DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

Cepheid
Scott Campbell, Ph.D., MBA
Executive Director, Clinical Affairs
904 Caribbean Drive
Sunnyvale CA 94089-1189

November 26, 2014

Re: K142501
Trade/Device Name: Xpert® Norovirus
Regulation Number: 21 CFR 866.3990
Regulation Name: Gastrointestinal Microorganism Multiplex Nucleic Acid-Based Assay
Regulatory Class: II
Product Code: PIQ, OOI
Dated: September 4, 2014
Received: September 5, 2014

Dear Dr. Campbell:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

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

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

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Uwe Scherf -S for

Sally A. Hojvat, M. Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K142501

Device Name
Xpert Norovirus

Indications for Use *(Describe)*
The Cepheid Xpert Norovirus Assay, performed on the GeneXpert® 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.

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

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

PLEASE DO NOT WRITE BELOW THIS LINE – CONTINUE ON A SEPARATE PAGE IF NEEDED.

FOR FDA USE ONLY

Concurrence of Center for Devices and Radiological Health (CDRH) *(Signature)*

510(k) Summary

As required by 21 CFR Section 807.92(c).

Submitted by:	Cepheid 904 Caribbean Drive Sunnyvale, CA 90489 Phone number: (408) 400-8460 Fax number: (847) 510-0539
Contact:	Kerry J. Flom, Ph.D.
Date of Preparation:	October 28, 2014
Device:	
Trade name:	Xpert® Norovirus
Common name:	Xpert Norovirus Assay
Type of Test:	Automated real-time reverse transcription-polymerase chain reaction (RT-PCR) assay intended for the *in vitro* qualitative detection and differentiation of norovirus genogroup I and genogroup II RNA.
Regulation number/ Classification name/ Product code:	866.3990/Gastrointestinal pathogen panel multiplex nucleic acid-based assay system /PIQ Instrumentation for clinical multiplex test systems/OOI
Classification Advisory Panel	Microbiology (83)
Prescription Use	Yes
Predicate Devices Name(s):	Luminex Molecular Diagnostics, Inc., xTAG® Gastrointestinal Pathogen Panel (GPP) (510(k) #K121894)

Device Description:

The Xpert Norovirus Assay is an automated *in vitro* diagnostic test for detection and differentiation of nucleic acid sequences for norovirus genogroup I and genogroup II from raw or unpreserved unformed (liquid or soft) 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 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.

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. Because the cartridges are self-contained and specimens never come into contact with working parts of the instrument modules, cross-contamination between samples is minimized.

The Xpert Norovirus Assay includes reagents for the detection and differentiation of nucleic acid sequences for norovirus genogroup I and genogroup II from raw or unpreserved unformed human stool specimens collected from patients with signs and 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[1], and a

[1] Although sonication is a fundamental capability of every GeneXpert module, sonication is not used in the Xpert Norovirus Assay.

proprietary I-CORE® 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® testing area for processing. The specimen may be stored at 2–8 ºC 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.

Device Intended Use:

The Cepheid Xpert Norovirus Assay, performed on the GeneXpert® 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.

Substantial Equivalence:

The Xpert Norovirus Assay is substantially equivalent to the Luminex Molecular Diagnostics, Inc., xTAG® Gastrointestinal Pathogen Panel (Luminex xTAG GPP) [510(k) #K121894]. The Xpert Norovirus Assay and the Luminex xTAG GPP both detect norovirus genogroup I and genogroup II from human stool specimens using multiplex nucleic acid-based technology. A multi-center clinical study was conducted to determine the performance characteristics of the Xpert Norovirus Assay relative to a composite comparator method that consisted of a combination of Center for Disease Control and Prevention (CDC) RT-PCR assays and bi-directional sequencing for norovirus. The Luminex xTAG GPP used the same composite comparator method in a clinical study to determine its performance characteristics. The Xpert Norovirus Assay study results showed the Xpert Norovirus Assay is acceptable for its intended use and is as safe and effective as the predicate device.

Table 5-1 shows the similarities and differences between the Xpert Norovirus Assay and the predicate device.

Table 5-1: Comparison of Similarities and Differences of the Xpert Norovirus Assay with the Predicate Device

Similarities		
	Device	**Predicate Device**
Item	**Xpert Norovirus (**K142501)	**Luminex xTAG GPP (**K121894)
Regulation	21 CFR 866.3990	21 CFR 866.3990
Device Class	Class II	Class II
Technology Principle of Operation	Real-time reverse transcriptase polymerase chain reaction (RT-PCR)	Real-time reverse transcriptase polymerase chain reaction (RT-PCR)
General Intended Use	The Cepheid Xpert Norovirus Assay, performed on the GeneXpert® 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.	The xTAG®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® GPP: ● *Campylobacter* (*C. jejuni*, *C. coli* and *C. lari* only) ● *Clostridium difficile* (*C. difficile*) toxin A/B ● *Cryptosporidium* (*C. parvum* and *C. hominis* only) ● *Escherichia coli* (*E. coli*) O157 ● Enterotoxigenic *Escherichia coli* (ETEC) LT/ST ● *Giardia* (*G. lamblia* only - also known as *G. intestinalis* and *G. duodenalis*) ● Norovirus GI/GII ● Rotavirus A ● *Salmonella* ● Shiga-like Toxin producing *E. coli* (STEC) stx 1/stx 2 ● *Shigella* (*S. boydii*, *S. sonnei*, *S. flexneri* and *S. dysenteriae*) The detection and identification of

Similarities		
	Device	**Predicate Device**
Item	**Xpert Norovirus (**K142501)	**Luminex xTAG GPP (**K121894)
		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 aids in the detection and identification of acute gastroenteritis in the context of outbreaks. **xTAG® GPP positive results are presumptive and must be confirmed by FDA-cleared tests or other acceptable reference methods.** 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 xTAGGastrointestinal 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. **xTAG® GPP is not intended to monitor or guide treatment for *C. difficile* infections.** The xTAG® GPP is indicated for use with the Luminex® MAGPIX® instrument.

Similarities		
	Device	**Predicate Device**
Item	**Xpert Norovirus (**K142501)	**Luminex xTAG GPP (**K121894)
Specimen Types	Human stool	Human stool

Differences		
	New Device	**Predicate Device**
Item	**Xpert Norovirus (**K142501)	**Luminex xTAG GPP (**K121894)
Product Code	PIQ	PCH
Assay Results	Qualitative detection of Norovirus GI/GII	Qualitative detection of norovirus
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® EasyMAG extraction Kit (BioMerieux).
Instrument Systems	Cepheid GeneXpert Dx Systems and GeneXpert Infinity Systems	Nucleic Acid Purification System PCR Thermocycler Luminex® 100/200® or MAGPIX® instruments

Differences		
	New Device	**Predicate Device**
Item	**Xpert Norovirus (**K142501)	**Luminex xTAG GPP (**K121894)
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.

Analytical Performance:

Analytical Sensitivity (Limit of Detection)

The limit of detection (LoD) study was performed to evaluate the analytical sensitivity of the Xpert Norovirus Assay with positive clinical stool specimens containing Norovirus GI.3 or Norovirus GII.4 diluted into a pooled negative stool matrix. The LoD is defined as the lowest concentration (copies/mL) per sample that can be reproducibly distinguished from negative samples with 95% confidence. Replicates of at least 23 were evaluated at seven concentrations for Norovirus GI.3 and Norovirus GII.4 and LoDs were estimated by probit analysis. The estimated LoDs were confirmed by testing at least 20 replicate samples with virus diluted to the estimated LoD concentrations.

The LoD point estimates and confirmed LoD for each genogroup tested are summarized in Table 5-2.

Table 5-2: Limit of Detection of the Xpert Norovirus Assay

Norovirus Genogroup/strain	Limit of Detection (95% CI)
GI.3	5.7×10^5(copies/mL) ($4.64 \times 10^5 – 6.67 \times 10^5$)
GII.4	3.0×10^5 (copies/mL) ($1.25 \times 10^5 – 1.78 \times 10^5$)

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 fungi, 9 viruses, and 4 parasites representing common gastroenteritis pathogens or those potentially encountered in stool. A minimum of three replicates of all bacterial and fungal strains were tested at concentrations $\geq 10^6$ CFU/mL. A minimum of three replicates of all viruses were tested at concentrations $\geq 10^5$ $TCID_{50}$/mL with the exception of two viruses obtained from clinical samples with unknown concentrations. A minimum of three replicates of all parasites were tested at concentrations $\geq 10^6$ copies/mL. All organisms tested were correctly reported as NORO GI NOT DETECTED; NORO GII NOT DETECTED by the Xpert Norovirus Assay. The analytical specificity was 100%. Results are shown in Table 5-3.

Table 5-3: Analytical Specificity of Xpert Norovirus

Organism	Strain ID	Concentration
Acinetobacter baumannii	CCUG 3477	$>3.0 \times 10^8$ CFU/mL
Anaerococcus prevotii[a]	ATCC 9321	6.7×10^8 CFU/mL
Bacteriocides fragilis[a]	ATCC 25285	1.4×10^9 CFU/mL
Campylobacter coli	ATCC 43478	1.8×10^8 CFU/mL
Campylobacter jejuni	ATCC 33560	1.3×10^8 CFU/mL
Campylobacter lari	ATCC 35221	3.4×10^7 CFU/mL
Citrobacter freundii	ATCC 33128	1.5×10^9 CFU/mL
Clostridium difficile[a]	ATCC 9689	2.2×10^8 CFU/mL

Organism	Strain ID	Concentration
Clostridium sordelli[a]	DSMZ 2141	2.0 x 10^8 CFU/mL
Eggerthella lenta	ATCC 43055	>3.0 x 10^7 CFU/mL
Enterobacter cloacae	ATCC 70021	1.0 x 10^9 CFU/mL
Enterococcus casseliflavus	ATCC 25788	1.0 x 10^9 CFU/mL
Enterococcus faecalis	ATCC 29212	5.4 x 10^8 CFU/mL
Enterococcus faecium	ATCC 9756	8.2 x 10^8 CFU/mL
Enterococcus gallinarium	ATCC 49573	4.5 x 10^8 CFU/mL
Escherichia coli O157:H7	ATCC 43888	8.4 x 10^8 CFU/mL
Escherichia coli O26:H11	CDC 033014	7.4 x 10^8 CFU/mL
Escherichia coli O45:H2	CDC 003039	3.3 x 10^8 CFU/mL
Escherichia coli O103:H11	CDC 063008	5.4 x 10^8 CFU/mL
Escherichia coli O11	CDC 201114	6.9 x 10^8 CFU/mL
Escherichia coli O121	CDC 023211	1.4 x 10^9 CFU/mL
Escherichia coli O145	CDC 993311	7.1 x 10^8 CFU/mL
Escherichia hermannii	ATCC 33650	1.5 x 10^9 CFU/mL
Fusobacterium necrophorum[a]	ATCC 31647	9.6 x 10^8 CFU/mL
Helicobacter pylori	CCUG 1784	1.5 x 10^8 CFU/mL
Klebsiella pneumoniae	ATCC 70063	1.2 x 10^9 CFU/mL
Lactobacillus jensenii	ATCC 25258	4.0 x 10^8 CFU/mL
Listeria monocytogenes	CCUG 3358	1.2 x 10^9 CFU/mL
Micrococcus luteus	ATCC 4698	1.8 x 10^8 CFU/mL
Morganella morganii	ATCC 49948	1.3x10^9 CFU/mL
Peptostreptococcus anaerobius[a]	CCUG 7835	1.5 x 10^9 CFU/mL
Plesiomonas shigelloides	ATCC 51903	3.1 x 10^8 CFU/mL
Prevotella oralis[a]	ATCC 33269	1.2 x 10^9 CFU/mL
Proteus mirabilis	ATCC 43071	1.1 x 10^9 CFU/mL
Proteus vulgaris	ATCC 49132	1.8 x 10^9 CFU/mL
Providencia alcalifaciens	CCUG 6325	1.8 x 10^9 CFU/mL
Providencia stuartii	ATCC 49809	1.3 x 10^9 CFU/mL

Organism	Strain ID	Concentration
Pseudomonas aeruginosa	ATCC 27853	6.3×10^8 CFU/mL
Pseudomonas fluorescens	ATCC 13525	$>3.0 \times 10^8$ CFU/mL
Pseudomonas putida	ATCC 49128	5.5×10^8 CFU/mL
Salmonella agona	ATCC 51957	1.2×10^9 CFU/mL
Salmonella bongori	ATCC 43975	1.7×10^9 CFU/mL
Salmonella enterica	ATCC 13314	9.2×10^8 CFU/mL
Serratia marcescens	ATCC 43862	3.8×10^8 CFU/mL
Shigella flexneri	ATCC 12022	8.1×10^8 CFU/mL
Shigella sonnei	ATCC 25931	$>3.0 \times 10^8$ CFU/mL
Staphylococcus aureus	ATCC 25923	8.8×10^8 CFU/mL
Staphylococcus epidermidis	ATCC 14990	$>3.0 \times 10^7$ CFU/mL
Streptococcus agalactiae (GBS)	ATCC 12386	9.6×10^8 CFU/mL
Streptococcus dysgalactiae	ATCC 43078	7.2×10^8 CFU/mL
Streptococcus pyogenes	ATCC 19615	5.5×10^8 CFU/mL
Vibrio cholerae[b]	CCUG 9118	5.2×10^9 copies/µL
Vibrio parahaemolyticus	ATCC 17802	3.8×10^8 CFU/mL
Yersinia enterocolitica	ATCC 9610	7.1×10^8 CFU/mL
Adenovirus	Type 31	3.6×10^5 $TCID_{50}$/mL
Adenovirus	Type 40	2.8×10^7 $TCID_{50}$/mL
Adenovirus	Type 41	4.6×10^7 $TCID_{50}$/mL
Astrovirus[d]	--	Not applicable[c]
Coxsackie virus	Type B5	1.4×10^5 $TCID_{50}$/mL
Echovirus	11	3.3×10^9 $TCID_{50}$/mL
Parechovirus	Type 6	1.9×10^7 $TCID_{50}$/mL
Rotavirus	Type Wa	1.0×10^6 $TCID_{50}$/mL
Sapovirus[d]	--	Not applicable[e]
Candida albicans	ATCC 10231	$>3.0 \times 10^7$ CFU/mL
Blastocystis hominis[b]	BT1	1.0×10^9 copies/mL
Cryptosporidium parvum[b]	Iowa	6.1×10^9 copies/mL
Giardia lamblia[b]	Portland-1	3.05×10^9 copies/mL
Entamoeba histolytica[b]	ATCC 30459D	4.9×10^6 copies/mL

[a] Strictly anaerobic bacteria.
[b] Tested as genomic DNA.
[c] The concentration is not known for the Astrovirus clinical samples that were obtained from

KUL; the Ct values according to KUL assay were in the range of 12-27.
[d] Clinical sample.
[e] *The concentration is not known for the Sapovirus clinical samples that were obtained from KUL; the Ct values according to KUL assay were in the range of 19-23.*

Analytical Reactivity (Inclusivity)

The analytical reactivity of the Xpert Norovirus Assay was evaluated against thirty-one genotypes representing both norovirus genogroups (GI and GII). The thirty-one norovirus strains evaluated in this study were tested near the LoD concentration of the assay (Table 5-4). Three replicates were tested for each strain.

Table 5-4: Analytical Reactivity Results of the Xpert Norovirus Assay

Norovirus Strain	Estimated Concentration (copies/mL)[a]	Result	
		GI	GII
GI.1	9.0×10^6	POS	NEG
GI.2	3.7×10^8	POS	NEG
GI.3	1.4×10^6	POS	NEG
GI.4	1.0×10^5	POS	NEG
GI.5[b]	2.5×10^5	POS	NEG
GI.6[b]	2.5×10^5	POS	NEG
GI.7[b]	2.5×10^5	POS	NEG
GI.8	3.7×10^5	POS	NEG
GI.14	3.0×10^6	POS	NEG
GII.1	3.6×10^6	NEG	POS
GII.2	1.1×10^5	NEG	POS
GII.3[b]	1.3×10^3	NEG	POS
GII.4 (2006a)	1.2×10^5	NEG	POS
GII.4 (2006b)	2.4×10^5	NEG	POS
GII.4 (2008)	4.3×10^5	NEG	POS
GII.4 (2009) New Orleans	1.7×10^5	NEG	POS
GII.4 (2010)	9.6×10^4	NEG	POS
GII.4 (2012) Sydney	1.2×10^5	NEG	POS
GII.5[b]	1.3×10^3	NEG	POS
GII.6[b]	1.3×10^3	NEG	POS
GII.7	8.0×10^4	NEG	POS

Norovirus Strain	Estimated Concentration (copies/mL)[a]	Result	
		GI	GII
GII.8[b]	1.3×10^3	NEG	POS
GII.9[b]	1.3×10^3	NEG	POS
GII.10[b]	1.3×10^3	NEG	POS
GII.11	2.6×10^5	NEG	POS
GII.12	5.7×10^5	NEG	POS
GII.13	6.9×10^5	NEG	POS
GII.14	1.5×10^5	NEG	POS
GII.15	1.7×10^5	NEG	POS
GII.16[b]	1.3×10^3	NEG	POS
GII.17[b]	1.3×10^3	NEG	POS

[a] 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.

[b] Naked RNA transcripts were used for these strains; clinical samples were not available at the time of testing.

Potentially Interfering Substances

Potentially interfering substances that may be present in the stool were evaluated directly relative to 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 Table 5-5.

Negative samples were tested in replicates of 8 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 8 per substance with one Norovirus GI and one Norovirus GII clinical isolate near the LoD.

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 Barium sulfate (5% w/w), a statistically significant inhibitory effect was observed on the Norovirus GII Ct in positive samples relative to the control (p-value <0.05). No statistically significant effect was observed on the Norovirus GII Ct relative to the control in the presence of Barium sulfate (1% w/w).

No other potential interfering substances were found to be inhibitory and no false-negatives were reported for these substances at the concentrations tested.

Table 5-5: Potentially Interfering Substances in Xpert Norovirus Assay

Endogenous substances		
Substance	**Description /Active Ingredient**	**Concentration Tested**
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

Exogenous substances		
Substance	**Description /Active Ingredient**	**Concentration Tested**
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 % w/w, 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
$CaCO_3$	Calcium Carbonate	5 % w/v
Hydrocortisone	Hydrocortisone	50 % w/v
Ibuprofen	Ibuprofen	5% w/v
Imodium	Loperamide HCl	5 % v/v
Kaopectate	Attapulgite	5 mg/mL
Metronidazole	Metronidazole	5 % w/v
Mycostatin	Nystatin	50 % w/w
Naprosyn	Naproxen Sodium	2.2 µmol/mL

Exogenous substances		
Substance	**Description /Active Ingredient**	**Concentration Tested**
Novaluzid	$Mg(OH)_2$, $Al(OH)_3$ and $MgCO_3$	5 % w/v
Polymyxin B sulfate Bacitrin 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

Carry-Over Contamination

A study was conducted to demonstrate that single-use, self-contained GeneXpert cartridges prevent carry-over contamination in negative samples followed by very high positive samples in the same GeneXpert module. The study consisted of a negative sample processed in the same GeneXpert module immediately followed by a highly positive Norovirus GII sample. This testing scheme was repeated 21 times between two GeneXpert modules for a total of 42 runs for 20 positive and 22 negative specimens. Nineteen (19) positive samples were correctly reported as NORO GI NOT DETECTED; NORO GII DETECTED and one positive sample was reported as an ERROR. All 22 negative samples were correctly reported as NORO GI NOT DETECTED; NORO GII NOT DETECTED.

Linearity

Not applicable, the Xpert Norovirus Assay is a qualitative assay.

Clinical Performance

Clinical Studies: Performance characteristics of the Xpert Norovirus Assay were evaluated at seven institutions in the U.S. and the E.U. 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 CDC (Atlanta, GA).

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.

On fresh, prospectively collected specimens, the Xpert Norovirus Assay demonstrated 100% PPA and 99.6% NPA for detection of Norovirus GI , relative to the composite

reference test (Table 5-6). The Xpert Norovirus Assay demonstrated 98.5% PPA and 98.8% NPA for detection of Norovirus GII (Table 5-7).

On 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 (Table 5-8). The Xpert Norovirus Assay demonstrated 100% PPA and 96.8% NPA for detection of Norovirus GII (Table 5-9).

Table 5-6. 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 % (95% CI)	100% (95% CI: 73.5-100)	
		NPA % (95% CI)	99.6% (95% CI: 98.9-99.9)	

Table 5-7. 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 % (95% CI)	98.5% (95% CI: 91.7-100)	
		NPA % (95% CI)	98.8% (95% CI: 97.8-99.4)	

Table 5-8. 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 % (95% CI)		98.1% (95% CI: 93.2-99.8)		
NPA % (95% CI)		94.6% (95% CI: 91.8-96.6)		

Table 5-9. 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 % (95% CI)		100% (95% CI: 96.7-100)		
NPA % (95% CI)		96.8% (95% CI: 94.5-98.3)		

Reproducibility Study

A panel of 7 specimens with varying concentrations of Norovirus GI and Norovirus GII 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. The Xpert Norovirus Assay was performed according to the Xpert Norovirus Assay procedure. Results are summarized in Table 5-10.

Table 5-10: Summary of Reproducibility Results

Sample ID	Site 1	Site2	Site3	Overall
Neg	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
GI - High Neg	30.0% (6/20)	15.0% (3/20)	30.0% (6/20)	25.0% (15/60)
GI - Low Pos	100% (20/20)	85.0% (17/20)	95.0% (19/20)	93.3% (56/60)
GI - Mod Pos	100% (19/19)	100% (20/20)	100% (20/20)	100% (59/59)[a]
GII - High Neg	25.0% (5/20)	30.0% (6/20)	35.0% (7/20)	30.0% (18/60)
GII - Low Pos	100% (20/20)	95.0% (19/20)	90.0% (18/20)	95.0% (57/60)
GII - Mod Pos	95.0% (19/20)	100% (20/20)	100% (20/20)	98.3% (59/60)

[a] One sample 2x indeterminate.

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 Table 5-11.

Table 5-11: Summary of Reproducibility Data

Sample	Assay Channel (Analyte)	N[a]	Mean Ct	Between-Site		Between-Day		Between-Operator		Within-Assay		Total	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Neg	SPC	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 Neg	GI	60	39.4	0	0	0.46	1.2	0	0	1.80	4.6	1.86	4.7
GI - Low Pos	GI	59	37.9	0.29	0.8	0	0	0.36	1.0	1.03	2.7	1.13	3.0
GI - Mod Pos[b]	GI	57	34.7	0.09	0.2	0.07	0.2	0	0	0.41	1.2	1.01	1.2
GII - High Neg	GII	54	38.9	0	0	0	0	0.77	2.0	1.77	4.5	1.93	5.0
GII - Low Pos	GII	60	37.3	0	0	0	0	0.58	1.6	1.33	3.6	1.45	3.9
GII - Mod Pos[b]	GII	59	34.3	0.22	0.6	0	0	0	0	0.45	1.3	0.50	1.5

[a] Results with non-zero Ct values out of 60.
[b] n=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 of samples 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 summarized in Table 5-12.

Table 5-12. 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	
Neg	100% (48/48)	100% (48/48)	100% (96/96)	100% (48/48)	100% (48/48)	100% (96/96)	100% (192/192)
GI - High Neg	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 Pos	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 Pos	100%[a] (47/47)	100% (48/48)	100% (95/95)	100% (48/48)	100% (48/48)	100% (96/96)	100% (191/191)
GII - High Neg	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 Pos	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 Pos	100% (48/48)	100% (48/48)	100% (96/96)	100% (48/48)	100%[b] (47/47)	100% (95/95)	100% (191/191)

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 Table 5-13.

Table 5-13: Summary of Precision Data

Sample	Assay Channel (Analyte)	N[a]	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 (%)
Neg	SPC	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 Neg	GI	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 Pos	GI	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 Pos	GI	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 Neg	GII	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 Pos	GII	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 Pos	GII	191	34.3	0	0	0.09	0.2	0	0	0.17	0.5	0.42	1.2	0.46	1.3

[a] Results with non-zero Ct values out of 192.

Conclusions

The results of the nonclinical analytical and clinical performance studies summarized above demonstrate that the Xpert Norovirus Assay is safe and effective for its intended use and is substantially equivalent to the predicate device.