510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K142033

B. Purpose for Submission

The purpose of the submission is for clearance of an additional two analytes, Norovirus GI/GII and Rotavirus A, to the already cleared Verigene Enteric Pathogens Nucleic Acid Test (EP) (k140083) which is run on the Verigene System.

C. Measurand

The Verigene Enteric Pathogens Nucleic Acid Test is performed on the automated Nanosphere Verigene System utilizing reverse transcription (RT), polymerase chain reaction (PCR), and array hybridization to detect specific gastrointestinal microbial nucleic acid gene sequences for the following organisms:

- *Campylobacter* Group (comprised of *C. coli*, *C. jejuni*, and *C. lari*)
- *Shigella* species (including *S. dysenteriae*, *S. boydii*, *S. sonnei*, and *S. flexneri*)
- *Vibrio* Group (comprised of *V. cholerae* and *V. parahaemolyticus*)
- *Yersinia enterocolitica*
- Shiga toxin 1 gene and Shiga toxin 2 gene virulence markers
- Norovirus GI/GII
- Rotavirus A

D. Type of Test:

The Verigene Enteric Pathogens Nucleic Acid Test is a qualitative molecular assay which relies on detection of specific nucleic acid targets in a microarray format. For each of the bacterial nucleic acid sequences detected by Enteric Pathogens Nucleic Acid Test, unique Capture and Mediator oligonucleotides are utilized, with gold nanoparticle probe-based endpoint detection. The Capture oligonucleotides are covalently bound to the microarray substrate and hybridize to a specific portion of the nucleic acid targets. The Mediator oligonucleotides have a region which bind to a different portion of the same nucleic acid targets and also have a sequence which allows binding of a gold nanoparticle probe. Catalytic enhancement of the bound gold nanoparticle probes at the capture sites results in gold-silver aggregates that scatter light with high efficiency and provide accurate detection of target capture.

E. Applicant:

Nanosphere, Inc.

F. Proprietary and Established Names:

Verigene® Enteric Pathogens Nucleic Acid Test (EP)

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3990 – Gastrointestinal microorganism multiplex nucleic acid-based assay

2. Classification:

   Class II

3. Product code:

   PCH, PCI, OOI

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended Use:

   The Verigene Enteric Pathogens Nucleic Acid Test (EP) is a multiplexed, qualitative test for simultaneous detection and identification of common pathogenic enteric bacteria, viruses and genetic virulence markers from liquid or soft stool preserved in Cary-Blair medium, collected from individuals with signs and symptoms of gastrointestinal infection. The test is performed on the automated Nanosphere Verigene System utilizing reverse transcription (RT), polymerase chain reaction (PCR), and array hybridization to detect specific gastrointestinal microbial nucleic acid gene sequences associated with the following pathogenic bacteria and viruses:

   - *Campylobacter* Group (comprised of *C. coli*, *C. jejuni*, and *C. lari*)
   - *Salmonella* species
   - *Shigella* species (including *S. dysenteriae*, *S. boydii*, *S. sonnei*, and *S. flexneri*)
   - *Vibrio* Group (comprised of *V. cholerae* and *V. parahaemolyticus*)
   - *Yersinia enterocolitica*
   - *Norovirus GI/GII*
   - *Rotavirus A*

In addition, EP detects the Shiga toxin 1 gene and Shiga toxin 2 gene virulence markers. Shiga toxin producing *E. coli* (STEC) typically harbor one or both genes that encode for Shiga Toxins 1 and 2.

EP is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness, in conjunction with other clinical, laboratory, and epidemiological information; however, is not to be used to monitor these infections. EP also aids in the detection and identification of acute gastroenteritis in the context of outbreaks.

Due to the limited number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for *Yersinia enterocolitica*, *Vibrio* Group and *Shigella* species were primarily established with contrived specimens.

Concomitant culture is necessary for organism recovery and further typing of bacterial agents.

EP results 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 EP 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.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For Prescription Use Only

4. Special instrument requirements:

   The assay is run on the Verigene System.

I. Device Description:

The Enteric Pathogens Nucleic Acid Test is performed on the Verigene System which is a fully automated, bench-top molecular diagnostics workstation. The System enables automated nucleic acid extraction from unformed stool specimens (liquid or soft) preserved in Cary-Blair media and detection of bacterial and viral-specific target DNA. The Verigene System consists of two components: the Verigene Reader and the Verigene Processor *SP*.

The Reader is the Verigene System's user interface, which serves as the central control unit for all aspects of test processing, imaging, and result generation using a touch-screen control panel and a barcode scanner. The Verigene Processor *SP* executes the test procedure,

automating the steps of sample preparation, target amplification hybridization to the detection microarray. Detection and identification of bacterial and viral-specific DNA is performed in a microarray format by using gold nanoparticle probe-based technology. Once the specimen is loaded by the operator, all other fluid transfer steps are performed by an automated pipette that transfers reagents between wells of the trays and finally loads the specimen into the Test Cartridge for hybridization. Single-use disposable test consumables and a self-contained Verigene Test Cartridge are utilized for each sample tested with the Enteric Pathogens Nucleic Acid Test assay.

To obtain the test results after test processing is complete, the user removes the Test Cartridge from the Processor *SP*, and inserts the substrate holder into the Reader for analysis. Light scatter from the capture spots is imaged by the Reader and intensities from the microarray spots are used to make a determination regarding the presence (Detected) or absence (Not Detected) of a bacterial or viral nucleic acid sequence/analyte. This determination is made by means of software-based decision algorithm resident in the Reader.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   xTAG Gastrointestinal Pathogen Panel (GPP) by Luminex Molecular Diagnostics, Inc.

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

   K121894/K121454

3. Comparison with predicate:

Similarities		
Element	**New Device: Enteric Pathogens Nucleic Acid Test (EP)**	**Predicate: xTAG® Gastrointestinal Pathogen Panel (GPP) K121894**
Intended Use	The Verigene Enteric Pathogens Nucleic Acid Test (EP) is a multiplexed, qualitative test for simultaneous detection and identification of common pathogenic enteric bacteria, viruses and genetic virulence markers from liquid or soft stool preserved in Cary-Blair medium, collected from individuals with signs and symptoms of gastrointestinal infection. The test is performed on the automated Nanosphere Verigene System utilizing reverse transcription (RT), polymerase chain reaction (PCR), and array hybridization to detect specific gastrointestinal microbial nucleic acid gene sequences associated with the following pathogenic bacteria and	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.*

Similarities		
Element	**New Device:** **Enteric Pathogens Nucleic Acid Test (EP)**	**Predicate:** **xTAG® Gastrointestinal Pathogen Panel (GPP)** **K121894**
	viruses: • *Campylobacter* Group (comprised of *C. coli*, *C. jejuni*, and *C. lari*) • *Salmonella* species • *Shigella* species (including *S. dysenteriae*, *S. boydii*, *S. sonnei*, and *S. flexneri*) • *Vibrio* Group (comprised of *V. cholerae* and *V. parahaemolyticus*) • *Yersinia enterocolitica* • *Norovirus GI/GII* • *Rotavirus A* In addition, EP detects the Shiga toxin 1 gene and Shiga toxin 2 gene virulence markers. Shiga toxin producing *E. coli* (STEC) typically harbor one or both genes that encode for Shiga Toxins 1 and 2. EP is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness, in conjunction with other clinical, laboratory, and epidemiological information; however, is not to be used to monitor these infections. EP also aids in the detection and identification of acute gastroenteritis in the context of outbreaks. Due to the limited number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for *Yersinia enterocolitica*, *Vibrio* Group and *Shigella* species were primarily established with contrived specimens. Concomitant culture is necessary for organism recovery and further typing of bacterial agents. EP results should not be used as the sole	*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 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,

Similarities		
Element	**New Device:** **Enteric Pathogens Nucleic Acid Test (EP)**	**Predicate:** **xTAG® Gastrointestinal Pathogen Panel (GPP)** **K121894**
	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 EP 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.	treatment, or other patient management decisions. Confirmed positive results do not rule out coinfection 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. 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.
DNA Amplification	PCR	Same
Organisms/NA Targets Detected	*Campylobacter* Group (*C. coli, C. jejuni,* and *C. lari)* *Salmonella* species *Shigella* species (*S. dysenteriae, S. boydii, S. sonnei,* and *S. flexneri)* *Yersinia enterocolitica* Norovirus GI/GII Rotavirus A Shiga toxin 1 gene and Shiga toxin 2 gene virulence markers	Same with additional analytes (excluding *Vibrio* Group and *Yersinia enterocolitica*)

Differences		
Element	**New Device: Enteric Pathogens Nucleic Acid Test (EP)**	**Predicate: xTAG® Gastrointestinal Pathogen Panel (GPP) K121894**
Time to Result	~ 2 hours	5 hours
Specimen Type	Stool in Cary Blair Media	Unpreserved Stool
Sample prep	On-board, automated NA extraction and amplification	Off-line NA Extraction and amplification
Detection Method	Gold/Silver nanoparticle probe detection of bacterial-specific DNA on complementary oligo- microarray	Specific microbial target or control bead populations coupled to sequences from Universal Array, streptavidin, R-phycoerythrin conjugate
Optical Detection	Light scatter	Multi-color fluorescence

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

CLSI EP5-A2; Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition

CLSI EP12-A2; User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline - Second Edition

CLSI MM3-A2; Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline - Second Edition

CLSI EP15-A2 - User Verification of Performance for Precision and Trueness; Approved Guideline – second edition

CLSI EP9-A2-IR - Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – second edition (Interim Revision)

CLSI EP17-A - Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

Guidance on Informed Consent for *In Vitro* Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable

Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices

Class II Special Controls Guidance Document: Instrumentation for Clinical Multiplex Test Systems

Establishing the Performance Characteristics of *In Vitro* Diagnostic Devices for the Detection of *Clostridium Difficile* – Draft Guidance for Industry and FDA Staff

In Vitro Diagnostic (IVD) Device Studies – Frequently Asked Questions

L. Test Principle:

The Enteric Pathogens Nucleic Acid Test is performed on samples of stool preserved in Cary-Blair media, collected from individuals suspected of having acquired an enteric infection. The provided flocked swab is dipped into the specimen until flocked tip is fully immersed. Once evenly coated, the swab is transferred to the Stool Prep Buffer tube and broken at the pre-formed scored breakpoint. The Stool Prep Buffer tube is sealed with a screw cap and thoroughly vortexed and centrifuged to ensure a proper mixture of the specimen. A 200 µL aliquot of the prepared Stool Prep Buffer (SLB) is pipetted into the Specimen Well of the Extraction Tray and the Drawer Assembly of the Verigene SP is closed to initiate the test. An automated pipettor performs all further fluidic transfers during the Sample Extraction and Amplification steps.

The steps automated by the Verigene System include:

- Sample Preparation—Cell lysis and magnetic bead-based bacterial and viral NA isolation from liquid or soft stool samples preserved in Cary-Blair media
- Target Amplification—Multiplex PCR-based amplification to generate specific amplicons
- Hybridization and Detection—Hybridization of analyte-specific NA to capture oligonucleotides on a microarray, using gold nanoparticle probe-based technology to aid detection.

For each of the bacterial or viral nucleic acid sequences/analytes detected by the EP test, two sets of oligonucleotides are required for the endpoint gold nanoparticle probe-based process: (i) Capture oligonucleotides (or captures) and (ii) Mediator oligonucleotides (or mediators). The Capture oligonucleotides are printed on the Test Substrate (or the microarray) and are designed to specifically bind to one part of the analyte-specific target oligonucleotide (or target). The Mediator oligonucleotides bind to a complementary portion of the same target which is now hybridized to the capture and enable binding of gold nanoparticle probes. Silver enhancement of the bound gold nanoparticle probes at the capture sites results in gold-silver aggregates that scatter light with high efficiency. Light scatter from the capture spots is imaged by the Verigene Reader and intensities from the microarray spots are processed by a decision algorithm to make calls regarding the presence (“Detected”) or absence (“Not Detected”) of a nucleic acid sequence/analyte.

M. Performance Characteristics:

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   Refer to K140083 for Precision/Reproducibility Study results for previously cleared EP

bacterial analytes.

Preparation of Samples for Precision/Reproducibility Studies (and other analytical studies):

For preparation of samples for analytical studies, organisms were spiked into a Negative Stool Matrix (NSM) pool. The NSM was prepared by pooling individual de-identified residual stool specimens preserved in Cary-Blair medium which were determined to be negative by standard culture methods as well as by the EP assay. These individual "Cary-Blair specimens" were originally prepared by diluting unpreserved stool specimens (obtained from individuals in the intended use population) in Cary-Blair medium such that the stool comprises 25% by volume of the resulting specimen. NSM was formulated by pooling the negative Cary-Blair specimens, combining with Enteric Stool Prep Buffer in a 1:5 ratio, and clarifying by centrifugation and collection of the supernatant. The NSM pool was again tested with the Enteric Pathogens Nucleic Acid Test in replicates of 20 and confirmed as negative.
Analytical samples were prepared using Rotavirus strains obtained from the American Type and Culture Collection (ATCC) and Norovirus strains obtained from the Center for Disease Control and Prevention (CDC). Viral strains were characterized and quantified and then diluted and added to the NSM to achieve appropriate organism concentrations. These simulated specimens were either used immediately (within 30 min of preparation) or stored frozen at <-70°C until use.

Precision Study

A Precision Study for the Norovirus and Rotavirus targets was conducted as part of a full 20-sample EP study (bacteria and viruses) previously performed for K140083. The panel was composed of simulated specimens prepared with three characterized and quantified viral strains as presented in the table below. In addition, two negative samples were also run (Negative Stool Matrix and *Clostridium difficile*). For each EP viral strain the panel included, a "Low Positive" sample at 1-2x the Limit of Detection (LoD), which would be expected to produce a positive result approximately 95% of the time, and a "Moderate Positive" sample at 2-5x LoD, which would be expected to yield a positive result approximately 99% of the time. Panel members were tested daily in duplicate by two operators for four non-consecutive days for a total of 16 tests per sample (2 operators/day x 2 replicates/operator x 4 days = 16 tests per sample). Positive and negative controls were run once per combination of consumable lots received.

Precision and Reproducibility Test Panel Composition

Description	Strain ID.	LOD*	Label	Concentration*	Expected Result
Rotavirus	VR-2550	$1.11x10^3$	Moderate	$5.55x10^3$	Rotavirus
			Low	$2.22x10^3$	Rotavirus
Norovirus GI	CDC 2142	$4.12x10^5$	Moderate	$2.06x10^6$	Norovirus
			Low	$1.03x10^6$	Norovirus
Norovirus GII	D17219	$1.67x10^6$	Moderate	$8.35x10^6$	Norovirus
			Low	$3.34x10^6$	Norovirus

*Rotavirus strains quantified as $TCID_{50}$/mL. Norovirus strains quantified as copies/mL

The Precision Study yielded the expected results for all 96 samples containing the targeted viral analytes. With the inclusion of the Norovirus and Rotavirus targets to the EP test, the results of the positive bacterial as well as negative samples tested in the full Precision Study performed for K140083 were assessed with the viral target results unmasked. Across all of the 224 bacterial and negative control samples tested, there were no cases of false positive results for Norovirus or Rotavirus and therefore the Precision study results presented in K140083 are unchanged with the addition of the viral targets.

Two samples returned initial No Calls, giving an initial call rate for viral samples of 97.9% (94/96). In addition, three samples returned pre-analysis (pre-AE) errors for a rate of 3% (3/101 tests). All initial no-calls and pre-AE errors gave the expected results upon repeat testing.

Study results as shown in the following table demonstrated acceptable within-laboratory precision for the EP test.

Summary of Precision Study Results		
Sample	**Concentration**	**Final Percent Agreement with Expected Result (95 % CI)**
Rotavirus A	**Moderate**	16/16 100% (79.4-100)
	Low	16/16 100% (79.4-100)
Norovirus GI	**Moderate**	16/16 100% (79.4-100)
	Low	16/16 100% (79.4-100)
Norovirus GII	**Moderate**	16/16 100% (79.4-100)
	Low	16/16 100% (79.4-100)
Negative Stool Matrix	**Negative for Targeted Analytes**	100%[a] 16/16 (79%-100%)
***Clostridium difficile* (Negative Control)**		100%[a] 16/16 (79%-100%)

a) Performance is for Negative results

<u>Reproducibility Study</u>:

The inter-laboratory reproducibility of the EP test for Norovirus and Rotavirus was evaluated in a study performed for the initial clearance of the EP test (K140083) at three external clinical sites. The viral panel members for the Reproducibility study were the same as described above for the internal Precision Study. Samples were prepared in NSM with each strain at two concentrations; low positive and moderate positive. In addition two negative samples were evaluated (NSM and *Clostridium difficile*). The study included five days of testing incorporating the following sources of potential variance: sites, operators, days, consumable lots and runs. Samples were tested daily in triplicate by two operators for five non-consecutive days at three sites for a total of 90 tests per sample (3 sites x 2 operators / site x 3 replicates / operator x 5 days = 90 tests per sample). Positive and negative controls were run at each site according to the study protocol. Samples which yielded an initial 'No Call' or Pre-Analysis Error test result with the Enteric Pathogens Nucleic Acid Test were repeated from the original sample (new aliquot) per the package insert instructions and the repeat result was considered the final result.

The results of the Reproducibility Study for the Norovirus and Rotavirus targets are summarized in the tables below. In addition to the results presented, a single false positive Salmonella result occurred for a Rotavirus sample.

Reproducibility Study Results

Sample	**Concentration**	**Site 1**	**Site 2**	**Site 3**	**Total Final Percent Agreement with Expected Result (95 % CI)**
Rotavirus	**Moderate**	30/30 100% (88.4-100)	30/30 100% (88.4-100)	30/30 100% (88.4-100)	90/90 100% (96-100)
	Low	30/30 100% (88.4-100)	27/30 90.0% (73.5-97.9)	30/30 100% (88.4-100)	87/90 96.7% (90.6-99.3)
Norovirus GI	**Moderate**	30/30 100% (88.4-100)	30/30 100% (88.4-100)	26/28 92.9% (76.5-99.1)	86/88 97.7% (92.0-99.7)
	Low	30/30 100% (88.4-100)	28/30 93.3% (77.9-99.2)	28/30 93.3% (77.9-99.2)	86/90 95.6% (89.0-98.8)
Norovirus GII	**Moderate**	28/28 100% (87.7-100)	30/30 100% (88.4-100)	28/29 100% (82.2-99.9)	86/87 98.9% (93.8-100)
	Low	30/30 100% (88.4-100)	30/30 100% (88.4-100)	29/29 100% (88.1-100)	89/89 100% (95.9-100)

Of the 540 tests performed for the viral targets, there were 25 initial No Call results (4.6%) and after repeat testing, six samples yielded a final No Call result (1.1%). In addition, three samples gave initial pre-AE errors (0.3%) all of which gave the expected result after repeat testing.

Initial No Call Rates Observed by Type

Initial No Call Type	Observed Rate
No Call - INT CTL	1.5% (8/540)
No Call - INT CTL 1	1.1% (6/540)
No Call - INT CTL 2	0.9% (5/540)
No Call - VARIATION	1.1% (6/540)
Total	**4.6% (25/540)**

With the inclusion of the Norovirus and Rotavirus targets to the EP test, the results of the positive bacterial as well as negative samples tested in the full Reproducibility Study performed for K140083 were assessed with the viral target results unmasked. Across all samples tested in the original reproducibility study, there were no false positive results for Norovirus or Rotavirus and therefore the Reproducibility Study results in K140083 are unchanged with the addition of the viral targets.

b. Linearity/assay reportable range:

Not applicable

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

Internal Assay Controls

Enteric Pathogens Nucleic Acid Test (EP) is a 'specimen-to-result' detection system wherein nucleic acids are isolated and identified by an automated process. In order to decrease the likelihood of false results due to processing errors, all reagents are prepackaged in single-use disposables. The following controls are built into the system so that processing failures are identified and the appropriate actions are taken.

INTL CTL 1: An artificial DNA construct serves as the internal hybridization control or "*positive control for detection*." It is a synthetic DNA construct present in the sample buffer and is hybridized in parallel with every test sample.

INTL CTL 2: An MS2 Phage serves as a specimen isolation and amplification control and is referred to as the Internal Processing Control 2. This control is added to each specimen prior to the extraction step.

For a valid Negative test result, both IC1 and IC2 must be "Detected." If IC1 or IC2 are not detected a "No Call – INT CTL 1" or a "No Call – INT CTL 2" is provided respectively. If both IC1 and IC2 are not detected, a "NO CALL – INT CTL" result is provided. The recommended action for any "No Call" decision is to repeat the test

according to the instructions package insert. These internal controls are not utilized for the detection of positive samples.

Imaging Controls:

These controls comprise a set of oligonucleotide spots on the Test Substrate that act as a quality check for proper fluid control and movement between the Test Cartridge and the Reagent Pack. Inability to detect the imaging controls results in a "No Call – No Grid." The Imaging Control signal is generated when a signal probe hybridizes to an oligonucleotide on the substrate. Signal at these spots indicates that the steps of probe hybridization and signal enhancement were performed as intended.

In summary, the detection algorithm requires that all internal controls are valid before decisions regarding the presence or absence of any other target on the panel can be reported. If all controls are not within the predefined specification, a no call result will be obtained and further action should be taken according to the product labeling.

Positive and Negative External Controls

External controls are not provided with the EP test; however the package insert indicates that external quality control testing should be performed in conformance with local, state, and federal regulations or accreditation organizations as applicable and users should follow their laboratory's standard quality control procedures. Refer to clinical the study section below for results of external quality control testing performed during the clinical study.

d. Detection limit:

Refer to K140083 for Limit of Detection (LoD) Study results for the previously cleared EP bacterial analytes.

The LoD for the viral analytes was evaluated using samples prepared with previously characterized and quantified preparations of Norovirus and Rotavirus strains serially diluted in Negative Stool Matrix (NSM). Each concentration in the dilution series was tested in replicates of four and the preliminary LoD for each targeted organism was estimated as the concentration level with the lowest concentration level for which all four replicates were positive. The LoD was then confirmed by testing 20 additional replicates at the preliminary LoD concentration to establish that the strain was detected in at least 95% of the measurements. If an organism was detected in all replicates (20/20), the next lower concentration was also tested to verify a detection rate below 95% (19/20). If the initial results yielded a 19/20 correct result, no further testing was conducted and the level was confirmed as the LoD. The validated LoD for the EP test Norovirus and Rotavirus targets is summarized in the following tables:

LoD Established for Rotavirus and Norovirus Strains

Organism	***Source ID***	***LoD/mL***
Norovirus – GI (CDC 2142)	Clinical Sample (CDC)	4.12×10^5 copies

Norovirus – GII (D17219)	Clinical Sample (CDC)	1.67×10^6 copies
Rotavirus – DS1 (VR-2550)	VR-2550 (ATCC)	1.11×10^3 $TCID_{50}$
Rotavirus – W161 (VR-2551)	VR-2551 (ATCC)	3.70×10^2 $TCID_{50}$

Final LoD Established for the EP Test Viral Analytes

Reportable Target	***LoD/mL***
Norovirus	4.12×10^5 – 1.67×10^6 copies
Rotavirus	3.70×10^2 – 1.11×10^3 $TCID_{50}$

e. Analytical Reactivity (Inclusivity):

Refer to K140083 for Analytical Reactivity Study results for previously cleared EP bacterial analytes.

An Analytical Reactivity study was performed to determine whether the EP test is able to detect a variety of organisms that represent the genetic diversity of Norovirus and Rotavirus stains. This study included different strains that were used to prepare samples at low concentrations (near the LoD). The study included a panel of 41 viral strains, representing temporal, geographical, and phylogenetic diversity of the organisms. As propagating Noroviruses *in vitro* is challenging, the Norovirus strains evaluated in the study were patient stool samples, characterized and quantitated by the CDC using a validated real-time RT- PCR reference assay. The Norovirus samples were prepared by diluting the stool samples to intermediate concentrations in viral transport media and spiked into NSM to yield the desired concentration. Rotavirus samples were prepared using titered stocks diluted in viral transport media and then subsequently added to NSM to give the appropriate sample concentration. All viral strains were tested in triplicate.

All tested strains of Rotavirus and Norovirus GI were detected at a concentration of 3x LoD. All strains of Norovirus GII were detected; however for some strains, increased organism concentrations (10x and 50x LoD) were required to achieve detection of all replicates. The strains tested are presented in the following table:

Inclusivity Results for Norovirus and Rotavirus

Reportable Target	**Strain #/Unique Identifier**	**Inclusive Organisms**	**Inclusivity Titer**	**Multiple of LoD**
Norovirus GI	CDC GI.1	Norovirus	1.65×10^6 copies/mL	3x
	CDC GI.2-1	Norovirus	1.24×10^6 copies/mL	3x
	CDC GI.2-2	Norovirus	1.24×10^6 copies/mL	3x
	CDC GI.3-1	Norovirus	1.24×10^6 copies/mL	3x
	CDC GI.3-2	Norovirus	1.24×10^6 copies/mL	3x
	CDC GI.4-1	Norovirus	1.24×10^6 copies/mL	3x
	CDC GI.4-2	Norovirus	1.24×10^6 copies/mL	3x
	CDC GI.5	Norovirus	1.24×10^6 copies/mL	3x
	CDC GI.6	Norovirus	1.24×10^6 copies/mL	3x
	CDC GI.7-1	Norovirus	1.24×10^6 copies/mL	3x
	CDC GI.7-2	Norovirus	1.24×10^6 copies/mL	3x

	CDC GI.8-1	Norovirus	1.24×10^{6} copies/mL	3x
	CDC GI 8-2	Norovirus	1.24×10^{6} copies/mL	3x
Norovirus GII	CDC GII.1-1	Norovirus	1.67×10^{7} copies/mL	10x
	CDC GII.2	Norovirus	1.67×10^{7} copies/mL	10x
	CDC GII.3-1	Norovirus	5.01×10^{6} copies/mL	3x
	CDC GII.3-2	Norovirus	1.67×10^{7} copies/mL	10x
	CDC GII.3-3	Norovirus	1.67×10^{7} copies/mL	10x
	CDC GII.4-2	Norovirus	1.67×10^{7} copies/mL	10x
	CDC GII.4-3	Norovirus	1.67×10^{7} copies/mL	10x
	CDC GII.4-4	Norovirus	5.01×10^{6} copies/mL	3x
	CDC GII.4-5	Norovirus	1.67×10^{7} copies/mL	10x
	CDC GII.5	Norovirus	5.01×10^{6} copies/mL	3x
	CDC GII.7	Norovirus	8.35×10^{7} copies/mL	50x
	CDC GII.10	Norovirus	1.67×10^{7} copies/mL	10x
	CDC GII.12-1	Norovirus	5.01×10^{6} copies/mL	3x
	CDC GII.12-2	Norovirus	1.67×10^{7} copies/mL	10x
	CDC GII.16	Norovirus	8.35×10^{7} copies/mL	50x
	CDC GII.17-1	Norovirus	8.35×10^{7} copies/mL	50x
Rotavirus	VR-2272	Group A, G1	3.33×10^{3} $TCID_{50}$/mL	3x
	VR-2275	Group A, G1	3.33×10^{3} $TCID_{50}$/mL	3x
	VR-2417	Group A, G2P[3]	3.33×10^{3} $TCID_{50}$/mL	3x
	VR-2550	Group A, G2P[1]	3.33×10^{3} $TCID_{50}$/mL	3x
	VR-2551	Group A, G9P[1]	3.33×10^{3} $TCID_{50}$/mL	3x
	0810041CF	Group A, G1P[1]	3.33×10^{3} $TCID_{50}$/mL	3x
	MCRI G6P14 #528	Group A, G6P[14]	3.33×10^{3} $TCID_{50}$/mL	3x
	MCRI G8P14 #511	Group A, G8P[14]	3.33×10^{3} $TCID_{50}$/mL	3x
	MCRI G12P4 #262	Group A, G12P[4]	3.33×10^{3} $TCID_{50}$/mL	3x
	MCRI G1P8 #102	Group A, G1P[8]	3.33×10^{3} $TCID_{50}$/mL	3x
	MCRI G2P4 #95	Group A, G2P[4]	3.33×10^{3} $TCID_{50}$/mL	3x
	MCRI G3P8 #XX 434	Group A, G3P[8]	3.33×10^{3} $TCID_{50}$/mL	3x

In silico Results for Norovirus:

Based on *in silico* analysis, the majority of genotypes of Norovirus GII are expected to be detected by the EP test with the exception of GII.11 which is not expected to be detected by EP. Additionally, GII.6 and GII.13 are expected to either be not detected or to be detected with reduced sensitivity (i.e., detected only at higher concentrations). Norovirus GII.2, GII.3, and GII.12 genotypes are predicted to be detected with reduced sensitivity. Norovirus strains GII.9, GII.14, and GIV.1 are predicted to be detected by EP and Norovirus strains GII.8 could not be evaluated due to lack of available sequence information.

The need for higher concentrations for detection of some Norovirus GII strains with the EP test is likely a reflection of the high genetic diversity within Norovirus GII genotypes resulting in reduced sensitivity due to inefficient amplification or hybridization or both.

The following limitations are included in the draft EP test Package Insert to address the detection or predicted detection of various Norovirus genogroups/genotypes:

- **EP** is expected to be inclusive to most strains of the Norovirus GI, GII, and GIV genotypes known to cause disease in humans based on empirical testing and supplemented by *in silico* analysis. However, due to the high genetic diversity within Noroviruses, some strains may not be detected or may be detected with reduced sensitivity by **EP**. Refer to the Analytical Sensitivity (Inclusivity) section and Table 16 for details.
- **EP** inclusivity to Norovirus strains GII.9, GII.14, and GIV.1 was evaluated by *in silico* analysis only. Rare Norovirus genotypes GII.6 and GII.13 were determined to either be detected at reduced sensitivity or predicted to not be detected by **EP** based on *in silico* analysis. For GII.8, **EP** inclusivity is unknown as, in the absence of sequence information, *in silico* analysis could not be performed.
- Norovirus GII.11 is not expected to be detected by EP based on *in silico* analysis.

<u>*In silico* Results for Rotavirus</u>:

Rotavirus genotypes G4, G5, G10, G11, and G15 were not wet-tested but are predicted to be detected based on *in silico* data. Sequence data regarding genotypes G7, G21, and G24 was not available and therefore it is not known if these strains would be detected by the EP test. The following limitation is included in the EP test package insert.

- **EP** inclusivity to Rotavirus A genotypes, G4, G5, G10, G11, and G15 was evaluated based on *in silico* analysis only. Inclusivity of **EP** to Rotavirus A genotypes G7, G21, and G24 is unknown; representative strains were not available for empirical testing and in the absence of sequence information, *in silico* analysis could not be performed.

f. Analytical specificity (exclusivity):

Refer to K140083 for Analytical Specificity Study results for previously cleared EP bacterial analytes.

The specificity of the Norovirus and Rotavirus probes in the EP Test was addressed through the "unmasking" of the viral target results generated in the original analytical specificity study performed for K140083. In the study, a panel of 158 organisms comprised of 34 bacterial organisms, 18 viruses, four parasites, one fungal organism (*C. albicans*) and one human cell line were evaluated. Samples were prepared by spiking NSM with bacterial strains and *Candida albicans* at 10^7 CFU/mL and viral strains at 10^6 PFU/mL. If an organism could not be accurately titered or was unavailable in whole organism form, genomic DNA was tested at approximately 10^7 CFU/mL. All samples were tested in triplicate. Results for the Norovirus and Rotavirus targets yielded a "Not Detected" results in all three replicates for all organisms evaluated demonstrating no cross-reactivity with the EP test. The organisms

evaluated in the analytical specificity study are presented in the following tables:

Bacteria and Yeast (tested at 1x10^7 CFU/mL unless otherwise noted)					
Genus	**Species**	**ATCC Source ID**	**Genus**	**Species**	**ATCC Source ID**
Abiotrophia	*defectiva*	49176	*Enterococcus*	*faecalis*	51299
Acinetobacter	*baumannii*	19606		*faecium*	700221
	lwoffii	17925	*Escherichia*	*coli*	23511
Acrobacter	*butzleri*	49942		*coli* (non-pathogenic)	25922
	cryaerophilus	43157			35218
Aeromonas	*allosaccharophila*	35942		*coli* (EAEC)	23501
	bestiarum	BAA-231		*coli* (EPEC)	BAA-1704
	caviae	13136		*coli* (EPEC)	12014
	encheleia	51929		*coli* (ETEC)	43886
	enteropelogenes	49803		*coli* (ETEC)	23519
	eucrenophila	23309		*fergusonii*	35469
	hydrophilia	7966		*hermannii*	33650
	jandaei	49568	*Fusobacterium*	*varium*	8501
	salmonicida subsp *masoucida*	27013	*Helicobacter*	*hepaticus**	51449
				pylori	43504
	salmonicida subsp *salmonicida*	14174		*pylori*	700392
		33658		*pylori*	49503
	veronii	9071		*pylori*	51652
Alcaligenes	*faecalis*	15554	*Klebsiella*	*oxytoca*	43165
Bacillus	*cereus*	10702		*pneumoniae*	13883
Bacteroides	*caccae*	43185	*Lactobacillus*	*acidophilus*	11975
	fragilis	25285		*reuteri*	23272
	merdae	43184		*rhamnosus*	53103
	stercoris	43183	*Lactococcus*	*lactis*	11454
Candida	*albicans*	10231	*Leminorela*	*grimontii*	33999
Cedecea	*davisae*	33431	*Listeria*	*grayi*	19120
Citrobacter	*amalonaticus*	25407		*monocytogenes*	7644
	freundii	8090	*Morganella*	*morganii*	25830
	sedlakii	51115	*Peptostreptococcus*	*anaerobius*	27337
Clostridium	*bifermentans*	638	*Plesiomonas*	*shigelloides*	14029
	bolteae	BAA-613	*Porphyromonas*	*asaccharoluticus*	25260
	butyricum	19398	*Prevotella*	*melaniogenica*	25845
	difficile	BAA-1805	*Proteus*	*mirabilis*	25933
	difficile	17857		*vulgaris*	29905
	difficile, non-tox	BAA-1801		*penneri*	35198
	haemolyticum	9650	*Providencia*	*stuartii*	33672
	methylpentosum	43829		*alcalifaciens*	9886
	nexile	27757		*rettgeri*	9250
	noyvi	19402	*Pseudomonas*	*aeruginosa*	35554
	orbiscindens	49531		*fluorescens*	13525
	perfringens	13124		*putida*	12633
	scindens	35704		*aeruginosa*	27853
	septicum	12464	*Ruminococcus*	*bromii*	27255
	sordellii	9714	*Serratia*	*liquefacians*	27592
	spiroforme	29899		*marcescens*	13880

Bacteria and Yeast (tested at 1x10^7 CFU/mL unless otherwise noted)					
Genus	**Species**	**ATCC Source ID**	**Genus**	**Species**	**ATCC Source ID**
	sporogenes	15579	*Staphylococcus*	*aureus*	43300
Colinsella	*aerofaciens*	25986		*epidermidis*	700583
Desulfovibrio	*piger*	29098	*Streptococcus*	*agalactiae, O90R*	12386
Edwardsiella	*tarda*	15947		*dysgalactiae*	12394
Enterobacter	*aerogenes*	13048		*mutans*	25175
	cloacae	29006			

Viral, Parasites and Human DNA.			
Virus	**Strain #/Unique Identifier/Source**	**Serovars/ Groups**	**Titer Tested**
Adenovirus	0810050CF	Type 1/Group C	1.0x106 TCID50/mL
	0810110CF	Type 2/Group C	
	0810062CF	Type 3/Group B1	
	0810070CF	Type 4/Group E	
	0810020CF	Type 5/Group C	
	0810108CF	Type 14/Group B2	
	0810117CF	Type 26/Group D	
	0810073CF	Type 31/Group A	
	0810119CF	Type 37/Group D	
	ATCC VR-931	Type 40/Group F	1.58x105 TCID50/mL
	VR-1572D	Human 4	1.0x106 TCID50/mL
Astrovirus	P#711/24/08	-	1.0x107 copies/mL
Coxsackievirus B4	ATCC VR-184	-	3.16x105 TCID50/mL
Cytomegalovirus	0810003-CF	-	7.24x105 TCID50/mL
Echovirus 11	0810023-CF	-	1.0x 106 TCID50/mL
Enterovirus 68	VR-213	-	1.0x106 TCID50/mL
Sapovirus	2008729730	-	1x107 copies/mL
	2009726567		
Human Cell Line			
Colon epithelial cells (colorectal adenocarcinoma)	ATCC CCL-218	-	1.0x105cells/mL
Parasites			
Blastocystis hominis	ATCC 50608-D	-	1x107 copies/mL
Cryptosporidium parvum	PRA-67-D		
Entamoeba histolytica	ATCC 30459-D		

Viral, Parasites and Human DNA.			
Virus	**Strain #/Unique Identifier/Source**	**Serovars/ Groups**	**Titer Tested**
Giardia lamblia	ATCC 50803-D		

Species of Campylobacter, Vibrio, and Yersinia not detected by EP			
Genus	***Species***	***Source Designation***	***Titer tested (CFU/mL)***
Campylobacter	*concisus*	ATCC BAA-1457	5.5x106
	curvus	ATCC BAA-1459	6.1x106
	fetus	ATCC 25936	3.9x107
	gracilis	ATCC 33236	1.0x107
	hominis (gDNA)	ATCC BAA-381D-5	1.0x107 copies/mL
	hyointestinalis	ATCC 35217	1.0x107
	insulaenigrae	CCUG 48653	
	lanienae	CCUG 44467	
	mucosalis	ATCC 49352	
	rectus	ATCC 33238	
	showae	ATCC 51146	
	sputorum	ATCC 35980	
	upsaliensis	ATCC BAA-1059	7.5x106
Vibrio	*alginolyticus*	ATCC 17749	1.0x107
	campbellii	ATCC 25920	
	cincinnatiensis	ATCC 35912	
	fluvialis	ATCC 33809	
	furnissii	ATCC 11218	
	harveyi	ATCC 14126	
	metschnikovii	ATCC 7708	
	mimicus	ATCC 33653	
	*tubiashii**	ATCC 18106	
	vulnificus	ATCC BAA-86	
		ATCC 27562	
		ATCC 33815	
Yersinia	*aldovae*	ATCC 35236	
	aleksiciae	CCUG 52872	
	bercovieri	ATCC 43970	
	frederiksenii	ATCC 33644	
	intermedia	ATCC 33647	
	kristensenii	ATCC 33639	
	mollaretii	ATCC 43969	
	pseudotuberculosis	ATCC 29910	
	ruckeri	ATCC 29473	
	rohdei	ATCC 43380	

Potential "within panel" cross-reactivity was evaluated using representative targeted viral and bacterial analytes tested in the Carryover/Cross-Contamination Study performed for the initial clearance of the EP Test (K140083). The study yielded no EP false positive results, demonstrating that cross-reactivity does not occur between EP targets. The strains evaluated are presented in the following table.

Organism	Strain	Expected EP Test	Concentration Tested
Yersinia enterocolitica subsp. enterocolitica	ATCC 23715	Yersinia enterocolitica	5×10^6 CFU/mL
Shigella dysenteriae, Stx1	ATCC 29026	Shigella spp., Shiga	5×10^6 CFU/mL
Escherichia coli, Stx2	ATCC BAA-176	Shiga toxin 2	5×10^6 CFU/mL
Salmonella enterica subsp. enterica serovar Typhi	ATCC 7251	Salmonella spp.	5×10^6 CFU/mL
Campylobacter jejuni subsp. jejuni	ATCC 43429	Campylobacter spp.	5×10^6 CFU/mL
Vibrio cholerae	ATCC 39315	Vibrio spp.	5×10^6 CFU/mL
Norovirus GI	CDC 2142	Norovirus	1.0×10^8 copies/mL
Norovirus GII	CDC 2012	Norovirus	3.0×10^6 copies/mL
Rotavirus A	VR-2551	Rotavirus	1.6×10^6 $TCID_{50}$/mL

For viral strains that were difficult to obtain, the analytical specificity of the EP Test was evaluated via *in silico* analysis. This analysis demonstrated that Norovirus genotype GIV.2, Norovirus genogroups GIII, GV and Rotavirus genogroups B, C, D, and NADRV (Novel Adult Diarrheal Rotavirus) are not predicted to cross-react with the EP test. The following product limitations are included in the package insert:

- Norovirus GIII, GIV.2, and GV are not expected to be detected based on *in silico* analysis.
- Rotavirus genogroups B, D, and NADRV are not expected to be detected by EP based on in silico analysis. In addition, Rotavirus genogroup C strains are not expected to be detected by EP, with the exception of porcine strains within this genogroup.

g. Matrix equivalence Study

N/A

h. Interference Study

Refer to K140083 for Interference Study results for previously cleared EP bacterial analytes.

A study was performed to assess the potential inhibitory effect of endogenous and exogenous substances commonly found in clinical stool specimens. Two representative strains for both Norovirus and Rotavirus were evaluated as shown in the following table:

Viral strains tested Interfering Substance Study

Organism	*Source/Strain*	*Expected Calls*	*LoD in Stool*
Norovirus (GI)	CDC 2142	Norovirus Detected	4.12×10^5 copies/mL
Rotavirus A	ATCC VR-2550	Rotavirus Detected	1.11×10^3 $TCID_{50}$/mL

Samples were prepared with each potentially interfering substance at high, medically relevant concentrations in NSM with Norovirus and Rotavirus strains at 3x the LoD. Samples containing NSM/interferent only were tested as controls. Each sample was tested in replicates of three. The potential interfering substances that were evaluated are provided in the following table.

Potentially Interfering Substances Evaluated

Interferent	Active Ingredient	Interferent Concentration in stool
Intralipid	Triglyceride (Fecal Fat)	5% v/v
Cholesterol	Cholesterol (Fecal Fat)	5% w/v
Whole Blood	Glucose, Hormones, Enzymes, Ions, Iron etc.	40% v/v
Mucus (Nasopharyngeal swab sample in UTM)	Immunoglobulins, Lysozyme, Polymers	40% w/v
Nystatin Suspension	Nystatin	30% w/v
Preparation H® Anti-itch Hydrocortisone 1%	Hydrocortisone	30% w/v
Desitin Maximum Strength Original Paste	Zinc Oxide	30% w/v
Preparation H® Hemorrhoidal Ointment	Phenylephrine	30% w/v
Options Conceptrol®Vaginal Contraceptive Gel	Nonoxynol-9	30% w/v
Wet Ones®Antibacterial Hand Wipes	Benzalkonium Chloride, Ethanol	30% v/v
K-Y®Personal Lubricant Jelly	Glycerin	30% w/v
Vaseline Original 100% Pure Petroleum Jelly	Petroleum	30% w/v
Tums Antacid with Calcium Extra Strength 750	Calcium Carbonate	10% w/v
Gaviscon Extra Strength Liquid Antacid	Aluminum Hydroxide, Magnesium Hydroxide	10% w/v
Mesalazine	S. Amino Salicylic Acid	10% w/v
Immodium® AD Anti-Diarrheal	Loperamide Hydrochloride	10% w/v
Pepto-Bismol Max Strength	Bismuth subsalicylate	10% v/v
MetronidazoleTopical Cream (0.75%)	Metronidazole	10% w/v
Naproxen Sodium	Naproxen Sodium	10% w/v
Mucin from bovine submaxillary glands, Type I-S (Dehydrated)	Mucin	10% w/v
Barium Sulfate	Barium Sulfate	10% w/v
Amoxicillin (Antibiotic)	Amoxicillin	1% w/v
Control (no interferent)	N/A	N/A

The EP test correctly detected Rotavirus and Norovirus for all samples; however the following two samples yielded false positive results:

- One of three replicates of low-titer Rotavirus with Loperamide HCl gave a false positive result for Norovirus; however the Rotavirus was detected correctly for this sample.

- One of three replicates of low titer Rotavirus with Zinc Oxide gave a false positive for Salmonella; however the Rotavirus was detected correctly for this sample.

Six additional replicates were tested for both of these samples and the additional testing yielded no further false positive results and therefore Loperamide HCl and Zinc Oxide are not considered to interfere with the EP test. In summary, the Interference Study results indicate that the substances evaluated should not affect the performance of the EP test for detection of Norovirus and Rotavirus.

i. Fresh versus Frozen Study

Refer to K140083 for Fresh versus Frozen Study results for previously cleared EP bacterial analytes.

A fresh versus frozen equivalency study was performed to support inclusion of frozen samples in analytical studies as well as inclusion of frozen archived specimens in the clinical study. The study included samples prepared with three viral strains at five concentrations prepared in a 3-fold serial dilution series in NSM. Baseline testing of the freshly-prepared samples was performed in replicates of four; the four remaining vials were stored frozen at ≤70ºC then thawed and tested. For demonstration of equivalence, the study acceptance criteria required detection of each targeted analyte in fresh and frozen samples with no higher than a 3-fold difference in the lowest concentration detected in fresh versus frozen samples.

Study results for both Norovirus strains demonstrated that the lowest concentration for which all replicates were detected was identical for fresh and frozen samples. For Rotavirus, frozen samples were detected at a 3-fold higher concentration than fresh samples, thereby meeting the predefined acceptance criteria. Therefore the fresh versus frozen study results shown in the table below in combination with study results for the bacterial analytes (K140083) support the use of simulated samples subjected to a single freeze/thaw cycle interchangeably with fresh/unfrozen simulated samples in the analytical studies. Additionally, these study results support the use of archived frozen specimens in the clinical study.

Summary of Results for Fresh Versus Frozen Study – NSM Samples

Organism	**Strain**	**Established LoD**	**Lowest Concentration where all Replicates were "Detected" (/mL)**	
			Fresh	*Frozen*
Norovirus, GI	CDC 2142	4.12×10^5 copies/mL	1.37×10^5 copies	1.37×10^5 copies
Norovirus, GII	D17219	1.67×10^6 copies/mL	1.67×10^6 copies	1.67×10^6 copies

Rotavirus	ATCC VR-2550	1.11×10^3 TCID50/mL	3.70×10^2 $TCID_{50}$	1.11×10^3 $TCID_{50}$

j. Carryover/Cross-Contamination

Refer to K140083 for Carryover/Cross-Contamination Study results for previously cleared EP bacterial analytes.

In order to assess the EP assay for potential carryover or cross-contamination from high positive Norovirus and Rotavirus samples, positive samples (100x LoD) prepared in NSM were tested in an alternating pattern with negative samples comprised of NSM only. The following Norovirus and Rotavirus strains were evaluated on three different Verigene Processor SP instruments as shown in the following tables.

Organisms Tested for the Carryover/Cross-Contamination Study

Organism	***Strain***	***Concentration***	***Expected EP Test Result***
Norovirus GI	CDC 2142	4.12×10^7 copies/mL	*Norovirus*
Norovirus GII	D17219	1.67×10^8 copies/mL	*Norovirus*
Rotavirus	ATCC VR-2550	1.11×10^5 $TCID_{50}$/mL	*Rotavirus*

Sample Testing Order for the Carryover/Cross-Contamination Study

	Verigene Processor SP		
Run No.	1	2	3
1	Rotavirus	Norovirus GI	Norovirus GII
2	NEG	NEG	NEG
3	Rotavirus	Norovirus GI	Norovirus GII
4	NEG	NEG	NEG
5	Rotavirus	Norovirus GI	Norovirus GII
6	NEG	NEG	NEG

The study results demonstrated that all nine negative samples yielded the expected "Not Detected" call for all EP analytes. These results combined with the initial carryover study results for the EP bacterial analytes (K140083) demonstrate that the carryover/cross contamination rate for the EP test is acceptable.

k. Competitive Inhibition Study:

Refer to K140083 for Competitive Inhibition Study results for previously cleared EP bacterial analytes.

<u>Within Panel Competitive Inhibition</u>

In order to assess the EP test for potential competitive inhibition for the Norovirus and Rotavirus targets, samples were tested that contained various combinations of each viral target with other EP bacterial and viral EP test panel organisms. Contrived samples were prepared in NSM with Norovirus and Rotavirus present at approximately 3x LoD mixed with high concentrations of each potentially interfering organism (>10^6 CFU/mL). Testing was performed in triplicate for each unique sample combination. The EP test correctly detected both expected targets for all sample combinations with one exception in which one of three replicates of the low titer Rotavirus/high titer *Y. enterocolitica* sample gave a false negative result for Rotavirus. Testing of an additional six replicates of this combination yielded correct calls for both organisms suggesting that high concentrations of *Y. enterocolitica* should not interfere with the detection of Rotavirus by EP.

Evaluation of Potential Interference from Non-targeted Microorganisms

A study was performed to evaluate whether 14 microorganisms commonly present in stool may interfere with the EP test for detection of Norovirus and Rotavirus. Samples for this study were prepared in NSM with Norovirus GI and Rotavirus A strains at 3x LoD mixed with each potentially interfering organism at >10^7 CFU/mL for bacteria, and 9×10^6 cells/mL and 7×10^5 cells/mL respectively for parasites *Blastocystis hominis* and *Entamoeba histolytica.* Additional samples containing only Norovirus or Rotavirus were included as control samples. Testing was performed in triplicate with the organisms listed in the following table.

Organisms tested	
Genus	**Species**
Bacteroides	*fragilis*
Prevotella	*oralis*
Prevotella	*melaninogenicus*
Bifidobacterium	*bifidum*
Clostridium	*perfringens*
Enterobacter	*aerogenes*
Enterococcus	*faecalis*
Escherichia	*coli*
Klebsiella	*pneumonia*
Lactobacillus	*acidophilus*
Staphylococcus	*aureus*
Blastocystis	*hominis*
Entamoeba	*histolytica*
Candida	*albicans*

The EP test correctly identified the expected analyte for all replicates of each organism combination, demonstrating that high concentrations of non-targeted organisms did not interfere with the ability of the EP test to detect Norovirus and Rotavirus.

l. Assay cut-off:

The presence or absence of each EP target analyte is determined by the mean intensity of target capture spots relative to the Signal Detection Threshold. The capture, mediator,

and PCR primer oligonucleotides in the EP test are designed to eliminate sequence-related cross-reactivity, resulting in non-specific target signal intensities at capture spots that are similar to the microarray background signal. In contrast, target amplicon hybridization to complementary capture and mediator probes are expected to give signals that are well-separated from negative capture spots. When reading a test slide, multiple images of each array are taken at increasing exposures times and the final target group mean intensity value for an analyte is assigned at the shortest exposure at which the intensity exceeds the Signal Detection Threshold. If none of the target signal exceeds the threshold for any exposure, the mean spot intensity is evaluated at the longest exposure taken. With this imaging and analysis design, a signal detection threshold of 30,000 was established to generate a "Detected" call for the eight bacterial target spot groups, four virus target spot groups, and two controls of the EP test.

In order to demonstrate the appropriateness of the cut-off value for the selected threshold, the target mean intensity values observed with the EP test were examined for the final LoD confirmatory tests for the bacterial and viral targets. Data from 16 bacterial strains, four viral strains, and two negative samples were evaluated. With replicates of 20 for each of 22 samples and 14 unique target spot groups evaluated per test, a total of 6160 data points (1320 expected positive) were assessed in the study.

A logistic fit analysis of Expected Results by Target Mean Intensity for the chosen threshold demonstrated that expected positive signals are well separated from the expected negative target signal and therefore the chosen threshold value distinguishes the "True Positives" from the "True Negatives". The selected threshold was then validated in the pivotal clinical study.

2. <u>Comparison studies:</u>

   a. *Method comparison with predicate device:*

      Not applicable. Refer to Clinical Study Section of this document.

   b. *Matrix comparison:*

      Not applicable

3. <u>Clinical study</u>

   Refer to K140083 for additional details regarding Clinical Study results for previously cleared EP bacterial analytes.

   The clinical evaluation of the EP test for Norovirus and Rotavirus was performed concurrently with the clinical evaluation of EP bacterial targets for the initial clearance of the EP test for bacterial analytes (K140083). Due to insufficient positive specimens for Norovirus and Rotavirus, the viral targets were not included for the initial clearance of the EP test and therefore the original clinical study was extended at one clinical site in

order to obtain additional performance data for these viral analytes. For this submission the Norovirus and Rotavirus results from the original clinical study were "unmasked" and the performance data provided below includes study results from the original study combined with results from the additional testing.

The clinical study was conducted at eight clinical study testing sites and was conducted under IRB supervision at each testing and specimen acquisition site. De-identified Cary-Blair specimens were enrolled from individuals for whom standard of care testing was ordered. A portion of each leftover residual unformed stool specimen in Cary-Blair media was obtained for testing.

The clinical study utilized the following four categories of specimens to establish performance for the EP bacterial and viral targets:

- Fresh: Prospectively-collected fresh Cary-Blair specimens enrolled and tested at the study test sites;
- Frozen: Prospectively-collected frozen Cary-Blair specimens enrolled and tested at the study test sites;
- Selected: De-identified archived frozen specimens collected and stored in Cary-Blair media were obtained from the specimen acquisition sites. These specimens were shipped frozen from each specimen acquisition site to the Sponsor, blinded and sent to clinical testing sites. In parallel, each specimen was tested by analyte-specific PCR amplification and bi-directional sequencing (BDS) to confirm the original result. If the original analyte identification could not be confirmed, the specimen was excluded from the study dataset.
- Contrived specimens (for selected bacterial analytes)

Reference/Comparator methods for Bacterial Analytes: Each specimen was cultured and suspected colonies were isolated and identified using an FDA Cleared Automated System. All samples were enriched in MacConkey Broth for suspected Shiga toxin producing organisms followed by EHEC EIA testing. In addition, bi-directional sequencing (BDS) assays were developed to confirm the identity of targeted analytes. Overall, these BDS systems were used in the EP test Clinical Study in various capacities: (i) for species-level identification of clinical positives, if applicable, (ii) typing of virulence markers (Stx1, Stx2), and (iii) discordant analysis between the EP test and the reference method identification. An overview of comparator methods performed for the bacterial analytes are listed in the following table:

Comparator Methods for Bacterial Analytes

EP Analyte	Comparator Methods
Campylobacter	Bacterial Culture and Automated Phenotypic Identification using FDA Cleared Methods
Salmonella	

EP Analyte	Comparator Methods
Shigella	
Vibrio	
Y. enterocolitica	
Stx1	MacConkey Broth Enrichment followed by EHEC EIA and PCR amplification//bi-directional sequencing for Confirmation and Typing
Stx2	MacConkey Broth Enrichment followed by EHEC EIA and PCR amplification//bi-directional sequencing for Confirmation and Typing

Comparator Methods for Viral Analytes: For both the Norovirus and Rotavirus targets, the comparator methods used for prospectively collected fresh and frozen specimens were a composite of one real-time PCR and two conventional PCR assays. Positive conventional PCR tests were confirmed with bi-directional sequencing. Specimens that were positive by real-time PCR and negative by the first conventional PCR assay were tested with a second conventional PCR assay and bi-directional sequencing, if applicable. Specimens were characterized as negative when both the real-time RT-PCR assay and the first conventional PCR assay were negative. The algorithm used to determine the composite comparator result is shown in the following table:

Composite Test Algorithm for Norovirus and Rotavirus

Real-Time RT-PCR Result	***Conventional PCR1 w/BDS Result***	***Conventional PCR2 w/BDS Result***	***Final Composite Comparator Result***
Positive	Positive	N/A	Positive
Negative	Positive	N/A	Positive
Positive	Negative	Positive	Positive
Positive	Negative	Negative	Negative
Negative	Negative	N/A	Negative

The Norovirus comparator assays included three well-established, validated and published assays from the CDC CalciNet Laboratory: a real-time PCR assay and two conventional PCR assays. These assays were also used to quantitate and characterize all Norovirus GI and GII strains used for LoD and analytical reactivity testing. Validation of the three assays was performed by CDC and the acceptability of these assays for use in the Norovirus composite comparator algorithm was supported by literature references provided by the sponsor.

For the Rotavirus comparator assays, primers for the real-time PCR and one of the conventional PCRs were obtained from published protocols. The second conventional PCR was developed and validated by Nanosphere. None of the primers used for each of three reference assays overlap with the EP test primers. Validation of the three comparator Rotavirus assays included evaluation of each assay's LoD and as well as inclusivity testing.

Clinical Study Results

The clinical study included a total of 1940 evaluable specimens comprised of 1328 prospectively collected fresh and frozen specimens, 203 selected archived specimens and 409 contrived specimens.

Following the clearance of K140083, confirmatory PCR sequencing results were received for an additional nine selected archived specimens positive for EP bacterial analytes that were not included in the original submission. These specimens included one specimen for *Stx1*/*Stx2*, two specimens positive for *Shigella spp*., and six specimens positive for *Salmonella spp*. For this submission, EP test results for these nine specimens have been added to the valid clinical dataset evaluated for K140083.

A total of 2102 specimens were tested with the EP test at the eight study sites. Excluded specimens included 60 fresh prospectively-collected, 73 selected specimens, and three simulated specimens. Of the remaining 1966 specimens, 26 specimens had a final No Call resulting in 26 indeterminate specimens. The following tables show the number of clinical specimens, arranged by site, determined to be valid, indeterminate, and excluded and additional information regarding the reasons for exclusion of 136 specimens.

Breakdown of Clinical Specimens by Site

Site	Enrolled Specimens	Excluded Specimens	Indeterminate Specimens	Valid Specimens
1	66	2	0	64
2	123	8	1	114
3	858	6	13	839
4	380	24	5	351
5	405	51	6	348
6	135	15	1	119
7	106	21	0	85
8	29	9	0	20
Total	**2102**	**136**	**26**	**1940**

Non-evaluable Clinical Specimens and Reasons for Exclusion from Dataset

Site	No. Excluded	Time Inclusion Criteria Not Met	Operator Error	Shipping Issue	Invalid QC	No/Inconclusive Reference Result	Invalid Specimens
1	2	1	1	0	0	0	0
2	8	2	2	0	2	2	0
3	6	5	0	0	0	1	0
4	24	3	1	4	0	9	7
5	51	0	4	4	14	22	7
6	15	7	2	5	0	1	0
7	21	0	0	3	0	18	0
8	9	0	0	0	0	9	0
Total	**136**	**18**	**10**	**16**	**16**	**62**	**14**

The initial No Call rate for the clinical study was 4% (78/1940 specimens). Of the 78

initial "No Calls," 52 yielded a valid test result upon retesting for a final No-call rate of 1.3% (26/1946). Additionally, there were an initial 16 specimens that gave Pre-AE results and three specimens that gave Pre-AE results upon repeat testing, for a total of 19 Pre-AE occurrences in the Method Comparison study (19/2063 tests run = 0.9% pre-AE rate). Combining the No Call and Pre-AE results showed an initial invalid test rate of 4.9% (104/1940) and a final invalid test rate of 1.3% (26/1940). The 26 specimens yielding a final No Call result were not included in the valid dataset.

The following table provides a summary of patient age for prospectively collected specimens (patient age was unknown for 15 specimens):

Valid Specimens Stratified by Patient Age (n=1313)

Age Range	*No. of Specimens*	*Percentage*
0-1	63	4.8%
>1-5	49	3.7%
>5-12	85	6.5%
>12-21	146	11.1%
>21-65	636	48.4%
>65	334	25.4%
Total	**1313**	**100%**

The following clinical performance tables provide a summary of the clinical performance of the EP test for the detection of the five bacterial targets, the Shiga toxin 1 gene and Shiga toxin 2 gene targets, and the Norovirus GI/GII and Rotavirus A targets.

Summary of Bacterial Target Clinical Test Performance (n=1940) - Compared to Reference Methods (Culture and Conventional Biochemical and Automated Phenotypic Identification

	Specimen Type			*n*	*% Agreement (95% CI)*	
					Positive	***Negative***
Campylobacter spp.	Clinical Specimens	Prospectively Collected	Fresh	1294	90.9% 20/22[a] (79.8-98.9)	98.7% 1255/1272[d,q] (97.9-99.2)
			Frozen	34	100% 2/2 (15.8-100)	100% 32/32 (89.1-100)
		Selected		203	97.5% 39/40[c] (86.8-99.9)	99.4% 162/163[e] (96.6-100)
	Simulated			409	98.5% 67/68[b] (92.1-100)	100% 341/341 (98.9-100)
***Shigella* spp.**	Clinical Specimens	Prospectively Collected	Fresh	1294	66.7% 2/3[k,r] (9.4-99.2)	98.8% 1275/1291[l] (98.0-99.3)
			Frozen	34	-	97.1% 33/34[m] (84.7-99.9)

	Specimen Type			*n*	*% Agreement (95% CI)*	
					Positive	***Negative***
***Salmonella* spp.**	Clinical Specimens	Prospectively Collected	Fresh	1294	86.4% 19/22[f,r] (65.1-97.1)	99.4% 1265/1272[h] (98.9-99.8)
			Frozen	34	100% 1/1 (2.5-100)	97.0% 32/33[i] (84.2-99.9)
		Selected		203	98.3% 58/59[g] (90.9-100)	99.3% 143/144[j] (96.2-100)
	Simulated			409	100% 67/67 (94.6-100)	100% 342/342 (98.9-100)
***Vibrio* spp.**	Clinical Specimens	Prospectively Collected	Fresh	1294	100% 1/1 (2.5-100)	100% 1293/1293 (99.7-100)
			Frozen	34	100% 1/1 (2.5-100)	100% 33/33 (89.4-100)

		Selected		203	100% 8/8 (63.1-100)	99.5% 194/195[n] (97.2-100)			Selected	203	100% 1/1 (2.5-100)	100% 202/202 (98.2-100)
	Simulated			409	100% 50/50 (92.9-100)	100% 359/359 (99.0-100)		Simulated		409	91.1% 51/56[o] (80.4-97.0)	99.7% 352/353[p] (98.4-100)
Y. enterocolitica	Clinical Specimens	Prospectively Collected	Fresh	1294	-	100% 1294/1294 (99.7-100)						
			Frozen	34	-	100% 34/34 (89.7-100)						
		Selected		203	100% 1/1 (2.5-100)	100% 202/202 (98.2-100)						
	Simulated			409	100% 59/59 (93.9-100)	100% 350/350 (99.0-100)						

Footnoted Information for Previous Table

	No.	*Type*	*EP Test Result*	*Reference Method Result(s)*	*PCR Amp/BDS Results*
a.	1	Fresh	Not Detected	*C. jejuni subsp. jejuni*	*Positive for Campylobacter jejuni*
	2	Fresh	Not Detected	*C. jejuni subsp. jejuni & Proteus spp.*	*Negative for Campylobacter spp.*
b.	1	Simulated (2X)	Not Detected	*C. lari*	*Positive for Campylobacter lari*
c.	1	Select	Not Detected	*Campylobacter*	*Low-Level Positive for Campylobacter jejuni (at LoD; Negative upon repeat))*
d.	1	Fresh	Campylobacter	Negative	*Positive for Campylobacter coli*
	2	Fresh	Campylobacter	*M. morganii subsp. morganii & N. cinerea*	*Positive for Campylobacter jejuni*
	3	Fresh	Campylobacter	*P. aeruginosa*	*Positive for Campylobacter jejuni*
	4	Fresh	Campylobacter	*E. coli*	*Positive for Campylobacter jejuni*
	5	Fresh	Campylobacter	Negative	*Positive for Campylobacter jejuni*
	6	Fresh	Campylobacter	*E. coli*	*Positive for Campylobacter jejuni*
	7	Fresh	Campylobacter	Negative	*Positive for Campylobacter jejuni*
	8	Fresh	Campylobacter	*M. morganii subsp. morganii*	*Positive for Campylobacter jejuni.*
	9	Fresh	Campylobacter	*C. braakii & E. cloacae subsp dissolvens & N. cinerea*	*Positive for Campylobacter jejuni*
	10	Fresh	Campylobacter	Negative	*Positive for Campylobacter spp.*
	11	Fresh	Campylobacter	Negative	*Positive for Campylobacter spp.*
	12	Fresh	Campylobacter	Negative	*Positive for Campylobacter jejuni*
	13	Fresh	Campylobacter	Negative	*Positive for Campylobacter jejuni*
	14	Fresh	Campylobacter	Negative	*Positive for Campylobacter jejuni*
	15	Fresh	Campylobacter	*E. coli*	*Positive for Campylobacter jejuni*
	16	Fresh	Campylobacter	*Proteus spp.*	*Positive for Campylobacter coli*
	17	Fresh	Campylobacter	*M. morganii subsp. morganii*	*Positive for Campylobacter jejuni*
e	1	Select	Campylobacter and Salmonella	*Salmonella*	*Positive for Campylobacter jejuni and Salmonella enterica*
f.	1	Fresh	Not Detected	*Salmonella spp.*	*Positive for Salmonella enterica*
	2	Fresh	Not Detected	*Salmonella spp.*	*Positive for Salmonella enterica*
	3	Fresh	Not Detected	*Salmonella spp.*	*Positive for Salmonella enterica*
g.	1	Select	Not Detected	*Salmonella spp.*	*Low-Level Positive for Salmonella enterica (at LoD; Negative upon repeat)*
h.	1	Fresh	Salmonella	Negative	*Negative for Salmonella spp.*
	2	Fresh	Salmonella	Negative	*Positive for Salmonella enterica*
	3	Fresh	Salmonella	*E. coli*	*Positive for Salmonella enterica*
	4	Fresh	Salmonella	*C. freundii & Proteus spp.*	*Negative for Salmonella spp.*
	5	Fresh	Salmonella	Negative	*Positive for Salmonella enterica*
	6	Fresh	Salmonella	*P. alcalifaciens*	*Negative for Salmonella spp.*
	7	Fresh	Salmonella	Negative	*Positive for Salmonella enterica*
i.	1	Frozen	Salmonella	*Proteus spp.*	*Positive for Salmonella enterica*
j.	1	Select	Salmonella	Campylobacter	*Positive for Campylobacter jejuni*
k.	1	Fresh	Not Detected	*Shigella spp.*	*Positive for Shigella/EIEC*
l.	1	Fresh	Shigella	Negative	*Positive for Shigella/EIEC*
	2	Fresh	Shigella	Negative	*Positive for Shigella/EIEC*
	3	Fresh	Shigella	Negative	*Positive for Shigella/EIEC*
	5	Fresh	Shigella	Negative	*Positive for Shigella/EIEC*
	6	Fresh	Shigella	Negative	*Positive for Shigella/EIEC*

	7	Fresh	Shigella	Negative	*Positive for Shigella/EIEC*
	8	Fresh	Shigella	*A. hydrophila/caviae & P. putida*	*Positive for Shigella/EIEC*
	9	Fresh	Shigella	Negative	*Positive for Shigella/EIEC*
	10	Fresh	Shigella	Negative	*Positive for Shigella/EIEC*
	11	Fresh	Shigella	*E. coli*	*Positive for Shigella/EIEC*
	12	Fresh	Shigella	Negative	*Not performed*
	13	Fresh	Shigella	Negative	*Positive for Shigella spp.*
	14	Fresh	Shigella	Negative	*Positive for Shigella spp.*
	15	Fresh	Shigella	Negative	*Positive for Shigella spp.*
	16	Fresh	Shigella	Negative	*Positive for Shigella/EIEC*
m.	1	Frozen	Shigella	*P. rettgeri*	*Positive for Shigella/EIEC*
n.	1	Select	Shigella	Shiga toxin	*Positive for Shiga toxin 1*
o.	1	Simulated (2X)	Not Detected	*Vibrio parahaemolyticus*	*Positive for V. parahaemolyticus*
	2	Simulated (2X)	Not Detected	*Vibrio cholerae*	*Positive for V. cholera*
	3	Simulated (40X)	Not Detected	*Vibrio cholerae*	*Negative for V. cholera*
	4	Simulated (40X)	Not Detected	*Vibrio parahaemolyticus*	*Negative for V. parahaemolyticus*
	5	Simulated (40X)	Not Detected	*Vibrio cholerae*	*Positive for V. cholerae*
p.	1	Simulated (30X)	Campylobacter and Vibrio	*Campylobacter lari*	*Not performed*
q.	1	One FP "Campylobacter" and one FN "Campylobacter" were processed together at the central reference testing site and may be a result of a sample mix-up.			
r.	1	One TP "Salmonella" and one FN "Salmonella" were processed together at the study testing site and may be a result of a sample mix-up.			

Summary of Clinical Test Performance (n=1940 - Compared to Broth Enrichment/EHEC EIA).

Stx Combined

Footnoted Information

	Specimen Type			***n***	***% Agreement (95% CI)***	
					Positive	***Negative***
***STX* Combined**	Clinical Specimens	Prospectively Collected	Fresh	1294	100% 7/7 (59.0-100)	99.7% 1283/1287[a, e] (99.2-99.9)
			Frozen	34	-	100% 34/34 (89.7-100)
		Selected		203	100% 13/13 (75.3-100)	99.5% 189/190[b] (97.1-100)
	Simulated			409	98.2% 107/109[d] (93.5-99.8)	98.7% 296/300[c, f] (96.6-99.6)

for Previous Table

	No.	***Fresh, Frozen or Simulated (xLoD)***	***Identified by EP test as:***	***Identified by Reference Method(s) as:***	***PCR Amp/BD Sequencing Results (if applicable)***
a.	1	Fresh	Shiga Toxin 1	Negative	*Positive for Stx 1 gene*
	2	Fresh	Shiga Toxin 1 and Norovirus	*Escherichia coli*	*Positive for Stx 1 gene*
	3	Fresh	Shiga Toxin 1 and Shiga Toxin 2	*Citrobacter youngae*	*Positive for Stx 1 gene and Stx 2 gene*
b.	1	Select	Shiga Toxin 1 and *Campylobacter*	*Campylobacter*	*Positive for Stx 1 gene*
c.	1	Simulated (13X)	Shiga Toxin 1and *Salmonella*	*Salmonella enterica subsp. enterica*	*Negative for Stx 1 gene and Stx 2 gene*
	2	Simulated (30X)	Shiga Toxin 1and *Campylobacter*	*Campylobacter jejuni subsp. jejuni*	*Not performed*
	3	Simulated (2X)	Shiga Toxin 1, Shiga Toxin 2 and *Yersinia enterocolitica*	*Yersinia enterocolitica*	*Positive for Stx 1 gene & Stx 2 gene*
d.	1	Simulated (30X)	Not Detected	*Escherichia coli*, Shiga Toxin 2	*Positive for Stx 2 gene*
	2	Simulated (2X)	Not Detected	*Escherichia coli*, Shiga Toxin 2	*Positive for Stx 2 gene*
e.	1	Fresh	Shiga Toxin 2	Negative	*Positive for Stx 2 gene*
	2	Fresh	Shiga Toxin 1 and Shiga Toxin 2	*Citrobacter youngae*	*Positive for Stx 1 gene and Stx 2 gene*
f.	1	Simulated (31X)	Shiga Toxin 2 and *Yersinia enterocolitica*	*Yersinia enterocolitica*	*Negative for Stx 1 gene and Stx 2 gene*
	2	Simulated (2X)	Shiga Toxin 1, Shiga Toxin 2 and *Yersinia enterocolitica*	*Yersinia enterocolitica*	*Positive for Stx 1 gene & Stx 2 gene*

Summary of Clinical Test Performance (n=1940 - Compared to Broth Enrichment/EHEC EIA and Stx 1 and Stx 2 typing).

	Specimen Type			n	% Agreement (95% CI)			Specimen Type			n	% Agreement (95% CI)	
					Positive	Negative						Positive	Negative
Stx1	Clinical Specimens	Prospectively Collected	Fresh	1294	100% 4/4 (39.8-100)	99.8% 1287/1290[a] (99.3-100	*Stx2*	Clinical Specimens	Prospectively Collected	Fresh	1294	100% 6/6 (54.1-100)	99.8% 1286/1288[e] (99.4-100)
			Frozen	34	-	100% 34/34 (89.7-100)				Frozen	34	-	100% 34/34 (89.7-100)
		Selected		203	100% 9/9 (66.4-100)	99.5% 193/194[b] (97.2-100)			Selected		203	100% 10/10 (69.2-100)	100% 193/193 (98.1-100)
	Simulated			409	100% 50/50 (92.9-100)	99.2% 356/359[c] (97.6-99.8)		Simulated			409	96.6% 57/59[d] (88.3-99.6)	99.4% 348/350[f] (98.0-99.9)

Footnoted Information for Previous Table

	No.	Fresh, Frozen or Simulated (xLoD)	Identified by EP test as:	Identified by Reference/ComparatorMethod(s) as:	PCR Amp/BD Sequencing Results (if applicable)
a.	1	Fresh	Shiga Toxin 1	Negative	*Positive for Stx 1 gene*
	2	Fresh	Shiga Toxin 1 and Norovirus	*Escherichia coli*	*Positive for Stx 1 gene*
	3	Fresh	Shiga Toxin 1 and Shiga Toxin 2	*Citrobacter youngae*	*Positive for Stx 1 gene and Stx 2 gene*
b.	1	Select	Shiga Toxin 1 and *Campylobacter*	*Campylobacter*	*Positive for Stx 1 gene*
c.	1	Simulated (13X)	Shiga Toxin 1and *Salmonella*	*Salmonella enterica subsp. enterica*	*Negative for Stx 1 gene and Stx 2 gene*
	2	Simulated (30X)	Shiga Toxin 1 and *Campylobacter*	*Campylobacter jejuni subsp. jejuni*	*Not performed*
	3	Simulated (2X)	Shiga Toxin 1, Shiga Toxin 2 and *Yersinia enterocolitica*	*Yersinia enterocolitica*	*Positive for Stx 1 gene & Stx 2 gene*
d.	1	Simulated (30X)	Not Detected	*Escherichia coli*, Shiga Toxin 2	*Positive for Stx 2 gene*
	2	Simulated (2X)	Not Detected	*Escherichia coli*, Shiga Toxin 2	*Positive for Stx 2 gene*
e.	1	Fresh	Shiga Toxin 2	Negative	*Positive for Stx 2 gene*
	2	Fresh	Shiga Toxin 1 and Shiga Toxin 2	*Citrobacter youngae*	*Positive for Stx 1 gene and Stx 2 gene*
f.	1	Simulated (31X)	Shiga Toxin 2 and *Yersinia enterocolitica*	*Yersinia enterocolitica*	*Negative for Stx 1 gene and Stx 2 gene*
	2	Simulated (2X)	Shiga Toxin 1, Shiga Toxin 2 and *Yersinia enterocolitica*	*Yersinia enterocolitica*	*Positive for Stx 1 gene & Stx 2 gene*

Summary of Viral Target Clinical Test Performance (n=1940) - Compared to Composite Comparator Method (real-time RT-PCR and two conventional PCRs with bi-directional sequencing)

	Specimen Type			n	% Agreement (95% CI)			Specimen Type			n	% Agreement (95% CI)	
					Positive	Negative						Positive	Negative
Norovirus GI/GII	Clinical Specimens	Prospectively Collected	Fresh	1294	94.9% 37/39[a] (82.7-99.4)	99.6% 1250/1255[d] (99.0-99.9)	*Rotavirus A*	Clinical Specimens	Prospectively Collected	Fresh	1294	66.7% 2/3[e] (9.4-99.2)	99.9% 1290/1291[g] (99.6-100)
			Frozen	34	0% 0/1[b] (0.0-97.5)	100% 33/33 (89.7-100)				Frozen	34	-	100% 34/34 (89.7-100)
		Selected		203	100% 18/18 (81.5-100)	99.5% 184/185[c] (97.0-100)			Selected		203	98.0% 50/51[f] (89.6-100)	100% 152/152 (97.6-100)

	Simulated	409	-	100% 409/409 (99.1-100)		Simulated	409	-	100% 409/409 (99.1-100)

Footnoted Information for Previous Table

	No.	*SID*	*Specimen Type*	*EP test result*	*Composite Comparator Method Result*:*
a	1	0611271	Fresh	Not Detected	Norovirus GII
	2	0611381	Fresh	Not Detected	Norovirus GII
b	1	065602	Frozen	Not Detected	Norovirus GI
c	1	973241	Selected	Rotavirus, Norovirus	Rotavirus,
d	1	052401	Fresh	Norovirus	Negative
	2	0610451	Fresh	Norovirus	Negative
	3	0611191	Fresh	Norovirus	Negative
	4	066181	Fresh	Norovirus	Negative
	5	069051	Fresh	Norovirus Shiga Toxin 1,	Stx1
e	1	067641	Fresh	Not Detected	Rotavirus
f	1	970381	Selected	Not Detected	Rotavirus
g	1	067631	Fresh	Rotavirus	Negative

*Composite Comparator method positive for Norovirus and Rotavirus: RT- PCR (endpoint PCR) with subsequent bi-directional sequencing must be positive.

Summary of Genus/Group-level Test Performance versus Reference Method(s) – Stratified by Species.

Campylobacter Genus					***Shigella Genus***				
Organism	***Prospective Fresh/Frozen***	***Selected***	***Simulated***	***Analytical***	***Organism***	***Prospective Fresh/Frozen***	***Selected***	***Simulated***	***Analytical***
Combined Campylobacter	**91.7% 22/24 (72.0-99.0)**	**97.5% 39/40 (86.8-99.9)**	**98.5% 67/68 (86.3-100)**	**100% 15/15 (78.2-100)**	***Combined Shigella***	**66.7% 2/3 (9.4-99.2)**	**100% 8/8 (54.1-100)**	**100% 50/50 (92.9-100)**	**100% 20/20 (83.2-100)**
Campylobacter coli	100% 3/3 (29.2-100)	100% 3/3 (29.2-100)	100% 18/18 (81.5-100)	100% 5/5 (47.8-100)	*Shigella boydii*	N/A	100% 2/2 (15.8-100)	100% 14/14 (76.8-100)	100% 5/5 (47.8-100)
Campylobacter jejuni	100% 1/1 (2.5-100)	97.3% 36/37 (85.8-99.9)	100% 9/9 (66.4-100)	100% 4/4 (40.0-100)	*Shigella dysenteriae*	N/A	N/A	100% 9/9 (66.4-100)	100% 5/5 (47.8-100)
Campylobacter jejuni subsp. doylei	N/A	N/A	100% 5/5 (47.8-100)	100% 1/1 (2.5-100)	*Shigella flexneri*	N/A	N/A	100% 16/16 (79.4-100)	100% 5/5 (47.8-100)
Campylobacter jejuni subsp. jejuni	90.0% 18/20 (68.3-99.8)	N/A	100% 21/21 (83.9-100)	N/A	*Shigella sonnei*	100% 2/2 (15.8-100)	N/A	100% 11/11 (71.5-100)	100% 5/5 (47.8-100)
Campylobacter lari	N/A	N/A	93.3% 14/15 (68.1-99.8)	100% 5/5 (47.8-100)	*Shigella spp not identified*	0% 0/1 (0-97.5)	100% 6/6 (54.1-100)	*N/A*	*N/A*
Salmonella Genus					***Vibrio Genus***				
Organism	***Prospective Fresh/Frozen***	***Selected***	***Simulated***	***Analytical***	***Organism***	***Prospective Fresh/Frozen***	***Selected***	***Simulated***	***Analytical***
Combined Salmonella	**87.0% 20/23 (66.4-97.2)**	**98.3% 58/59 (90.9-100)**	**100% 67/67 (94.6-100)**	**100% 31/31 (88.8-100)**	***Combined Vibrio***	**100% 2/2 (15.8-100)**	**100% 1/1 (2.5-100)**	**91.1% 51/56 (80.4-97.0))**	**100% 10/10 (69.2-100)**
Salmonella "non-typhi"	N/A	N/A	100% 2/2 (15.8-100)	N/A	*Vibrio cholerae*	N/A	N/A	84.2% 16/19 (60.4-96.6)	100% 5/5 (47.8-100)

Salmonella bongori	N/A	N/A	100% 2/2 (15.8-100)	100% 1/1 (2.5-100)	*Vibrio parahaemolyticus*	100% 2/2 (15.8-100)	100% 1/1 (2.5-100)	94.6% 35/37 (81.8-99.3)	100% 5/5 (47.8-100)
Salmonella enterica	N/A	98.3% 58/59 (90.9-100)	100% 1/1 (2.5-100)	N/A					
Salmonella enterica subsp. arizonae	N/A	N/A	N/A	100% 1/1 (2.5-100)					
Salmonella enterica subsp. diarizonae	N/A	N/A	100% 3/3 (29.2-100)	100% 1/1 (2.5-100)					
Salmonella enterica subsp. enterica	N/A	N/A	100% 52/52 (93.2-100)	100% 25/25 (86.3-100)					
Salmonella enterica subsp. houtenae	N/A	N/A	100% 2/2 (15.8-100)	100% 1/1 (2.5-100)					
Salmonella enterica subsp. indica	N/A	N/A	100% 3/3 (29.2-100)	100% 1/1 (2.5-100)					
Salmonella enterica subsp. salamae	N/A	N/A	100% 2/2 (15.8-100)	100% 1/1 (2.5-100)					
Salmonella spp not identified	87.0% 20/23 (66.4-97.2)	N/A	*N/A*	N/A					

The following two tables list the distinct mixed specimen combinations observed during the clinical study. In summary, there were a total of 26 mixed specimens that were detected by either the EP test or the reference methods or by both. All mixed specimens that were detected by the reference methods were detected by the EP test.

Clinical Mixed Specimen Combinations Detected by EP

Multiple Target Combinations Detected by EP			***Reference/Comparator Test***		
Target 1	*Target 2*	*Target 3*	*Total Specimens*	*Discrepant Specimens*	*Discrepant Identification*
Y. enterocolitica	*Shiga Toxin 1*	*Shiga Toxin 2*	1	1	Stx 1 gene, Stx 2 gene
Campylobacter	*Shiga Toxin 1*	N/A	2	2	*Stx 1 gene*
Campylobacter	*Salmonella*	N/A	2	1	*Salmonella*
				1	*Campylobacter*
Campylobacter	*Vibrio*	N/A	1	1	*Vibrio*
Salmonella	*Shiga Toxin 1*	N/A	1	1	*Stx 1 gene*
Shigella	*Shiga Toxin 1*	N/A	1	1	*Shigella*
Y. enterocolitica	*Shiga Toxin 2*	N/A	1	1	*Stx 2 gene*
Shiga Toxin 1	*Shiga Toxin 2*	N/A	10	1	*Stx 1 gene* *Stx 2 gene*
Salmonella	*Norovirus*	N/A	2	0	*N/A*
Shiga Toxin 1	*Norovirus*	N/A	2	1	*Norovirus*
				1	*Stx 1 gene*
Norovirus	*Rotavirus*	N/A	2	1	*Norovirus*
TOTAL			***25***	***13***	

Clinical Mixed Specimen Combinations Detected by Reference/Comparator Methods

Multiple Target Combinations by Reference/Comparator Test			***Detected by EP***		
Target 1	*Target 2*	*Target 3*	*Total Specimens*	*Discrepant Specimens*	*Discrepant Targets*
Shiga Toxin 1	*Shiga Toxin 2*	N/A	9	0	N/A
Salmonella	*Norovirus*	N/A	2	0	N/A
TOTAL			***11***	***0***	

External Quality Control Testing: The clinical study protocol specified running QC samples each day of testing, utilizing one external negative control and one of eleven external positive controls (tested on a rotating basis) representing all target analytes. The following table summarizes the positive and negative control test runs performed during the clinical study.

Summary of QC Performance during the Clinical Study

Control	***Control Type***	***Total***	***No Calls***	***Call Rate***	***Mis-calls***	***Accuracy***	***Pre-AE***	***Pre-AE Rate***
N01	Negative	254	9	96.5%	11	95.7%	1	0.4%
P01	Campylobacter	21	0	100.0%	0	100.0%	0	0.0%
P02	Salmonella	20	0	100.0%	0	100.0%	0	0.0%
P03	Shigella	16	0	100.0%	0	100.0%	0	0.0%
P04	Vibrio	24	0	100.0%	0	100.0%	0	0.0%
P05	Y. enterocolitica	26	0	100.0%	0	100.0%	0	0.0%
P06	Shigella + Shiga Toxin 1	28	1	96.4%	1	96.4%	1	3.6%
P07	Shiga Toxin 2	29	0	100.0%	1	96.6%	0	0.0%
P08	Rotavirus	31	2	93.5%	0	100.0%	0	0.0%
P09	Norovirus GI	28	1	96.4%	1	96.4%		0.0%
P10	Norovirus GII	16	0	100.0%	1	93.8%	0	0.0%
P11	Adenovirus	26	0	100.0%	2	92.3%	1	3.8%
Total		**509**	**13**	**97.4%**	**17**	**96.7%**	**3**	**0.6%**

4. Clinical cut-off**:**

   N/A

5. Expected values/Reference range:

   In the Verigene EP Methods Comparison study, 1328 prospectively collected fresh and frozen specimens were obtained from multiple medium to large-sized healthcare institutions geographically distributed across the United States. The number and percentage of positive cases (positivity rate) determined by Verigene EP stratified by geographic region for each of the organisms detected by the test are presented in the table below. Overall, EP detected at least one target in 11.2% (149/1328) of prospectively collected specimens. In routine practice, prevalence rates may vary depending on the institution, geographical location, and patient population.

Prevalence of Organisms Detected by Verigene EP – Clinical Study Observations

		US Geographic Region/Division*					***Total***
Target	***Region***	*Midwest*		*South*	*Northeast*	*West*	
	Division	*West North Central*	*East North Central*	*W. South Central*	*Middle Atlantic*	*Pacific*	
	State	*MO*	*WI*	*TX*	*NY*	*CA*	
	Total n	10	196	119	232	771	1328
Campylobacter	POS n	0	7	5	5	22	39
	% Prev.	-	3.6	4.2	2.2	2.8	2.9
Salmonella	POS n	1	2	1	6	18	28
	% Prev.	10.0	1.0	0.8	2.6	2.3	2.1
Shigella	POS n	1	0	4	1	13	19
	% Prev.	10.0	-	2.5	0.4	1.7	1.4
Vibrio	POS n	0	0	0	0	2	2
	% Prev.	-	-	-	-	0.3	0.2
Y. enterocolitica	POS n	0	0	0	0	0	0
	% Prev.	-	-	-	-	-	-
Stx1	POS n	0	1	1	1	4	7
	% Prev.	-	0.5	0.8	0.4	0.5	0.5
Stx2	POS n	0	1	1	1	5	8
	% Prev.	-	0.5	0.8	0.4	0.6	0.6
Norovirus	POS n	0	2	2	4	34	42
	% Prev	-	1.0	1.7	1.7	4.5	3.2
Rotavirus	POS n	0	0	0	0	3	3
	% Prev	-	-	-	-	0.4	0.2

N. Instrument Name:

Verigene® System

O. System Descriptions:

1. Modes of Operation:

   The Verigene® System has one mode of operation available to the User. Prior to initiating a test run the User places the sample into the sample loading well, loads the consumable, and enters identifying information into the system. After the Processor SP run has completed, the test cartridge is removed from the instrument and the reagent pack is manually separated from the Substrate Holder. The barcode on the substrate holder is scanned prior insertion into the Reader. The Reader images the Substrate and automatically determines the test result without further user intervention.

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes __X_____ or No _____

3. Specimen Identification:

All tests must be ordered through the Verigene Reader. No tests can be processed on the Verigene Processor *SP* without the user entering the Test Cartridge ID and Sample ID into the Verigene Reader.

The User enters the Test Cartridge ID by scanning the barcode using the barcode scanner attached to the Reader. The user may manually enter in the Test Cartridge ID by selecting MENU and 'Enter Barcode' and then keying in the Test Cartridge ID number with the Reader's keyboard. The User has the option to scan the Test Cartridge Cover's 2D barcode using a barcode gun-style scanner to display the Test Cartridge's Reference Number, Expiration Date, and Lot Number on reports. The User enters the Sample ID by scanning or manually entering the Sample ID using the Reader's touch-screen keyboard then confirming the Sample ID in the software.

4. Specimen Sampling and Handling:

The user is instructed to perform the following steps after which all specimen handling is performed by the automated Verigene System:

- Put on fresh gloves.
- For each Cary-Blair preserved specimen to be tested, place one sterile flocked swab and one uncapped Stool Prep Buffer tube (place the cap to the side for recapping later) into a biological safety cabinet (BSC).
- Wipe down the outside of the specimen vial with a lint-free decontaminating wipe.
- Invert the vial containing the Cary-Blair preserved specimen twice and vortex the specimen for 5-10 seconds to ensure homogeneity.
- To prepare the Stool Prep Buffer tube, dip the provided flocked swab into either the primary Cary-Blair preserved specimen vial or the secondary tube until the flocked tip is fully immersed in specimen. Once evenly coated, transfer the swab to the Stool Prep Buffer tube and break swab at the pre-formed scored breakpoint. Leave the swab in the Stool Prep Buffer tube and screw the cap finger tight on to Stool Prep Buffer tube.
- Recap the original, primary Cary-Blair preserved specimen container and set aside.
- Repeat steps 1-6 for each specimen, changing gloves between each specimen.
- Vortex each Stool Prep Buffer tube for 15-20 seconds.

- Spin all prepared Stool Prep Buffer tubes in the Mini Centrifuge for 30-35 seconds at a MAXIMUM of 2200 rcf.
- Pipette 200 µL of the prepared supernatant into the Extraction Tray.

5. Calibration:

   There is no calibration required by the user.

6. Quality Control:

   See Section M.1c above.

P. Proposed Labeling:

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

Q. Conclusion:

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