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

A. 510(k) Number:

k102416

B. Purpose for Submission:

The BD GeneOhm™ VanR Assay was submitted to obtain a substantial equivalence determination for vancomycin-resistance (*vanA* and *vanB*) from perianal or rectal swab specimens

C. Measurand:

Vancomycin resistant (*VanA* and *vanB)* genes

D. Type of Test:

Qualitative Nuclei Acid Amplification Test using Real-Time PCR technology, to detect *vanA* and *vanB* genes directly from perianal or rectal swabs

E. Applicant:

BD Diagnostics (GeneOhm Sciences, Inc)

F. Proprietary and Established Names:

BD GeneOhm™ VanR Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.1640

2. Classification:

   Class II

3. Product code:

   NIJ- System, test, genotypic detection, resistant markers, *Enterococcus* species
   OOI- Nucleic acid amplification systems, real time

4. Panel:

   83 Microbiology

H. Intended Use:

1. Intended use(s):

   The BD GeneOhm™ VanR Assay is a qualitative *in vitro* test for the rapid detection of vancomycin-resistance (*vanA* and *vanB*) genes directly from perianal or rectal swabs. The BD GeneOhm™ VanR Assay detects the presence of the *vanA* and *vanB* genes that can be associated with vancomycin-resistant enterococci (VRE). The assay is performed on an automated real-time PCR instrument with perianal or rectal swabs from individuals at risk for VRE colonization. The BD GeneOhm™ VanR Assay can be used as an aid to identify, prevent and control vancomycin-resistant colonization in healthcare settings. The BD GeneOhm™ VanR Assay is not intended to diagnose VRE infections nor to guide or monitor treatment for VRE infections. Concomitant cultures are necessary to recover organisms for epidemiological typing, susceptibility testing and for further confirmatory identification.

2. Indication(s) for use:

   The BD GeneOhm™ VanR Assay is a qualitative *in vitro* test for the rapid detection of vancomycin-resistance (*vanA* and *vanB*) genes directly from perianal or rectal swabs. The BD GeneOhm™ VanR Assay detects the presence of the *vanA* and *vanB* genes that can be associated with vancomycin-resistant enterococci (VRE). The assay is performed on an automated real-time PCR instrument with perianal or rectal swabs from individuals at risk for VRE colonization. The BD GeneOhm™ VanR Assay can be used as an aid to identify, prevent and control vancomycin-resistant colonization in healthcare settings. The BD GeneOhm™ VanR Assay is not intended to diagnose VRE infections nor to guide or monitor treatment for VRE infections. Concomitant cultures are necessary to recover organisms for epidemiological typing, susceptibility testing and for further confirmatory identification.

3. Special conditions for use statement(s):

   Prescription use only

4. Special instrument requirements:

   Automated SmartCycler® System (instrument, Dx Software version 1.7b, 3.0a or 3.0b)

I. Device Description:

The test uses a perianal and/or rectal swab specimen, which is resuspended in a sample preparation buffer tube. The suspension is added to a lysis tube. The lysate is then added to a PCR master mix including *vanA* and *vanB* specific primers and an internal control (IC) template. The reaction takes place in a disposable reaction tube placed in the SmartCycler® analyzer. Discrimination of amplicons indicative of drug resistance is done by using a molecular beacon with different fluorometric properties than those of the beacon for the detection of the internal control.

The SmartCycler® software simultaneously monitors the fluorescence emitted by each molecular beacon probe, interprets all data, and provides a final result at the end of the cycling program.

The operation of the SmartCycler® is based on the proprietary microprocessor-controlled I-CORETM (Intelligent Cooling/Heating Optical Reaction) module (SmartCycler® Diagnostic, Operator Manual).

BD GeneOhm™ VanR Assay Reagents

- **Master Mix**
- **Control DNA**
- **Diluent**

Materials Provided

- Sample buffer
- lysis tube
- Master Mix
- Control DNA
- Diluent
- SmartCycler reaction tubes, 25µL
- Specimen identification labels

Materials Required but not Provided

- Vortex Genie 2 (Fisher) with 1.5 mL microtube holder or equivalent; for processing multiple samples, an adapter capable of holding multiple tubes can be used
- Micropipettors (accurate range between 1-10 µL, 10-100 µL and 100-1000 µL)
- Sterile DNAse·free filter-blocked or positive displacement micropipettor tips
- DNAse-free microcentrifuge tubes
- Scissors
- Gauze
- Disposable gloves, powder less
- Microcentrifuge for low speed centrifugation
- Dry heating block specific for 1.5 mL tubes or water bath
- Ice or cooling block specific for 1.5 mL tubes

Interpretation of Results

The decision algorithm for the BD GeneOhm VanR Assay is embedded in the SmartCycler software. The interpretation of assay results is provided by clinical specimen type, instrument reported assay result, and instrument reported IC result.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Remel Bile Esculin Azide agar with 6 µg/mL vancomycin

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

   k972359

3. Comparison with predicate:

Similarities

Item	BD GeneOhm™ VanR Assay	Remel Bile Esculin Azide agar with 6µg/mL vancomycin, k972359)
Intended Use	Screening for vancomycin resistance associated with *Enterococcus*	Same
Sample type	Perianal or rectal swab	Same
Type of Test	Qualitative	Same

Differences

Mode of detection	*vanA, vanB* gene	Growth/no growth in the presence of 6µg/mL vancomycin
Assay format	Amplification: PCR Detection: Fluorogenic target-specific hybridization	Phenotypic detection based on growth
Interpretation of test results	Diagnostic software of SmartCycler® version 1.7b, 3.0a or 3.0b	Visual interpretation

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

N/A

L. Test Principle:

The rectal or perianal swab is eluted in sample buffer and the specimen is lysed. Following specimen lysis, amplification of the *vanA* and *vanB* targets occurs. Amplification of the internal control (IC), a DNA fragment of 294-bp including a 254-bp sequence not found in VRE, will also take place unless there are PCR

inhibitory substances. The amplified DNA targets are detected with molecular beacons, hairpin-forming single stranded oligonucleotides labeled at one end with a quencher and at the other end with a fluorescent reporter dye (fluorophore). In the absence of target, the fluorescence is quenched. In the presence of target, the hairpin structure opens upon beacon/target hybridization, resulting in emission of fluorescence. For the detection of *vanA* amplicons, the molecular beacon contains the fluorophore FAM at the 5' end and the non-fluorescent quencher moiety DABCYL at the opposite end of the oligonucleotide. For the detection of the *vanB* amplicons, the molecular beacon contains the fluorophore Texas Red at the 5' end and the quencher DABCYL at the 3' end. For the detection of the Internal Control (IC) amplicons, the molecular beacon contains the fluorophore TET at the 5' end and the quencher DABCYL at the 3' end. Each beacon-target hybrid fluoresces at a wavelength characteristic of the fluorophore used in the particular molecular beacon. The amount of fluorescence at any given cycle, or following cycling, depends on the amount of specific amplicons present at that time. The SmartCycler® software simultaneously monitors the fluorescence emitted by each beacon, interprets all data, and provides a final result at the end of the cycling program.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   The reproducibility panel consisted of low positives near the LoD, moderate and blank (negative) specimens. Panel members were prepared from samples containing negative matrix inoculated with organisms harboring the targeted genes. Specimen Processing Controls (SPC) and Run Controls (Positive and Negative) were included. Each panel member was tested in triplicate per panel run, over five days, with two runs per day and two technologists per site. Two external and one internal, with one lot of reagents, were used to assess site-to-site reproducibility. One clinical site participated in an extended study where two additional lots of reagents were tested to assess lot-to-lot reproducibility with low and moderate positive and negative members.

   Site-to-site and lot-to-lot demonstrated >95% reproducibility across negative, low positive and moderate positive categories for both *vanA* and *vanB*.

Site-to-site Reproducibility study Results (one lot)

	Site						Overall %Agreement		Ct Values		
	Site 1 %Agreement		Site 2 %Agreement		Site 3 % Agreement				Overall Mean	SD	%CV
NEG	30/30	100%	29/30	96.7%	30/30	100%	89/90	98.9%	35.7†	0.3†	0.9%†
Low pos *vanA*	30/30	100%	30/30	100%	30/30	100%	90/90	100%	35.1	1.0	2.9%
Low pos *vanB*	30/30	100%	26/30	86.7%	30/30	100%	86/90	95.6%	38.4	1.4	3.6%
Mod pos* *vanA*	30/30	100%	30/30	100%	30/30	100%	90/90	100%	34.0	1.1	3.3%
Low pos* *vanB*	30/30	100%	30/30	100%	30/30	100%	90/90	100%	37.2	1.4	3.7%

† represent values from the internal control

* A single panel member contained both *vanA* and *vanB* containing VRE

Lot-to-lot Reproducibility study Results (three lots)

	Lot						Overall %Agreement		Ct Values		
	Lot 1 %Agreement		Lot 2 %Agreement		Lot 3 % Agreement				Overall Mean	SD	%CV
NEG	30/30	100%	30/30	100%	30/30	100%	90/90	100%	35.6†	0.3†	1.0%†
Low pos *vanA*	30/30	100%	30/30	100%	30/30	100%	90/90	100%	35.1	1.0	1.9%
Low pos *vanB*	30/30	100%	30/30	100%	30/30	100%	90/90	100%	38.4	1.4	2.0%
Mod pos* *vanA*	30/30	100%	30/30	100%	30/30	100%	90/90	100%	34.0	1.1	2.0%
Low pos* *vanB*	30/30	100%	30/30	100%	30/30	100%	90/90	100%	37.2	1.4	2.0%

† represent values from the internal control

* A single panel member contained both *vanA* and *vanB* containing VRE

An additional reproducibility study was conducted to assess high negative specimens below LoD. 100-fold and 10-fold dilutions were prepared to obtain the two high negative panel members. The more dilute panel member (i.e. 100-fold below the LoD) contained lower levels of target, demonstrated a higher % agreement for negative test results.

High negatives, site-to-site Reproducibility study Results

High Negatives Panel	Site						Overall %Agreement		Ct Values		
	Site 1 %Agreement*		Site 2 %Agreement*		Site 3 % Agreement*				Overall Mean	SD	%CV
1:100 *vanA*	22/30	73.3%	21/30	70%	23/30	76.7%	66/90	73.3%	39.2	0.9	2.2%
1:100 *vanB*	21/30	70.0%	27/30	90%	25/30	83.3%	73/90	81.1%	41.4	0.6	1.4%
1:10 *vanA*	0/30	0.0%	2/30	6.7%	1/30	3.3%	3/90	3.3%	38.0	1.1	2.9%
1:10 *vanB*	1/30	3.3%	3/30	10.0%	4/30	13.3%	8/90	8.9%	39.9	1.0	2.4%

* Percent agreement for a negative result

Precision

Within-laboratory precision was evaluated for the BD GeneOhm VanR Assay at one site. Samples included simulated specimens representing low and moderate positive *vanA* and *vanB* containing VRE, high negative specimens (100-fold and 10-fold dilutions of a sample prepared at the LoD), and samples negative for VRE. The study was performed over 12 days, with two (2) runs per day and two (2) sample replicates (negative specimens) or three (3) sample replicates (low/moderate positives and high negatives) per run. All samples produced reportable results. Precision study results for negative samples, low and moderate positive samples demonstrated agreement for 100% of the replicates. The more dilute (1:100) high negative panel member, containing lower levels of target, demonstrated a higher percent agreement (68.1% and 69.4% for *vanA* and *vanB* respectively) for negative test results than the less dilute (1:10) panel member (1.4% and 4.2% for *vanA* and *vanB* respectively), which contains higher levels of target.

The following tables show the mean CT values and %CV obtained for the different types of samples.

Variance component analysis POSITIVE results

Category	Mean CT	Within Run CV	Between Run CV	Between Day CV	Overall CV
Low Pos *vanA*	35.6	1.3%	0.0%	0.0%	1.3%
Low Pos *vanB*	37.2	1.2%	0.0%	0.1%	1.2%
Mod Pos *vanA*	33.5	0.9%	0.3%	0.0%	1.0%
Mod Pos *vanB*	36.3	1.3%	0.0%	0.0%	1.4%

Variance components analysis NEGATIVE results

Category	Mean CT	Within Run CV	Between Run CV	Between Day CV	Overall CV
1:100 dil. *vanA*	36.0	0.9%	0.0%	0.0%	0.9%
1:100 dil. *vanB*	35.9	0.8%	0.5%	0.0%	1.0%
1:10 dil. *vanA*	35.8	NA	NA	NA	NA
1:10 dil. *vanB*	35.8	NA	NA	NA	NA
NEG	35.6	0.9%	0.4%	0.0%	1.0%

NA: Not enough data for calculation

b. Linearity/assay reportable range:

Not applicable

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

Positive and negative controls are assay controls (run controls). Assay controls were performed at each site demonstrating acceptable QC results most of the time. The overall invalid result (i.e. failure of the positive and/or negative run controls) rates were 3.4% (6/177). The overall initial unresolved

results (i.e. failure of the internal control) rate was 1.1%.

External specimen processing controls (*E. faecalis* ATCC 51299 for VRE, and *E. faecalis* ATCC 29212 as VSE) were also performed. There were a total of 156 SPC runs for the clinical trial, with an acceptable valid SPC rate of 91.7% (143/156).

Stability Studies

Reagents Stability

The reagents in their protective sealed pouches for the stability claim of 2-25ºC were tested at 5±3 º C for 21 months and 25±2 º C for 12 months on three lots. Data supported that the reconstituted Master Mix and Control DNA were stable:

a) In the original container, in an ice bucket or cooling block at ambient temperature, for up to 3 hours.
b) In the SmartCycler tubes, in an ice bucket or cooling block at ambient temperature, for up to 1 hour.
c) Outside their protective pouch before reconstitution, for up to 2 hours at ambient temperature.

Specimen Stability

The DNA amplifiability results showed that the four transport media allowed the sample eluate storage at ambient temperature for 48 hrs or 4ºC for 5 days.

d. Detection limit:

The limit of detection (LOD) was determined in rectal and perianal matrix with one vancomycin-resistant *Enterococcus faecium* containing the *vanA* gene (ATCC 700221) and with three vancomycin-resistant *Enterococcus faecalis*, each containing one of the $vanB_1$ (ATCC 51299), $vanB_2$ (TUH1-75) or $vanB_3$ (TUH7-68) gene variants. Quantitative culture diluted into negative or perianal matrix was tested in 24 replicates. The LOD was determined using statistical modeling of the lowest concentration of viable organisms achieving 95% detection with the assay. The LoD was determined as follows:

	Rectal		Perianal	
	CFU/rxn	CFU/swab	CFU/rxn	CFU/swab
E. faecium vanA	3	2500	1	833
E. faecalis $vanB_1$	2	1449	5	3623
E. faecalis $vanB_2$	3	2174	2	1449
E. faecalis $vanB_3$	5	3623	14	10145

Analytical Reactivity

A total of 156 vancomycin resistant strains (representing 19 countries) from well characterized c1inical isolates or public collections were evaluated using the BD GeneOhm™ VanR Assay. The detection rate was 99.4% (155/156).

Challenge Study

A challenge set comprising of 50 well characterized *Enterococci* spp. was evaluated. The study included 30 vancomycin resistance due to the *vanA* or *vanB*, 10 intrinsically vancomycin resistant (i.e. *vanC*), and10 vancomycin susceptible *Enterococci*. All results were as expected.

e. Analytical specificity:

Genomic DNA or culture lysate were tested for the cross-reactivity study. It included one human DNA specimen, 98 closely related organisms, normal and pathogenic perianal and/or rectal flora, 27 strains of vancomycin-susceptible enterococci and vancomycin-resistant enterococci other than *vanA/vanB* (i.e. *vanC, vanC*$_2$*, vanD, vanE,* and *vanG*). All results were negative.

Vancomycin resistant Staphylococcus aureus (VRSA) strains

Genomic DNA of five VRSA strains from Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) were tested with the BD GeneOhm™ VanR Assay to determine if amplification/detection of *vanA* and *vanB* genes occurred. The GeneOhm™ VanR Assay detected the *vanA* gene in all the VRSA strains; *vanB* was not detected in any of these strains. They were also positive on agarose gel for *vanA.*

Potentially interfering substances

Potentially interfering substances were tested using materials that may be found in the rectum, such as petroleum jellies, creams, blood, suppositories. Undiluted hydrocortisone cream USP gave unresolved results; blood demonstrated an inhibitory effect and could cause a negative result for a low positive signal.

f. Assay cut-off:

Acceptance Criteria for the BD GeneOhm™ VanR Assay were established during assay development and pre-validated at one external site with 216 specimens prior to the clinical trial. The acceptance criteria included: Endpoint threshold, 2nd derivative threshold, minimum cycle threshold, maximum cycle threshold, and IC% endpoint threshold.

The following table demonstrates the acceptance criteria for the assay:

Acceptance Criteria for BD GeneOhm™ VanR Assay

Acceptance criteria	Clinical specimens			Positive Control		Negative Control
	Assay		Internal Control	Assay		Internal Control
	vanA	vanB		vanA	vanB	
Endpoint threshold	15	10	30	40	70	30
2nd derivative threshold	25	25	30	25	25	30
Minimum cycle threshold	10	10	31	31	33	32
Maximum cycle threshold	45	45	42	39	41	41
% IC NC endpoint	N/A	N/A	50%	N/A	N/A	N/A

2. Comparison studies:

The reference method consisted of direct culture complemented by enriched culture. Enriched culture analysis was completed for all specimens that were negative for VRE by direct culture. Direct culture was performed by inoculating specimens onto a primary isolation media containing Bile Esculin Azide agar supplemented with 6µg/mL vancomycin (BEAV). Enriched culture was performed by inoculating 300 µL of sample buffer containing the specimen into BEA broth or BEAV broth (BEA broth supplemented with 6µg/mL vancomycin). Vancomycin resistance was determined for confirmed enterococcal colonies by using a MIC method. Confirmed vancomycin-resistant enterococcal isolates were further tested for genotypic characterization (i.e. *vanA* and *vanB* genes) by use of an alternative PCR method.

a. Method comparison with predicate device:

See clinical studies below

b. Matrix comparison:

Not Applicable

3. Clinical studies:

The clinical study was conducted at five medical centers. A total of 2156 specimens were tested using Direct/Enriched culture with alternative PCR and the BD GeneOhm VanR™ Assay, producing 2150 (1316 perianal and 834 rectal specimens) reportable results. Two hundred and twenty-two specimens (123 perianal and 99 rectal) were culture negative but positive for *vanB* only by the BD GeneOhm VanR™ PCR and may represent detection of the *vanB* gene in non-enterococcal organisms. Further investigation for non-enterococcal organisms

that might contain the *vanB* gene was not performed.

In comparison to Direct/Enriched culture with alternative PCR, the BD GeneOhm™ VanR Assay identified 92.9% and 93.1% of the perianal and rectal positive specimens, respectively, and identified 86.0% and 82.2% of the perianal and rectal negative specimens, respectively.

Clinical Performance by Perianal Specimens in comparison to Direct/Enriched culture with Alternative PCR

	Sensitivity (95% CI[1])	Specificity (95% CI[1])	VRE Prevalence	PPV (95% CI[1])	NPV (95% CI[1])
vanA	88.2% (134/152) (81.9% - 92.8%)	96.6% (1124/1164) (95.3% - 97.5%)	11.1% (152/1372)	77.0% (134/174) (70.0% - 83.0%)	98.4% (1124/1142) (97.5% - 99.1%)
vanB	100.0% (3/3) (29.2% - 100.0%)	87.6% (1150/1313) (85.7% - 89.3%)	0.2% (3/1372)	1.8% (3/166) (0.4% - 5.2%)	100.0% (1150/1150) (99.7% - 100.0%))
VanR	92.9% (144/155) (87.7% - 96.4%)	86.0% (998/1161) (83.8% - 87.9%)	11.3% (155/1372)	46.9% (144/307) (41.2% - 52.7%)	98.9% (998/1009) (98.1% - 99.5%)

[1] Binomial 95% exact confidence intervals.

Clinical Performance by Rectal Specimens in comparison to Direct/Enriched culture with Alternative PCR

	Sensitivity (95% CI[1])	Specificity (95% CI[1])	VRE Prevalence	PPV (95% CI[1])	NPV (95% CI[1])
vanA	86.4% (108/125) (79.1% - 91.9%)	96.1% (681/709) (94.3% - 97.4%)	15.4% (133/863)	79.4% (108/136) (71.6% - 85.9%)	97.6% (681/698) (96.1% - 98.6%)
vanB	100.0% (7/7) (59.0% - 100.0%)	82.5% (682/827) (79.7% - 85.0%)	1.0% (9/863)	4.6% (7/152) (1.9% - 9.3%)	100.0% (682/682) (99.5% - 100.0%)
VanR	93.1% (122/131) (87.4% - 96.8%)	82.2% (578/703) (79.2% - 85.0%)	16.3% (141/863)	49.4% (122/247) (43.0% - 55.8%)	98.5% (578/587) (97.1% - 99.3%)

[1] Binomial 95% exact confidence intervals.

A total of 2152 specimens were tested using Direct Culture with alternative PCR and the BD GeneOhm VanR™ Assay, producing 2146 (1314 perianal, 832 rectal) reportable results. Two hundred and thirty-one specimens (129 perianal and 102 rectal) were culture negative but positive for *vanB* only by the BD GeneOhm VanR™ PCR and may represent detection of the *vanB* gene in non-enterococcal organisms. Further investigation for non-enterococcal organisms that might contain the *vanB* gene was not performed.

In comparison to direct culture with alternative PCR, the BD GeneOhm™ VanR Assay identified 95.0% and 95.5% of the perianal and rectal positive specimens, respectively, and identified 83.9% and 81.0% of the perianal and rectal negative specimens, respectively.

Clinical Performance by Perianal Specimens in comparison to Direct culture with Alternative PCR

	Positive Percent Agreement (95% CI[1])	Negative Percent Agreement (95% CI[1])
vanA	92.4% (109/118) (86.0%-96.5%)	94.7% (1133/1196) (93.3%-95.9%)
vanB	100.0% (1/1) (2.5%-100.0%)	87.4% (1148/1313) (85.5%-89.2%)
VanR	95.0% (113/119) (89.3%-98.1%)	83.9% (1003/1195) (81.7%-86.0%)

[1] Binomial 95% exact confidence intervals.

Clinical Performance by Rectal Specimens in comparison to Direct/Enriched culture with Alternative PCR

	Positive Percent Agreement (95% CI[1])	Negative Percent Agreement (95% CI[1])
vanA	91.5% (97/106) (84.5%-96.0%)	94.9% (689/726) (93.0%-96.4%)
vanB	100.0% (7/7) (59.0%-100.0%)	82.5% (681/825) (79.8%-85.1%)
VanR	95.5% (107/112) (89.9%-98.5%)	81.0% (583/720) (77.9%-83.8%)

[1] Binomial 95% exact confidence intervals.

a. *Clinical Sensitivity:*

See perianal and rectal clinical performance studies above "…in comparison to Direct/Enriched culture with Alternative PCR"

*b. Clinical specificity**:***

See perianal and rectal clinical performance studies above "…in comparison to Direct/Enriched culture with Alternative PCR"

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:

Perianal Specimens

Health System	Total N	Number of VRE with *vanA*	Number of VRE with *vanB*	Number of VRE with *vanA* and/or *vanB*	Prevalence of VRE with *vanA*	Prevalence of VRE with *vanB*	Prevalence of VRE with *vanA* and/or *vanB*
Multi-Institutional	789	73	2	75	9.3% (73/789)	0.3% (2/789)	9.5% (75/789)
Academic	583	79	1	80	13.6% (79/583)	0.2% (1/583)	13.7% (80/583)
Total	1372	152	3	155	11.1% (152/1372)	0.2% (3/1372)	11.3% (155/1372)

Rectal Specimens

Health System	Total N	Number of VRE with *vanA*	Number of VRE with *vanB*	Number of VRE with *vanA* and/or *vanB*	Prevalence of VRE with *vanA*	Prevalence of VRE with *vanB*	Prevalence of VRE with *vanA* and/or *vanB*
Multi-Institutional	417	79	9	87	18.9% (79/417)	2.2% (9/417)	20.9% (87/417)
Academic	446	54	0	54	12.1% (54/446)	0.0% (0/446)	12.1% (54/446)
Total	863	133	9	141	15.4% (133/863)	1.0% (9/863)	16.3% (141/863)

N. Instrument Name:

Automated SmartCycler® System (instrument, Dx Software version 1.7b, 3.0a or 3.0b)

O. System Descriptions:

1. Modes of Operation:

   The operation of the Smart Cycler instrument is based on the proprietary microprocessor –controlled I-CORE (Intelligent Cooling/Heating Optical Reaction) module. Each Smart Cycler processing block contains 16 independently controlled, programmable I-CORE modules, each with one reaction site. Thermally optimized proprietary reaction tubes combined with the design of the I-CORE modules allow very rapid temperature cycling and rapid amplification. Up to 6 Smart Cycler processing blocks can be daisy-chained together, allowing simultaneous analysis of 96 discrete samples. Information regarding data acquisition, data analysis and diagnostic algorithm was submitted and cleared under k022504 and k042357

2. Software:

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

Yes __X_____ or No _____

3. Specimen Identification:

   A liquid or soft stool specimen is collected and transported to the laboratory. A sterile dry swab is dipped into the liquid or soft stool material and processed.

4. Specimen Sampling and Handling:

   For testing, the swab is eluted in sample buffer and the specimen is lysed. An aliquot of the lysate is added to PCR reagents which contain the *vanA* and *vanB* specific primers used to amplify the genetic target, if present. Amplified targets are detected with hybridization probes labeled with quenched fluorophores (molecular beacons). The amplification, detection and interpretation of the signals are done automatically by the Cepheid SmartCycler software. The entire procedure takes about 60 to 75 minutes

5. Calibration:

   N/A

6. Quality Control:

   Positive and Negative Controls

   Quality control procedures are designed to monitor assay performance. The positive control is intended to monitor substantial reagent failure. The negative control is used to detect reagent or environmental contamination (or carry-over) by either DNA containing *vanA* or *vanB* genes or amplicons. Positive and negative controls are assay controls (run controls). An invalid control invalidates the run. Finally, an internal control incorporated into each reaction mixture is intended to monitor the reagent integrity and PCR inhibition in each specimen.

   One positive control and one negative control must be included in each assay run on the SmartCycler®. The software automatically assigns the position of the controls on the instrument.

   Specimen Processing Controls

   A reference VRE strain (e.g. American Type Culture Collection, ATCC 51299) or a well characterized VRE clinical isolate may be used as a positive specimen processing control. A vancomycin sensitive enterococci (e.g. ATCC 29212) or *non-vanA*/*vanB* VRE (e.g. *Enterococcus gallinarum* ATCC 700425) may be used as a negative specimen processing control.

   Re-suspend isolated colonies from an 18 to 24 h 5% sheep blood agar plate in saline to a turbidity of 0.5 McFarland (approximately1.5 x 10^5 CFU/mL). Dilute

with saline to obtain a suspension of approximately 10^6 CFU/mL. Dip a recommended swab into the bacterial suspension and press out the excess fluid. Process and test as a clinical specimen. The specimen processing controls should yield valid results.

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

Not Applicable

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.