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Prepared by QA Committee		
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## Introduction

The presence of acquired vancomycin resistance ( $V^R$ ) in Gram positive cocci has both clinical and epidemiological implications. The genes encoding  $V^R$  are on mobile elements called transposons that are transmitted between strains on conjugative plasmids. These plasmids are found in *E. faecium* (>98%) and *E. faecalis* (VRE) but may also be found in other species of enterococci, *S. aureus* (VRSA) or anaerobes. The most common  $V^R$  clusters in circulation are *vanA/vanB* (includes variants: *vanB1*, *vanB2* and *vanB3*). The Cepheid Xpert *vanA/vanB* Assay, performed on the Cepheid GeneXpert<sup>®</sup> Dx System, is a qualitative *in vitro* diagnostic test for rapid detection of these  $V^R$  genes, regardless of their host species. Other related  $V^R$  determinants that occur less frequently, *vanD*, *vanE* and *vanG*, are not detected by this PCR assay.

This method detects  $V^R$  genes directly from rectal swabs or bacterial strains.

## Specimen Collection, Transport and Storage

Collect rectal swab in Eswab transport container and send the specimen to the Microbiology laboratory as soon as possible.

Collect stool in a sterile container and send the specimen to the Microbiology laboratory. The specimen is stable for up to 5 days when stored at 2–8°C. Alternatively, specimens can be kept at room temperature (20 – 30°C) for up to 24 hours. Stool collected in Enteric Transport Medium, or in SAF is not suitable for this assay.

Bacterial colonies isolated on culture media.

## Materials & Equipment



- GeneXpert Dx System
- Vortex mixer
- COPAN CLASSIQswabs Cat. No. 138CS01
- Pipette that can deliver 30  $\mu$ L
- Pipette tips.
- Xpert *vanA/vanB* kit – each kit contains 10 Xpert *vanA/vanB* cartridges and buffer

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Each Xpert *vanA/vanB* gene Assay Cartridge with integrated reaction tubes contain:

Bead 1 (freeze-dried)

- Polymerase
- dNTPs
- BSA (bovine serum albumin)
- Probe

Bead 2 (freeze-dried)

- Primers
- Probes
- BSA

Bead 3 (freeze-dried)

- Sample Processing Control (SPC) non-infectious sample preparation control spores

Reagent 1 (Tris Buffer, EDTA, surfactants)

Reagent 2 (Sodium Hydroxide)

Xpert *vanA/vanB* reagent pouch

; Buffer, EDTA, surfactants)

## **Procedure**

GeneXpert Dx System

### **GeneXpert Cartridge Preparation**



1. The disposable single-use GeneXpert DX cartridge holds the samples and reagents that you want to process in the GeneXpert DX System. Do not reuse spent cartridges.
2. Store new GeneXpert DX cartridge at 2 – 28°C. The cartridge and reagents are stable for up to 7 days after the package has been opened.
3. The test must be started within 30 minutes of adding reagents to the cartridge.

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4. Use one cartridge and one sample reagent vial for each sample that needs to be tested. Label the cartridge with the corresponding LIS Lab no. barcode. Do not touch the reaction tube of the cartridge.

**For Bacterial Colonies:**

5. Label a tube of 0.5 mL of 0.45% saline with the LIS Lab No. Use the dry COPAN CLASSIQSwab to touch 5-10 colonies and immerse the swab into the pre-labeled saline tube.  
\*\*\*Use this suspension for inoculation of any subculture media (i.e. BVRE/SUBBA)
6. Using the same swab, immerse into the elution vial containing the Sample Reagent.
7. Hold the swab by the stem near the rim of the vial, lift the swab a few millimeters from the bottom of the tube and push the stem against the edge of the vial to break it. Make sure the swab is short enough to allow the cap to close tightly.
8. Close the lid and vortex at high speed for 10 seconds.
9. Open the cartridge lid. Using a clean transfer pipette, transfer the entire contents of the Sample Reagent into the “S” chamber of the Xpert *vanA/vanB* Assay cartridge.
10. Close the cartridge lid.

**For Rectal Swabs:**


11. Use the fluid of the Eswab transport fluid.
12. Vortex at high speed for 10 seconds
13. Pipette 30µL of the Eswab fluid into the elution vial containing the Sample Reagent.
14. Close the lid and vortex at high speed for 10 seconds.
15. Open the cartridge lid. Using a clean transfer pipette, transfer the entire contents of the Sample Reagent into the “S” chamber of the Xpert *vanA/vanB* Assay cartridge.

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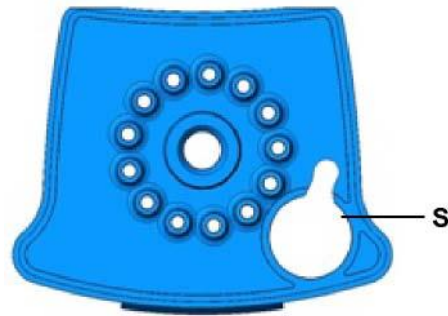
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16. Close the cartridge lid.





**Figure 1.** Xpert *vanA/vanB* cartridge (top view)

### **Assay Method**

1. Turn on the computer, and then turn on the GeneXpert Dx instrument. Log into windows using the password “cphd”.
2. On the Windows® desktop, double-click the GeneXpert Dx shortcut icon.
3. Log on to the GeneXpert Dx System software using user name and password (refer to GeneXpert Log In in the “Application Login Information” manual)
4. The Database Management dialog box appears on top of the GeneXpert Dx System window once the system starts up. Click “**NO**” in the Database Management dialog box if you do not want to perform any database management tasks.
5. If a test archive is overdue, the Test Archive Reminder dialog box appears. If you do not want to archive click “**No**” and if you do want to archive click “**Yes**”.
6. In the GeneXpert Dx System window, click **Create Test**. The Scan Sample ID Barcode dialog box appears. Scan the Sample ID barcode using the barcode scanner or you can manually enter the sample ID by clicking “Manual Entry”. Type in the Sample ID into the Manually Patient ID Barcode Entry dialog box that appears.

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7. Scan the barcode on the Xpert *vanA/vanB* Assay cartridge. The **Create Test** window appears. Using the barcode information, the software automatically fills in the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.
8. Click **Start Test**. (bottom of Create Test dialog box) and the module on the GeneXpert to be used stars to blink green. In the Check Status Window, the selected instrument module progress changes to Waiting.
9. Open the instrument module door with the blinking green light and load the cartridge.
10. Close the door. The test starts and the green light stops blinking.
11. When the test is complete the instrument module door unlocks and the green light turns off. Wait until the system releases the door lock before opening the module door and removing the cartridge.
12. Dispose of the used cartridge in an appropriate specimen waste container.
13. Once testing is complete the report is automatically printed. To view the result, in the GeneXpert DX System window, click **View Results** on the menu bar. The View Results window appears.
14. Click View Test. The Select Test To Be Viewed dialog box appears. Select the test of interest and click **OK**. The results of the selected test appear in the View Results window.
15. The View Results window contains three tabs: **Results**, **Errors**, and **Support**. The **Results** tab displays the information for a test such as the Patient ID, Sample ID, Assay name and the test Result. The Errors tab list the errors encountered during the test process.
16. Monthly Maintenance Procedure:
  - a. Disinfect the instrument surfaces
    1. Dampen a paper towel with 10% bleach solution and wipe the instrument surface thoroughly with the paper towel.
    2. Wait 10 minutes
    3. Dampen a paper towel with 70% alcohol solution and wipe the instrument thoroughly with the paper towel.

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- b. Disinfect the cartridge bay interior
  1. Dip a swab into 70% alcohol solution. Press the swab against the wall of the container to remove excess solution.
  2. Wipe the same surface with the new swab.
  3. Repeat steps 1 and 2 two times with a new swab.
  4. Close the instrument module door.
  
- c. Disinfecting the plunger rod
  1. In the GeneXpert DX System window, click **Maintenance** on the menu bar. The **Maintenance** window appears.
  2. On the **Maintenance** menu, click **Plunger Maintenance**. The Plunger Maintenance dialog box appears.
  3. In the **Module** table, select the module you want to clean, and then click **Clean** or select **Clean All** to clean all modules simultaneously. The Plunger Cleaning dialog box then appears.
  4. Follow the directions in the Plunger Cleaning dialog box, then click **OK**. In the Plunger Maintenance dialog box, the **Clean** button changes to Move Up (if you clicked Clean All button, it changes to Move Up All). In the instrument, the plunger rod in the selected module (or all modules if you clicked Clean All button) lowers into the cartridge bay.
  5. Dip swab into 70% alcohol solution. Press the swabs against the inside wall of the container to remove excess solution.
  6. Wipe the plunger rods with the swabs. Use a fresh swab for each plunger rod.
  7. In the Plunger Maintenance dialog box, click Move Up (or Move Up All). The plunger rod moves back into its resting position.
  8. Click Close to dismiss the Plunger Maintenance dialog box.
  
- d. Clean fan filters - if present
  
- e. The GeneXpert DX instrument needs to be recalibrated annually or after 2000 test per instrument module, whichever comes first. The system monitors the number of tests since last calibration. To check whether the system requires calibration:
  1. In the Maintenance window, look at the **ICORE Starts Since Cal** column. On the Maintenance menu, click Module Reports. The Module Reports dialog box appears.
  2. Check the calibration date. If calibration is required contact the Cepheid Technical Support to schedule a calibration.

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## Assay Validation

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

1. **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 organism is 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.
2. **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.

## Interpretation of Results

The results are interpolated by the GeneXpert Dx System from measured fluorescent signals and embedded calculation algorithms and will be shown in the **View Results** window. Possible results are:

### 1. *vanA* POSITIVE

*vanA* target DNA is detected

- *vanA* POSITIVE – the *vanA* target has a Ct within the valid range and endpoint above the minimum setting.
- SPC — NA (not applicable); SPC is ignored since *vanA* amplification may compete with this control
- Probe Check – PASS; all probe check results pass.
- Inoculate BVRE agar and BHIB broth if this is a “New” positive patient (no positive from this patient within the last 3 month).

### 2. *vanB* POSITIVE



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*vanB* target DNA is detected

- *vanB* POSITIVE – the *vanB* target has a Ct within the valid range and endpoint above the minimum setting.
- SPC — NA (not applicable); SPC is ignored since *vanB* amplification may compete with this control
- Probe Check – PASS; all probe check results pass.
- Inoculate BVRE agar and BHIB broth if this is a “New” positive patient (no positive from this patient within the last 3 month).

### 3. *vanA* POSITIVE, *vanB* POSITIVE

*vanA* and *vanB* target DNA are detected

- *vanA* POSITIVE – the *vanA* target has a Ct within the valid range and endpoint above the minimum setting.
- *vanB* POSITIVE – the *vanB* target has a Ct within the valid range and endpoint above the minimum setting.
- SPC — NA (not applicable); SPC is ignored since *vanA* and/or *vanB* amplification may compete with this control
- Probe Check – PASS; all probe check results pass.
- Inoculate BVRE agar and BHIB broth if this is a “New” positive patient (no positive from this patient within the last 3 month).

### 4. NEGATIVE

*vanA* and *vanB* target DNA are not detected. SPC meets acceptance criteria.

- No *vanA* or *vanB* 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.

## Reporting



### Direct from Swab:

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**Negative Report:** “No vanA or vanB gene detected by Cepheid Xpert vanA/vanB Assay. This is a research test.”

**Positive report:** Report as ISOLATE code:

“vanA” – “vanA gene DETECTED.” Add ISOLATE COMMENT “by Cepheid Xpert vanA/vanB Assay. This is a research test.”

“vanB” – “~vanB gene DETECTED.” Add ISOLATE COMMENT “~by Cepheid Xpert vanA/vanB Assay. This is a research test.”

"Presence of a vanB gene on direct testing may or may not indicate the presence of VRE. Please consult infection control about whether additional precautions are necessary."

Note: Positive reports for Sinai Health patients (MSH and Bridgepoint Health) should have the following comment automatically added  
**ICPR** “THIS PATIENT IS TO BE MANAGED IN “CONTACT PRECUATIONS” UNTIL FURTHER NOTICE”

Phone positive result to ward (in-patient) or physician (Out-patient), and notify Infection Control Practitioner as per.

### From colonies:

**Negative Report:** none; continue with workup as per culture protocol

**Positive report:** Report as ISOLATE code: “entvre”

“*Enterococcus* species -vancomycin resistant”



ISOLATE COMMENT:

“This organism is positive for the vanAorB gene as tested by the Cepheid vanA/B GenXpert Assay (for research only).

~Phenotypic confirmation to follow.” Isolate Comment Code **\vaAg** or **\vaBg**

Phone positive result to ward (in-patient) or physician (Out-patient), and also notify Infection Control Practitioner as per

[Isolate Notification and Freezing Table QPCMI16003](#).

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## Quality Control


Each test includes a Sample Processing Control (SPC) and Probe Check Control (PCC). Refer to a senior technologist if control results are outside of limits or for any other problems with running or reporting the assay.

Run external control (*vanA* and *vanB* VRE from Positive VRE cultures) with each new lot, QC and instrument problems. Result in Soft Total QCIf result is negative, the run is invalid. Inform Charge/senior technologist, and repeat testing.

CAP provides external proficiency testing.

## Reference

Xpert *vanA/vanB* PCR Assay package insert

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***Record of Edited Revisions***

**Manual Section Name:** VRE vanA and vanB PCR by Cepheid GeneXpert

Page Number / Item	Date of Revision	Signature of Approval
Annual Review	July 12, 2013	Dr. T. Mazzulli
Added Roche run schedule for <i>vanB</i> positives	July 14, 2014	Dr. T. Mazzulli
Annual Review	July 14, 2014	Dr. T. Mazzulli
Inserted new UHN/MSH logo into header Added vanB reporting message "Presence of a vanB gene on direct testing may or may not indicate the presence of VRE. Please consult infection control about whether additional precautions are necessary."	August 6, 2014	Dr. T. Mazzulli
Annual Review Remove step 10 "for bacterial colonies & step 17 for rectal swabs: "add reagent 1 and 2 to corresponding wells. Insert link to qualitative pcr external control log Changed phone ICP to notify ICP	July 13, 2015	Dr. T. Mazzulli
-Manual moved from IC manual to Molecular manual. Previous manual: Cepheid Xpert <i>vanA/vanB</i> Assay MI/IC/XpertVREPCR/v05 -Added Stool in sterile container. To stool section in Specimen collection section. -Remove bleach from cleaning maintenance inside GeneXpert	February 10, 2016	Dr. T. Mazzulli
Annual Review	August 30, 2017	Dr. T. Mazzulli
Annual Review	August 15, 2018	Dr. T. Mazzulli
Annual Review	September 11, 2019	Dr. T. Mazzulli
Annual Review	October 02, 2020	Dr. T. Mazzulli

**Full document review included in all updates. Biennial review conducted when no revision had been made within 2 years.**

Page Number / Item	Date of Revision	Edited by:
Minor formatting change	April 11, 2021	Jessica Bourke
Updated positive reporting to include contact precautions comment for Sinai Health samples.	May 26, 2021	Jessica Bourke



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