GeneXpert
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Xpert® vanA/vanB

REF **GXVANA/B-CE-10**

Cepheid®

In Vitro Diagnostic Medical Device IVD

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Xpert® *van*A/*van*B

In Vitro Diagnostic Use Only

1. Proprietary Name

Xpert® *van*A/*van*B

2. Common or Usual Name

Xpert *van*A/*van*B Assay

3. Intended Use

The Cepheid Xpert *van*A/*van*B Assay, performed on GeneXpert® Instrument Systems, is a qualitative *in vitro* diagnostic test designed for rapid detection of vancomycin-resistance (*van*A/*van*B) genes from rectal and perianal swab specimens in patients at risk for intestinal colonization of vancomycin-resistant bacteria. The test utilizes automated real-time polymerase chain reaction (PCR) to detect the *van*A and *van*B genes that can be associated with Vancomycin-Resistant Enterococci (VRE). The Xpert *van*A/*van*B Assay is intended to aid in the recognition, prevention and control of vancomycin-resistant organism colonization in healthcare settings. The Xpert *van*A/*van*B Assay is not intended to diagnose VRE nor to guide or monitor treatment for VRE infections. Concomitant cultures are necessary only to recover organisms for epidemiological typing, susceptibility testing and for further confirmatory identification of VRE.

4. Summary and Explanation

Vancomycin-Resistant Enterococci (VRE) has become a major cause of nosocomial infections specifically in the Intensive Care Units (ICU). Enterococci accounts for over one third of infections within the ICU according to a 2004 National Nosocomial Infection Survey[1]. Infections caused by VRE have been associated with greater morbidity, mortality, length of stays, and hospital costs. The risk of VRE colonization has been attributed to the use of multiple antimicrobial classes including glycopeptides, third generation cephalosporins, and antibiotics with potent anti-anaerobic activity. The spread of VRE is through contact with colonized or infected individuals within a healthcare facility. Thus, many facilities are putting into place active surveillance programs to identify carriers of VRE and to isolate them appropriately to reduce the transmission of the pathogen. Active surveillance screening programs test patients via perianal or rectal swabs at admission, once a week while in the ICU, after receipt of antimicrobial therapy, and upon discharge.

5. Principle of the Procedure

The GeneXpert Instrument Systems automate and integrate sample purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples using real-time PCR and RT-PCR assays. The systems consist of an instrument, personal computer, and preloaded software for running tests on collected samples and viewing the results. The systems require the use of single-use disposable Xpert cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is eliminated. For a full description of the system, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual.*

Xpert *van*A/*van*B Assay includes reagents for the detection of the *van*A and *van*B resistance genes as well as a sample processing control (SPC) to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The primers and probes in the Xpert *van*A/*van*B assay detect sequences in the genes for vancomycin/teicoplanin resistance (*van*A, *van*B).

6. Reagents and Instruments

6.1 Material Provided

The Xpert *van*A/*van*B Assay kit contains sufficient reagents to process 10 specimens or quality control samples.

The kit contains the following:

Xpert *van*A/*van*B Assay Cartridges with integrated reaction tubes	**10**
• Bead 1, 2, and 3 (freeze-dried)	1 each per cartridge
• Reagent 1	3.0 mL per cartridge
• Reagent 2 (Sodium Hydroxide)	3.0 mL per cartridge
Sample Reagent	**1 x 1.7 mL**
CD	**1 per kit**

- Assay Definition File (ADF)
- Instructions to import ADF into GX software
- Instructions for Use (Package Insert)

Note Safety Data Sheets (SDS) are available at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab.

Note The bovine serum albumin (BSA) in the beads within this product was produced exclusively from bovine plasma sourced in the United States. The manufacturing of the BSA is also performed in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and post-mortem testing. During processing, there was no mixing of the material with other animal materials

6.2 Storage and Handling

- Store the Xpert *van*A/*van*B cartridges and reagents at 2 – 28 °C.
- Do not use reagents or cartridges that have passed the expiration date.
- Do not open the cartridge lid until you are ready to perform testing.
- Use the cartridge and reagents within 30 minutes after opening the lid.
- Do not use any reagents that have become cloudy or discolored.

7. Materials Required but Not Provided

- GeneXpert Dx System or GeneXpert Infinity System (catalog number varies by configuration): GeneXpert instrument, computer with proprietary GeneXpert Software Version 1.6b or higher, barcode wand reader and Operator Manual
- Printer: If a printer is required, contact Cepheid Customer Support to arrange for the purchase of a recommended printer.
- Vortex mixer
- Disposable, sterile transfer Pipettes
- Cepheid sample collection device (Cepheid Part Number 900-0370)

8. Warnings and Precautions

- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be treated with standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention and the Clinical and Laboratory Standards Institute [2, 3].
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- The Xpert *van*A/*van*B Assay does not provide susceptibility results. Additional time is required to culture and perform susceptibility testing.
- Do not substitute Xpert *van*A/*van*B reagents with other reagents.

- Do not open the Xpert *van*A/*van*B cartridge lid except when adding sample or performing a retest.
- Do not use a cartridge that has been dropped or shaken after you have added the sample.
- Do not use a cartridge that has a damaged reaction tube.
- Each single-use Xpert *van*A/*van*B cartridge is used to process one test. Do not reuse spent cartridges.
- Biological specimens, transfer devices and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution's environmental waste procedure for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific national or regional disposal procedures. If national or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.
- Store the Xpert *van*A/*van*B kit at 2 – 28 °C.
- Do not open the cartridge lid until you are ready to perform testing.

9. Chemical Hazards[7,8]

- UN GHS Hazard Pictogram:
- Signal Word: WARNING
- **UN GHS Hazard Statements**
  - Harmful if swallowed
  - Causes skin irritation
  - Causes serious eye irritation
- **UN GHS Precautionary Statements**
  - **Prevention**
    - Wash thoroughly after handling.
    - Do not eat, drink, or smoke when using this product.
    - Avoid release to the environment.
    - Wear protective gloves/protective clothing/eye protection/face protection
  - **Response**
    - IF ON SKIN: Wash with plenty of soap and water.
    - Take off contaminated clothing and wash before reuse.
    - Specific treatment, see the supplemental first aid information.
    - If skin irritation occurs: Get medical advice/attention.
    - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
    - If eye irritation persist: Get medical advice/attention
    - IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician if you feel unwell.
    - Rinse mouth.
  - **Storage Disposal**
    - Dispose of content and/or container in accordance with local, regional, national, and/or international regulations.

10. Specimen Collection, Transport and Storage

To obtain adequate specimen, follow the instructions in this section closely.

For rectal specimens:

1. Collect the swab specimen using the Cepheid sample Collection Device (Cepheid Part Number 900-0370).
2. Carefully insert the swab approximately 2.5 cm beyond the anal sphincter (so that the cotton tip is no longer visible) and gently rotate 3 times to ensure uniform sample on both swabs.
3. Place the swabs back in the sample container.
4. Label with Sample ID and send to the laboratory.

5. Store swab specimen at 2 – 8 °C. The swab specimen is stable up to 5 days when stored at 2 – 8 °C.

For perianal specimens:

1. Collect the swab specimen using the Cepheid sample Collection Device (Cepheid Part Number 900-0370).
2. Press the buttocks apart to expose the perianal region, then using both of the swabs, fully swab around the perianal surface making sure to swab as much of the surface as possible.
3. Place the swabs back in the sample container.
4. Label with Sample ID and send to the laboratory.
5. Store swab specimen at 2 – 8 °C. The swab specimen is stable up to 5 days when stored at 2 – 8 ºC.

11. Procedure

11.1 Preparing the Cartridge

Important **Start the test within 30 minutes of adding the Sample reagent to the cartridge.**

For rectal/perianal swabs:

Note Only one swab is required.

To add the sample into the cartridge (Xpert *van*A/*van*B):

1. Remove the cartridge and Sample Reagent from the kit.
2. Remove one swab from the transport container.
3. Insert the swab into the tube containing the Sample Reagent.

Note Use sterile gauze to minimize risks of contamination.

4. Hold the swab by the stem near the rim of the tube, lift the swab a few millimeters from the bottom of the tube and push the stem against the edge of the tube to break it. Make sure the swab is short enough to allow the cap to close tightly.
5. Close the lid and vortex at high speed for 10 seconds.
6. Open the cartridge lid. Using a sterile disposable transfer pipette, transfer the entire contents of the Sample Reagent to the sample chamber of the Xpert *van*A/*van*B cartridge.
7. Close the cartridge lid.

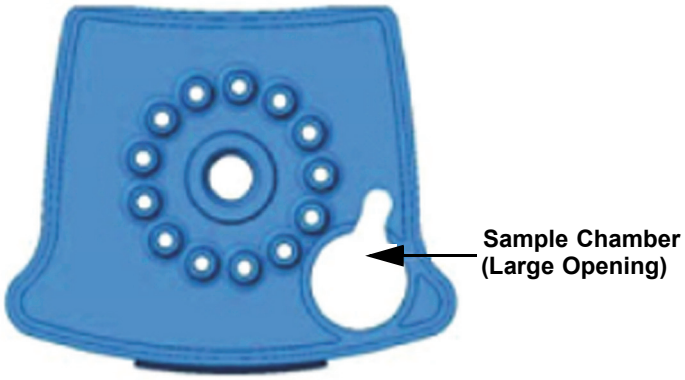

Figure 1. Xpert *van*A/*van*B Cartridge (Top View)

11.2 Starting the Test

Important **Before you start the test, make sure the Xpert *van*A/*van*B assay definition is imported into the software.**

This section lists the basic steps for running the test. For detailed instructions, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending on the model that is being used.

1. Turn on the GeneXpert instrument:
   - If using the GeneXpert Dx instrument, first turn on the GX Dx instrument, and then turn on the computer. The GeneXpert software will launch automatically or may require double-clicking the GeneXpert Dx software shortcut icon on the Windows® desktop..

   or

   - If using the GeneXpert Infinity instrument, power up the instrument. The GeneXpert software will launch automatically or may require double-clicking the Xpertise software shortcut icon on the Windows desktop.
2. Log on to the GeneXpert Instrument System software using your user name and password.
3. In the GeneXpert System window, click **Create Test** (GeneXpert Dx) or **Orders** and **Order Test** (Infinity).
4. Scan or type the Patient ID (optional).If typing the Patient ID, make sure the Patient ID is typed correctly. The patient ID is associated with the test results and is shown in the View Results window.
5. Scan or type the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is associated with the test results and is shown in the View Results window and all the reports. The Scan Cartridge Barcode dialog box appears.
6. Scan the barcode on the Xpert *van*A/*van*B Assay cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.
7. Click **Start Test** (GeneXpert Dx) or **Submit** (Infinity). In the dialog box that appears, type your password.
8. For the GeneXpert Infinity System, place the cartridge on the conveyor belt. The cartridge will be automatically loaded, the test will run and the used cartridge will be placed into the waste container.

   or

   For the GeneXpert Dx Instrument:

   A. Open the instrument module door with the blinking green light and load the cartridge.

   B. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.

   C. Wait until the system releases the door lock before opening the module door and removing the cartridge.

   D. The used cartridges should be disposed in the appropriate specimen waste containers according to your institution's standard practices.

12. Viewing and Printing Results

This section lists the basic steps for viewing and printing results. For more detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual* or *GeneXpert Infinity Operator Manual* depending upon the instrument being used.

1. Click the **View Results** icon to view results.
2. Upon completion of the test, click the **Report** button of the View Results window to view and/or generate a PDF report file.

13. Quality Control

13.1 Built-in Quality Controls

CONTROL

Each test includes a Sample Processing Control (SPC) and Probe Check Control (PCC).

- **Sample processing control (SPC)** — Ensures the sample was correctly processed. The SPC contains spores of *Bacillus globigii* in the form of a dry spore cake that is included in each cartridge to verify adequate processing of the sample bacteria. The SPC verifies that lysis of vancomycin-resistant bacteria has occurred if the organisms are present and verifies that specimen processing is adequate. Additionally this control detects specimen-associated inhibition of the real-time PCR assay. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.
- **Probe check control (PCC)** — Before the start of the PCR reaction, the GeneXpert® Dx System measures the fluorescence signal from the probes to monitor bead rehydration, reaction-tube filling, probe integrity and dye stability. Probe Check passes if it meets the assigned acceptance criteria.

14. Interpretation of Results

The results are interpolated by the GeneXpert System from measured fluorescent signals and embedded calculation algorithms, and are shown in the View Results window (Figure 2, Figure 3, Figure 4, and Figure 5). Possible results are:

Table 1. Results and Interpretation

Result	Interpretation
***van*A POSITIVE** Figure 2	*van*A target DNA is detected. • *van*A POSITIVE—the *van*A target has a Ct within the valid range and endpoint above the minimum setting. • SPC—NA (not applicable); SPC is ignored since *van*A amplification may compete with this control. • Probe Check—PASS; all probe check results pass.
***van*B POSITIVE** Figure 4	*van*B target DNA is detected. • *van*B POSITIVE—the *van*B target has a Ct within the valid range and endpoint above the minimum setting. • SPC—NA (not applicable); SPC is ignored since *van*B amplification may compete with this control. • Probe Check—PASS; all probe check results pass.
***van*A POSITIVE, *van*B POSITIVE**	*van*A and *van*B target DNA are detected. • *van*A POSITIVE—the *van*A target has a Ct within the valid range and endpoint above the minimum setting. • *van*B POSITIVE—the *van*B target has a Ct within the valid range and endpoint above the minimum setting. • SPC—NA (not applicable); SPC is ignored since *van*A and/or *van*B amplification may compete with this control. • Probe Check – PASS; all probe check results pass.
NEGATIVE Figure 3	*van*A and *van*B target DNA are not detected. SPC meets acceptance criteria. • NEGATIVE—No *van*A or *van*B target DNA are detected. • SPC—PASS; SPC has a Ct within the valid range and endpoint above the endpoint minimum setting. • Probe Check—PASS; all probe check results pass.
INVALID	Presence or absence of *van*A/*van*B cannot be determined, repeat test according to the instructions in the Retest Procedure section below. SPC does not meet acceptance criteria, the sample was not properly processed, or PCR is inhibited. • INVALID— presence or absence of *van*A or *van*B DNA cannot be determined. • SPC—FAIL; *van*A and *van*B target results are negative and the SPC Ct is not within valid range and endpoint below minimum setting. • Probe Check—PASS; all probe check results pass.
ERROR Figure 5	Presence or absence of *van*A/*van*B cannot be determined, repeat test according to the instructions in the Retest Procedure section below. The Probe Check control failed probably due to reaction tube was filled improperly, a probe integrity problem was detected or because the maximum pressure limits were exceeded. • *van*A—NO RESULT • *van*B—NO RESULT • SPC—NO RESULT • Probe Check—FAIL*; all or one of the probe check results fail • *If the probe check passed, the error is caused by a system component failure.

Table 1. Results and Interpretation

Result	Interpretation
NO RESULT	Presence or absence of *van*A/*van*B cannot be determined, repeat test according to the instructions in the Retest Procedure section below. Insufficient data were collected to produce a test result (for example, the operator stopped a test that was in progress). • *van*A—NO RESULT • *van*B—NO RESULT • SPC—NO RESULT • Probe Check—NA (not applicable)

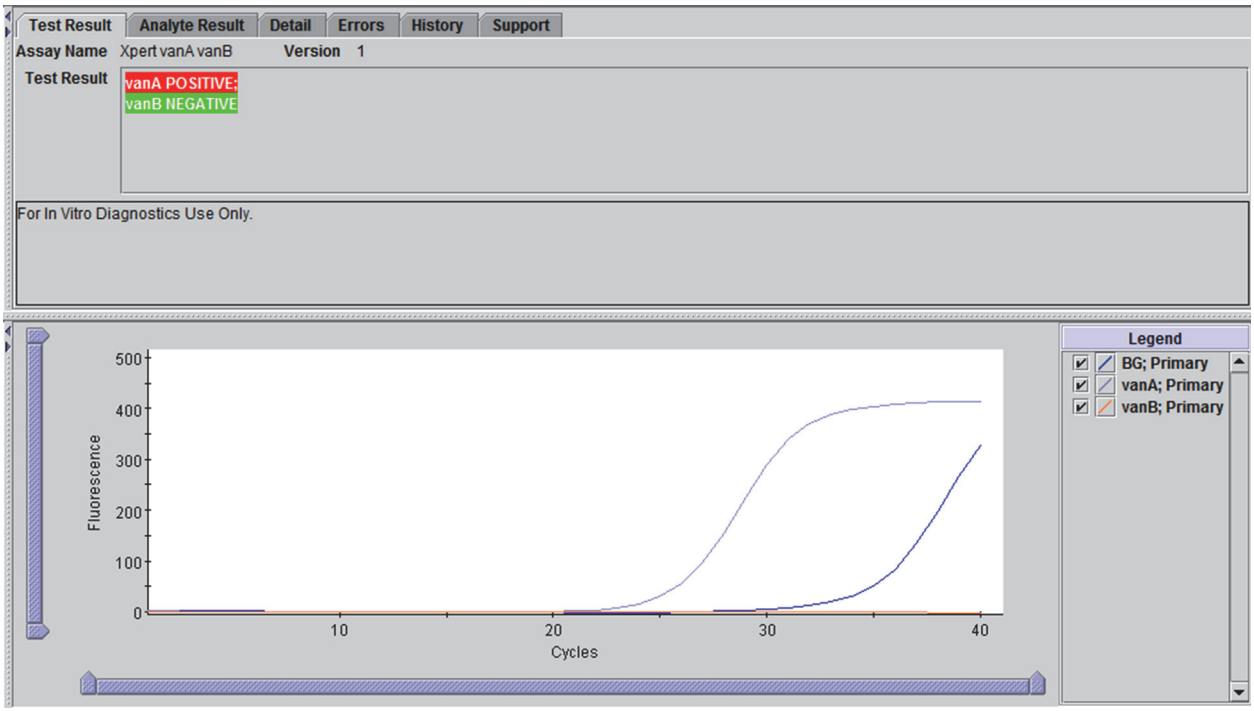

Figure 2. Example of *van*A Positive Result and *van*B Negative Result

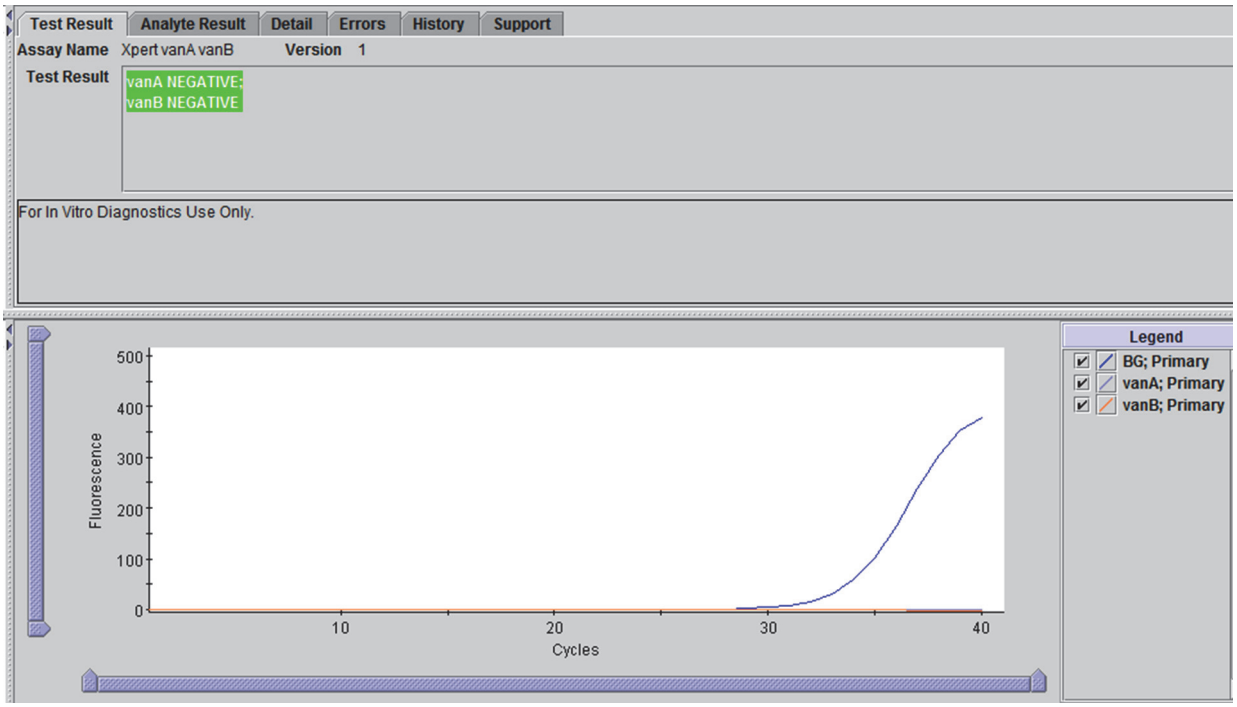

Figure 3. Example of *van*A and *van*B Negative Result

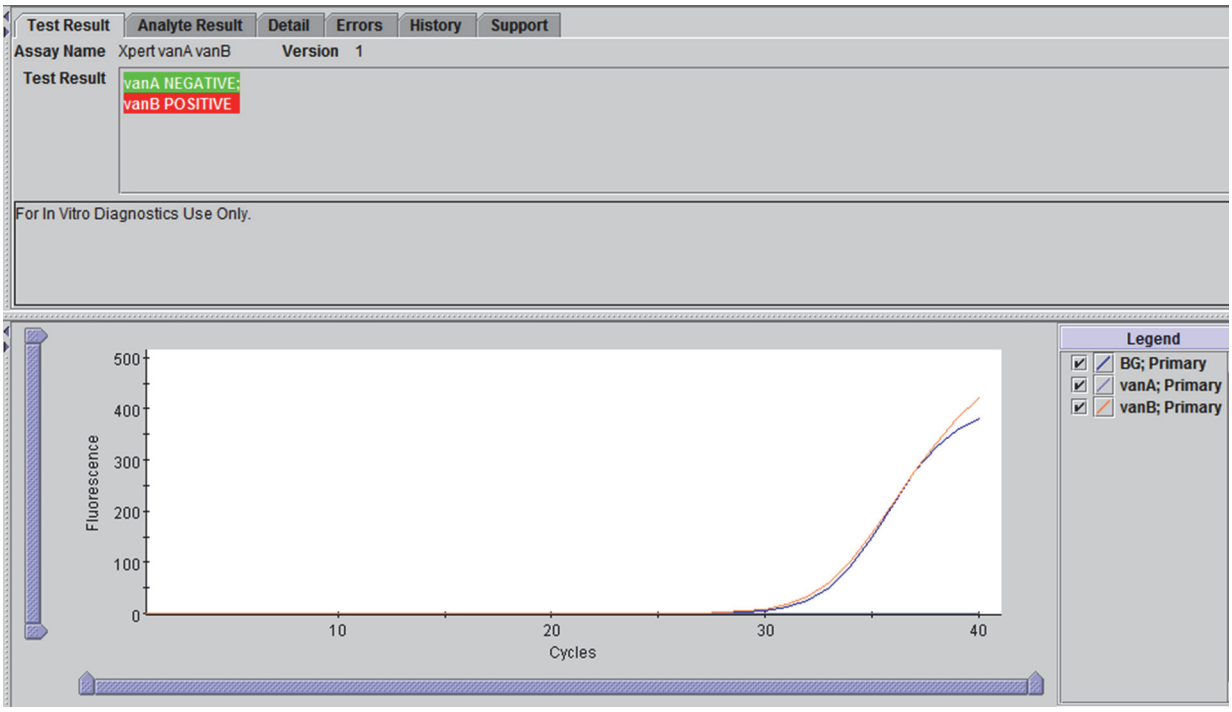

Figure 4. Example of *van*A Negative Result and *van*B Positive Result

Figure 5. An Example of an Error Result

15. Retests

15.1 Reasons to Repeat the Test

If any of the test results mentioned below occur, repeat the test according to instructions in the following section titled Section 15.2, Retest Procedure.

An **INVALID** result indicates that the controls SPC failed. The sample was not properly processed or PCR is inhibited.

An **ERROR** result indicates that the Probe Check control failed and the assay was aborted possibly due to the reaction tube being filled improperly, a reagent probe integrity problem was detected, or because the maximum pressure limits were exceeded.

A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.

15.2 Retest Procedure

For retest within 3 hours of an indeterminate result, use a new Xpert *van*A/*van*B cartridge (do not re-use the cartridge) and new Sample Reagent vial. Transfer all remaining contents from Chamber S to a new Sample Reagent. Vortex and add the entire contents of the Sample Reagent to the sample chamber of the new Xpert *van*A/*van*B cartridge.

16. Limitations

- The performance of the Xpert *van*A/*van*B Assay was validated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test. Results from the Xpert *van*A/*van*B Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
- Erroneous test results might occur from improper specimen collection, failure to follow the recommended sample collection, handling and storage procedures, technical error, sample mix-up, or because the number of organisms in the specimen is too low to be detected by the test. Careful compliance with the instructions in this insert is necessary to avoid erroneous results.
- Because the detection of VRE is dependent on the number of organisms present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.
- Rerunning the Xpert *van*A/*van*B Assay when results are **INVALID**, **ERROR**, or **NO RESULT** should depend on practices and policies within each facility. Alternate procedures should be available. For culturing, remaining swab specimens should be placed in appropriate transport systems and cultured within 4 days.
- A positive test result does not necessarily indicate the presence of viable organism. It is however, presumptive for the presence of VRE.

- Positive Xpert *van*A/*van*B results for *van*B in the absence of *van*A may be due to organisms other than VRE. It is recommended to perform culture confirmation on these organisms.
- As described in the literature, some aerobic and anaerobic bacteria containing the *van*B gene may be found[4,5,6] and would be detected by this assay, however, the clinical relevance of such findings is unknown. Anaerobic bacteria positive for the *van*B gene have been suggested to constitute a reservoir of vancomycin resistance determinants[1], but this hypothesis remains to be proven.
- Testing with the Xpert *van*A/*van*B Assay should be used as an adjunct to other methods available. Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown VRE variants resulting in a false negative result.
- The use of any other specimen collection and transport system other than Cepheid Sample Collection Device is not recommended and has not been qualified.
- The Xpert *van*A/*van*B Assay detects *van*A gene, not microorganism; therefore, *van*A genes carried by non-enterococci, such as vancomycin-resistant Staphylococcus aureus strains, may also give a positive result.
- Because of the dilution factor associated with the retest procedure, it is possible that *van*A and *van*A positive specimens, very near or at the limit of detection (LoD) of the Xpert *van*A*van*B assay, may result in a false negative result upon retest.
- Test results may also be affected by the concurrent antibiotic therapy, or the number of organisms in the specimen which may be below the limit of detection of the test.

17. Performance Characteristics

17.1 Clinical Performance

Performance characteristics of the Xpert *van*A/*van*B Assay were determined in a multi-site prospective investigation study at four institutions in the United States and one site in Europe by comparing the Xpert *van*A/*van*B Assay on the GeneXpert System (Xpert *van*A/*van*B Assay) with culture. To be enrolled in the study, specimens had to be from individuals for whom cultures were indicated and/or ordered, according to institutional practices.

One swab sample was used for testing with the Xpert *van*A/*van*B Assay. The second swab was sent to the central culture laboratory except for the site in Europe. Upon receipt at the central culture site the swab was used to inoculate a Bile Esculin azide agar plate with vancomycin and was then placed into bile esculin broth containing vancomycin. After 24 hours incubation at + 35°C the broth was sub-cultured on bile esculin azide agar with vancomycin and examined at 24 hours and 48 hours. Following Gram-staining and PYR-test, presumptive VRE were identified using API20S strips (BioMérieux, France). Determination of *van*A and/or *van*B was done using E-tests (AB Biodisk, Sweden) for vancomycin and teicoplanin.

17.2 Overall Results

A total of 878 rectal swab specimens were tested for VRE by the Xpert *van*A/*van*B Assay and compared to the direct culture method. A total of 878 rectal swab specimens were tested for VRE by the Xpert *van*A/*van*B Assay and compared to the enriched culture method.

A total of 429 perianal swab specimens were tested for VRE by the Xpert *van*A/*van*B Assay and compared to the direct culture method. A total of 430 perianal swab specimens were tested for VRE by the Xpert *van*A/*van*B Assay and compared to the enriched culture method.

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the Xpert *van*A/*van*B Assay are provided in Table 2 and Table 3.

Table 2. Performance Characteristics of the Xpert *van*A/*van*B Assay by Specimen Type and the Direct Culture Method

Xpert *van*A/*van*B vs. Direct Culture *van*A with 95% CI				
	Sensitivity	**Specificity**	**PPV**	**NPV**
Perianal	98.1% (51/52) (89.7% - 100.0%)	93.4% (352/377) (90.4% - 95.7%)	67.1% (51/76) (55.4% - 77.5%)	99.7% (352/353) (98.4% - 100.0%)
Rectal	96.5% (83/86) (90.1% - 99.3%)	91.5% (725/792) (89.4% - 93.4%)	55.3% (83/150) (47.0% - 63.4%)	99.6% (725/728) (98.8% - 99.9%)
Total	97.1% (134/138) (92.7% - 99.2%)	91.2% (1077/1169) (90.4% - 93.6%)	59.3% (134/226) (52.6% - 65.8%)	99.6% (1077/1081) (99.1% - 99.9%)
Xpert *van*A/*van*B vs. Direct Culture *van*B with 95% CI				
	Sensitivity	**Specificity**	**PPV**	**NPV**
Perianal	25% (1/4) (0.6% - 80.6%)[a]	94.1% (400/425) (91.4% - 96.2%)	3.8% (1/26) (0.1% - 19.6%)	99.3% (400/403) (97.8% - 99.8%)
Rectal	100% (13/13) (79.4% - 100.0%)	83.9% (726/865) (81.3% - 86.3%)	8.6% (13/152) (4.6% - 14.2%)	100% (726/726) (99.6% - 100.0%)
Total	82.4% (14/17) (56.6% - 96.2%)	87.3% (1126/1290) (85.3% - 89.1%)	7.9% (14/178) (4.4% - 12.8%)	99.7% (1126/1129) (99.2% - 99.9%)
Xpert *van*A/*van*B vs. Direct Culture *van*A/*van*B with 95% CI				
	Sensitivity	**Specificity**	**PPV**	**NPV**
Perianal	92.9% (52/56) (82.7% - 98.0%)	88.7% (331/373) (85.1% - 91.8%)	55.3% (52/94) (44.7% - 65.6%)	98.8% (331/335) (97.0% - 99.7%)
Rectal	99.0% (96/97) (94.4% - 100.0%)	79.3% (619/781) (76.2% - 82.1%)	37.2% (96/258) (31.3% - 43.4%)	99.8% (619/620) (99.1% - 100.0%)
Total	96.7% (148/153) (92.5% - 98.9%)	82.3% (950/1154) (80.0-% - 84.5%)	42.0% (148/352) (36.8% - 47.4%)	99.5% (950/955) (98.8% - 99.8%)

[a] The primary factor which contributed to the lower sensitivity of 25% for *van*B detection in perianal specimens was the low number of samples that were positive for *van*B by culture. Overall, there was a low prevalence of *van*B in the study population.

Table 3. Performance Characteristics of the Xpert *van*A/*van*B Assay by Specimen Type and the Enriched Culture Method

Xpert *van*A/*van*B vs. Enriched Culture *van*A with 95% CI				
	Sensitivity	**Specificity**	**PPV**	**NPV**
Perianal	90.6% (58/64) (80.7%- 96.5%)	95.1% (348/366) (92.3% - 97.1%)	76.3% (58/76) (65.2% - 85.3%)	98.3% (348/354) (96.3% - 99.4%)
Rectal	92.0% (103/112) (85.3% - 96.3%)	94.0% (720/766) (92.1% - 95.6%)	69.1% (103/149) (61.0% - 76.4%	98.8% (720/729) (97.7% - 99.4%)
Total	91.5% (161/176) (86.3% - 95.2%)	94.3% (1068/1132) (92.8% - 95.6%)	71.6% (161/225) (65.2% - 77.4%)	98.6% (1068/1083) (97.7% - 99.2%)
Xpert *van*A/*van*B vs. Enriched Culture *van*B with 95% CI				
	Sensitivity	**Specificity**	**PPV**	**NPV**
Perianal	25% (1/4) (0.6% - 80.6%)[a]	94.1% (401/426) (91.5% - 96.2%)	3.8% (1/26) (0.1% -18.6%)	99.3% (401/404) (97.8% - 99.8%)
Rectal	100% (13/13) (79.4% - 100.0%)	83.9% (726/865) (81.3% - 86.3%)	8.6% (13/152) (4.6% - 14.2%)	100% (726/726) (99.6% - 100.0%)
Total	82.4% (14/17) (56.6% - 96.2%)	87.3% (1127/1291) (85.4% - 89.1%)	7.9% (14/178) (4.4% - 12.8%)	99.7% (1127/1130) (99.2% - 99.9%)
Xpert *van*A/*van*B vs. Enriched Culture *van*A/*van*B with 95% CI				
	Sensitivity	**Specificity**	**PPV**	**NPV**
Perianal	86.8% (59/68) (76.4% - 93.8%)	90.3% (327/362) (86.8% - 93.2%)	62.8% (59/94) (52.2% - 72.5%)	97.3% (327/336) (95.0% - 98.8%)
Rectal	94.3% (115/122) (88.5% - 97.7%)	81.2% (614/756) (78.2% - 83.9%)	44.7% (115/257) (38.6% - 51.1%)	98.9% (614/621) (97.7% - 99.5%)
Total	91.6% (174/190) (86.6% - 95.1%)	84.2% (941/1118) (81.9% - 86.3%)	49.6% (174/351) (44.2% - 54.9%)	98.3% (941/957) (97.3% - 99.0%)

[a] The primary factor which contributed to the lower sensitivity of 25% for *vanB* detection in perianal specimens was the low number of samples that were positive for *vanB* by culture. Overall, there was a low prevalence of *vanB* in the study population.

18. Analytical Specificity

Forty-two bacterial and fungal strains were collected, quantitated and tested using the Xpert *van*A/*van*B Assay. The strains originated from the American Type Culture Collection (ATCC), Culture Collection University of Goteborg (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, two (2) vancomycin-sensitive strains representing *E. faecalis* and *E. faecium* were included.

Each strain was tested in triplicate at concentrations ranging from $8.5x10^8$ to 2.3 $x10^{10}$ CFU/swab. Yeasts were tested at approximately 10^7 cells per swab. Positive and negative controls were included in the study. Under the conditions of the study, all isolates were reported "vanA NEGATIVE" and "vanB NEGATIVE". The analytical specificity was 100%.

19. Analytical Sensitivity

Studies were performed to determine the 95% confidence intervals for the analytical limit of detection (LoD) of *Enterococcus faecium* (*van*A) and *Enterococcus faecalis* (*van*B) diluted into a fecal matrix of human origin that can be detected by the Xpert *van*A/*van*B Assay. The fecal matrix consisted of autoclaved human liquid feces (*van*A negative and *van*B negative by the Xpert *van*A/*van*B Assay) diluted 1:10 in Tris buffer. The LoD is defined as the lowest number of colony forming units (CFU) per swab that can be reproducibly distinguished from negative samples with 95% confidence.

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 *van*A/*van*B Assay on a simulated rectal swab specimen is 37 CFU for the *van*A target and 112 CFU for the *van*B target.

20. Interfering Substances

Sixteen exogenous substances occasionally used or found in stool were tested for interference with the Xpert *van*A/*van*B Assay. The substances tested are listed in Table 1. None of the 16 substances tested showed detectable interference for *van*A. However, two of the sixteen exogenous substances, Hydrocortisone cream (1% Hydrocortisone) and Pepto-Bismol® (1 – 5% Bismuth subsalicylate), may potentially interfere with *vanB*. When tested in the Interference study, Hydrocortisone cream and Pepto-Bismol® resulted in slightly higher Ct values relative to the buffer control.

Table 4. Substances Tested for Interference for Xpert *vanA/vanB*

Substance	Substance
Whole Blood Karolinska University Hospital	Vaseline Unilever
Mucin (porcine) Sigma	Dulcolax® Boehringer Ingelheim Pharmaceuticals
Kaopectate® Chattem	Preparation H ® Portable Wipes Wyeth Consumer Healthcare
Imodium®McNeil-PPC	Vancomycin Fluka
Fleet® CB Fleet Company	Metronidazole Actavis
Fecal fats Karolinska University Hospital	Anusol® Plus TM Warner-Lambert Company
K-Y Jelly/Gelée® McNeil-PPC	E-Z-HDTM High Density Barium Sulfate for suspension E-Z-EM Canada
Hydrocortisone Cream Longs Drugs[a]	Pepto-Bismol® Proctor &Gamble[a]

[a] When tested in the Interference study, results showed slightly higher Ct values relative to the buffer control.

21. References

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7. REGULATION (EO) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on the classification labeling and packaging of substances and mixtures amending and repealing. List of Precautionary Statements, Directives 67/548/EEC and 1999/EC (amending Regulations (EO) No 1907/2007)
8. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R, pt. 1910, subpt. Z).

22. Cepheid Headquarters Locations

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23. Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

- Product name
- Lot number
- Serial number of the instrument
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag number

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24. Table of Symbols

Symbol	Meaning
	Catalog number
	*In vitro* diagnostic medical device
	Do not reuse
	Batch code
	Consult instructions for use
	Caution
	CE marking – European Conformity
	Authorized Representative in the European Community
	Manufacturer
	Contains sufficient for <n> tests
	Control
	Expiration date
	Temperature limitation
	Biological risks
	Warning

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