**MICRO.CULT.2.7 BLOOD CULTURE NUCLEIC ACID TEST**

**PRINCIPLE**

The Verigene Gram-Positive Blood Culture Nucleic Acid Test (BC-GP) and Gram-Negative Blood Culture Nucleic Acid Test (BC-GN) are performed using the sample-to-result Verigene System. The Verigene System is a qualitative, multiplexed in vitro diagnostic test for the simultaneous detection and identification of potentially pathogenic gram-positive and gram-negative bacteria which may cause bloodstream infection (BSI). BC-GP and BC-GN are performed directly on blood culture bottles identified as positive by a continuous monitoring blood culture system and which contain bacteria.

BC-GP detects and identifies the following bacterial genera and species: *Staphylococcus spp., S. aureus, S. epidermidis, S. lugdunensis, Streptococcus spp., S. pneumoniae, S. pyogenes, S. agalactiae, S. anginosus* group*, Enterococcus faecalis, Enterococcus faecium* and *Listeria spp.*

In addition, BC-GP detects the mecA, resistance marker, inferring mecA-mediated methicillin resistance to either *S. aureus* or *S. epidermidis* and the vanA and vanB resistance markers, inferring vanA/vanB-mediated vancomycin resistance to either *E. faecalis* or *E. faecium*.

BC-GN detects and identifies the following bacterial genera and/or species: *Acinetobacter* spp., *Citrobacter* spp., *Enterobacter* spp., *Proteus* spp., *Escherichia* *coli*, *Klebsiella* *pneumoniae*, *Klebsiella* *oxytoca* and *Pseudomonas* *aeruginosa*.

In addition, BC-GN detects resistance markers: CTX-M (ESBL), KPC (carbapenemase), NDM (carbapenemase), VIM (carbapenemase), IMP (carbapenemase), and OXA (oxacillinase).

BC-GP and BC-GN are indicated for use in conjunction with other clinical and laboratory findings to aid in the diagnosis of bacterial bloodstream infections.

**RELATED DOCUMENTS**

MICRO.CULT.2.0 BLOOD CULTURE

MICRO.CULT.2.8 BLOOD CULTURE NUCLEIC ACID TEST QC ROTATION

MICRO.CULT.2.9 BLOOD CULTURE NUCLEIC ACID TEST GN QC ROTATION

**OWNERS**

Manager, Regional Microbiology

**SPECIMEN**Positive blood culture bottle media can be stored at 2-37 ºC for up to 24 hours after bottle positivity prior to testing.  
See MICRO.CULT.2.0

**REAGENTS**

A. Verigene Consumables

1. BC-GP
   1. Test Cartridge (stored at 2-8oC)
   2. Extraction Tray (stored at 2-8oC)
   3. Tip Assembly (stored at 2-8oC)
   4. Utility Tray (stored at 2-8oC)
2. BC-GN
   1. Test Cartridge (stored at 2-8oC)
   2. Extraction Tray (stored at 2-8oC)
   3. Tip Assembly (stored at 2-8oC)
   4. Utility Tray (stored at **≤-20oC**)

**EQUIPMENT**

1. Verigene Processor SP
2. Verigene Reader
3. Micro-pipette and tips
4. Vortex mixer
5. Alcohol wipes
6. 70% ethanol
7. Lint free wipes
8. See MICRO.CULT.2.0

**CALIBRATION**Not applicable

**QUALITY CONTROL**

1. For each test performed, both internal controls (INT CTL 1 and INT CTL 2) must yield correct results to enable reporting of valid test results. On each patient’s instrument result printout, verify internal control result. If the internal control result is acceptable (PASS or NO FAILURE), write down “INT QC OK, <tech initial>” by the internal control field or in SVEV Verigene Result Log. If the internal control fails, do not report patient results and repeat the test.
2. External controls are performed on a monthly basis with a rotating set of 2-3 organisms or using the Microbiologics Verigene Blood Culture Control Panel. Each target must produce a positive result at least once annually. See MICRO.CULT.2.8 and MICRO.CULT.2.9for external QC schedule and expected targets.
   1. Aliquots of multiple QC organisms may be pooled together and run on a single test. Each expected target for all pooled QC organisms must be detected in order for QC to pass.
3. Preparing QC material

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| --- | --- |
| **Step** | **Action** |
| 1. | Select an organism that has been culture confirmed positive for desired target(s) or select a QC organism that is positive for desired target(s). |
| 2. | Subculture selected organism to appropriate supportive media (BAP or CHOC) for 18-24 hours at appropriate temperature and environment for that organism. |
| 3. | Obtain a negative blood culture bottle and culture tubes with 3.0 mL of sterile 0.9% saline for each selected organism. |
| 4. | Select 3-4 colonies using a sterile swab and elute bacteria in the saline tube to create an 0.5 McFarland standard inoculum of selected organism. |
| 5. | Sterilize the negative blood culture bottle top by wiping with an alcohol wipe, using a sterile syringe transfer 0.1mL of the inoculum into the negative blood culture bottle, invert bottle several times. |
| 6. | Incubate bottle(s) in BACTEC instrument using a new barcode label. Incubate on BACTEC until instrument flags bottle(s) as positive. Remove bottle(s) from instrument once positive. |
| 7. | Keep bottle at room temperature until aliquots can be made, no longer than 12 hours. |
| 8. | Sterilize the bottle top again by wiping with an alcohol wipe. Invert the bottle 4-5 times to homogenize the specimen, draw fluid by using a 10mL syringe and transfer to a secondary sterile vessel. |
| 9. | Vortex secondary vessel to homogenize specimen, dispense 1.0mL aliquots into cryovials. |
| 10. | Freeze aliquots at -70C to be used for future QC. |

1. Each new lot and each new shipment must be have an acceptable QC before being used for patient testing. QC for new lot/shipments of BC-GP: *S. aureus* ATCC 43300 and *E. faecalis* ATCC 51299; for BC-GN*: K. pneumoniae* ATCC 1705 and *P. mirabilis* ATCC 7002
2. If expected control results are not obtained, do not report patient results. Notify shift lead tech or supervisor immediately.

**PROCEDURE**

1. General Safety Considerations

Pathogenic microorganisms, including Hepatitis B Virus and Human Immunodeficiency Virus, may be present in specimens. “Universal Precautions” must be followed in handling all items contaminated with blood or other body fluids.

1. Wear gloves at all times while handling inoculated vials or culture plates.
2. Perform all blood culture vial processing in a biological safety cabinet.
3. Properly dispose of all contaminated materials. Place syringes, needles, and sharp contaminated materials in a puncture proof container.

B. Performing Nucleic Acid Testing on Blood Cultures:

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| --- | --- |
| **If** | **Then** |
| **All** the following requirements are met:   * The Gram stain of the bottles show pure Gram positive cocci, Gram positive rods, or aerobic Gram negative rods. * Patient is currently admitted to a hospital that has requested nucleic acid testing, see Addendum A. * Patient is NOT a pediatric patient (<18 years old)\*\* * Patient does NOT have a previous blood culture with that organism within 4 days | Perform nucleic acid testing as described below |
| **Any** of the following are met:   * Gram stain shows multiple organism types (mixed) * Gram stain is NOT Gram positive cocci, Gram positive rod or Gram negative rod. * Gram negative rod is ONLY present in anaerobic bottle. * Patient is NOT currently admitted * Patient is admitted to a hospital that has NOT requested nucleic acid testing * Patient is < 18 years old\*\* * Patient has history (within 4 days) of same organism seen in Gram stain of a blood culture | Do NOT perform nucleic acid testing |

\*\*Regional Microbiology has validated the BC-GN and BC-GP performance with pediatric bottles and patients. Regional Microbiology may omit the age requirement when determining if a culture qualifies for NAT.

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| **Step** | **Action** |
| 1. | With a lint-free wipe and 70% ethanol, wipe all Verigene work surfaces or trays that may be used to hold, transport or come in contact with consumables and/or specimen. Wipe pipette to be used for dispensing sample. |
| 2. | Remove Extraction Tray, Tip Holder Assembly, and Test Cartridge from refrigerator or freezer, allow consumables 10 minutes to warm to room temp.   |  |  | | --- | --- | | **If** | **Then** | | Gram positive organisms are seen | Obtain BC-GP testing consumables | | Gram negative organisms are seen | Obtain BC-GN testing consumables |   Note: Do not mix any BC-GN with BC-GP consumables or vice versa, each testing kit has specific consumables for that test. |
| 3. | Aliquot sample   1. Mix blood culture bottle by inverting several times (>4) 2. Using a syringe, extract 1mL into a 1.5mL sample tube 3. Label the sample tube with the accession number |
| 4. | Open the Drawer Assembly and lift the Drawer Clamp |
| 5. | Extraction Tray:   1. Shake the Extraction Tray to resuspend magnetic beads – visually verify complete resuspension 2. Tap the Extraction Tray to settle reagents 3. Insert Extraction Tray into the Extraction Module |
| 6. | Load Tip Assembly   1. Double check for O-rings and Tip Seal 2. Insert Tip Assembly into Drawer Assembly |
| 7. | Load Utility Tray   1. Gently vortex (at least 5 seconds) the Utility Tray to mix reagents if performing BC-GN skip to part ‘e’. If performing BC-GP continue to part ‘b.’ 2. Tap the Utility Tray to settle the reagents - visually verify pellet location for BC-GP, BC-GN does not have a PC tube. 3. Remove the cap of the PC tube – save cap for post procedure 4. Push the PC tube into the Utility Tray 5. Insert the Utility Tray into the Utility Module |
| 8. | Lower and latch the Drawer Clamp over Trays |
| 9. | Order test – Use the Reader to order tests   1. Login (Operator: **BLC** or personal login (SVEV)) 2. Select current Session using the Session Tab 3. Scan the Test Cartridge ID by scanning the barcode using the barcode scanner attached to the Reader 4. Scan the Test Cartridge Cover’s 2D barcode using the barcode scanner to display the Test Cartridge’s Reference Number, Expiration Date, and Lot Number on reports. |
| 10. | Load Test Cartridge   1. Remove the cover from the top of the Test Cartridge  or 2. Tap the Test Cartridge on the barcode to settle the reagents 3. Insert the Test Cartridge into the Hybridization Module |
| 11. | Load Sample   1. Enter Sample identification information on Reader by scanning the patient barcode or using the Reader’s touch screen keyboard. Press Yes to confirm the sample ID. Ensure Hybridization and Extraction options are selected. 2. Confirm **all** assay targets are to be reported, press Yes to confirm. 3. Gently vortex sample tube (at least 5 seconds) 4. Follow the appropriate step based on which test (BC-GP or BC-GN) is being performed  |  |  | | --- | --- | | **If** | **Then** | | BC-GP is being performed | Pipette **350uL** of sample into the bottom of the Sample Well (the large open well on the Extraction Tray) | | BC-GN is being performed | Pipette **700uL** of sample into the bottom of the Sample loading well (small open well, see picture) |  1. Close the drawer Assembly to begin test processing. The Processor will automatically verify that each consumable is properly loaded and begin sample processing. 2. Confirm countdown started on the Processor SP display screen |
| 12. | Allow testing to complete   1. The Reader will ring to notify the user when the test is completed and the Processor SP will display a message indicating the test is finished. The Test Cartridge should be removed from the Processor SP upon completion of the test. |
| 13. | Open the Drawer Assembly |
| 14. | BC-GP only: Cap the PC tube |
| 15. | Remove the Test Cartridge   1. Remove the Test Cartridge from the Hybridization Module 2. Keeping the cartridge on its side, remove and discard the reagent pack 3. Leave the Test Substrate on its side for 30-60 seconds |
| 16. | Result analysis:   1. Remove silver tape from the back of the Test Substrate 2. Scan the barcode on the Test Substrate and immediately insert the Substrate holder into the Reader 3. To properly insert the substrate into the Reader hold the substrate by the handle with barcode facing away from you. Next, insert the Substrate holder into the substrate compartment. The compartment is designed to place the holder in the correct position. Do not force the holder in, but do insert it into the compartment as far as it will go comfortably. Close the door of the substrate compartment. 4. The analysis will automatically begin. A small camera icon will appear on the Reader letting the user know analysis has begun. 5. The analysis is completed by the Reader when the camera icon is replaced with an upward facing arrow and the Reader rings. 6. Confirm that a result other than ‘No Call – NO GRID’ has been generated. A substrate producing a ‘No Call – NO GRID’ should be rescanned and reanalyzed. If ‘No Call,’ INT CTL 1, or INT CTL 2 are reported repeat testing, do no repeat if specimen has produced an INT CTL 1 or INT CTL 2 twice. Do not report BC-GP or BC-GN if a specimen produced INT CTL twice. 7. Dispose of used Test Substrate. |
| 17. | Print results   1. Touch the substrate icon in the Sessions’ Processing screen. A window displaying the results will open, touch the ‘Print’ option on this screen to print a Detail Report. |
| 18. | Once results are printed and determined valid, discard all consumables in a biohazard waste container. |

C. Daily maintenance – end of day

* 1. With a lint-free wipe and 70% ethanol
     1. Wipe the heat block inside the Processor SP’s Hybridization Module
     2. Wipe the Process Control Tube Well and surrounding area (area where Utility Tray is seated)
     3. Wipe the Drawer Clamp and arms
     4. Wipe the outer surfaces of the Processor SP and Reader

D. Procedure note

1. If patient’s sample is required to store for future or repeated testing, dispense 0.5mL blood sample into cryovials. And freeze the aliquot at -70C to be used for future testing.

**REPORTING RESULTS**

1. Result Entry for BC-GP: Blood culture nucleic acid test is only applied to Sunquest patients. At the **“Other Tests”** tab in GUI Sunquest, follow the instructions below to enter results. Select Keyboard: **RX2-BLC GM POS.** Only report the targets that have results on the instrument printout. For further assistance, press **F8** key to show resulting keyboard.

|  |  |
| --- | --- |
| **Test Code** | **Result** |
| **BLCRX** (default)  =Blood Culture Nucleic Acid Test | Remove “HIDE” and enter “**BGPOS**” |
| **IORGM** (Press **1** key )  = Indicated Organism | See section **B. Interpretation for BC-GP** |
| **STAPY** (Press **2** key)  = Staphylococcus spp. | D = Detected  N = Not Detected |
| **SAURE** (Press **3** key)  = Staphylococcus aureus | D = Detected  N = Not Detected |
| **MECA** (Press **4** key)  = mecA gene | D = Detected  N = Not Detected |
| **SLUGD** (Press **5** key)  = Staphylococcus lugdunensis | D = Detected  N = Not Detected |
| **SEPID**(Press **6** key)  = Staphylococcus epidermidis | D = Detected  N = Not Detected |
| **LISTR** (Press **7** key)  = Listeria spp. | D = Detected  N = Not Detected |
| **STRPT** (Press **8** key)  = Streptococcus spp. | D = Detected  N = Not Detected |
| **SPNEU** (Press **9** key)  = Streptococcus pneumoniae | D = Detected  N = Not Detected |
| **SPYOG** (Press **0** key)  = Streptococcus pyogenes (Group A) | D = Detected  N = Not Detected |
| **SAGAL** (Press **Q** key)  = Streptococcus agalactiae (Group B) | D = Detected  N = Not Detected |
| **SANGI** (Press **W** key)  = Streptococcus anginosus group | D = Detected  N = Not Detected |
| **EFAEL** (Press **E** key)  = Enterococcus faecalis | D = Detected  N = Not Detected |
| **EFAEI** (Press **R** key)  = Enterococcus faecium | D = Detected  N = Not Detected |
| **VANA** (Press **T** key)  = vanA gene | D = Detected  N = Not Detected |
| **VANB** (Press **Y** key)  = vanB gene | D = Detected  N = Not Detected |

1. Interpretation for BC-GP: At the **IORGM** (indicated organism) field, enter result according to the following interpretations.

|  |  |
| --- | --- |
| **If** | **Then** |
| **Genus : Staphylococcus** | |
| Staphylococcus = Detected  Staph. aureus = Detected | Enter **PRESP** <tab> **STA** <tab> **BNAT** <tab> |
| Staphylococcus = Detected  Staph. aureus = Detected  mecA gene = Detected | Enter **PRESP** <tab> **MRSA** <tab> **BNAT** <tab> |
| Staphylococcus = Detected  Staph. lugdunensis = Detected  mecA gene = Detected or Not Detected | Enter **PRESP** <tab> **STLU** <tab> **BNAT** <tab> |
| Staphylococcus = Detected  Staph. epidermidis = Detected  mecA gene = Detected or Not Detected | Enter **PRESP** <tab> **SCN** <tab> **BNAT** <tab> |
| Staphylococcus = Detected  mecA gene = Detected or Not Detected | Enter **PRESP** <tab> **SCN** <tab> **BNAT** <tab> |
| **Genus: Listeria** | |
| Listeria = Detected | Enter **PRESP** <tab> **LIST** <tab> **BNAT** <tab> |
| **Genus: Streptococcus** | |
| Streptococcus = Detected  Strep. pneumoniae= Detected | Enter **PRESP** <tab> **SPNE** <tab> **BNAT** <tab> |
| Streptococcus = Detected  Strep. pyogenes = Detected | Enter **PRESP** <tab> **BSA** <tab> **BNAT** <tab> |
| Streptococcus = Detected  Strep. agalactiae = Detected | Enter **PRESP** <tab> **BSB** <tab> **BNAT** <tab> |
| Streptococcus = Detected  Strep. anginosus group = Detected | Enter **PRESP** <tab> **SVIRG** <tab> **BNAT** <tab> |
| Streptococcus = Detected only | Enter **PRESP** <tab> **STS** <tab> **BNAT** <tab> |
| **Genus: Enterococcus** | |
| Ent. faecalis = Detected | Enter **PRESP** <tab> **EFL** <tab> **BNAT** <tab> |
| Ent. faecium = Detected | Enter **PRESP** <tab> **EFM** <tab> **BNAT** <tab> |
| Ent. faecalis = Detected  vanA **and/or** vanB = Detected | Enter **PRESP** <tab> **VREFL** <tab> **BNAT** <tab> |
| Ent. faecium = Detected  vanA **and/or** vanB = Detected | Enter **PRESP** <tab> **VREFM** <tab> **BNAT** <tab> |
| **No Targets** | |
| All targets = Not Detected | Enter **NSSEL** |
| **Mixed Genus** | |
| When mixed genus are detected | Based on the interpretation above, report each genus accordingly. |

1. Result Entry for BC-GN: Blood culture nucleic acid test is only applied to Sunquest patients. At the **“Other Tests” tab** in GUI Sunquest, follow the instructions below to enter results. Select Keyboard: **RX2-BLC GM NEG.** Only report the targets that have results on the instrument printout. For further assistance, press **F8** key to show resulting keyboard.

|  |  |
| --- | --- |
| **Test Code** | **Result** |
| **BLCRX** (default)  =Blood Culture Nucleic Acid Test | Remove “HIDE” and enter “**BGPOS**” |
| **IORGM** (Press **1** key )  = Indicated Organism | See section **D. Interpretation for BC-GN** |
| **ACINT** (Press **2** key)  = Acinetobacter spp. | D = Detected  N = Not Detected |
| **CITRO** (Press **3** key)  = Citrobacter spp. | D = Detected  N = Not Detected |
| **ENTEB** (Press **4** key)  = Enterobacter spp. | D = Detected  N = Not Detected |
| **PROT** (Press **5** key)  = Proteus spp. | D = Detected  N = Not Detected |
| **ECOL** (Press **6** key)  = E. coli | D = Detected  N = Not Detected |
| **PAERU** (Press **7** key)  = P. aeruginosa | D = Detected  N = Not Detected |
| **KOXYT** (Press **8** key)  = K. oxytoca | D = Detected  N = Not Detected |
| **KPNEU** (Press **9** key)  = K. pneumoniae | D = Detected  N = Not Detected |
| **VIM** (Press **0** key)  = VIM | D = Detected  N = Not Detected |
| **OXAA** (Press **Q** key)  = OXA | D = Detected  N = Not Detected |
| **CTXM** (Press **W** key)  = CTX-M | D = Detected  N = Not Detected |
| **KPCA** (Press **E** key)  = KPC | D = Detected  N = Not Detected |
| **NDM** (Press **R** key)  = NDM | D = Detected  N = Not Detected |
| **IMPA** (Press **T** key)  = IMP | D = Detected  N = Not Detected |

1. Interpretation for BC-GN: At the **IORGM** (indicated organism) field, enter result according to the following interpretations.

|  |  |
| --- | --- |
| **If** | **Then** |
| **Organism Interpretation** | |
| Acintobacter spp. = Detected | Enter **PRESP** <tab> **ACINE** <tab> Resistance interp <tab> **BNAT** <tab> |
| Citrobacter spp. = Detected | Enter **PRESP** <tab> **CITSP** <tab> Resistance interp <tab> **BNAT** <tab> |
| Enterobacter spp. = Detected | Enter **PRESP** <tab> **ENTSP** <tab> Resistance interp <tab> **BNAT** <tab> |
| Proteus spp. = Detected | Enter **PRESP** <tab> **PROSP** <tab> Resistance interp <tab> **BNAT** <tab> |
| E. coli = Detected \*include additional ECDIS comment | Enter **PRESP** <tab> **EC** <tab> Resistance interp <tab> **BNAT** <tab>  **ECDIS** <tab> |
| P. aeruginosa = Detected | Enter **PRESP** <tab> **PSAR** <tab> Resistance interp <tab> **BNAT** <tab> |
| K. oxytoca = Detected | Enter **PRESP** <tab> **KOX** <tab> Resistance interp <tab> **BNAT** <tab> |
| K. pneumoniae = Detected | Enter **PRESP** <tab> **KPNE** <tab> Resistance interp <tab> **BNAT** <tab> |
| All organism targets = Not detected | Enter **NBCGN** |
| **Resistance Interpretations** | |
| CTX-M = detected | Enter Resistance interp: **CTXMA** (shortcut key C) |
| KPC= detected | Enter Resistance interp: **KPCR** (shortcut key K) |
| NDM= detected | Enter Resistance interp: **NDMB** (shortcut key M) |
| VIM= detected | Enter Resistance interp: **VIMB** (shortcut key V) |
| IMP= detected | Enter Resistance interp: **IMPB** (shortcut key I) |
| OXA= detected | Enter Resistance interp: **OXAB** (shortcut key O) |
| **Multiple Organisms** | |
| When multiple organism are detected | Enter: **PRESP** <tab> organism code as described above <tab> **PRESP** <tab> organism code as described above <tab>  Any resistance interpretations as described above <tab>  **BNAT** <tab> |

1. Notification and Charging
   * + 1. Call blood culture nucleic acid result along with blood culture Gram stain result to patient’s unit. SVEV Gram stain results should be called at time of smear. If nucleic acid testing is going to be delayed (greater than 3 hours from time of Gram stain) call the Grain stain result to patient’s unit, then call nucleic acid results once they are completed.
       2. When the following markers are detected from Verigene’s BC-GN panel, please perform the following actions.

|  |  |
| --- | --- |
| **Detected Marker** | **Actions** |
| CTX-M | * When call results to unit nurse, state “this is a potential multi-drug resistant organisms. Follow your hospital’s infection control protocol.” * Call results to hospital infection control. |
| KPC |
| NDM |
| VIM |
| IMP |
| OXA |

* + - 1. Refer to MICRO.CULT.2.0 Blood Culture procedure for additional requirements on reporting and notification.
      2. Once the blood culture nucleic acid test is completed, add “**BGPNA**” charge for BC-GP or “**BGNNA**” for BC-GN at the Billing Tab.

**PROCEDURE NOTES**

1. CTX-M: CTX-M-type enzymes are a group of class A extended-spectrum beta-lactamases (ESBLs) that are rapidly spreading among *Enterobacteriaceae* worldwide, replacing TEM-type and SHV-type ESBLs as the predominant ESBLs in many countries. Some carbapenemases have become associated with strains that have great epidemic potential, spreading across countries and continents. This enzyme is acquired through the plasmid acquisition of beta-lactamase genes rather than via genetic mutation. There are more than 80 CTX-M enzymes that have been identified.
2. KPC: The class A *Klebsiella pneumoniae* carbapenemase (KPC) has rapidly spread in the United States and is increasing elsewhere in the world. At least ten variants have been identified and are distinguished by 1-2 amino acid substitutions. Class B metallo-beta-lactamases (MBLs) of the IMP, VIM and NDM types have been reported worldwide, and their genes carried by plasmids and integrons, hydrolyzing all beta lactams with the exception of aztreonam.
3. NDM: New Delhi metallo-beta-lactamase (NDM) is a novel broad spectrum cabapenemase. Since its first description, NDM carbapenemases -- with DNM-1 presenting as the dominate type—has been reported for many countries worldwide. NDM enzymes are present largely in *Enterobacteriaceae*, but also in non-fermenters.
4. VIM: Verona integrin-encoded metallo-beta-lactamase (VIM) belongs to a growing family of carbapenemase enzymes that include at least 10 known members, of which VIM-2 is the predominate variety. VIM enzymes have been found mainly in non-fermenting Gram-negative bacteria such as *P. aeruginosa*, but their numbers are increasing in *Enterobacteriaceae*.
5. IMP: Imipenem-resistant metallo-beta-lactamase (IMP) is a plasmid mediated IMP-type carbapenemase, with at least 17 known varieties. IMP enzymes are most frequently seen in *Pseudomonas* and *Acinetobacter* species.
6. OXA: Class D beta-lactmases are also called oxacillinases, or OXA-type beta-lactamases (OXA). Genes encoding OXAs are known to be intrinsic in many Gram-negative rods, including *Acintobacter baumannii* and *P. aeruginosa*, but play a minor role in natural resistance phenotypes. The acquired OXAs possess either a narrow spectrum or an expanded spectrum of hydrolysis, including carbapenems in several instances. None of these carbapenem-hydrolyzing class D beta-lactamases (CHDLs) significantly hydrolyze expanded-spectrum cephalosporins, therefore indicating that currently known OXAs are unable to combine extended-spectrum and carbapenem-hydrolyzing properties. Three groups of acquired CHDLs have been identified in *Acinetobacter* species, other CHDL are mostly identified in *K. pneumoniae* and *E. coli*, but also in various other enterobacterial species.

**LIMITATIONS**

1. In mixed cultures containing gram-positive or gram-negative bacteria and other organisms, BC-GP and BC-GN may not identify all the detectable organisms in the specimen, depending upon the concentration of each target present
2. The detection of bacterial nucleic acid is dependent on proper specimen collection, handling, transport, storage, and preparation, including extraction. Failure to observe proper procedures in any of these steps could lead to incorrect results.
3. A negative result for *S. aureus, S. epidermidis,* or mecA should not be used as the sole basis for diagnosis, treatment or patient management decisions.
4. Vancomycin resistance can be caused by genes other than vanA and vanB.
5. Some anaerobic bacteria containing the vanB gene have been described in literature and may give a positive vanB result in mixed cultures of *E. faecalis* or *E. faecium.*
6. Pediatric patient specimens were not differentiated from adult patient specimens in the clinical study; therefore, the performance characteristics of the assay with specimens obtained from pediatric patients have not been determined.
7. There is a risk of false negative results due to sequence variants in the bacterial targets of the assay.
8. The assay detects the presence of the mecA gene in a sample, but does not determine which *Staphylococcus* spp (*S. aureus* and/or *S. epidermidis*) produced the gene.
9. The assay detects the presence of the vanA or vanB gene in a sample, but does not determine which *Enterococcus* spp (*E. faecalis* and/or *E. faecium*) produced the gene.
10. The assay does not differentiate *Staphylococcus* spp. other than *S. aureus, S. epidermidis, S. lugdunensis*. If, for example, *S. hominis* and/or *S. capitis* are present the BC-GP result will be ‘*Staphylococcus’* detected.
11. The assay does not differentiate *Streptococcus* spp or groups other than *S. agalactiae, S. anginosus* group, *S. pneumoniae* and *S. pygones*. If, for example, *S. sanguinis* and/or *S. salivarius* are present, the BC-GP result will be ‘Streptococcus’ detected.
12. Detection of CTX-M, KPC, NDM, VIM, IMP and/or OXA gene(s) in a specimen does not confirm that the resistant marker is associated with the organism(s) detected. Subculturing and subsequent testing of the isolated organism is necessary to definitively link antimicrobial resistance with a specific organism.
13. Based on the sequence homology analysis and analytical testing, a low likelihood of cross-reactivity exists between BC-GN probes which detect *K. oxytoca* and the nucleic acid sequence for *K. pneumoniae*. Therefore, on rare occasions, both a “*K. oxytoca* Detected” result and a “*K. pneumoniae* Detected” result may be obtained when *K. pneumoniae* is present in the specimen.
14. BC-GN will not distinguish *E. coli* from *Shigella* spp. including *S. dysenteriae*, *S. flexneri,* *S. boydii,* and *S. sonnei.*
15. *Buttiauxella gaviniae* and *Enteric* Group 137 (ATCC BAA-69) cross-react with BC-GN *Citrobacter* spp. probes which will cause a false positive “Citrobacter spp. Detected” result.
16. *Escherichia albertii* stains cross-react with BC-GN E. coli probes, which will cause a false positive “E. coli Detected” result.
17. *Kluyvera ascorbata, Raoultella ornithinolytica, Raoultella planticola, and Cedecea davisae* cross react BC-GN *K. oxytoca* probes, which will cause false positive “K. oxytoca Detected” result.
18. *Leminorella grimontii, Enterococcus raffinosus* and *Candida parapsilossi* cross-react with BC-GN CTX-M probes, which will cause false positive “CTX-M Detected” result.
19. BC-GN does not detect *Acinetobacter tartarogenes, Enterobacter gergoviae, Enterobacter kobei,* and *Enterobacter pyrinus.*
20. Rare strains of *K. pneumoniae, K. variicola,* and *Leclercia adecarboxylata* may cross-react with BC-GN *Enterobacter* spp. probes, which will case a false positive “Enterobacter Detected”

**REFERENCES**

Verigene Gram-Positive Blood Culture Nucleic Acid Test (BC-GP) package insert. Nanosphere 027-00030-01, Rev. B; October 2012

Verigene Gram-Negative Blood Culture Nucleic Acid Test (BC-GN) package insert. Nanosphere. 027-00039-01, Rev B; February 2014

Blood Culture Bottle Spiking Protocols. Nanosphere. 13-0014-C

**Addendum A**

Hospitals that have requested NAT:

|  |  |
| --- | --- |
| HID (Hospital ID in SunQuest) | Hospital name |
| SVEV | St. Vincent Evansville (St. Mary’s) |
| CHE | Community Hospital East |
| CHS | Community Hospital South |
| CHN | Community Hospital North |
| CHH | Community Heart Hospital |
| SVIN (including St. Vincent Women’s Hospital) | St. Vincent Hospital (86th Street) St. Vincent Women’s Hospital  Payton Manning Children’s Hospital |
| SVCR | St. Vincent Hospital Carmel |
| SVHH | St. Vincent Heart Hospital |
| SVAN | St. Vincent Hospital Anderson |
| SVJO | St. Vincent Hospital Kokomo (St. Joseph) |
| IOH | Ortho Indy Hospital |