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

A. 510(k) Number:

k092953

B. Purpose for Submission:

To obtain substantial equivalence determination for a new 510k

C. Measurand:

The *vanA* gene sequence associated with vancomycin resistance in bacteria

D. Type of Test:

Qualitative nucleic acid amplification test of the *vanA* gene directly from rectal swabs

E. Applicant:

Cepheid

F. Proprietary and Established Names:

Xpert® *vanA* Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.1640 Antimicrobial susceptibility test powder

2. Classification:

   Class II

3. Product code:

   NIJ
   OOI

4. Panel:

   83 Microbiology

H. Intended Use:

1. Intended use(s):

   The Cepheid Xpert® *vanA* Assay performed in the GeneXpert® Dx System is a qualitative *in vitro* diagnostic test designed for rapid detection of the *vanA* gene sequence associated with vancomycin resistance in bacteria obtained from rectal swab specimens from patients at risk for intestinal colonization with vancomycin-resistant bacteria. The test utilizes automated real-time polymerase chain reaction (PCR) to detect the *vanA* gene that is frequently associated with vancomycin-resistant *enterococci* (VRE). The Xpert *vanA* Assay is intended to aid in the recognition, prevention, and control of vancomycin resistant organisms that colonize patients in healthcare settings. The Xpert *vanA* Assay is not intended to diagnose infections caused by vancomycin-resistant bacteria nor to guide or monitor treatment for vancomycin-resistant bacterial infections. Concomitant cultures are necessary to recover organisms for confirmatory identification of vancomycin-resistant bacteria, antimicrobial susceptibility testing, and for epidemiological typing.

2. Indication(s) for use:

   The Cepheid Xpert® *vanA* Assay performed in the GeneXpert® Dx System is a qualitative *in vitro* diagnostic test designed for rapid detection of the *vanA* gene sequence associated with vancomycin resistance in bacteria obtained from rectal swab specimens from patients at risk for intestinal colonization with vancomycin-resistant bacteria. The test utilizes automated real-time polymerase chain reaction (PCR) to detect the *vanA* gene that is frequently associated with vancomycin-resistant *enterococci* (VRE). The Xpert *vanA* Assay is intended to aid in the recognition, prevention, and control of vancomycin resistant organisms that colonize patients in healthcare settings. The Xpert *vanA* Assay is not intended to diagnose infections caused by vancomycin-resistant bacteria nor to guide or monitor treatment for vancomycin-resistant bacterial infections. Concomitant cultures are necessary to recover organisms for confirmatory identification of vancomycin-resistant bacteria, antimicrobial susceptibility testing, and for epidemiological typing.

3. Special conditions for use statement(s):

   For Prescription Use only

4. Special instrument requirements:

   GeneXpert® Dx System (GX-4 or GX-16 instruments, and the GeneXpert® Dx System Version 2.1 software)

I. Device Description:

The Cepheid Xpert *vanA* Assay is a rapid, automated *in vitro* diagnostic test for qualitative detection of the *vanA* gene sequence associated with vancomycin resistance in bacteria obtained directly from rectal swab specimens. The Xpert *vanA* Assay system performs real-

time multiplex polymerase chain reaction (PCR) for detection of DNA after an initial sample processing step. The assay is performed on the Cepheid GeneXpert® Dx System.

The specimen is collected on a double swab, one of which is placed in a tube containing elution reagent. Following brief vortexing, the eluted material and two single-use reagents (Reagent 1 and Reagent 2) that are provided with the assay are transferred to different, uniquely-labeled chambers of the disposable fluidic cartridge (the Xpert *vanA* cartridge). The user initiates a test from the system user interface and places the cartridge into the GeneXpert® Dx System instrument platform, which performs hands-off realtime, multiplex polymerase chain reaction (PCR) for detection of DNA. In this platform, additional sample preparation, amplification, and real-time detection are all fully automated and completely integrated.

The GeneXpert® System consists of a GeneXpert instrument, personal computer, and the multi-chambered fluidic cartridges that are designed to complete sample preparation and real-time PCR for detection of the *vanA* gene that is associated with vancomycin-resistant *enterococci* (VRE) in less than 45 minutes. Each instrument system has 1 to 16 randomly accessible modules that are each capable of performing separate sample preparation and real-time PCR tests. Each module contains a syringe drive for dispensing fluids, an ultrasonic horn for lysing cells or spores, and a proprietary I-CORE® thermocycler for performing real-time PCR and detection.

The Xpert *vanA* Assay includes reagents for the detection of the *vanA* resistant gene as well as an internal sample processing control (SPC) to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR assay. The SPC also ensures the PCR conditions (temperature and time) are appropriate for the amplification reaction and that the PCR reagents are functional. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   IDI-VanR Assay
   Remel Bile Esculin Azide agar with 6 µg/mL vancomycin (BEAV)

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

   K061686
   K972359

3. Comparison with predicate:

Similarities			
	Device	**Predicate**	
Item	**Xpert *vanA* Assay**	**Remel Bile Esculin Azide agar with 6 µg/mL vancomycin (BEAV)**	**IDI-VanR Assay**
Intended Use	The Cepheid Xpert® *vanA* Assay performed in the GeneXpert® Dx System is a qualitative in vitro diagnostic test designed for rapid detection of the *vanA* gene sequence associated with vancomycin resistance in bacteria obtained from rectal swab specimens from patients at risk for intestinal colonization with vancomycin-resistant bacteria. The test utilizes automated real-time polymerase chain reaction (PCR) to detect the *vanA* gene that is frequently associated with vancomycin resistant *enterococci* (VRE). The Xpert *vanA* Assay is intended to aid in the recognition, prevention, and control of vancomycin resistant organisms that colonize patients in healthcare settings. The Xpert *vanA* Assay is not intended to diagnose infections caused by vancomycin-resistant bacteria nor to guide or monitor treatment for vancomycin resistant bacterial infections. Concomitant cultures are necessary to recover organisms for confirmatory identification of vancomycin-resistant bacteria, antimicrobial susceptibility testing, and for epidemiological typing.	Remel Bile Esculin Azide agar with 6 µg/mL vancomycin is a plated medium recommended for use in qualitative procedures as a selective and differential medium for the primary isolation of vancomycin-resistant *enterococci* from surveillance cultures. This product is not intended for use as a method of antimicrobial susceptibility testing. Confirmation of vancomycin resistance by an approved method is recommended as some organisms on initial isolation may overcome the inhibitory effects of the medium.	The IDI-VanR® Assay is a qualitative in vitro test for the rapid detection of vancomycin-resistance *enterococci* (VRE). The assay is performed on an automated real-time PCR instrument with rectal swabs from patients at risk for VRE colonization. The IDI-VanR® Assay can be used as an aid to identify, prevent and control vancomycin-resistant colonization in healthcare settings. Concomitant cultures are necessary to recover organisms for epidemiological typing, susceptibility testing and for further confirmatory identification. The IDI VanR® Assay is not intended to diagnose VRE infections nor to guide or monitor treatment for VRE infections.
Type of test	Qualitative	Same	Same
Technological Principles	Fully-automated nucleic acid amplification (DNA); real-time PCR	N/A	Same
Specimen Type	Rectal swabs	N/A	Same
Test Cartridge	Disposable single-use, multichambered, fluidic cartridge.	N/A	Disposable single-use PCR tube
Probes	TaqMan® Probes	N/A	Molecular Beacons

Similarities			
	Device	**Predicate**	
Item	**Xpert *vanA* Assay**	**Remel Bile Esculin Azide agar with 6 µg/mL vancomycin (BEAV)**	**IDI-VanR Assay**
Controls	Internal sample processing control (SPC) and probe check control (PCC). External controls available.	N/A	One internal reagent control and external positive and negative controls required per run
DNA Target Sequence	Detects gene sequences for the *vanA* encoded resistance to vancomycin / teicoplanin	N/A	Detects gene sequences for VanR (*vanA* and *vanB*) encoded resistance to vancomycin / teicoplanin.
Rapid test results	Less than 45 minutes to results.	48 hours	Approximately 120 minutes.
Interpretation of test results	Diagnostic software of the Cepheid GeneXpert DX system	Visual interpretation	Diagnostic software of the Cepheid SmartCycler DX system

Differences			
	Device	Predicate	
Item	**Xpert *VanA* Assay**	**Remel Bile Esculin Azide agar with 6 µg/mL vancomycin (BEAV)**	**IDI-VanR Assay**
Instrument System	Cepheid GeneXpert Dx System	N/A	Cepheid SmartCycler
Technological Principles	Fully-automated nucleic acid amplification (DNA); real-time PCR	Phenotypic detection of vancomycin-resistant *enterococci* (VRE) based on culture growth	N/A
Mode of Detection	Presence of *vanA* gene	Growth or no growth on 6ug/mL vancomycin agar	Presence of VanR (*vanA* and *vanB*) gene
Specimen Type	Rectal swabs	Culture grown direct from rectal swab or stool	Rectal swabs
Controls	Internal sample processing control (SPC) and probe check control (PCC). External controls available.	Internal Controls – N/A	One internal reagent control and external positive and negative controls required per run
DNA Target Sequence	Detects sequences for the *vanA* gene.	N/A	Detects sequences for vancomycin resistance [*vanR* (*vanA* and *vanB)]* gene, but does not differentiate *vanA* from *vanB*.
Sample Extraction / Fluidics	Self-contained and automated after swab elution and two single-dose reagent additions.	N/A	Manual

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

Not applicable

L. Test Principle:

The primers and probes in the Xpert *vanA* Assay detect the presence of unique sequences for *vanA* resistance gene. Rectal swabs are collected and transported to the GeneXpert® System area. The swab is placed in a tube containing 1.7 mL Sample Reagent. Following a brief vortex, the eluted material and two other liquid reagents are transferred to different chambers of the cartridge. The user initiates a test from the system user interface, the instrument signals the user where to place the cartridge by flashing a green light, and the cartridge is placed into the indicated module in the GeneXpert® Dx System instrument. The instrument moves the sample and reagents to and from different chambers within the Xpert *vanA* Assay cartridge. The GeneXpert® Dx System performs sample preparation by mixing the eluted sample with the Sample Preparation Control (*Bacillus globigii* in the form of a dry spore cake within the cartridge, is used as the SPC) and treatment reagents, capturing the bacterial cells on a filter, lysing the cells using glass beads and an ultrasonic horn, then eluting the released DNA. The DNA solution is then mixed with dry PCR reagents and transferred into the PCR tube for real-time PCR and detection. The Xpert *vanA* Assay completes sample preparation and real-time PCR in less than 45 minutes. Internal controls in Xpert *vanA* Assay check key automated steps.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility study was performed using a panel of 4 specimens with varying concentrations of *vanA* on 10 different days at each of the three sites (4 specimens x 2 operators/ day x 10 days x 3 sites). One lot of Xpert *vanA* assay was used at each of the 3 testing sites.

Summary of Reproducibility Results (all)[a]

	% Agreement			
Specimen ID	Site 1	Site 2	Site 3	Total Agreement
Neg	100% (20/20)	90% (18/20)	100% (20/20)	96.7% (58/60)
vanA High Neg	100% (20/20)	100% (20/20)	95% (19/20)	98.3% (59/60)
vanA Low Pos	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)

	% Agreement			
Specimen ID	Site 1	Site 2	Site 3	Total Agreement
vanA Moderate Pos	100% (20/20)	95% (19/20)	100% (20/20)	98.3% (59/60)
% Total Agreement by Site	100% (80/80)	96.3% (77/80)	98.8% (79/80)	98.3% (236/240)

[a]For negative and high negative samples, %Agreement = (#negative results/total samples run); for low and moderate positive samples, % Agreement = (# positive results/total samples run)

Summary of Ct Value Results by Sample Level and Target

Bg			
Level	**Mean**	**Std Dev**	**%CV**
vanA High Neg	32.88	0.60	1.83
vanA Low Pos	32.88	0.77	2.34
vanA Moderate Pos	32.80	0.78	2.38
Neg	33.15	0.65	196

***vanA*[1]**			
Level	**Mean**	**Std Dev**	**%CV**
vanA Low Pos	33.76	1.00	2.95
vanA Moderate Pos	30.35	1.33	4.40

[1]Ct cutoff for *vanA*=40

b. Linearity/assay reportable range:

Linearity was evaluated using *Enterococcus faecium* (*vanA*) cells serially diluted over 6 logs and processed using the Xpert *vanA* Assay. The diluted cells resulted in a cell concentration dose range of 50 CFU/test to $5x10^7$ CFU/test. Replicates of four (4) were tested at each concentration. For *enterococci* cells, under the conditions of this study, the Xpert *vanA* Assay responds linearly (r2 = 0.994) with respect to *vanA* detection as a function of *Enterococcus faecium* cell input over 6 logs (50 – $5x10^7$ CFU/test). The reportable Ct range is 12.1 to 35.3 (cutoff Ct = 40.0). PCR efficiency for the *vanA* reaction is 87.7 %.

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

The following external controls were evaluated for use with the GeneXpert Dx System and Xpert *vanA* assay.

External Controls

External Control ID	Organism	Control
MicroBioLogics 0366 (Kwik-Stik)	Vancomycin-sensitive *Enterococcus faecalis*	Negative
CCUG36804 (Culture Collection of Göteborg)	Vancomycin-resistant *Enterococcus faecium*	Positive (*vanA*)
Gibson Laboratories, LLC #CeVRE-01	Vancomycin-resistant *Enterococcus faecium*	Positive (*vanA*)
Gibson Laboratories, LLC #CeVRE-02	Vancomycin-sensitive *Enterococcus faecalis*	Negative

The external quality control organisms were tested at the different testing sites with the following results.

KWIK-STIK™ Negative Control Data

Sample ID	Test Result	SPC Ct	*vanA* Ct
Neg _01	*vanA* NEGATIVE	33.1	0
Neg _02	*vanA* NEGATIVE	33.4	0
Neg _03	*vanA* NEGATIVE	34.0	0
Neg _04	*vanA* NEGATIVE	33.4	0
Neg _05	*vanA* NEGATIVE	32.5	0
Neg _06	*vanA* NEGATIVE	33.5	0
Neg _07	*vanA* NEGATIVE	32.2	0
Neg _08	ERROR	0	0
Neg _09	*vanA* NEGATIVE	32.9	0
Neg _10	*vanA* NEGATIVE	33.2	0

One negative control result reported "ERROR" due to a probe check failure.

Control Data, CCUG36804 (*vanA* positive *E. faecium*)

Sample ID	Test Result	SPC Ct	*vanA* Ct
*van*A_01	*vanA* POSITIVE	32.1	29.2
*van*A _02	*vanA* POSITIVE	32.6	29.7
*van*A _03	*vanA* POSITIVE	33.7	29.6
*van*A _04	*vanA* POSITIVE	33.3	30.2
*van*A _05	*vanA* POSITIVE	33.8	30.3
*van*A _06	*vanA* POSITIVE	32.8	29.5
*van*A _07	*vanA* POSITIVE	34.7	30.2
*van*A _08	*vanA* POSITIVE	33.8	29.9
*van*A _09	*vanA* POSITIVE	32.5	29.4
*van*A _10	*vanA* POSITIVE	33.5	30.5

Control Data, Gibson Laboratories (*vanA* positive *E. faecium*)

Sample ID	Test Result	SPC Ct	*vanA* Ct
*van*A_01	*vanA* POSITIVE	32.5	23.3
*van*A _02	*vanA* POSITIVE	33.2	23.6

*van*A _03	*vanA* POSITIVE	32.4	23.8
*van*A _04	*vanA* POSITIVE	32.6	23.3
*van*A _05	*vanA* POSITIVE	31.5	23.2
*van*A _06	*vanA* POSITIVE	33.4	23.5
*van*A _07	*ERROR*	0	0
*van*A _08	*vanA* POSITIVE	31.5	23.2
*van*A _09	*vanA* POSITIVE	32.3	24.4
*van*A _10	*vanA* POSITIVE	32.7	23.4

One sample result reported "ERROR" due to high syringe pressure.

Control Data, Gibson Laboratories, LLC (Negative Control, *E. faecalis*)

Sample ID	Test Result	SPC Ct	*vanA* Ct
Neg _01	*vanA* NEGATIVE	33.0	0
Neg _02	*vanA* NEGATIVE	32.1	0
Neg _03	*vanA* NEGATIVE	33.4	0
Neg _04	*vanA* NEGATIVE	34.0	0
Neg _05	*vanA* NEGATIVE	34.3	0
Neg _06	*vanA* NEGATIVE	32.4	0
Neg _07	*vanA* NEGATIVE	33.2	0
Neg _08	*vanA* NEGATIVE	33.1	0
Neg _09	*vanA* NEGATIVE	35.2	0
Neg _10	*vanA* NEGATIVE	32.5	0

External Controls may be used in accordance with accrediting institutions and government regulations. External Controls are not provided in the test kit; however they are available for purchase from outside sources. The outside source and the catalog numbers are provided to the costumer in the 'Materials Available but Not Provided' section of the Xpert vanA Assay Package Insert.

d. Analytical Sensitivity:

Limit of Detection

Limit of Detection (LoD) studies were performed using *Enterococcus faecium* (*vanA*) diluted into a fecal matrix of human origin that can be detected by the Xpert *vanA* Assay. The fecal matrix consisted of autoclaved human liquid feces (*vanA* negative) diluted 1:10 in Tris buffer.

The analytical LoD was estimated using 4 to 10 replicates at each dilution. The LoD was confirmed by running a total of 20 replicates at the estimated LoD concentration. Under the conditions of this study, the limit of detection for the Xpert *vanA* Assay on a simulated rectal swab specimen is 37 CFU.

Summary of Mean Ct

Strain	CFU*	Positives/Replicates Tested^	SPC	*vanA*
			Ct	Ct
	0	0/5	32.5	-
	16	5/5	33.6	35.1
	37	**20/20**	33.3	35.6

vanA^	68	5/5	34.3	34.9
	107	5/5	32.9	34.1
	219	5/5	32.4	32.9

(*) determined by the direct plate method
(^) CCUG36804 Strain Source: Culture Collection University of Göteborg (CCUG), Gothenburg, Sweden

Analytical Reactivity (Inclusivity) / *Challenge Panel*

Challenge panel included thirty vancomycin-resistant *enterococci* strains and 20 vancomycin sensitive *enterococci* strains, provided by the CDC, and tested using the Xpert *vanA* Assay. Of the 30 vancomycin-resistant *enterococci* strains, 10 were identified as *vanA* positive. *Enterococci* strains were selected to broadly represent the genetic diversity found in *enterococci*. Stock cultures were prepared by suspending the bacterial growth from agar plates in PBS buffer containing 15% glycerol. The concentration of each stock was adjusted to $5.6x10^9$ to $2.1x10^{10}$ CFU/mL. All strains were serially diluted to approximately 360 CFU/swab and tested in triplicate.

All 20 vancomycin sensitive strains were reported as "*vanA* NEGATIVE". Among the 10 *vanA* positive vancomycin-resistant *enterococci* strains tested, one strain was reported as "*vanA* NEGATIVE." When this strain was sequenced, it was reported to be a *vanB*. The remaining 9 *vanA* positive vancomycin resistant *enterococci* strains were correctly reported as "*vanA* POSITIVE.". Among the 20 non-*vanA* vancomycin resistant *enterococci* strains, all were reported as "*vanA* NEGATIVE."

Summary Table of Analytical Reactivity (Inclusivity) Results of the Xpert *vanA* Assay on a CDC-Supplied Panel of *Enterococci* Specimens

Sample ID	Organism	Genotype[A]	Xpert *vanA* Result
NJ-5	*E. faecalis*	Sensitive	*vanA* NEGATIVE
VA32	*E. casseliflavus*	Sensitive	*vanA* NEGATIVE
VS110	*E. faecalis*	Sensitive	*vanA* NEGATIVE
VS119	*E. faecalis*	Sensitive	*vanA* NEGATIVE
VS307	*E. faecalis*	Sensitive	*vanA* NEGATIVE
VS314	*E. faecalis*	Sensitive	*vanA* NEGATIVE
VS406	*E. faecium*	Sensitive	*vanA* NEGATIVE
VS413	*E. casseliflavus*	Sensitive	*vanA* NEGATIVE

Sample ID	Organism	Genotype[A]	Xpert *vanA* Result
VS414	*E. casseliflavus*	Sensitive	*vanA* NEGATIVE
VS418	*E. casseliflavus*	Sensitive	*vanA* NEGATIVE
VS517	*E. faecalis*	Sensitive	*vanA* NEGATIVE
VS604	*E. faecium*	Sensitive	*vanA* NEGATIVE
VS615	*E. faecium*	Sensitive	*vanA* NEGATIVE
VS719	*E. faecalis*	Sensitive	*vanA* NEGATIVE
VS804	*E. casseliflavus*	Sensitive	*vanA* NEGATIVE
NJ-4	*E. gallinarium*	Sensitive (vanC)	*vanA* NEGATIVE
VS106	*E. gallinarium*	Sensitive (vanC)	*vanA* NEGATIVE
VS411	*E. gallinarium*	Sensitive (vanC)	*vanA* NEGATIVE
VS608	*E. gallinarium*	Sensitive (vanC)	*vanA* NEGATIVE
VS807	*E. gallinarium*	Sensitive (vanC)	*vanA* NEGATIVE
E38-10	*E. faecalis*	vanB	*vanA* NEGATIVE
E6-1	*E. faecium*	vanB	*vanA* NEGATIVE
NJ-2	*E. faecium*	vanB	*vanA* NEGATIVE
VA16	*E. faecalis*	vanB	*vanA* NEGATIVE
VA36	*E. faecium*	vanB	*vanA* NEGATIVE
VA38	*E. faecium*	vanB	*vanA* NEGATIVE
VA63	*E. faecalis*	vanB	*vanA* NEGATIVE
VA8	*E. faecium*	vanB	*vanA* NEGATIVE
VA89	*E. faecalis*	vanB	*vanA* NEGATIVE
VS102	*E. faecalis*	vanB	*vanA* NEGATIVE
VS103	*E. faecium*	vanB	*vanA* NEGATIVE

Sample ID	Organism	Genotype[A]	Xpert *vanA* Result
VS111	*E. faecalis*	vanB	*vanA* NEGATIVE
VS112	*E. faecium*	vanB	*vanA* NEGATIVE
VS319	*E. faecalis*	vanB	*vanA* NEGATIVE
VS415	*E. faecalis*	vanB	*vanA* NEGATIVE
VS416	*E. faecalis*	vanB	*vanA* NEGATIVE
VS501	*E. faecalis*	vanB	*vanA* NEGATIVE
VS506	*E. faecium*	vanB	*vanA* NEGATIVE
VS514	*E. faecalis*	vanB	*vanA* NEGATIVE
VS605	*E. faecium*	vanB	*vanA* NEGATIVE
A256	*E. faecalis*	*vanA*	*vanA* POSITIVE
NJ-1	*E. faecium*	*vanA*	*vanA* POSITIVE
VA100[B]	*E. faecium*	*vanA*	*vanA* NEGATIVE
VA29	*E. faecium*	*vanA*	*vanA* POSITIVE
VA6	*E. faecium*	*vanA*	*vanA* POSITIVE
VS105	*E. faecium*	*vanA*	*vanA* POSITIVE
VS318	*E. faecium*	*vanA*	*vanA* POSITIVE
VS420	*E. faecium*	*vanA*	*vanA* POSITIVE
VS511	*E. faecium*	*vanA*	*vanA* POSITIVE
VS611	*E. faecalis*	*vanA*	*vanA* POSITIVE

[A] The genotype information contained in the grey column was provided by the CDC

[B] Sequencing confirmed that this specimen is a *vanB* subtype, not *vanA* as typed by CDC

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e. Analytical specificity:

Cross Reactivity

Forty-two bacterial and fungal strains were collected, quantitated and tested using the Xpert *vanA* Assay. The strains originated from the American Type Culture Collection (ATCC), Culture Collection University of Göteborg (CCUG), German Collection of Microorganisms and Cell Cultures (DSMZ), and the Centers for Disease Control and Prevention (CDC).

The organisms tested were identified as Gram-positive (22), Gram-negative (18), including antibiotic-resistant strains of *Pseudomonas spp*. and *Acinetobacter spp*., and yeast (2). The organisms were further classified as aerobic (24), anaerobic (14) or microaerophillic (2). Of the species tested, 2 vancomycin-sensitive strains of *E. faecalis* and *E. faecium* were included.

Each strain was tested in triplicate at concentrations ranging from $8.5x10^8$ to $2.3x10^{10}$ CFU/swab. Positive and negative controls were included in the study. Under the conditions of the study, all isolates were reported "*vanA* NEGATIVE".

Interference Study

Sixteen exogenous substances occasionally used or found in stool were tested for interference with the Xpert *vanA* Assay. These potentially interfering substances in rectal swab specimens include, but are not limited to, blood, mucus, fecal fats, laxatives, stool softeners, anti-diarrhea medications, anti-itch creams, lubricants, antibiotics and hemorrhoid ointments. The substances were tested at levels representing approximately 1 – 25% (v/v or w/v) final concentrations. Two (2) of the sixteen (16) exogenous substances tested in this study gave a slightly higher Ct relative to the buffer control (hydrocortisone cream and Pepto-Bismol®).

Mean Cts for the SPC and *vanA* Target per Potentially Interfering Substance

Substance	SPC Mean Ct	*vanA* Mean Ct
PBS 15% glycerol (Buffer Control)	33.3	32.0
Whole Blood	33.0	31.3
Mucin	32.7	30.7
Kaopectate®	34.0	32.7
Imodium®	33.3	32.4
Pepto-Bismol®	35.3	33.5
Fleet®	33.0	31.2
Fecal fats	32.9	30.2
Hydrocortisone Cream	34.4	34.4
K-Y Jelly/Gelée®	33.6	32.4
Vaseline	33.6	32.5
Dulcolax®	33.3	32.2
Preparation H Portable Wipes	33.4	32.6
Vancomycin	33.2	31.8

Metronidazole	33.0	31.9
Anusol® Plus	33.8	32.4
Barium sulfate	34.0	31.5

Carry-Over Contamination study consisted of a negative sample (no *enterococci* present) processed in the same GeneXpert module immediately following a very high positive sample made up of 10^6 CFU *Enterococcus faecium* (*vanA*) spiked into the elution buffer. This was repeated 20 times between two GeneXpert modules for a total of 40 runs. All 20 negative samples were correctly reported "*vanA* NEGATIVE." All 20 high positive samples were correctly reported "*vanA* POSITIVE."

f. Assay cut-off:

Lot Specific Parameters and Assay Settings

Lot specific assay settings are generated for every lot manufactured to account for slight variations in reagent production. The lot specific assay settings (LSP file) are incorporated into the 2-D barcode on each cartridge label and are transferred to the GeneXpert® Dx system via a hand-held barcode scanner prior to initiating the Xpert *vanA* Assay.

General Assay Settings

General assay settings are used for all reagent lots. They are fixed and not part of the LSP process. The following table lists general assay settings:

Attribute	Setting
Background Subtraction	Always ON
Background Minimum Cycle	Default setting = 5
Background Maximum Cycle	Manual setting = 30
Manual Threshold (*vanA* and SPC)	Manual setting = 30
Curve Analysis	Primary
Boxcar Average Cycles	Zero (Off)
Valid Minimum Ct (*vanA*)	Default setting = 5
Valid Minimum Ct (SPC)	Manual setting = 25
Valid Maximum Ct (SPC and *vanA*)	Manual setting = 40

The valid cycle range for the *vanA* target is 5 to 40 cycles based upon pre-clinical results to maximize percent sensitivity and percent specificity. The valid cycle range for the SPC is 25 to 40 cycles. The SPC ensures the PCR conditions (temperature and time) are appropriate for the amplification reaction and that the PCR reagents are functional. SPC performance is designed to respond to the presence of potentially inhibitory conditions and provide an invalid test result in lieu of a believable but erroneous test result.

To obtain a valid positive test result, the *vanA* Ct value must be reported within the valid cycle range. To obtain a valid negative test result, the SPC Ct must be reported within its

valid cycle range. If the SPC falls outside the valid cycle range, the test result is "Invalid" and must be repeated.

Valid minimum and maximum Ct settings (25 to 40 cycles) for the SPC were derived from analytical data (*vanA* lot specific parameter (LSP) testing of three production lots), inhibitory studies with potentially interfering substances (IS), and pre-clinical true negative *vanA* samples collected during the design and development phase.

A valid maximum SPC Ct of 40 cycles was used to differentiate between valid negative results and invalid results in the clinical study. The SPC was designed to be sensitive to the presence of inhibition, while at the same time limiting the number of invalid test results.

2. Comparison studies:

a. Method comparison with predicate device:

Performance characteristics of the Xpert *vanA* Assay were determined in a multi-site prospective investigation study at three US sites by comparing the Xpert *vanA* Assay to reference culture followed by bi-directional sequencing for confirmation on vancomycin-resistant *Enterococcus* isolates.

Subjects included individuals whose routine care called for VRE testing. One swab from a double swab set was used for patient management; the other swab was used for the Xpert *vanA* Assay testing. The leftover swab designated for patient management was sent to a central laboratory for reference culture.

Leftover specimen swabs designated for culture testing were stored at 2-8°C and shipped on ice packs to the central culture laboratory within 48 hours of collection. Reference culture was initiated within 16 hours of receipt or within 5 days of swab collection.

Each swab was subsequently placed into an enrichment broth. The plates were incubated at 35°C and examined at 48 and 72 hours. The broth was also incubated at 35°C for 48 hours and subcultured to a bile esculin azide agar with 6 μg/ml of vancomycin.

Small, gray colonies with a black halo were considered suspicious for VRE. Presumptive identification was accomplished by performing a Gram stain, catalase and PYR disc (L-pyrrolidonyl-beta-naphthylamide) test. Presumptive VRE specimens were Gram-positive *cocci* or *coccobacilli* and PYR positive. Presumptive VRE was definitively identified using the API20S strip (BioMérieux, France). Finally, VRE isolates were tested for their susceptibility to glycopeptides using vancomycin E-test strips (AB Biodisk, Sweden). Susceptibility to teicoplanin for the isolates was determined by agar dilution.

Following reference culture testing, DNA was prepared from vancomycin-resistant *Enterococcus* isolates, and sent to a second reference laboratory for bi-directional sequencing using alternative *vanA* specific primers (*i.e.*, different from those used in the Xpert *vanA* Assay).

Performance of the Xpert *vanA* Assay was calculated relative to the results of direct culture with bi-directional sequencing, and enriched culture with bi-directional sequencing.

Overall Results

A total of 1231 specimens were tested by Xpert *vanA* Assay, culture and bi-directional sequencing.

Xpert *vanA* Assay Performance vs.
Direct Culture with Bi-directional Sequencing

		Direct Culture + Sequencing		
Xpert *vanA* Assay		Pos	Neg	Total
	Pos	126	84	210
	Neg	2	1019	1021
	Total	128	1103	1231

% Positive Agreement:	98.4%
% Negative Agreement:	92.4%
Accuracy:	93.0%
PPV:	60.0%
NPV:	99.8%
Prevalence:	10.4%

Of the Xpert *vanA* Assays run on eligible specimens, 94.0% (1180/1255) of these specimens were successful on the first attempt. The remaining 75 gave indeterminate results on the first attempt (26 "INVALID", 49 "ERROR" and 0 "NO RESULT"). Sixty two (62) of the75 indeterminates on the first attempt had sufficient sample for retest, 82.3% (51/62) gave a result on the second the attempt. Overall assay success rate (combining the first and second attempts) was 98.1% (1231/1255).

Xpert *vanA* Assay Performance vs.
Enriched Culture with Bi-directional Sequencing

		Enriched Culture + Sequencing		
Xpert *vanA* Assay		Pos	Neg	Total
	Pos	141	69	210
	Neg	22	999	1021
	Total	163	1068	1231

% Positive Agreement:	86.5%
% Negative Agreement:	93.5%
Accuracy:	92.6%
PPV:	67.1%
NPV:	97.8%
Prevalence:	13.2%

Of the Xpert *vanA* Assays run on eligible specimens, 94.0% (1180/1255) of these specimens were successful on the first attempt. The remaining 75 gave indeterminate results on the first attempt (26 "INVALID", 49 "ERROR" and 0 "NO RESULT"). Sixty two (62) of the75 indeterminates on the first attempt had sufficient sample for retest, 82.3% (51/62) gave a result on the second the attempt. Overall assay success rate (combining the first and second attempts) was 98.1% (1231/1255).

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

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

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

   Not applicable

N. Instrument Name:

GeneXpert Dx System

- GeneXpert® instrument, computer with proprietary software,
- hand-held barcode scanner, and
- Operator Manual

O. System Descriptions:

1. Modes of Operation:

   The GeneXpert® Dx System automates and integrates sample preparation, nucleic acid amplification, and detection of the target DNA sequence in patient specimens using realtime Polymerase Chain Reaction (PCR).

   Each GeneXpert instrument is similar in that the GeneXpert I (GX I), GeneXpert IV (GX IV) and GeneXpert XVI (GX XVI) all contain the same modules. The difference between the GeneXpert models is that the GX I contains one module, the GX IV can hold up to four modules, and the GX XVI can hold up to sixteen modules, each of which processes one sample at a time. Once the cartridge is loaded in the instrument, all fluids are completely contained within the disposable, single-use plastic Xpert™ Assay cartridges throughout the sample handling, amplification and detection processes.

2. Software:

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

   Yes __X____ or No _____

   The software that controls the operation of the sample processing and the I-CORE module, and collects, analyzes and interprets the acquired optical data is the GeneXpert Dx Version 2.1 software.

3. Specimen Identification: Barcode

4. Specimen Sampling and Handling: Automated

GeneXpert Dx System Hardware Components for Automated Sample Processing

Module Hardware Components	Function
Valve Drive	Rotates the cartridge valve body to address the different cartridge chambers.
Syringe Pump drive	Dispenses fluids to and from the different cartridge chambers.
Ultrasonic horn	Lyses the bacterial cells and sample prep control.
I-CORE® module	Performs PCR amplification and detection. As the user inserts the cartridge into the system, the reaction tube component of the cartridge is inserted into the I-CORE module. After sample preparation within the cartridge, the sample and reagent mixture is transferred from the cartridge chamber into the reaction tube. During the amplification process, the I-CORE heater heats up and the fan cools down the reaction tube contents. Two optical blocks positioned within the ICORE excite the dye molecules that make up the probes and detect the fluorescence emitted. The system uses calibration and data analysis algorithms to determine a relative fluorescence value for each reporter dye after each thermal cycle.
Hand-held Barcode Scanner	Scans cartridge barcode and optional Patient or Sample ID barcode into the GeneXpert Dx System.

5. Calibration:

Optical and thermal calibration of the GeneXpert Dx System is performed by Cepheid at the time of manufacture prior to installation and once yearly or after 1000 runs per module. The user does not calibrate or perform any serviceable functions on the instrument. The normalization function compensates for any optical degradation between calibrations.

The thermal reaction chamber thermistors are calibrated to ± 0.50ºC using National Institute of Standards and Technology (NIST)-traceable standards. During the manufacturing process, the temperature of the heating system is measured at two temperatures: 60ºC and 95ºC. Calibration coefficients that correct for small errors in the raw thermistor readings of the heaters are stored in the memory of each I-CORE module.

The optical system is calibrated using standard concentrations of individual unquenched fluorescent dye-oligos. For each optical channel, the signal produced by a tube alone (the blank signal) is subtracted from the raw signal produced by the dye-oligo standard to determine the spectral characteristics. Using the individual spectral characteristics of the pure dye-oligos, signals from an unknown mixture of dye-oligos can be resolved into corrected signals for the individual dye-oligos in the mixture.

6. Quality Control:

The Xpert *vanA* Assay includes a system control, referred to as the Probe Check Control (PCC), and an internal control, referred to as the Sample Processing Control (SPC). These internal controls are contained within the Xpert *vanA* cartridge. An additional control is the System Control Check for Temperature. This check ensures that the GeneXpert® Dx Instrument is operating within validated heating and cooling specifications.

The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability. The PCC is considered to PASS if the fluorescence generated meets the validated acceptance criteria. If the PCC fails for *vanA* target or SPC, a probe check error is reported and the test will not continue.

The SPC verifies that conditions adequate for processing the target bacteria containing the *vanA* gene have occurred. It consists of *Bacillus globigii* spores formulated into a dry reagent bead included in each Xpert *vanA* cartridge. In addition, the SPC verifies the effectiveness of each sample preparation step, including reaction tube filling, that all reaction components are present and functioning, and monitoring for the presence of potential inhibitor(s) in the PCR Assay.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Failure Modes Testing was performed to determine the effect of failure modes that might occur with the *vanA* Assay. Assay failures may be attributed to operator error, manufacturing error, or instrument malfunction.

Operator error may include failing to add a liquid reagent to the cartridge or adding a liquid reagent to the wrong chamber. The following table includes results of the study describing possible operator errors

Test Results – Possible Operator Errors

Condition	Liquid Reagent Addition	Xpert Results	
		Pos	**Neg**
A (Control)	**All liquid reagents added correctly**	**Pos**	**Neg**
B	Omit addition of both Reagents 1 and 2	*Error*	*Error*
C	Omit addition of Reagent 1 only	*Error*	*Error*
D	Omit addition of Reagent 2 only	Pos	Neg
E	Swap Reagent 1 and 2 addition locations	*Error*	*Error*

A manufacturing error may result in beads being loaded incorrectly into the cartridge before packaging (missing beads or double beads), or a cartridge being assembled incorrectly (missing the filter required for cell capture). Results of the manufacturing error study is presented in the table below

Test Results – Possible Manufacturing Errors

Condition	Bead			Xpert Results	
	EZR	**TSR**	**SPC**	**Pos**	**Neg**
A (Control)	1	1	1	**Pos**	**Neg**
F	1	0	1	*Error*	*Error*
G	0	1	1	*Error*	*Error*
H	1	1	0	Pos	*Invalid*
I	1	1	2	Pos	Neg
J	2	1	1	*Error*	*Error*
K	1	2	1	*Error*	*Error*
L*	1	1	1	*Invalid*	*Invalid*

(*) cartridges intentionally assembled without capture filter material

Some examples of instrument malfunctions include ultrasonic horn failure, motion of the syringe drive not detected, syringe pressure reading exceeds protocol limit, the system failed to find the plunger home position, a valve positioning error was detected, digital temperature reading of thermistor(s) not within acceptable range, and the optical signal from the detector(s) did not reach the expected value. Because the software performs self-check procedures prior to the start of each test, if any of the instrument malfunctions described above occur the test is aborted; no assay results are reported. Operation-terminated error messages are stored for each test and appear in the View Results screen. Instrument malfunctions are not part of this failure mode effect and criticality testing. However, failure mode effect and criticality analysis (FMECA) and hazard analysis has been completed on the GeneXpert Dx system.

Thermal Calibration Study was performed to determine if the thermal calibration is stable for 2,000 runs regardless of the time period of instrument use. The data from the study reported that a 2,000 run thermal stability claim can be supported with a predicted failure rate of 0.7%. The current labeling for the GeneXpert Dx System is 1 year or 1000 runs/module, whichever comes first.

Calibrator Variation Study was conducted to quantify possible effects of degradation of GeneXpert optical calibrations on assay performance. At 95% confidence, the data from the altered calibrations are the same as the data from the nominal calibration. These data provide evidence that the Xpert assays will continue to function effectively even when the instrument calibrations are off by as much as ±50%.

Decay of GeneXpert Optical Calibration study was performed to determine the rate of degradation of the GeneXpert optical calibration. Based on the average measured rate of optical calibration decay following 2000 runs and the demonstrated effectiveness of probe check normalization to mitigate optical calibration decay, Xpert assays will meet specifications for optical performance with 99% confidence. No adverse impact on assay performance is expected. If assay-specific probe check acceptance criteria are not met, Xpert tests are aborted prior to initiation of PCR.

Q. Proposed Labeling:

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

R. Conclusion:

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