# Assay Training: Xpert® vanA/vanB

For CE-IVD product only

CE IVD In Vitro Diagnostic Medical Device



302-1916 Rev. A October 2019

CE-IVD. For *in vitro* diagnostic use.

# Training Agenda

- **Xpert® vanA/vanB Training**
  - Reagents
  - Sample collection
  - Kit storage and handling
  - Preparing the cartridge
  - Quality Controls
  - Results analysis
- **Discussion**

# Training Objectives

- **At the end of the training, users will be able to:**
  - Store and handle the Xpert® vanA/vanB cartridge kit and sample collection kits
  - Follow proper laboratory safety precautions
  - Collect and transport appropriate specimens
  - Prepare a cartridge and run the assay
  - Report the various software-generated results
  - Understand the assay control strategy

# The Cepheid Solution


- Detection of *vanA* and *vanB* genes
- On-board internal controls
  - Probe Check Control (PCC)
  - Specimen Processing Control (SPC)
- Closed cartridge system minimizes risk of contamination
- On-demand results
- Random access



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# Intended Use

- **The Cepheid Xpert® vanA/vanB Assay performed in the GeneXpert® Instrument Systems is a qualitative in vitro diagnostic test designed for rapid detection of vancomycin-resistance (*vanA/vanB*) 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 *vanA* and *vanB* genes that can be associated with vancomycin-resistant enterococci (VRE).**

- **The Xpert® vanA/vanB Assay is intended to aid in the recognition, prevention and control of vancomycin-resistant organism colonization in healthcare settings. The Xpert® *vanA/vanB* 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.**

# Targets and Probes

## Target(s)

- *vanA*
- *vanB*

## Probes

- 1 probe for *vanA* Resistance to vancomycin and teicoplanin (plasmid-mediated)
- 1 probe for *vanB* Resistance to vancomycin (transposon)
- 1 probe for SPC Sample Prep Control (SPC): *B. globigii* spores

# Xpert® vanA/vanB Requirements

## Test Kits (CE-IVD)

- GXVANA/B-CE-10

## Sample Collection

- Cepheid sample collection device (Cepheid Part Number 900-0370)

## Other materials

- Personal Protective Equipment (PPE)
- Disposable, sterile transfer pipettes
- Vortex mixer
- 1:10 dilution of household bleach
- 70% ethanol or denatured ethanol

## Optional

- Uninterruptible Power Supply /Surge Protector
- Printer

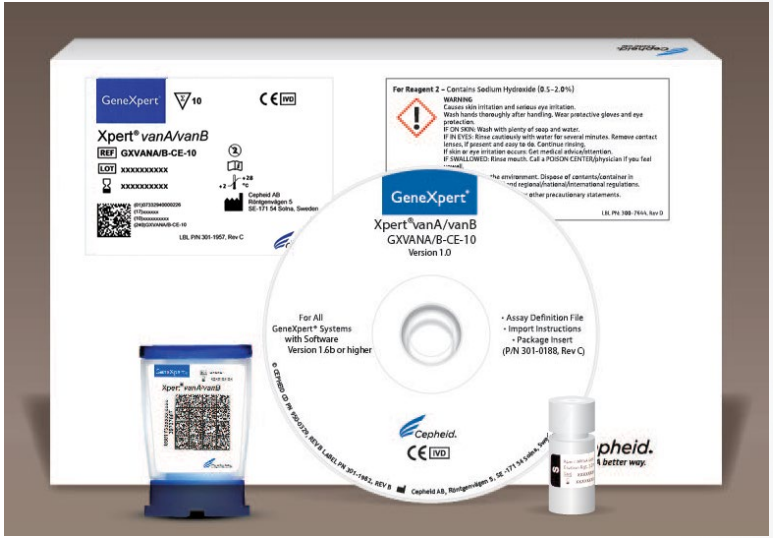
# Good Laboratory Practice

| | |
|---|---|
| **Personel Protective Equipment (PPE)** | • Wear clean lab coats, gloves, and safety glasses<br>• Change gloves between processing samples |
| **Lab Bench area** | • Clean work surfaces routinely with:<br>✓ 1:10 dilution of household bleach*<br>✓ 70% Ethanol Solution<br>** Final Active Chlorine concentration should be 0.5% regardless of the household bleach concentration in your country*<br>• After cleaning, ensure work surfaces are dry |
| **Specimens, Samples, and Kits Storage** | • Store specimens and sample away from kit to prevent contamination |
| **Equipment** | • Use filtered pipette tips when recommended<br>• Follow the manufacturer's requirements for calibration and maintenance of equipment(s) |

# Kit Handling

# Xpert® vanA/vanB Kit Contents

| Catalog Number | GXVANA/B-CE-10 |
|---|---|
| **Cartridges Per Kit** | 10 |
| **Reagent Vials** | 10 |
| **Kit CD** | Assay Definition File (ADF) |
| | Assay Name Import Instructions |
| | Package Insert (PDF) |
| **Storage** | 2-28 ˚C |




*Cartridges contain chemically hazardous substances-please see Package Insert and Safety Data Sheet for more detailed information.*

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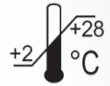
# Xpert® vanA/vanB Kit Storage and Handling

- Store the Xpert® vanA/vanB cartridges and reagents at 2–28˚C




- Follow your institution's safety procedures for working with chemicals and handling biological samples
- Do not use collection devices that have not been validated by Cepheid
- Open the Xpert® vanA/vanB cartridge lid only when adding the sample, close the lid, and proceed with processing

# Warnings and Precautions

- Do not shake the cartridge
- Do not use a cartridge that… :
  - appears wet, has leaked, or if the lid seal appears to have been broken
  - appears damaged
  - has been dropped after removing it from packaging
  - has been dropped or shaken after adding the sample to it
  - has a damaged reaction tube
  - has been used; each cartridge is single-use to process one test
  - is expired
  - Do not reuse spent disposable pipettes

*Dispose Xpert® vanA/vanB cartridges and reagents according to your institution's and country's guidelines for disposal of hazardous materials*

# Waste Disposal

- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions.

- Follow your institution's environmental waste procedures 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.

# Limitations

- A positive test result does not necessarily indicate the presence of a viable organism. It is, however, presumptive for the presence of VRE.
- Positive Xpert® *vanA/vanB* results for *vanB* in the absence of *vanA* may be due to organisms other than VRE. It is recommended to perform culture confirmation for these organisms.
- As described in the literature, some aerobic and anaerobic bacteria containing the *vanB* gene may be found and would be detected by this assay, however, the clinical relevance of such findings is unknown. Anaerobic bacteria positive for the *vanB* gene have been suggested to constitute a reservoir of vancomycin resistance determinants, but this hypothesis remains to be proven.
- Testing with the Xpert® *vanA/vanB* 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.

# Specimen Collection and Storage

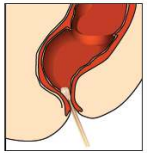
# Specimen Collection

## Rectal/Perianal Specimen Collection

1 Use Cepheid Collection Device #900-0370 to collect the specimen.

**Rectal Swab Sample**

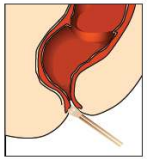
Carefully insert both swabs approximately 2.5 cm beyond the anal sphincter (so that the fiber tips are no longer visible) and gently rotate 3 times to ensure uniform sample on both swabs.



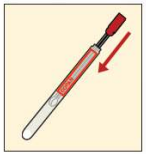
2 OR

**Perianal Swab Sample**

Press the buttocks apart to expose the perianal region. Fully swab around the perianal surface making sure to swab as much of the surface as possible.



3 Place the swabs back in the tube.



4 The specimen may be stored at 2-8°C for up to 5 days.



In Vitro Diagnostic Medical Device CE IVD

302-1918, Rev A May, 2019

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# Specimen Storage

| Sample type | Storage |
|---|---|
| Rectal/ Perianal swabs | +2 +8 °C for 5 days |


Use the Cepheid Sample Collection Device (P/N 900-0370)

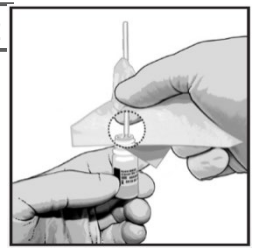
# Cartridge Preparation

# Cartridge Preparation

1

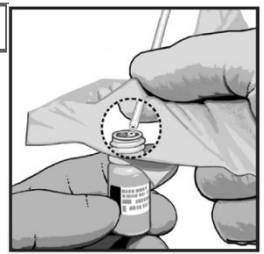
Obtain one Xpert® cartridge, sample reagent vial for each sample.

2



Insert the swab into the Sample Reagent vial.

3

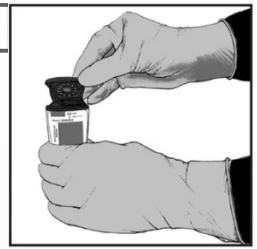


Break the swab at the score mark near the opening of the vial.
Note: Do not hold the swab below the score mark.
Use gauze or its equivalent to minimize the risk of contamination.

4

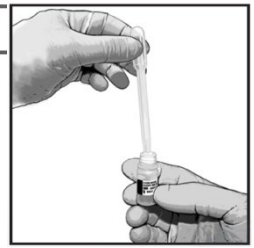
Recap the Sample Reagent vial and vortex for 10 seconds.

5



Open the cartridge lid.

6



Aspirate all of the Sample Reagent vial contents with a disposable transfer pipette.

7

Empty the pipette into the sample chamber.

8

Close the lid firmly.
Start the test within the time frame specified in the package insert.

*Adapted from 301-1049*

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# Run a Test

## 1 Create Test

**GeneXpert**

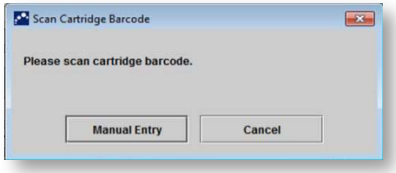

Start the test within **30** minutes after adding the sample to the cartridge

**GeneXpert Infinity**

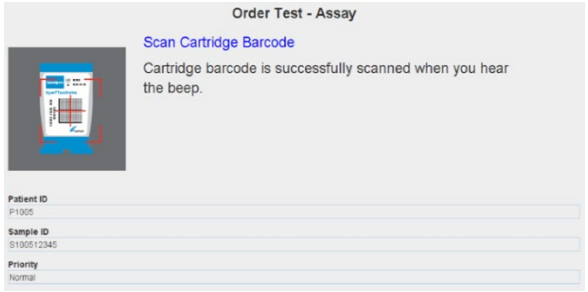

Place the cartridge on the conveyor within **30** minutes of adding the sample.

## 2 Scan barcode messages:

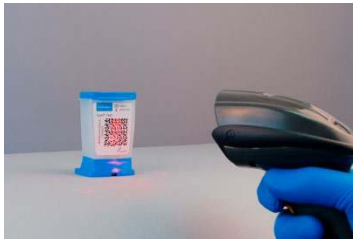
Cartridge/ Patient and/or Sample ID

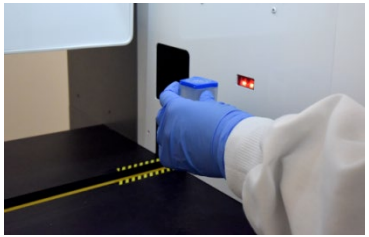



*By default, do not click on* **Manual Entry** or **Cancel**




## 3 Scan the cartridge





*"For complete details on how to run a test, refer to the Package Insert and the GeneXpert® Dx or Xpertise™ Operator Manuals.*

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# Create a Test on GeneXpert® Dx Software

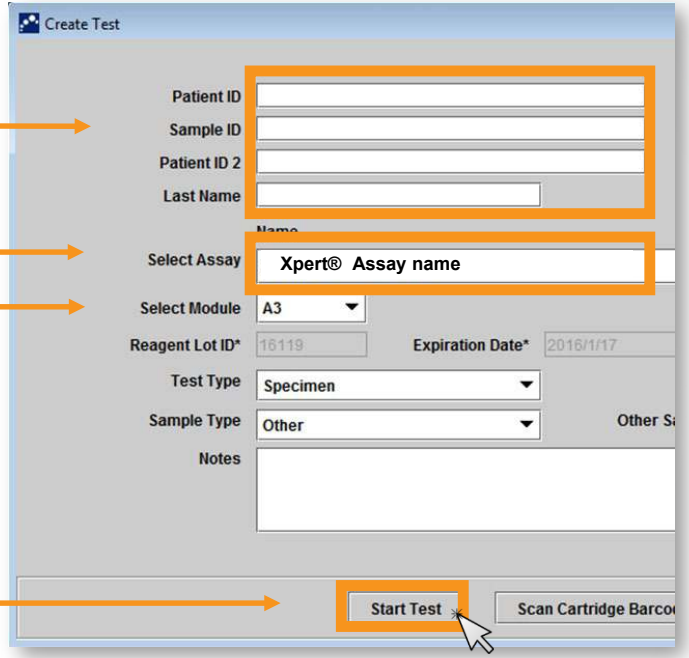
4 Complete the fields as required

5 The Assay Protocol is selected automatically

6 The module is selected automatically

7 Click on Start Test

8 A green light will flash on the module
Load the cartridge into module and close the door



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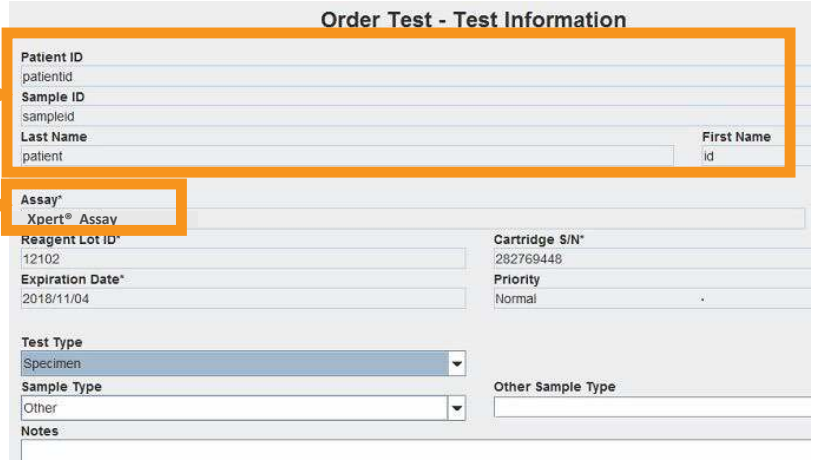
# Create a Test on Xpertise™ Software

4 Complete the fields as required

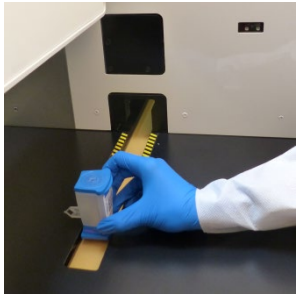
5 The Assay Name Protocol is selected automatically

6 Click on SUBMIT





7 Place the cartridge onto the conveyor belt



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# Automated Xpert® Protocol

1. Sample is added to the cartridge
2. The cartridge is loaded into the System
3. Nucleic acids are purified
4. Purified nucleic acids mix with the PCR reagents
5. Simultaneous amplification and detection occurs
6. Results are ready to view


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# Quality Controls

# Assay Control Strategy

CONTROL

- **Xpert® vanA/vanB Quality Controls**
  - Each Xpert® cartridge is a self-contained test device
  - Cepheid designed specific molecular methods to include internal controls that enable the system to detect specific failure modes within each cartridge
    - Specimen Processing Control (SPC)
    - Probe Check Controls (PCC)

*Refer to 301-4868 GeneXpert® Quality Control Features for All Cepheid Xpert® Assays*

# Internal Quality Controls

- **Probe Check Controls (PCC)**
  - Before the PCR step, fluorescence signal is measured on all probes and compared with default factory settings to monitor
    - bead rehydration
    - reaction tube filling
    - probe integrity
    - dye stability

- **Sample Processing Controls (SPC)**
  - non-infectious spore in each cartridge
    - Verifies adequate sample processing
    - Verifies lysis, presence of the organism and detects PCR inhibition
    - Should be positive in a negative sample
    - Can be positive or negative in a positive sample

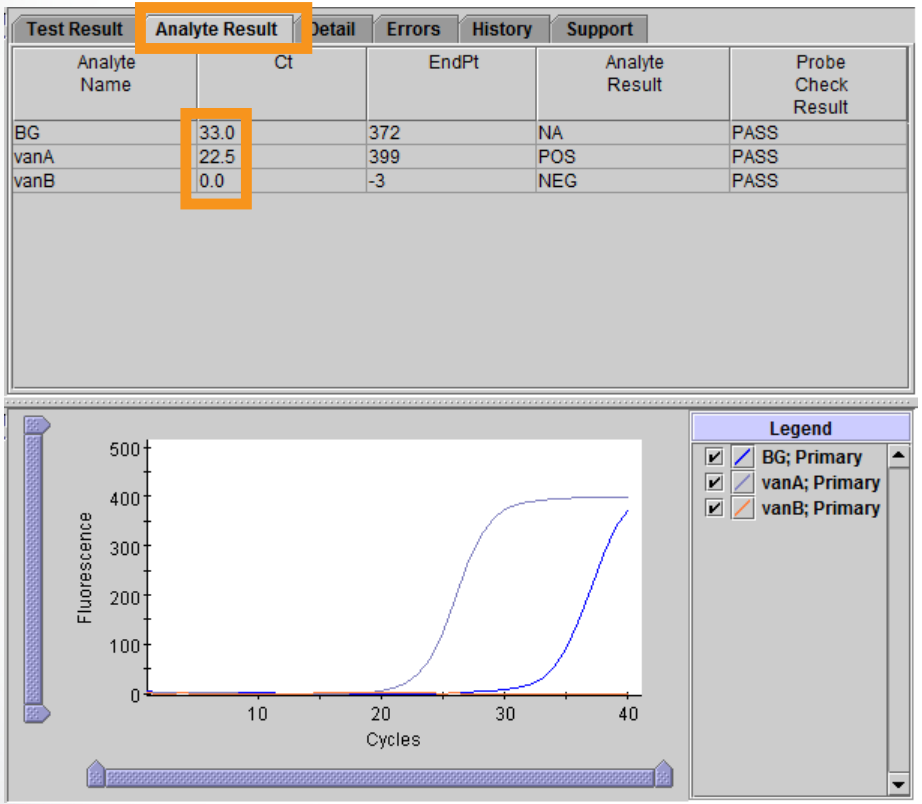
# Result Interpretation

# Xpert® vanA/vanB– All Possible Results

| Result displayed | van A | van B | SPC |
|---|---|---|---|
| Van A Positive<br>Van B Negative | + | - | +/- |
| Van A Negative<br>Van B Positive | - | + | +/- |
| Van A Positive<br>Van B Positive | + | + | +/- |
| Van A Negative<br>Van B Negative | - | - | + |
| INVALID | - | - | - |
| ERROR | NO RESULT | NO RESULT | NO RESULT |
| NO RESULT | NO RESULT | NO RESULT | NO RESULT |

# vanA Positive; vanB Negative

vanA POSITIVE;
vanB NEGATIVE




- *vanA* target DNA is detected.
- *vanB* target DNA is not detected

- *vanA* POSITIVE: *vanB* NEGATIVE

The *van*A target has a Ct within the valid range and endpoint above the minimum setting.

- SPC: NA (not applicable)

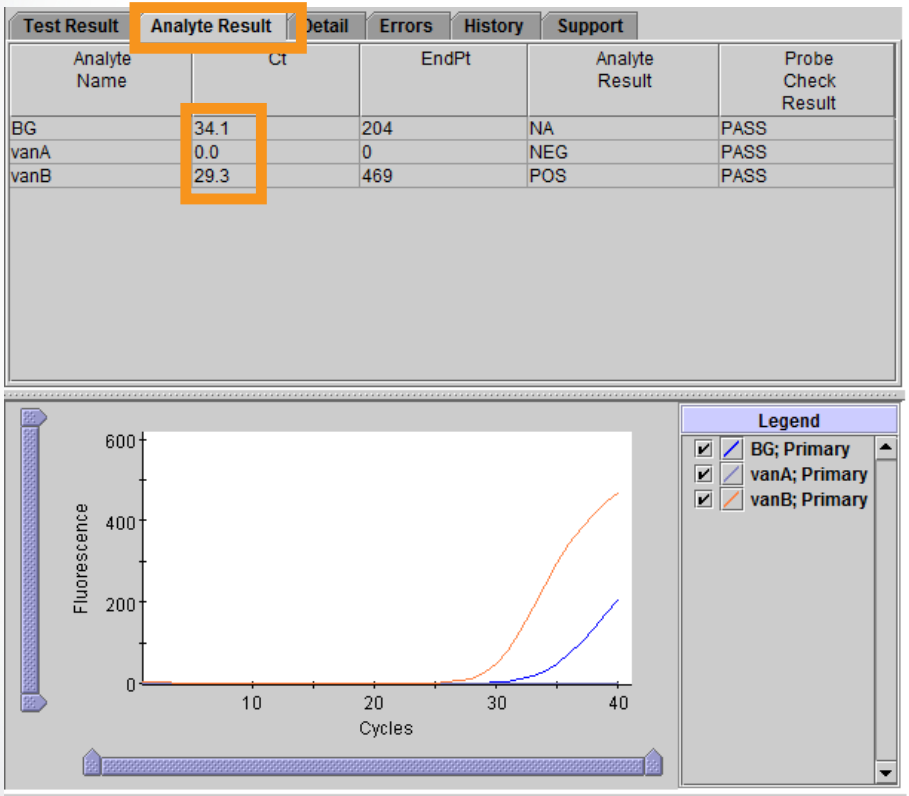
SPC is ignored because vanA and/or vanB amplification may compete with this control.

- Probe Check: PASS

All probe check results pass.

# vanA Negative; vanB Positive

vanA NEGATIVE;
vanB POSITIVE




- *vanA* target DNA is not detected.
- *vanB* target DNA is detected.

- *vanA* NEGATIVE: *vanB* POSITIVE

The *van*B target has a Ct within the valid range and endpoint above the minimum setting."

- SPC: PASS

SPC has a Ct within the valid range and endpoint above the endpoint minimum setting.

- Probe Check: PASS

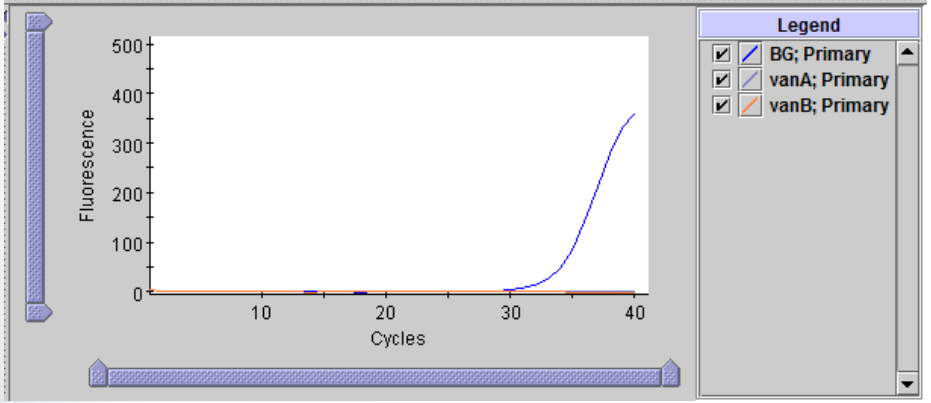
All probe check results pass.

# vanA Negative; vanB Negative

vanA NEGATIVE; vanB NEGATIVE

Test Result | Analyte Result | Detail | Errors | History | Support

| Analyte Name | Ct | EndPt | Analyte Result | Probe Check Result |
|---|---|---|---|---|
| BG | 33.3 | 360 | PASS | PASS |
| vanA | 0.0 | 1 | NEG | PASS |
| vanB | 0.0 | -4 | NEG | PASS |




- *vanA* and *vanB* target DNA is not detected.
- *vanA* NEGATIVE: *vanB* target NEGATIVE
- 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.

# Troubleshooting

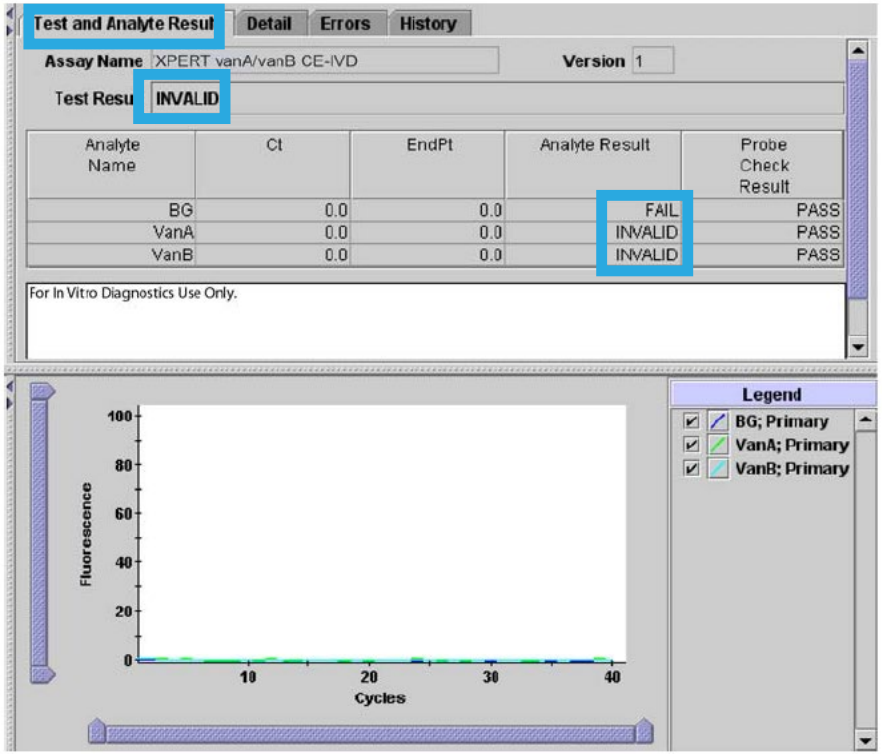
 

# Factors That Negatively Affect Results

- Improper specimen collection
  - The bacterial load in the specimen is below the detection limit of the test
- Improper transport or storage of collected specimen
  - Storage and transport conditions are specimen specific
  - Refer to the Package Insert for the appropriate handling instructions
- Improper testing procedure
  - Modification to the testing procedures may alter the performance of the test
  - Careful compliance with the package insert is necessary to avoid erroneous results

# INVALID Result

INVALID




The presence or absence of *vanA/vanB* target cannot be determined

- SPC: FAIL. The SPC does not meet the acceptance criteria.
- Probe Check: PASS

## Possible Causes

- Improper sample collection
- Incorrect sample preparation
- Improper storage of the cartridges
- Presence of interfering substances in the sample

## Solution

- Repeat the test with a new cartridge.
- Refer to the package insert for details.

# Interfering Substances

- Hydrocortisone cream (1 % Hydrocortisone) and Pepto-Bismol®

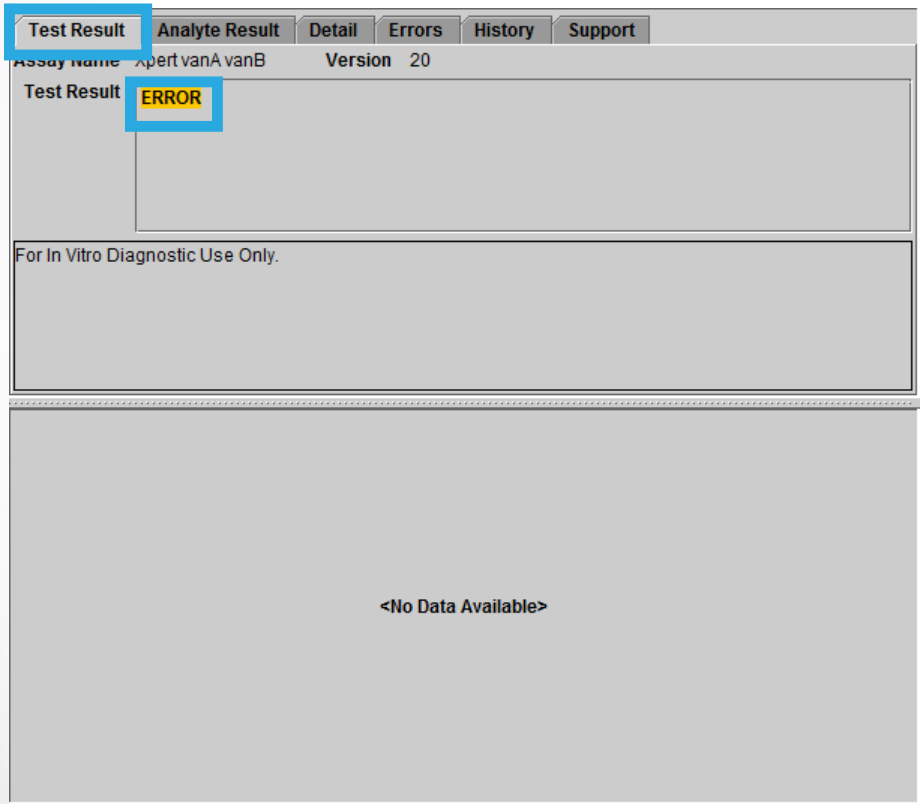
(1 – 5% Bismuth subsalicylate), may potentially interfere with *vanB*.

- When tested in the Interference study, Hydrocortisone cream and

Pepto-Bismol® resulted in slightly higher Ct values relative to the buffer control.

# ERROR Result

ERROR




- The presence or absence of *vanA/vanB* cannot be determined
  - *vanA*: NO RESULT
  - *vanB*: NO RESULT

## Possible Causes

If Probe Check: FAIL
- Improper Sample collection
- Incorrect Sample volume added to the cartridge

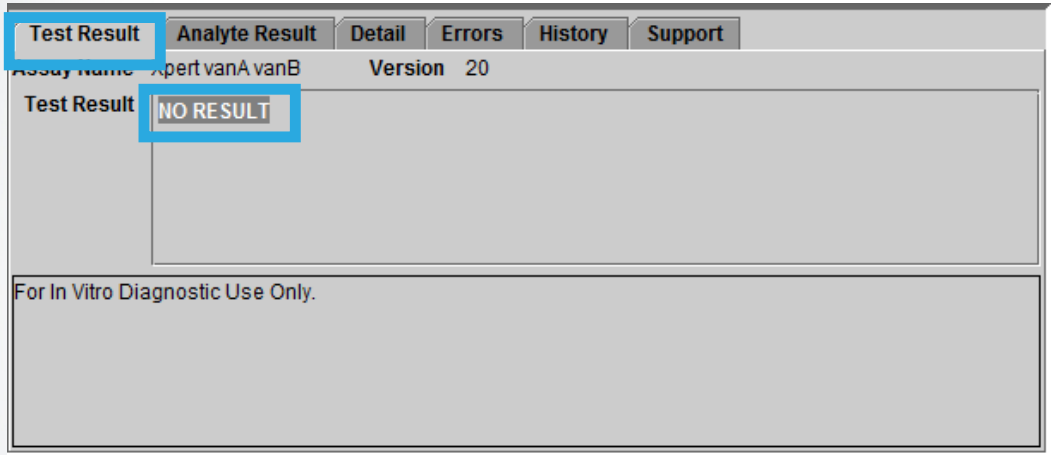
If Probe Check: PASS
- Check the GeneXpert® System module

## Solution

- Repeat the test with a new cartridge

# NO RESULT

NO RESULT




- The presence or absence of *vanA/vanB* cannot be determined.
  - *VanA*: NO RESULT
  - *VanB*: NO RESULT
- Probe Check: NA (not applicable)

## Possible Causes

A NO RESULT indicates that insufficient data were collected.

- Test was stopped with stop test button
- Electrical failure

## Solution

- Secure the power
- Repeat the test with a new cartridge

# Retest Procedure

1

Retain the used cartridge. Obtain a new Xpert cartridge and a new Sample Reagent vial.

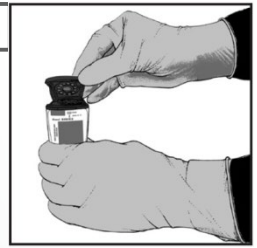
2

Transfer all of the remaining contents from the sample chamber of the used cartridge to a new sample reagent vial.

3

Recap the sample reagent vial and vortex for 10 seconds.

4



Open the cartridge lid.

5

Aspirate all of the Sample Reagent vial contents with a disposable transfer pipette.

6

Empty the pipette into the sample chamber.

7

Close the lid firmly.
Start the test within the time frame specified in the package insert.

*Adapted from 301-6925*

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# Technical Assistance

- Before contacting Cepheid Technical Support, collect the following information:
  - Product name
  - Lot number
  - Serial number of the System
  - Error messages (if any)
  - Software version and, if applicable, Computer Service Tag number
- Log your complaint using the following link ***http://www.cepheid.com/us/support*** ***- Create a Support Case***


Thank You.
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