GeneXpert®
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Xpert® vanA

REF **GXVANA-10**

Instructions For Use

IVD Rx only

Cepheid®

In Vitro Diagnostic Medical Device

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See Section 29 Revision History for a description of changes.

Xpert® vanA

In Vitro Diagnostic Use Only

℞only

1 Proprietary Name

Xpert® *van*A

2 Common or Usual Name

Xpert *van*A test

3 Intended Use

The Cepheid Xpert *van*A Test performed in the GeneXpert® Dx System is a qualitative *in vitro* diagnostic test designed for rapid detection of the *van*A 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 *van*A gene that is frequently associated with vancomycin-resistant enterococci (VRE). The Xpert *van*A Test is intended to aid in the recognition, prevention, and control of vancomycin-resistant organisms that colonize patients in healthcare settings. The Xpert *van*A Test 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 identification of vancomycin-resistant bacteria, antimicrobial susceptibility testing, and for epidemiological typing.

4 Summary and Explanation

Enterococci are Gram-positive facultative aerobic bacteria that are present in the human intestinal flora. Two of the most common species are *Enterococcus faecium* (*E. faecium*) and *Enterococcus faecalis* (*E. faecalis*).[1] Enterococci reportedly account for over one third of all infections within the ICU.[2] Vancomycin-resistant enterococci (VRE) have become a major cause of nosocomial infections, particularly in transplant units and the ICU.[3] Just like methicillin-resistant *Staphylococcus aureus* (MRSA), VRE infections have been associated with increased morbidity, mortality, lengths of stay, and hospital costs. Recent data from the National Healthcare Safety network show that the number of device related VRE infections equals the number of MRSA device related infections.[4]

The first isolates of enterococci resistant to the glycopeptide vancomycin were reported simultaneously from France and the United Kingdom in the late 1980s. Since then, the number of resistant isolates has increased steadily.[5] There are currently six different known genes mediating vancomycin resistance; *van*A, *van*B, *van*C, *van*D, *van*E and *van*G, although subtypes of *van*B and *van*D also have been recognized.[6] The two genes of greatest clinical importance are *van*A (conferring high-level resistance to teicoplanin and vancomycin) and *van*B (conferring moderate to high-level resistance to vancomycin and occasional resistance to teicoplanin). While the *van*A determinant has been identified in nine U.S. isolates of *Staphylococcus aureus*[7] and rare streptococcal species, the *van*B gene appears to be more widely disseminated among a variety of intestinal anaerobic species.[8-13] Thus, while the likelihood of recovering a *van*A-containing enterococcus from a stool sample that is positive for *van*A remains high according to published studies,[14] the predictive value of recovering *van*B-containing enterococci from stool samples positive for *van*B by PCR is much lower. This suggests that the use of a *van*B PCR test in the U.S. population, where the prevalence of *van*B-containing enterococci is low, may lead to the unnecessary isolation of patients who would be incorrectly identified as VRE carriers by a *van*B test.

Colonization with VRE is usually acquired by susceptible hosts in an environment in which there is a high proportion of other patients colonized or infected with VRE (e.g., intensive care units, oncology units, etc.). Whether the colonization leads to infection or not depends on the virulence characteristics of the organism and the health status of the individual. Immunocompetent patients are at lower risk for infection than those individuals with weakened immune systems; however, both groups may develop infection following colonization.[1]

The risk of VRE colonization has been attributed to the use of multiple antimicrobial classes including glycopeptides, third generation cephalosporins, beta-lactam/beta-lactamase inhibitor combinations, and antimicrobial agents with potent anti-anaerobic activity.[15] The spread of VRE occurs through contact with colonized or infected individuals usually within a healthcare facility, although transmission in nursing homes has also been reported. Thus, many facilities, including pediatric hospitals,[16] are implementing active surveillance programs to identify carriers of VRE and to isolate them appropriately to reduce the transmission of the organism.[17] As part of the active surveillance screening programs peri-rectal or rectal swabs are obtained from patients and tested for VRE at admission, once a week, after receipt of antimicrobial therapy, and upon discharge.[18]

Active surveillance programs in conjunction with infection control interventions, including hand washing and placing patients in contact precautions, are important components for preventing transmission of VRE.[16][17] The use of tests providing rapid results to identify patients who are VRE carriers is also an important factor for effective control and prevention of nosocomial outbreaks of VRE.[14]

5 Principle of the Procedure

The GeneXpert Dx System automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples using real-time PCR and RT-PCR tests. The system consists of an instrument, personal computer, and preloaded software for running tests and viewing the results. The system requires the use of single-use disposable 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*.

The Xpert *van*A test includes reagents for the detection of the *van*A resistance 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 test. 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.

The Cepheid Xpert *van*A test is a rapid, automated *in vitro* diagnostic test for qualitative detection of *van*A vancomycin-resistant gene sequences directly from rectal swab specimens. The Xpert *van*A test in the Cepheid GeneXpert Dx System performs real-time, multiplex polymerase chain reaction (PCR) for detection of DNA after an initial sample processing step.

6 Reagents

6.1 Materials Provided

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

The kit contains the following:

Xpert *van*A 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
Xpert *van*A Sample Reagent	**10**
• 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 and manufactured exclusively from bovine plasma sourced 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 cartridges and reagents at 2–28 °C.
- Do not use sample reagents or cartridges that have passed the expiration date.
- Do not use any sample reagent that has become cloudy or discolored.

7 Materials Required but Not Provided

- GeneXpert Dx System (catalog number varies by configuration): GeneXpert instrument, computer, barcode wand reader, and operator manual.
  - For GeneXpert Dx System: GeneXpert Dx Software Version 2.1 or higher
- 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 900-0370 (Copan Venturi Transystem® Culture Dual Swab Transport System) (139CFM LQ STUART)

8 Materials Available but Not Provided

Gibson Laboratories, LLC, catalog #CeVRE-01 (vancomycin-resistant Enterococcus faecium, vanA) as positive control and catalog #CeVRE-02 (vancomycin-sensitive Enterococcus faecalis) as negative control.

In addition, strains for validation studies may be obtained from the ATCC and the Centers for Disease Control and Prevention, Division of Healthcare Quality Promotion.

9 Warnings and Precautions

- For *In vitro* Diagnostic Use.
- For prescription use only.
- 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[19] and the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards).[20]
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- The Xpert *van*A test does not provide susceptibility results. A separate specimen aliquot and additional time is required to culture and perform susceptibility testing.
- Do not substitute Xpert *van*A sample reagents with other sample reagents.
- Do not open the Xpert *van*A 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 (e.g., bent or broken) reaction tube.
- Each single-use Xpert *van*A 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 kit at 2 – 28 °C.
- Reagent 2 contains sodium hydroxide (pH > 12.5); (H302, H315, H319) which is corrosive to eyes and skin requiring eye and skin protection.

10 Chemical Hazards[21, 22]

- 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.

11 Specimen Collection, Transport and Storage

Rectal swab specimens can be taken with the Cepheid Sample Collection Device (Part no. 900-0370, or equivalent) following the user institution's standard procedures. The specimen swabs are placed back in the plastic transport tube (liquid Stuarts medium, Cepheid Collection Device or Copan recommended) and sent to the GeneXpert testing area for processing. The swab specimen can be stored for 24 hours at room temperature or up to 5 days at 2–8 °C before testing. Specimens may be tested after one freeze / thaw cycle.

12 Procedure

12.1 Preparing the Cartridge

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

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

1. Remove the cartridge and Sample Reagent from the package.
2. Remove one swab from the transport container.

Note Only one swab is required.

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 clean transfer pipette (not supplied), transfer the entire contents of the Sample Reagent to the sample chamber of the Xpert *van*A cartridge.
7. Close the cartridge lid.

12.2 Starting the Test

Before you start the test, make sure that:

Important
- The system is running the correct GeneXpert Dx software version shown in section 3 Materials Required but Not Provided.
- The correct assay definition file 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*.

Note The steps you follow can be different if the system administrator changed the default workflow of the system.

1. Turn on the GeneXpert Dx System, then turn on the computer and log on. The GeneXpert software will launch automatically. If it does not, double-click the GeneXpert Dx software shortcut icon on the Windows® desktop.
2. Log on using your username and password.
3. In the **GeneXpert System** window, click **Create Test**.
   The **Create Test** window displays. The **Scan Patient ID barcode** dialog box displays.
4. Scan or type in the Patient ID. If typing the Patient ID, make sure the Patient ID is typed correctly.
   The Patient ID is associated with the test results and displays in the **View Results** window and all the reports. The **Scan Sample ID barcode** dialog box displays.
5. Scan or type in 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 displays in the **View Results** window and all the reports. The **Scan Cartridge Barcode** dialog box displays.
6. Scan the barcode on the 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.

Note If the barcode on the cartridge does not scan, then repeat the test with a new cartridge. If you have scanned the cartridge barcode in the software and the assay definition file is not available, a screen displays indicating the assay definition file is not loaded on the system. If this screen displays, contact Cepheid Technical Support.

7. Click **Start Test**. In the dialog box that displays, type your password, if required.
8. Open the instrument module door with the blinking green light and load the cartridge.
9. Close the door. The test starts and the green light stops blinking.
   When the test is finished, the light turns off.
10. Wait until the system releases the door lock before opening the module door, then remove the cartridge.
11. Dispose of the used cartridges in the appropriate specimen waste containers according to your institution's standard practices.

13 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*.

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.

14 Quality Control

14.1 Built-in Quality Controls

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 that is included in each cartridge verifies 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 test. 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.

External controls — External controls may be used in accordance with local, state, federal accrediting organizations requirements as applicable.

Sources for external controls:

- Microbiologics®, catalog # 0366 (Vancomycin-sensitive *Enterococcus faecalis*)
- University of Göteborg (Culture Collection of Göteborg) CCUG36804 Vancomycin-resistant *Enterococcus faecium*, *van*A)
- Gibson Laboratories, LLC, catalog #CeVRE-01 (vancomycin-resistant *Enterococcus faecium*, *van*A) and catalog #CeVRE-02 (vancomycin-sensitive *Enterococcus faecalis*)

15 Interpretation of Results

The results are interpolated by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms, and are shown in the View Results window (Figure 1, Figure 2, and Figure 3). Possible results are:

Table 1. Results and Interpretation

Result	Interpretation
POSITIVE Figure 1	*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.
NEGATIVE Figure 2	*van*A target DNA is not detected. SPC meets acceptance criteria. ● NEGATIVE—No *van*A 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 Figure 3	Presence or absence of *van*A 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 DNA cannot be determined. ● SPC—FAIL; *van*A 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	Presence or absence of *van*A cannot be determined, repeat test according to the instructions in the Retest Procedure section below. The Probe Check control failed probably because the reaction tube was filled improperly, a probe integrity problem was detected or because the maximum pressure limits were exceeded. ● *van*A—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.

Result	Interpretation
NO RESULT	Presence or absence of *van*A 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 ● SPC—NO RESULT ● Probe Check—NA (not applicable)

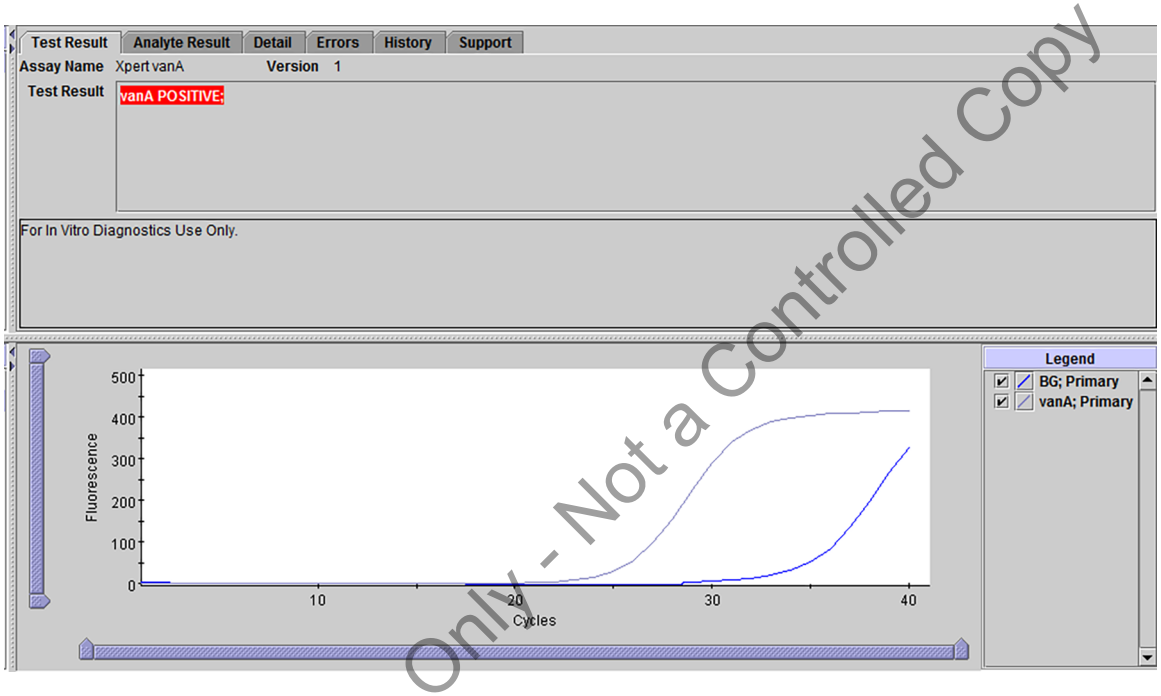

Figure 1. Example of vanA Positive Result

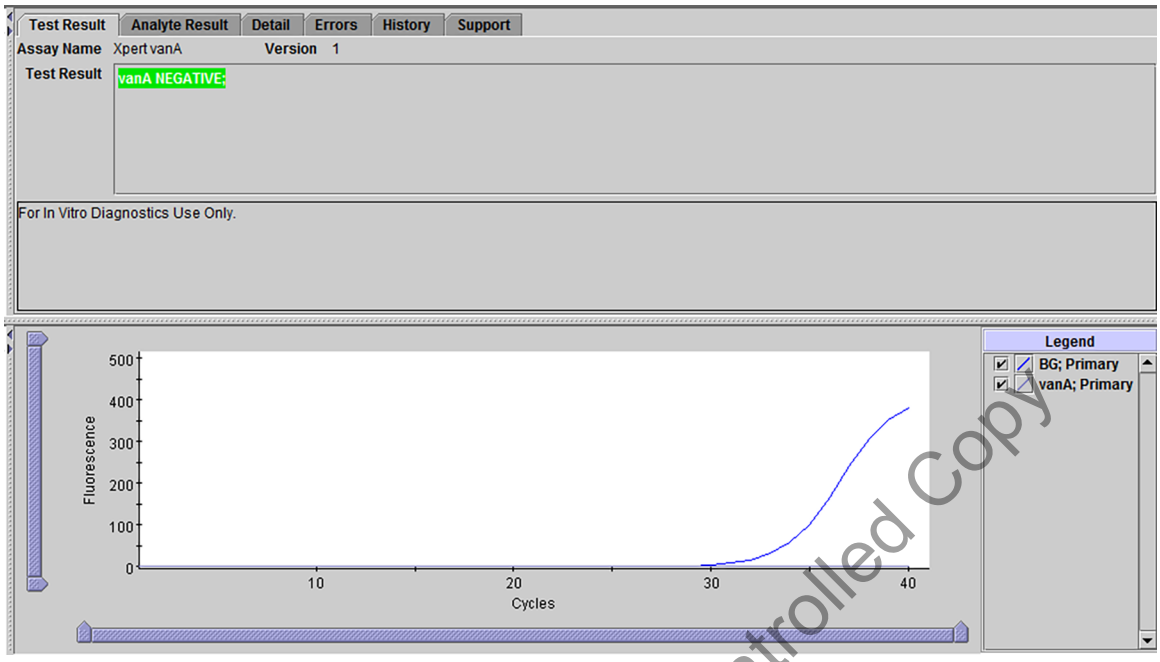

Figure 2. Example of vanA Negative Result

Figure 3. An Example of an Invalid Result

16 Retests

16.1 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 16.2.

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

An **ERROR** result indicates that the Probe Check control failed and the test 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.

16.2 Retest Procedure

For retest within 3 hours of an indeterminate result (i.e., **INVALID**, **ERROR** or **NO RESULT**), use a new Xpert *van*A 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 cartridge.

17 Limitations

- The performance of the Cepheid Xpert *van*A test to detect the *van*A gene sequence from microorganisms other than *Enterococcus* is unknown.
- The performance of the Xpert *van*A test was validated using the procedures provided in this instructions for use only. Modifications to these procedures may alter the performance of the test.
- The use of any other specimen collection and transport system other than Cepheid Sample Collection Device (Copan Venturi Transystem® Culture Dual Swab Transport System) (139CFM LQ STUART) is not recommended and has not been qualified.
- Hydrocortisone cream (1 % Hydrocortisone) and Pepto-Bismol® (1 - 5% Bismuth subsalicylate) may interfere with the Xpert *van*A test. When tested in the Interference study, Hydrocortisone cream and Pepto-Bismol® resulted in slightly higher Ct values relative to the buffer control.
- The Xpert *van*A test detects *van*A gene only, 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 positive specimens, very near or at the limit of detection (LoD) of the Xpert *van*A test, may result in a false negative result upon retest.
- 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 *van*A gene sequence is dependent on the number of organisms that contain the *van*A gene present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.
- A positive test result does not necessarily indicate the presence of viable organisms. It is however, presumptive for the presence of *van*A containing bacteria.
- Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown *van*A variants resulting in a false negative result.
- Positive and negative predictive values are highly dependent on prevalence. The Xpert *van*A test performance may vary depending on the prevalence and population tested.
- Tests results may also be affected by concurrent antibiotic therapy, or the number of organisms in the specimen which may be below the limit of detection of the test.

18 Performance Characteristics

18.1 Expected Values

In the Xpert *van*A Assay clinical study, a total of 1,231 rectal swab specimens were included from three study centers. The number and percentage of *van*A positive cases by enriched culture with bi-directional sequencing, calculated by age group are presented in Table 1.

Table 2. Observed Prevalence of vanA by Age Group

Age Group	N	vanA Prevalence

Age Group	N	vanA Prevalence
2-5	3	0% (0/3)
6-21	19	5.3% (1/19)
22-59	466	12.7% (59/466)
>60	743	13.9% (103/743)

18.2 Clinical Performance

Performance characteristics of the Xpert *van*A test were determined in a multi-site prospective investigation study at three US institutions by comparing the Xpert *van*A test 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 *van*A test 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 disc pyr (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 ε-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 *van*A specific primers (i.e., different from those used in the Xpert *van*A test).

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

18.3 Overall Results

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

Performance vs. Direct Culture

Relative to direct culture with bi-directional sequencing, the Xpert *van*A test demonstrated a percent positive agreement of 98.4% and a percent negative agreement of 92.4% (Table 3).

Table 3. Xpert *van*A test Performance vs. Direct Culture with Bi-directional Sequencing

		Direct Culture + Sequencing		
		Pos	**Neg**	**Total**
Xpert *vanV* test	**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 *van*A tests 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 test success rate (combining the first and second attempts) was 98.1% (1231/1255).

Performance vs. Enriched Culture

Relative to enriched culture with bi-directional sequencing, the Xpert *van*A test demonstrated a percent positive agreement of 86.5% and a percent negative agreement of 93.5% (Table 4).

Table 4. Xpert *van*A test Performance vs. Enriched Culture with Bi-directional Sequencing

		Enriched Culture + Sequencing		
Xpert *van*V test		**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 *van*A tests 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 test success rate (combining the first and second attempts) was 98.1% (1231/1255).

19 Antibiotic Usage

Among the 1231 cases included in the main dataset, antibiotic use within the 3 weeks prior to sample collection was reported for 414 and no antibiotic use was confirmed for 483; for 334 cases, antibiotic status was unknown. Antibiotic use did not cause a statistically significant difference in test performance.

20 Analytical Specificity

Forty-two bacterial and fungal strains were collected, quantitated and tested using the Xpert *van*A test. 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, two (2) vancomycin-sensitive strains representing *E. faecalis* and *E. faecium* were included.

Each strain was tested in triplicate at concentrations ranging from 8.5×10^8 to 2.3×10^{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 "*van*A NEGATIVE". The analytical specificity was 100%.

21 Analytical Reactivity (Inclusivity)/ Evaluation of a Well Characterized Challenge Strain Panel

Thirty vancomycin-resistant enterococci strains (*van*A and *van*B) and 20 vancomycin sensitive enterococci strains (all provided by the CDC) were tested using the Xpert *van*A test. Of the 30 vancomycin-resistant enterococci strains, 10 were identified as *van*A and 20 were identified as *van*B by the CDC. 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 5.6×10^9 to 2.1×10^{10} CFU/mL. All strains were serially diluted to approximately 360 CFU/swab and tested in triplicate.

Under the conditions of this study, all 20 vancomycin sensitive strains were correctly reported as "*van*A NEGATIVE". Among the 10 *van*A positive vancomycin-resistant enterococci strains tested, one strain was reported as "*van*A NEGATIVE." When this strain was sequenced the data matched 100% to a reference *van*B sequence, confirming that the Xpert *van*A test correctly reported the strain as "*van*A NEGATIVE." The remaining 9 *van*A positive vancomycin resistant enterococci strains were correctly reported as "*van*A POSITIVE". Among the 20 *van*B (non-*van*A) vancomycin resistant enterococci strains, all were correctly reported as "*van*A NEGATIVE". The analytical reactivity (inclusivity) study results are summarized in Table 5, the genotype information was provided by the CDC.

Table 5. Summary Table of Analytical Reactivity (Inclusivity) Results of the Xpert *van*A test on a CDC-Supplied Panel of Enterococci Specimens

Sample ID	Organism	Genotype[a]	Xpert *van*A 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
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

Sample ID	Organism	Genotype[a]	Xpert *van*A Result
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
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

Sample ID	Organism	Genotype[a]	Xpert *van*A Result
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 *van*B subtype, not *van*A as typed by CDC.

22 Analytical Sensitivity

Studies were performed to determine the 95% confidence intervals for the analytical limit of detection (LoD) of *Enterococcus faecium* (*van*A) diluted into a fecal matrix of human origin that can be detected by the Xpert *van*A test. The fecal matrix consisted of autoclaved human liquid feces (*van*A negative) 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 test on a simulated rectal swab specimen is 37 CFU.

23 Interfering Substances

Sixteen exogenous substances occasionally used or found in stool were tested for interference with the Xpert *van*A test. The substances tested are listed in Table 6. None of the 16 substances tested showed detectable interference for *van*A. However, Hydrocortisone cream (1 % Hydrocortisone) and Pepto-Bismol® (1 – 5% Bismuth subsalicylate) may slightly interfere with the Xpert *van*A test. When tested in the Interference study, Hydrocortisone cream and Pepto-Bismol® resulted in slightly higher Ct values relative to the buffer control.

Table 6. Substances Tested and Showing No Test Interference for *van*A

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

Substance	Substance
[a]Hydrocortisone Cream Longs Drugs	[a]Pepto-Bismol® Proctor & Gamble

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

24 Reproducibility

A panel of four specimens with varying concentrations of *van*A were tested on 10 different days by two different operators at each of the three sites (4 specimens × 2 operators/ day × 10 days × 3 sites). One lot of Xpert *van*A test was used at each of the 3 testing sites. Xpert *van*A tests were performed according to the Xpert *van*A test procedure. Results are summarized in Table 7 and Table 8.

Table 7. Summary of Reproducibility Results (all)[a]

	% Agreement[a]			
Specimen ID	**Site 1**	**Site 2**	**Site 3**	**% Total Agreement by Sample**
Neg	100% (20/20)	90% (18/20)	100% (20/20)	96.7% (58/60)
*van*A High Neg	100% (20/20)	100% (20/20)	95% (19/20)	98.3% (59/60)
*van*A Low Pos	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
*van*A 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).

Table 8. Summary of Ct Value Results by Sample Level and Target

Bg			
Level	**Mean**	**StdDev**	**CV**
*van*A high neg	32.88	0.60	1.83%
*van*A low pos	32.88	0.77	2.34%
*van*A mod pos	32.80	0.78	2.38%
Neg	33.15	0.65	1.96%
***van*A[a]**			
Level	**Mean**	**StdDev**	**CV**

Bg			
Level	**Mean**	**StdDev**	**CV**
*van*A low pos	33.76	1.00	2.95%
*van*A mod pos	30.35	1.33	4.40%

[a] Ct cutoff for *van*A=40

25 References

1. Fischetti VA, Novick RP, Ferretti JJ, Portnoy DA; Rood JI (editors). Gram-Positive Pathogens. ASM Press, Washington D.C. 2000.
2. deBruin MA, Riley LW. Does vancomycin prescribing intervention affect vancomycin-resistant enterococcus infection and colonization in hospitals? A systematic review. BMC Infect Dis. 2007;7:24.
3. Calderwood MS, Mauer A, Tolentino J, et al. Epidemiology of vancomycin-resistant enterococci among patients on an adult stem cell transplant unit: observations from an active surveillance program. Infect Control Hosp Epidemiol. 2008;29:1019-1025.
4. Hidron A, Edwards JR, Patel J, et al. NHSN Annual Update: Antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. Infect Control Hosp Epidemic. 2008;29:996-1011.
5. Cetinkaya Y, Falk P and Mayhall CG. Vancomycin-resistant enterococci. Clin Micro Rev. 2000;13:686-707.
6. Courvalin P. Vancomycin resistance in Gram-positive cocci. Clin Infect Dis. 2006;42 Supply 1: S25-34.
7. Sievert DM, Rudrik JT, Patel JB, et al. Vancomycin-resistant Staphylococcus aureus in the United States, 2002-2006. Clin Infect Dis. 2008;46:668-674.
8. Zirakzadeh A, Patel R. Vancomycin-resistant enterococci: Colonization, infection, detection and treatment. Mayo Clin Proc. 2006;81:529-536.
9. Ballard SA, Pertile KK, Lim M, et al. Molecular characterization of *van*B elements in naturally occurring gut anaerobes. Antimicrob Agents Chemother. 2005;49(5):1688-1694.
10. Petrich AK, Luinstra KE, Groves D, et al. Direct detection of *van*A and *van*B genes in clinical specimens for rapid identification of VRE using multiplex PCR. Mol Cellular Probes. 1999;13:275-281.
11. Domingo MC, Huletsky A, Bernal R, et al. Characterization of a Tn5382-like transposon containing the *van*B2 gene cluster in a Clostridium strain isolated from human faeces. J Antimicrob Chemother. 2005;55:466-474.
12. Domingo MC, Huletsky A, Giroux K, et al. High prevalence of glycopeptides resistance genes *van*B, *van*D, and *van*G not associated with enterococci in human fecal flora. Antimicrob Agents Chemother. 2005;49(11):4784-4786.
13. Ballard SA, Grabsch EA, Johnson PDR and Grayson ML. Comparison of three PCR primer sets for identification of *van*B gene carriage in feces and correlation with carriage of vancomycin-resistant enterococci: Interference by *van*B containing anaerobic bacillii. Antimicrob Agents Chemother. 2005;49(1):77-81.
14. Sloan LM, Uhl JR, Vetter EA, et al. Comparison of the Roche LightCycler *van*A/*van*B Detection Assay and culture for detection of vancomycin-resistant enterococci from perianal swabs. J Clin Micro. 2004;42:2636-2643.
15. Rice LB. Antibiotics and gastrointestinal colonization by vancomycin-resistant enterococci. Eur J Clin Microbiol Infect Dis. 2005;24:804-814.
16. Milstone AM, Song X, Beers C, et al. Unrecognized burden of Methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus carriage in pediatric intensive care units. Infect Control Hosp Epidemiol. 2008;29:1174-1176.
17. Huang SS, Rifas-Shiman SL, Pottinger JM, et al. Improving the assessment of vancomycin-resistant enterococci by routine screening. J Infect Dis. 2007;195:339-346.
18. Perencevich EN, Fisman DN, Lipsitch M, et al. Projected benefits of active surveillance for vancomycin-resistant enterococci in intensive care units. CID. 2004;38:1108-1115.
19. Centers for Disease Control and Prevention. Biosafety in microbiological and biomedical laboratories. Richmond JY and McKinney RW (eds) (1993). HHS Publication number (CDC) 93-8395.
20. Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards). Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (refer to latest edition).
21. 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)

22. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R, pt. 1910, subpt. Z).

26 Cepheid Headquarters Locations

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27 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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Contact information for all Cepheid Technical Support offices is available on our website: www.cepheid.com/en/support/contact-us.

28 Table of Symbols

Symbol	Meaning
	Catalog number
	For prescription use only
	*In vitro* diagnostic medical device
	Do not reuse
	Batch code
	Consult instructions for use
	Caution
	Manufacturer
	Country of manufacture
	Contains sufficient for *n* tests
	Control
	Expiration date
	Temperature limitation
	Biological risks
	Warning

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IVD

29 Revision History

Description of Changes: 300-9105, Rev. D to E

Purpose: Corrections

Section	Description of Change
Specimen Collection, Transport and Storage	Revised unintended changes to specimen type and specimen storage.
Expected Values	Returned Expected Values section to the IFU.