University of Washington Medical Center

Clinical Microbiology Laboratory Document # 601.U.125.01

Antibiotics Manual **Xpert Carba-R Assay**		Effective: 1/15/18
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Revises or supersedes:		Revised by:

ANNUAL REVIEW			
Reviewed by:	Date	Reviewed by:	Date

I. Purpose

The global spread of carbapenemase-producing Enterobacteriaceae, Pseudomonas aeruginosa, and Acinetobacter species is a critical medical and public health issue. These organisms, known alternately as carbapenem non-susceptible organisms, (CNSO), or carbapenem-resistant Enterobacteriaceae (CRE) when appropriate, are often resistant to all beta-lactam agents and frequently are co-resistant to multiple classes of other antimicrobial agents, leaving very few treatment options. Tracing the spread of CNSOs is complicated by the diversity of carbapenem-hydrolyzing enzymes that have emerged and the ability of the genes to spread among multiple bacterial species. Some of the resistance genes, such as the Klebsiella pneumoniae carbapenemase (KPC) determinants, are associated with successful clonal lineages of bacteria (e.g., K. pneumoniae ST258), which have a selective advantage in hospital settings where antimicrobial use is high. Opportunities for transmission of organisms are often frequent, with further dissemination of the resistance genes via transmissible plasmids and integrons. K. pneumoniae strain ST258 has caused multiple epidemics globally, especially in the United States and Israel.

A fast and accurate method of determining whether a carbapenem-non-susceptible bacterial isolate harbors one of these five common classes of carbapenem resistance genes would be a significant aid to infection control programs especially during outbreaks, since it has the potential to: 1) identify the specific resistance gene present in the organism, and 2) differentiate those organisms with the most common transmissible carbapenem resistance genes from organisms that are resistant due to other beta-lactamases and changes in the organism's cell wall, which do not necessarily require placement of the patient in contact precautions.

II. Principle

The GeneXpert System will automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequence in simple or complex samples using real-time PCR. The system consists of an instrument, personal computer, and preloaded software for performing 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 minimized. For a full description of the system, see the GeneXpert Dx System Operator Manual.

The Xpert Carba-R Assay includes reagents for the detection of blaKPC, blaNDM, blaVIM, blaOXA-48, and blaIMP gene sequences as well as a Sample Processing Control (SPC) to control for adequate processing of the target bacteria and to indicate the presence of inhibitor(s) in the PCR reaction. The SPC also ensures that the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction and that the PCR reagents are functional. An additional internal control, the Probe Check Control (PCC), verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The primers and probes in the Xpert Carba-R Assay detect proprietary sequences for the blaKPC (KPC), blaNDM (NDM), blaVIM (VIM), blaOXA-48 (OXA-48), and blaIMP (IMP) gene sequences associated with carbapenem-non-susceptibility in gram-negative bacteria.

III. Specimen

Testing is performed on Gram negative rods identified as Enterobacteriaceae, Pseudomonas aeruginosa, or Acinetobacter baumannii which have been grown on either blood agar or MacConkey agar at 35 °C for 18 - 24 hours in ambient air prior to testing. See section "Preparing isolate for testing" section for further information.

- Organisms should be identified and carbapenem non-susceptibility status should be determined in accordance with the current FDA approved drug package insert and the latest version of CLSI guideline M100 prior to testing on Xpert Carba-R Assay.

IV. Reagents/ Supplies/Equipment

1. Xpert Carba-R Assay kit (2 - 28 °C)
   Xpert Carba-R Assay Cartridges with Integrated Reaction Tubes
   a. Reagent 2 contains Guanidinium chloride - avoid release to the environment; may cause long lasting harmful effects to aquatic life. Dispose of contents/container in accordance with local and regional /national/ international regulations. Consult Safety Data Sheet for other precautionary statements.
   b. 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 commingling of the material with other animal materials.
2. Disposable 1.7mL Transfer Pipettes
3. Microbiologics Cepheid Xpert® Carba-R Helix Elite positive and negative swab control panel, catalog No. 8187. (2 - 8°C).
   Each positive control swab consists of:
   a. Escherichia coli derived from NCTC 13476 IMP-type positive.
   b. Klebsiella pneumoniae derived from NCTC 13440 VIM-1, Metallo-beta-lactamase positive.
   c. Klebsiella pneumoniae derived from NCTC 13443 NDM-1 positive, New Delhi metallo-beta-lactamase.
   d. Klebsiella pneumoniae derived from NCTC 13438 produces carbapenemase KPC-3.
   e. Klebsiella pneumoniae derived from NCTC 13442 OXA-48.
   Each negative control swab consists of a non- carbapenemase producing Escherichia coli derived from NCTC 11954.
4. 10 µg meropenem discs
5. Sterile forceps
6. Disposable, sterile 10 µL inoculating loops
7. Black marker
8. Transport container
9. Biological safety cabinet (BSC)
10. Vortex mixer
11. GeneXpert Dx System

V. Testing Procedure

Notes:

- If refrigerated, bring all components to room temperature prior to use.
- Do not use cartridges or reagents that have passed the expiration date.
- Do not store any of the kit components in the biological safety cabinet.

- Do not open the cartridge lid except when adding sample.
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- Do not use a cartridge that has been dropped or shaken after you have added the sample.
- To label a cartridge, write or affix an ID label to the sides. Do not put a label permanently on the lid of the cartridge or cover the existing 2D barcode on the cartridge.
- Do not use a cartridge that has a damaged reaction tube.
- Change gloves between patient samples. Always change gloves if contamination is evident or suspected.
- Start the test within 30 minutes of adding the sample to the cartridge.

A. Preparing the isolate for testing

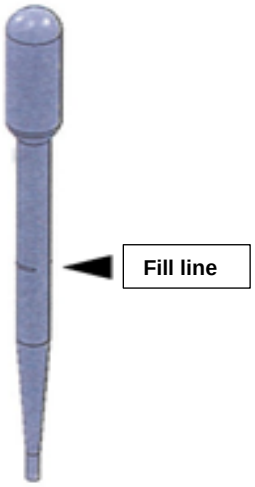
1. Consult with Lead, Micro fellow or Director if need for carbapenemase testing is questionable.
2. Use the Carbapenemase Testing Quality Control Sheet in the antibiotics section to record each specimen tested.
3. Organisms should be identified and carbapenem non-susceptibility status should be determined in accordance with the current FDA approved drug package insert and the latest version of CLSI guideline M100 prior to testing on Xpert Carba-R Assay.
4. Organisms should be tested from either a Blood or MacConkey agar plate incubated for 18 to 24 hours at 35 °C in ambient air. Ideally, but not required, sub-cultured colonies around a 10µg meropenem disk in the first streak quadrant should be selected.
5. Use the direct colony suspension method by touching isolated colonies with a swab or loop to prepare a 0.5 McFarland suspension of the bacterial isolate as outlined in the CLSI M07 Approved Standard. The steps are also described below.
    a. Make a suspension of isolated colonies selected from an agar plate (e.g., a nonselective medium such as blood agar that has been incubated for 18-hours to 24-hours) directly in saline.
    b. Adjust the suspension to achieve a turbidity equivalent to a 0.5 McFarland standard. This results in a suspension containing approximately 1 to 2 x 10^8 CFU/mL for E. coli ATCC (American Type Culture Collection) 25922.
    c. Either use a photometric device or, if performed visually, use adequate light to compare the inoculum tube and the 0.5 McFarland standard against a Wickerham card, a paper card with a white background and contrasting black lines.

B. Preparing the Cartridge for Testing

1. Wearing a new pair of gloves, place a cartridge inside the pre-cleaned biological safety cabinet. Label cartridge with patient/ control information. (Note: If more than one specimen is being tested, handle only one cartridge within the BSC at a time**. Change gloves between specimens**.)
2. Vortex the 0.5 McFarland suspension (for patient testing).
3. Using a 10 µL loop, transfer 10 µL of the 0.5 McFarland suspension to a 5mL vial of Xpert Carba-R Sample Reagent. Swirl the loop a minimum of three times in the sample reagent.
    - NOTE: Ensure that the 10 µL loop is filled with sample and the sample suspension in the loop does not burst when transferring the 0.5 McFarland suspension to the Xpert Carba-R Sample Reagent.
4. Cap the Sample Reagent vial tightly and vortex at high speed for 10 seconds.
5. Open the cartridge lid. Open the Sample Reagent cap.
6. Using the transfer pipette provided, aspirate the prepared Sample Reagent suspension up to the mark on the pipette (which is approximately1.7 mL; see Figure 1).
7. Transfer the material into the Sample Chamber (large opening) of the Xpert Carba-R Assay cartridge. See Figure 2.

8. The remaining sample in the Sample Reagent vial can be retained at 2–28°C for up to four days if a retest is required.

Figure 1. Transfer Pipette



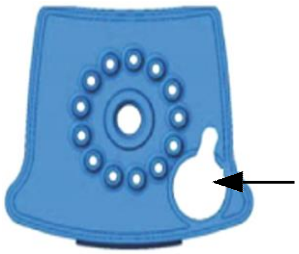


Figure 2

9. Close the cartridge lid firmly.
10. Transport the cartridge(s) to GeneXpert instrumentation area.

C. Starting the Test

Notes:

- See additional information on the operation of the GeneXpert Dx instrument by referring to the GeneXpert Dx System Operation.
- Once the test has been started, do not close the GeneXpert Dx System computer window.
- Keep the cartridge upright while scanning barcodes.

1. In the GeneXpert Dx system window, click **Create Test** on the toolbar. The Create Test window appears.
2. Click Manual Entry.
3. At the **Please scan sample ID barcode** prompt, manually enter the **patient's name/accession number.**.
4. There will be a popup window, click Abort Query. When the comment "No matching host order found" click OK.
5. The **Scan Cartridge Barcode** dialog box appears. Scan the product barcode on the Xpert Carba-R Assay cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.
6. Click **Start Test.**
7. Open the instrument module door with the blinking green light and load the cartridge. Insure the cartridge is seated properly.
8. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
9. Wait until the system releases the door lock before opening the module door and removing the cartridge.
10. Dispose of used cartridges in a biohazard waste container in an area outside of the area of testing.

D. Interpretation/ Reporting

1. The results are interpreted by the GeneXpert System from measured fluorescent signals and embedded calculation algorithms and will be shown in the View Results window. Interpretations for all possible combinations of results with the five target analytes in the Xpert Carba-R Assay are not shown; however, the following examples are indicative of the type of results that can be expected. A report prints when a test is completed. Add the results of testing patient's culture under the WCIM workup at the CARBAR workup code.
2. Give the report to the Antibiotic's Lead for review. Once these reports are reviewed for reporting accuracy, file in the CRE notebook. Printed reports are retained for two years.
3. See package insert for screenshots of the graphs generated for positive, negative, and invalid results.
4. See Table 1 at the end of this document for interpretation, and reporting information.
5. See the procedure 601.U.115 Carbapenem-Resistant Gram-Negative Rod Reporting and Surveillance for full information on reporting phenotypic and genotypic carbapenemase testing.

VI. Quality Control

Initial and Monthly Quality control is performed by using Microbiologics Xpert® Carba-R Helix Elite positive and negative swab control panel.

Initial Quality Control

External quality control is performed on each new lot number/ shipment prior to patient testing. The positive sample(s) must produce the appropriate positive reaction(s). The negative sample must produce a negative reaction.

Positive Controls = Microbiologics Xpert Carba-R Helix Elite Positive Control swab (1)
Negative Control = Microbiologics Xpert Carba-R Helix Elite Negative Control swab (1)

Microbiologics Xpert Carba-R Helix Elite Controls Use:

1. Tear open a swab pouch at the notch.
2. Remove the swab from the pouch.
3. Insert the swab into a Sample Reagent vial provided in the Cepheid assay and break the swab by snapping the shaft.
4. Close the Sample Reagent vial cap and vortex the vial for 10 seconds at full speed to mix.
5. Prepare one control cartridge at a time:
   A. Label the cartridge with the appropriate control information.
   B. Open the cartridge lid, and then open the associated control suspension:
      i. Negative control = Microbiologics Xpert Carba-R Helix Elite Negative Control
      ii. Positive control = Microbiologics Xpert Carba-R Helix Elite Positive Control
   C. Using a transfer pipette provided in the kit, transfer 1.7mL of the control to the sample chamber with large opening in the cartridge.
   D. Close the cartridge lid firmly.
6. To load these samples into the GeneXpert:
   A. At the **Please scan sample ID barcode** prompt, select Manual Entry:
      i. Enter the following for the positive control: **Initial Pos Control 1**
      ii. Enter the following for the negative control: **Initial Neg Control 1**
   B. Once at the **Test Type** field, select "Positive Control 1" for the positive control and "Negative Control 1" for the negative control from the drop-down menu (the default is "Specimen").
   C. See the GeneXpert Dx System Operation manual for more detail.
8. Document external quality control on the 601.U.488.01 Xpert Carba-R Initial Kit Lot QC sheet.

Monthly Quality Control

External quality control is performed at least every 31 days on the in-use lot number of product using the

Microbiologics Xpert Carba-R Helix Elite Controls (see instructions for use in the Initial Quality Control Section for the setup of controls for testing). To ensure that each module of the instrument generates both a positive and a negative external quality control result, the GeneXpert instrument automatically rotates testing of the controls and samples with each test.

Positive Controls = Microbiologics Xpert Carba-R Helix Elite Positive Control swab

Negative Control = Microbiologics Xpert Carba-R Helix Elite Negative Control swab

To load these samples into the GeneXpert:

1. At the **Please scan sample ID barcode** prompt, select Manual Entry.
   A. Enter the following for the positive control: **Monthly Pos Control 2**
   B. Enter the following for the negative control: **Monthly Neg Control 2**
2. Once at the **Test Type** field, select "Positive Control 2" for the positive control and "Negative Control 2" for the negative control from the drop-down menu (the default is "Specimen").
3. See the GeneXpert Dx System Operation manual for more detail.
4. Document monthly quality control results on the 601.U.489 Xpert Carba-R Monthly Lot QC Sheet.

<u>For All Testing</u>

Each test includes a Sample Processing Control (SPC) and Probe Check Control (PCC). These must perform satisfactorily in order to report results.

- Sample Processing Control (SPC): Ensures the sample was processed correctly. The SPC contains spores of *Bacillus globigii* in the form of a dry bead that is included in each cartridge to verify adequate processing of the sample. The SPC verifies that lysis of bacteria has occurred if the organisms are present and verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time PCR assay, ensures the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that the PCR reagents are functional.
  - 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, QC1, QC2): Before the start of the PCR reaction, the GeneXpert 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.

VII. Charging for testing

1. Add an ADCARB to the billing section of the test for positive and negative results.

Table 1: Results/ Interpretations/ Reporting				
Result	**Interpretation**	**Reporting**		
		LIS Code (Individual Codes)	**LIS Code Translation**	**LIS Workup Result Code (CARBAR)**
IMP DETECTED; VIM NOT DETECTED; NDM NOT DETECTED; KPC NOT DETECTED; OXA48 NOT DETECTED	IMP target DNA sequence is detected; VIM, NDM, KPC, and OXA-48 target DNA sequences are not detected.	IMPCAR (GNIMP-PCR-MDRO-ICCOMC-REPT0)	: Imipenem-resistant metallo-beta-lactamase (IMP) gene detected. Likely resistant to penicillins,cephalosporins, and carbapenems (but not aztreonam).(Methodology: real time PCR). Multi-Drug Resistant Organism – For inpatients, isolate using contact precautions per institutional policy. Contact Infection Control if you have any questions. - This result is a Washington state Notifiable condition. Contact public health authorities in accordance with WAC 246-101. http://www.doh.wa.gov/Portals/1/Documents/5100/210-001-Poster-HCP.pdf	GNIMP
IMP NOT DETECTED; VIM DETECTED; NDM NOT DETECTED; KPC NOT DETECTED; OXA48 NOT DETECTED	VIM target DNA sequence is detected; IMP, NDM, KPC, and OXA-48 target DNA sequences are not detected.	VIMCAR (GNVIM-PCR-MDRO-ICCOMC-REPT0)	: Verona integron-encoded metallo-beta-lactamase (VIM) gene detected. Likely resistant to penicillins, cephalosporins, and carbapenems (but not aztreonam). (Methodology: real time PCR). Multi-Drug Resistant Organism – For inpatients, isolate using contact precautions per institutional policy. Contact Infection Control if you have any questions. - This result is a Washington state Notifiable condition. Contact public health authorities in accordance with WAC 246-101. http://www.doh.wa.gov/Portals/1/Documents/5100/210-001-Poster-HCP.pdf	GNVIM
continued				

Table 1: Results/ Interpretations/ Reporting				
Result	**Interpretation**	**Reporting**		
		LIS Code (Individual Codes)	**LIS Code Translation**	**LIS Workup Result Code (CARBAR)**
IMP NOT DETECTED; VIM NOT DETECTED; NDM DETECTED; KPC NOT DETECTED; OXA48 NOT DETECTED	NDM target DNA sequences are detected; IMP, VIM, KPC, and OXA-48 target DNA sequences are not detected.	NDMCAR (GNNDM-PCR-MDRO-ICCOMC-REPT0)	: New Delhi metallo beta-lactamase (NDM) gene detected. Likely resistant to penicillins, cephalosporins, and carbapenems (but not aztreonam). (Methodology: real time PCR). Multi-Drug Resistant Organism – For inpatients, isolate using contact precautions per institutional policy. Contact Infection Control if you have any questions. - This result is a Washington state Notifiable condition. Contact public health authorities in accordance with WAC 246-101. http://www.doh.wa.gov/Portals/1/Documents/5100/210-001-Poster-HCP.pdf	GNNDM
IMP NOT DETECTED; VIM NOT DETECTED; NDM NOT DETECTED; KPC DETECTED; OXA48 NOT DETECTED	KPC target DNA sequences are detected; IMP,VIM, NDM, and OXA-48 target DNA sequences are not detected.	KPCCAR (GNKPC-PCR-MDRO-ICCOMC-REPT0)	: KPC (Klebsiella pneumonia carbapenemase) gene detected. Likely resistant to penicillins, cephalosporins, carbapenems, and aztreonam. (Methodology: real time PCR). Multi-Drug Resistant Organism – For inpatients, isolate using contact precautions per institutional policy. Contact Infection Control if you have any questions. - This result is a Washington state Notifiable condition. Contact public health authorities in accordance with WAC 246-101. http://www.doh.wa.gov/Portals/1/Documents/5100/210-001-Poster-HCP.pdf	GNKPC
continued				

Table 1: Results/ Interpretations/ Reporting				
Result	**Interpretation**	**Reporting**		
		LIS Code (Individual Codes)	**LIS Code Translation**	**LIS Workup Result Code (CARBAR)**
IMP NOT DETECTED; VIM NOT DETECTED; NDM NOT DETECTED; KPC NOT DETECTED; OXA48 DETECTED	OXA-48 target DNA sequences are detected; IMP, VIM, NDM and KPC target DNA sequences are not detected.	OXACAR (GNIMP-PCR-MDRO-ICCOMC-REPT0)	: OXA-type beta-lactamase (OXA) gene detected. Possibly resistant to penicillins, cephalosporins, and carbapenems (but not aztreonam). (Methodology: real time PCR). Multi-Drug Resistant Organism – For inpatients, isolate using contact precautions per institutional policy. Contact Infection Control if you have any questions. - This result is a Washington state Notifiable condition. Contact public health authorities in accordance with WAC 246-101. http://www.doh.wa.gov/Portals/1/Documents/5100/210-001-Poster-HCP.pdf	GNOXA
IMP NOT DETECTED; VIM NOT DETECTED; NDM NOT DETECTED; KPC NOT DETECTED; OXA48 NOT DETECTED	IMP, VIM, NDM, KPC, and OXA-48 target DNA sequences are not detected.			NRN (Negative)
continued				

Table 1: Results/ Interpretations/ Reporting				
Result	**Interpretation**	**Reporting**		
		LIS Code (Individual Codes)	**LIS Code Translation**	**LIS Workup Result Code (CARBAR)**
More than one carbapenemase detected	Multiple target DNA sequences are detected. ➢ **Lead, Micro fellow or Director regarding the reporting of multiple carbapenemase targets.**	(see above)	(see above)	(see above)
INVALID	Presence or absence of IMP, VIM, NDM, KPC, and OXA-48 target DNA sequences cannot be determined. • SPC: FAIL; No PCR amplification of the SPC DNA sequence or the SPC Ct is not within valid range and the fluorescence endpoint is below threshold setting. • PCC: PASS; all probe check results pass. • Use the instructions in the Retest Procedure section to repeat the test.			
continued				

Table 1: Results/ Interpretations/ Reporting				
Result	Interpretation	Reporting		
		LIS Code (Individual Codes)	LIS Code Translation	LIS Workup Result Code (CARBAR)
ERROR	Presence or absence of IMP, VIM, NDM, KPC, and OXA-48 target DNA sequences cannot be determined. • SPC: NO RESULT • PCC: FAIL*; one or more of the probe check results failed. The PCC probably failed because the reaction tube was filled improperly, or a probe integrity problem was detected. • Use the instructions in the Retest Procedure section to repeat the test. * If the probe check passed, the error is caused by a system component failure.			
NO RESULT	Presence or absence of IMP, VIM, NDM, KPC, and OXA-48 target DNA sequences cannot be determined. Insufficient data were collected to produce a test result (for example, the operator stopped a test that was in progress). • SPC: NO RESULT • PCC: Not applicable • Use the instructions in the Retest Procedure section to repeat the test.			

Retest Procedure:

1. Remove a new cartridge, a new Sample Reagent vial, and a new transfer pipette from the kit.
2. Transfer the entire contents of the leftover sample in the Sample Reagent vial to the new Sample Reagent vial.
3. Cap the new Sample Reagent vial tightly and vortex at high speed for 10 seconds.
4. Open the cartridge lid, and using the provided transfer pipette, aspirate the Sample Reagent to the mark on the pipette, and then transfer the material into the Sample Chamber of the Xpert Carba-R Assay cartridge.
5. Close the cartridge lid and place the cartridge into the GeneXpert instrument within 30 minutes following the previously detailed testing procedure.
6. Indicate this as a repeat test by adding **–R1** (-R1 = first repeat) to the accession number in the sample ID field.
    A. Enter or scan the accession number in the patient ID field.
    B. At the sample ID field enter the accession number with the –R1 suffix (e.g., W12345-R1).

Retest Interpretation:

1. Report the result according to Table 1.
2. If the repeat result is INVALID, ERROR, or NO RESULT record result in workup area of patient's culture and onto the Carba-R patient testing log. Consult with appropriate supervisory staff about further testing by an alternate method or sending to the State Department of Health laboratory.

Limitations/ Precautions

1. For optimal results, first inoculate the organism onto either a blood or MacConkey agar plate, streak for isolation, place a 10 µg meropenem disk in the first streak quadrant as a means to ensure that the isolate retains its non-susceptibility to carbapenem, and then test the organism following incubation for 18 to 24 hours at 35 °C in ambient air.
2. The Xpert Carba-R Assay detects *bla*KPC, *bla*NDM, *bla*VIM, *bla*OXA-48, and *bla*IMP from pure colonies, and is not for bacterial identification. The performance of the Xpert Carba-R Assay with bacteria other than Enterobacteriaceae, *Pseudomonas aeruginosa,* or *Acinetobacter baumannii* has not been evaluated. Organisms should be identified and carbapenem non-susceptibility status should be determined prior to testing on Xpert Carba-R Assay.
3. The Xpert Carba-R Assay is not a sub-typing tool and does not report variants of the *bla*IMP, *bla*VIM, *bla*NDM, *bla*KPC, or *bla*OXA-48 genes.
4. Certain bacterial species, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* have been shown to exhibit resistance to carbapenems due to intrinsic resistance mechanisms.
5. The detection of other OXA-carbapenemase genes, besides *bla*OXA-48 and *bla*OXA-181, has not been evaluated in the study.
6. The *in silico* analyses used to predict variants detected by the assay were based on a comparison of target gene sequences available in GenBank to the Xpert Carba-R Assay primer/probe oligonucleotides and amplicon sequence for each gene target. BLAST searches for in silico analysis were performed in 2014-2015. *In silico* analysis of new variant gene sequences deposited into the database after 2015 for the five target genes have not been performed.
7. Mutations or polymorphisms in primer or probe binding regions may affect detection of current, new or unknown *bla*KPC, *bla*NDM, *bla*VIM, *bla*OXA-48, and *bla*IMP variants, resulting in a false negative result.
8. The Xpert Carba-R Assay will generate a negative IMP result when testing bacterial isolates containing IMP-7, IMP-13, or IMP-14 gene sequences.
9. Performance of the Xpert Carba-R Assay with organisms harboring non-target carbapenemase genes, other than *bla*SPM, *bla*SME, and *bla*IMI, is unknown.
10. Good laboratory practices, including changing gloves between handling patient specimens, are recommended to avoid contamination of specimens or reagents.
11. Erroneous test results might occur from improper culture techniques, failure to follow the recommended procedure to prepare the 0.5 McFarland suspension, 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.

12. As the detection of *bla*KPC, *bla*NDM, *bla*VIM, *bla*OXA-48, and *bla*IMP gene sequences is dependent on the number of organisms present in the sample, reliable results are dependent on proper sample handling and storage.
13. Testing with the Xpert Carba-R Assay should be used as an adjunct to other available methods.
14. Xpert Carba-R Assay results may sometimes be INVALID due to a failed SPC control, or result in an ERROR or NO RESULT, and require retesting that can lead to a delay in obtaining final results.
15. The interpretation of test results requires trained clinical personnel who should use judgment, knowledge, and any additional testing/ information necessary before reporting.

References

Package Insert, Xpert® Carba-R, Cepheid, Sunnyvale, California, 301-2438, Rev. A March 2016.

Package Insert, External Controls Carba_R Microbiolologic Helix Elite Sept 7th 2016

Related Documents

601.U.488 Xpert Carba-R Initial Kit Lot QC sheet
601.U.489 Xpert Carba-R Monthly Lot QC Sheet
601.U.115 Carbapenem-Resistant Gram-Negative Rod Reporting and Surveillance procedure

Carba-R PCR Test Reference Card

Patient Isolate testing

1. Make a 0.5 McFarland of the isolate to be tested from a BA or Mac plate.
2. Clean BSC with bleach and 70% ETOH. Change gloves.
3. In BSC use a 10uL loop to inoculate a vial of Sample Reagent.
4. Vortex vial.
5. Using transfer pipette in the kit, transfer 1.7mL (to the line on pipette) into the large opening of the cartridge, and close lid of cartridge.
6. Program run: click on Create Test-> manual entry-> enter patient name and accession number.
7. Abort Query-> click ok-> scan cartridge barcode-> Start Test. Place the cartridge in the instrument and close door. When the test is finished green light will turn off. Discard the cartridge.
8. Clean BSC.
9. Log internal QC and test results onto CIM QC sheet.

Carba-R Initial Lot and Monthly QC-Positive and Negative controls

1. Clean BSC with bleach and 70% ETOH, change gloves.
2. Prepare one control at a time, label 2 cartridges and 2 sample vials with “pos or neg control”
3. Remove swab from Microbiologics Xpert Carba-R pouch. Insert into a sample reagent vial and break the swab by snapping the shaft.
4. Cap the vial and vortex for 10 sec.
5. Using transfer pipette in the kit, transfer 1.7mL (to the line on pipette) into the large opening of the cartridge, and close lid of cartridge.
6. Program runs similar for patient testing: at please scan sample ID barcode enter either:
   - Initial Pos Control 1 or Initial Neg Control 1 (for new lot QC) - or -
   - Monthly Pos Control 2 or Monthly Neg Control 2 (for Monthly QC)
7. In test type field select either:
   - Positive control 1 or Negative Control 1 (for new lot QC) -or-
   - Negative control 2 or Negative Control 2 (for monthly lot QC)
8. Scan cartridge barcode and start run.