8$ CFU/mL
<i>Enterococcus gallinarium</i>	ATCC 49573	$4.5 \times 10^8$ CFU/mL
<i>Escherichiacoli</i> O157:H7	ATCC 43888	$8.4 \times 10^8$ CFU/mL
<i>Escherichia coli</i> O26:H11	CDC 033014	$7.4 \times 10^8$ CFU/mL
<i>Escherichia coli</i> O45:H2	CDC 003039	$3.3 \times 10^8$ CFU/mL
<i>Escherichia coli</i> O103:H11	CDC 063008	$5.4 \times 10^8$ CFU/mL
<i>Escherichia coli</i> O11	CDC 201114	$6.9 \times 10^8$ CFU/mL

<b>Organism</b>	<b>Strain ID</b>	<b>Concentration</b>
<i>Escherichia coli</i> O121	CDC 023211	1.4 x 10 <sup>9</sup> CFU/mL
<i>Escherichia coli</i> O145	CDC 993311	7.1 x 10 <sup>8</sup> CFU/mL
<i>Escherichia hermannii</i>	ATCC 33650	1.5 x 10 <sup>9</sup> CFU/mL
<i>Fusobacterium necrophorum</i> <sup>a</sup>	ATCC 31647	9.6 x 10 <sup>8</sup> CFU/mL
<i>Helicobacter pylori</i>	CCUG 1784	1.5 x 10 <sup>8</sup> CFU/mL
<i>Klebsiella pneumonia</i>	ATCC 70063	1.2 x 10 <sup>9</sup> CFU/mL
<i>Lactobacillus jensenii</i>	ATCC 25258	4.0 x 10 <sup>8</sup> CFU/mL
<i>Listeria monocytogenes</i>	CCUG 3358	1.2 x 10 <sup>9</sup> CFU/mL
<i>Micrococcus luteus</i>	ATCC 4698	1.8 x 10 <sup>8</sup> CFU/mL
<i>Morganella morganii</i>	ATCC 49948	1.3x10 <sup>9</sup> CFU/mL
<i>Peptostreptococcus anaerobius</i> <sup>a</sup>	CCUG 7835	1.5 x 10 <sup>9</sup> CFU/mL
<i>Plesiomonas shigelloides</i>	ATCC 51903	3.1 x 10 <sup>8</sup> CFU/mL
<i>Prevotella oralis</i> <sup>a</sup>	ATCC 33269	1.2 x 10 <sup>9</sup> CFU/mL
<i>Proteus mirabilis</i>	ATCC 43071	1.1 x 10 <sup>9</sup> CFU/mL
<i>Proteus vulgaris</i>	ATCC 49132	1.8 x 10 <sup>9</sup> CFU/mL
<i>Providencia alcalifaciens</i>	CCUG 6325	1.8 x 10 <sup>9</sup> CFU/mL
<i>Providencia stuartii</i>	ATCC 49809	1.3 x 10 <sup>9</sup> CFU/mL
<i>Pseudomonas aeruginosa</i>	ATCC 27853	6.3 x 10 <sup>8</sup> CFU/mL
<i>Pseudomonas fluorescens</i>	ATCC 13525	>3.0 x 10 <sup>8</sup> CFU/mL
<i>Pseudomonas putida</i>	ATCC 49128	5.5 x 10 <sup>8</sup> CFU/mL
<i>Salmonella agona</i>	ATCC 51957	1.2 x 10 <sup>9</sup> CFU/mL
<i>Salmonella bongori</i>	ATCC 43975	1.7 x 10 <sup>9</sup> CFU/mL
<i>Salmonella enterica</i>	ATCC 13314	9.2 x 10 <sup>8</sup> CFU/mL
<i>Serratia marcescens</i>	ATCC 43862	3.8 x 10 <sup>8</sup> CFU/mL
<i>Shigella flexneri</i>	ATCC 12022	8.1 x 10 <sup>8</sup> CFU/mL
<i>Shigella sonnei</i>	ATCC 25931	>3.0 x 10 <sup>8</sup> CFU/mL
<i>Staphylococcus aureus</i>	ATCC 25923	8.8 x 10 <sup>8</sup> CFU/mL
<i>Staphylococcus epidermidis</i>	ATCC 14990	>3.0 x 10 <sup>7</sup> CFU/mL
<i>Streptococcus agalactiae</i> (GBS)	ATCC 12386	9.6 x 10 <sup>8</sup> CFU/mL

Organism	Strain ID	Concentration
<i>Streptococcus dysgalactiae</i>	ATCC 43078	7.2 x 10 <sup>8</sup> CFU/mL
<i>Streptococcus pyogenes</i>	ATCC 19615	5.5 x 10 <sup>8</sup> CFU/mL
<i>Vibrio cholerae</i> <sup>b</sup>	CCUG 9118	5.2 x 10 <sup>9</sup> copies/μL
<i>Vibrio parahaemolyticus</i>	ATCC 17802	3.8 x 10 <sup>8</sup> CFU/mL
<i>Yersinia enterocolitica</i>	ATCC 9610	7.1 x 10 <sup>8</sup> CFU/mL
<i>Adenovirus</i>	Type 31	3.6 x 10 <sup>5</sup> TCID <sub>50</sub> /mL
<i>Adenovirus</i>	Type 40	2.8 x 10 <sup>7</sup> TCID <sub>50</sub> /mL
<i>Adenovirus</i>	Type 41	4.6 x 10 <sup>7</sup> TCID <sub>50</sub> /mL
<i>Astrovirus</i> <sup>c</sup>	--	Not applicable <sup>d</sup>
<i>Coxsackievirus</i>	Type B5	1.4 x 10 <sup>5</sup> TCID <sub>50</sub> /mL
<i>Echovirus</i>	11	3.3 x 10 <sup>9</sup> TCID <sub>50</sub> /mL
<i>Parechovirus</i>	Type 6	1.9 x 10 <sup>7</sup> TCID <sub>50</sub> /mL
<i>Rotavirus</i>	Type Wa	1.0 x 10 <sup>6</sup> TCID <sub>50</sub> /mL
<i>Sapovirus</i> <sup>c</sup>	--	Not applicable <sup>e</sup>
<i>Candida albicans</i>	ATCC 10231	>3.0 x 10 <sup>7</sup> CFU/mL
<i>Blastocystis hominis</i> <sup>b</sup>	BT1	1.0 x 10 <sup>9</sup> copies/mL
<i>Cryptosporidium parvum</i> <sup>b</sup>	Iowa	6.1 x 10 <sup>9</sup> copies/mL
<i>Giardia lamblia</i> <sup>b</sup>	Portland-1	3.05 x 10 <sup>9</sup> copies/mL
<i>Entamoeba histolytica</i> <sup>b</sup>	ATCC 30459D	4.9 x 10 <sup>6</sup> copies/mL

<sup>a</sup> Strictly anaerobic bacteria.

<sup>b</sup> Tested as genomic DNA.

<sup>c</sup> Clinical sample.

<sup>d</sup> Ct values were in the range of 12-27.

<sup>e</sup> Ct values were in the range of 19-23.

#### f. Interference

Potentially interfering substances that may be present in stool were tested for effects on the performance of the Xpert Norovirus Assay. Potentially interfering substances included hemoglobin, mucin, cholesterol, triglycerides and whole blood, plus additional endogenous and exogenous substances listed in the following table.

Negative samples were tested in replicates of eight with each substance in a negative stool matrix to determine the effect on the performance of the sample processing control (SPC). Positive samples were tested in replicates of eight per substance with one Norovirus GI and one Norovirus GII clinical isolate near the LoD of the Xpert Norovirus Assay.

All results were compared to positive and negative controls prepared in negative stool matrix. All valid positive and negative control samples were correctly reported using the Xpert Norovirus Assay.

Inhibition of the Xpert Norovirus Assay was observed in the presence of benzalkonium chloride (1% (w/v), 0.2% (w/v), and 0.04% (w/v)). False negative test results were reported for the Norovirus GII target at 1% (w/v) benzalkonium chloride. In the presence of 5% (w/w) barium sulfate, a statistically significant inhibitory effect was observed on the Norovirus GII Ct in positive samples relative to the control sample (p-value <0.05). No statistically significant effect was observed on the Norovirus GII Ct relative to the control sample in the presence of 1% (w/w) barium sulfate.

No other potentially interfering substances were found to be inhibitory and no false-negatives were reported for these substances.

#### Potentially Interfering Substances in Xpert Norovirus Assay

<b>Endogenous substances</b>		
<b>Substance</b>	<b>Description /Active Ingredient</b>	<b>Concentration Tested</b>
Cholesterol	Fecal fat/Cholesterol	5 % (w/v)
Hemoglobin	Hemoglobin human	12.5 % (w/v)
Mucin	purified Mucin protein	5 % (w/v)
Steric acid/ Palmitic acid (1:1)	Fatty acids/Steric acid, Palmitic acid	5 % (w/w)
Triglyceride	Fecal fat/Triglyceride Mix	5 % (w/v)
Whole Blood	Human Whole Blood	10 % (v/v)
<b>Exogenous substances</b>		
<b>Substance</b>	<b>Description /Active Ingredient</b>	<b>Concentration Tested</b>
Acetaminophen	Acetaminophen	5 % (w/v)
Amoxicillin	Antibiotic/Amoxicillin	5 % (w/v)
Ampicillin	Ampicillin Sodium Salt	152 µmol/L
Aspartame	Aspartame	5 % (w/v)
Barium sulfate	Contrast medium/Barium sulfate	5 %, 1% (w/w)
Benzalkonium chloride Commercial alcohol	Antiseptic Towelettes/ Benzalkonium Chloride in ethanol	1 %, 0.2 %, 0.04 % (w/v)
Bismuth subsalicylate	Bismuth (III) Subsalicylate (an active ingredient in Peptobismol)	1 % (w/v)
CaCO <sub>3</sub>	Calcium Carbonate	5 % (w/v)
Hydrocortisone	Hydrocortisone	50 % (w/v)

Ibuprofen	Ibuprofen	5% (w/v)
Imodium	Loperamide HCl	5 % (v/v)
Kaopectate	Attapulgate	5 mg/mL
Metronidazole	Metronidazole	5 % (w/v)
Mycostatin	Nystatin	50 % (w/w)
Naprosyn	Naproxen Sodium	2.2 µmol/mL
Novaluzid	Mg(OH) <sub>2</sub> , Al(OH) <sub>3</sub> and MgCO <sub>3</sub>	5 % (w/v)
Polymyxin B sulfate Bacitracin zinc	Polysporin/Polymyxin B Sulfate and Bacitracin Zinc	50 % (w/v)
Pursennid	Sennaglycosides	5 % (w/v)
Rexall Mineral oil laxative	Mineral Oil	50 % (v/v)

*g. Carry-over Contamination Study*

A study was conducted to demonstrate that the single-use, self-contained GeneXpert cartridges prevent carry-over contamination in negative samples that are run immediately after very high positive samples have been run in the same GeneXpert module. The study consisted of a negative sample processed in a GeneXpert module immediately followed by a highly positive Norovirus GII sample processed in the same GeneXpert module. This testing scheme was repeated 21 times in two GeneXpert modules for a total of 42 runs for 20 positive and 22 negative specimens. All 19 positive samples were correctly reported as “Norovirus GI not Detected; Norovirus GII Detected” and one positive sample was reported as an Error. All 22 negative samples were correctly reported as “Norovirus GI not Detected; Norovirus GII not Detected.”

2. Comparison studies:

*a. Method comparison with predicate device:*

The clinical performance evaluation was performed against a composite reference test method.

*b. Matrix comparison:*

Not applicable

3. Clinical studies:

*a. Clinical Sensitivity:*

Not applicable

b. *Clinical specificity:*

Not applicable

c. *Other clinical supportive data (when a. and b. are not applicable):*

*Clinical Performance*

Performance characteristics of the Xpert Norovirus Assay were evaluated at seven institutions in the U.S. and Europe. The study specimens consisted of raw or unpreserved unformed stool specimens from subjects with symptoms of acute gastroenteritis. The Xpert Norovirus Assay performance was compared to a composite reference test method performed at the Centers for Disease Control and Prevention. A total of 1403 specimens were tested for norovirus GI by the Xpert Norovirus Assay and the composite reference test. Of the 1403 specimens, 914 were fresh, prospectively collected and 489 were frozen, archived specimens. A total of 1401 specimens were tested for norovirus GII by the Xpert Norovirus Assay and the composite reference test. Of the 1401 specimens, 914 were fresh, prospectively collected and 487 were frozen, archived specimens.

With fresh, prospectively collected specimens, the Xpert Norovirus Assay demonstrated a Positive Percent Agreement (PPA) of 100% and a Negative Percent Agreement (NPA) of 99.6% for detection of Norovirus GI, relative to the composite reference test. The Xpert Norovirus Assay demonstrated 98.5% PPA and 98.8% NPA for detection of Norovirus GII in fresh, prospectively collected specimens.

With frozen, archived specimens, the Xpert Norovirus Assay demonstrated 98.1% PPA and 94.6% NPA for detection of Norovirus GI, relative to the composite reference test. The Xpert Norovirus Assay demonstrated 100% PPA and 96.8% NPA for detection of Norovirus GII with frozen, archived specimens.

**Xpert Norovirus Assay Performance for GI  
vs. Composite Reference Test – Fresh Specimens**

		Composite Reference Test		
		POS	NEG	Total
Xpert Norovirus	POS	12	4	16
	NEG	0	898	898
	Total	12	902	914
		PPA	100% (95% CI: 73.5-100)	
		NPA	99.6% (95% CI: 98.9-99.9)	

**Xpert Norovirus Assay Performance for GII  
vs. Composite Reference Test – Fresh Specimens**

		Composite Reference Test		
		POS	NEG	Total
Xpert Norovirus	POS	64	10	74
	NEG	1	839	840
	Total	65	849	914
		PPA	98.5% (95% CI: 91.7-100)	
		NPA	98.8% (95% CI: 97.8-99.4)	

**Xpert Norovirus Assay Performance for GI  
vs. Composite Reference Test – Frozen Specimens**

		Composite Reference Test		
		POS	NEG	Total
Xpert Norovirus	POS	101	21	122
	NEG	2	365	367
	Total	103	386	489
		PPA	98.1% (95% CI: 93.2-99.8)	
		NPA	94.6% (95% CI: 91.8-96.6)	

**Xpert Norovirus Assay Performance for GII  
vs. Composite Reference Test – Frozen Specimens**

		Composite Reference Test		
		POS	NEG	Total
Xpert Norovirus	POS	109	12	121
	NEG	0	366	366
	Total	109	378	487
		PPA	100% (95% CI: 96.7-100)	
		NPA	96.8% (95% CI: 94.5-98.3)	

4. Clinical cut-off:

Not Applicable

5. Expected values/Reference range:

In the Xpert Norovirus Assay clinical study, a total of 914 prospectively collected, fresh, raw or unpreserved unformed stool specimens were included from seven study centers. The number and percentage of Norovirus GI and Norovirus GII positive cases, calculated by age group, are presented in the following table.

**Observed Prevalence of GI and GII by Age Group**

Age (Years)	No. of GI Positives	GI Observed Prevalence (%)	No. of GII Positives	GII Observed Prevalence (%)
0-1	0/8	0	0/8	0
>1-5	1/6	16.7	0/6	0
>5-12	0/10	0	1/10	10.0
>12-21	0/29	0	3/29	10.3
>21-65	9/520	1.7	35/520	6.7
>65	6/341	1.8	35/341	10.3
Total	16/914	1.8	74/914	8.1

**N. Proposed Labeling**

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

**O. Conclusion:**

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