BioGX
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Manual

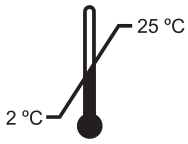
Carbapenem Resistance KNO – *OSR for BD MAX™*

Version 05

REF 400-015-C-MAX

Σ 24 reactions

For *In Vitro* Diagnostic Use
For use with BD MAX™ Open System Reagents on the BD MAX™ System

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PROPRIETARY NAME

BioGX Carbapenem Resistance KNO – OSR for BD MAX™

INTENDED USE

The BioGX Carbapenem Resistance KNO – OSR for BD MAX™ is an automated *in vitro* diagnostic test reagent. The open system reagent (OSR) is used for qualitative real-time PCR multiplex detection of DNA from the bla_{KPC}, bla_{NDM-1}, and bla_{OXA-48} resistance genes, and a DNA sample processing control (abbreviated as KNO and SPC, respectively). The assay is run on direct stool, Copan eSwab™, or Copan FecalSwab™ samples collected from individuals at risk for the presence of *Enterobacteriaceae* and other organisms resistant to carbapenem. Automated extraction of the sample DNA is done using the BD MAX™ ExK™ DNA-2 series extraction kits. The extraction kit contains the SPC DNA, which serves as both an extraction control and an internal amplification control (IAC), and thus precludes external addition of SPC. The multiplex PCR mix is provided in BioGX proprietary Sample-Ready™ lyophilized format and contains all PCR primers, probes, enzyme, dNTPs, $MgCl_2$, buffers, and other components required for real-time PCR-based analysis of one sample.

SUMMARY AND EXPLANATION

Carbapenemase Producing Enterobacteriaceae/Organisms (CPE/CPO, respectively) are bacteria that are difficult to treat because of multi-drug resistance that is associated with high mortality rates, often estimated at 40% or higher. Resistance to broad-spectrum carbapenem antibiotics stems from production of carbapenemases such as KPC, NDM, IMP, GES, VIM, and OXA-48. These enzymes are capable of breaking down almost all β-lactams, including the carbapenems, and rendering them ineffective. The majority of the genes encoding for carbapenemases are plasmid-mediated which allows for horizontal transmission among a variety of bacterial species and genera contributing to the aggressive spread of resistant organisms. Carbapenem is considered to be a “last-resort” antibiotic to treat multi-drug resistant infections.

Carbapenem resistant organisms can be found worldwide but infections usually occur in hospitals, nursing homes, and other healthcare settings and are not common amongst healthy individuals. Patients whose care requires devices like ventilators, urinary catheters, or intravenous catheters, and patients who are taking long courses of certain antibiotics are most at risk for infection. Infections with carbapenem resistant organisms can be very difficult to treat, and can be deadly which highlights the importance of controlling the spread of these organisms. Utilizing molecular testing for the detection of carbapenem resistant

organisms is an advantageous method for identifying and controlling the spread of these organisms.

The BioGX Carbapenem Resistance KNO – OSR for BD MAX™ is a real time multiplex qualitative *in vitro* test reagent intended to be used by laboratory personnel trained in the use of the BD MAX™ automated real-time PCR system. The test is intended to aid in the diagnosis of carbapenem resistant infection by detecting the presence of carbapenem resistant target DNA extracted from direct stool, Copan eSwab™, or Copan FecalSwab™ samples collected from individuals at risk of infection.

PRINCIPLES OF THE PROCEDURE

The BioGX Carbapenem Resistance KNO – OSR for BD MAX™ is to be used with the BD MAX™ Open System for automated patient sample processing and molecular analysis. The BD MAX™ System uses a combination of lytic and extraction reagents to perform cell lysis and nucleic acid extraction. Following enzymatic cell lysis at elevated temperature, the released nucleic acids are captured by magnetic affinity beads. To control for extraction efficiency, a DNA Sample Processing Control is included in each BD MAX™ DNA Extraction Tube. The beads with bound nucleic acids are washed and the nucleic acids are eluted by heat in an elution buffer. The eluted nucleic acid is then mixed with the BioGX Rehydration Buffer, which is then transferred to the BioGX Sample-Ready™ lyophilized master mix tube in order to rehydrate the Sample-Ready™ lyophilized master mix. The rehydrated mix of amplification reagent and nucleic acid is then dispensed into the BD MAX™ PCR Cartridge. Microvalves in the BD MAX™ PCR Cartridge are sealed by the system prior to initiating PCR to prevent evaporation and amplicon contamination.

The amplified DNA targets are detected using hydrolysis probes labeled at one end with a fluorescent reporter dye (fluorophore) and at the other end with a quencher moiety. Probes labeled with different fluorophores are used to detect specific amplicons originating from bla_{KPC}, bla_{NDM-1}, and bla_{OXA-48} and a Sample Processing Control in four different optical channels of the BD MAX™ System: bla_{KPC} amplicons are detected in the 475/520 channel, bla_{NDM-1} amplicons are detected in the 530/565 channel, bla_{OXA-48} amplicons are detected in the 585/630 channel, and the DNA Sample Processing Control is detected in the 680/715 channel. When the probes are in their native state, the fluorescence of the fluorophore is quenched due to its proximity to the quencher. However, in the presence of their specific target cDNA, the probes hybridize to their complementary sequences and are hydrolyzed by the 5'-3' exonuclease activity of the DNA polymerase as it synthesizes the nascent strand along the DNA template. As a result, the fluorophores are separated from their quencher

molecules and fluorescence is emitted. The amount of fluorescence detected in the four optical channels used for the BioGX Carbapenem Resistance KNO – OSR for BD MAX™ is directly proportional to the quantity of the corresponding probe that is hydrolyzed, and therefore proportional to the amount of synthesized target. The BD MAX™ System measures these signals at the end of each amplification cycle in real time, and interprets the data to provide a qualitative result for each of the above targets.

REAGENTS

Qty	REF	Contents	Tests
2	400-015-MAX	**BioGX Carbapenem Resistance KNO - OSR for BD MAX™** Sample-Ready™ lyophilized PCR Master Mix containing polymerase, nucleotides, specific molecular primers and probes, Sample Processing Control-specific molecular primers and probe.	12 tests per pouch
1	800-028-C	**Rehydration Buffer Tube (C) Open System Reagents for BD MAX™** Reagent tube containing a rehydration buffer for use in Lyophilized PCR Master Mix rehydration.	24 tests per pouch

NOTE: Safety Data Sheets (SDS) are available at www.biogx.com/eu or by request.

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- BD MAX™ ExK™ DNA-2 (BD catalog no. 442820)
  Extraction Kits include Sample Buffer Tubes (SBT), Septum Caps, Extraction Tubes, and Unitized Reagent Strips sufficient for 24 tests.
- BD MAX™ PCR Cartridges (BD catalog no. 437519)
- Direct Stool collection device, Copan eSwab™, or Copan FecalSwab™.
- Vortex Genie 2 Vortexer (VWR catalog no. 58815-234) or equivalent.
- Disposable nitrile gloves.

WARNINGS AND PRECAUTIONS

- Treat all biological specimens, including used Extraction Kits and PCR Cartridges, as if capable of transmitting infectious agents in accordance with safe laboratory procedures such as those described in CLSI Document M29[1] and in Biosafety in Microbiological and Biomedical Laboratories.[2]
- Performance characteristics of this test have been established only with the specimen types listed in “Intended Use” section. The performance of this assay with other specimen types or samples has not been evaluated.
- Do not use the reagents if the protective pouches are open or torn upon arrival.
- Close reagent protective pouches promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing and store at 2-25 °C.

- Do not remove desiccant from the PCR Master Mix pouches.
- Do not use Master Mix if the desiccant is not present or is broken inside the Master Mix pouches.
- Do not use reagent tubes if the foil seal has been opened or damaged.
- Do not mix reagents from different pouches and/or kits and/or lots.
- Do not use expired reagents and/or materials.
- Each Master Mix and Rehydration Buffer tube is used to process a single sample. Do not reuse Master Mix or Rehydration Buffer tubes.
- Refer to BD MAX™ ExK™ DNA-2 Extraction Kit Instructions for information about proper handling, cautions, and proper waste disposal.
- Do not mix septum caps between Sample Buffer Tubes or re-use septum caps as contamination may occur and compromise test results.
- Check BD Unitized Reagent Strips for proper liquid fills (ensure that the liquids are at the bottom of the tubes).
- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where specimens or kits are being handled.
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state, and local regulations.
- Use clean gloves when handling extraction kit components and PCR reagents and buffer tubes.

STORAGE AND STABILITY

- BioGX recommends long-term storage at 2-25°C.

- Reagents have been tested to demonstrate optimal performance when stored properly and consumed by the Expiration Date. Long-term stability studies are ongoing and the Expiration Date will be amended as additional data is available.

- Avoid exposing the reagents (lyophilized or rehydrated) to direct sunlight or long-term ambient lighting.
- Tightly reseal the pouch with unused reactions and immediately store the pouch in a dry location after opening.

- Avoid exposure to moisture and use the entire contents of the opened pouch within 1 month.

INSTRUCTIONS FOR USE

Install the BioGX Electronic User Defined Protocol on the BD MAX™

It will be necessary to import an Electronic User Defined Protocol (eUDP) onto the BD MAX™. The most current eUDP is available for download on **www.biogx.com/eu** by clicking on “Product Documentation” and selecting the appropriate platform and product

name. eUDPs can also be obtained by emailing BioGX at **eu@biogx.com**. Please refer to the BD MAX™ user manual for uploading instructions**.**

NOTE: eUDPs are specific to extraction kit type and are programmed to be used with 3-snap strips unless otherwise indicated. If a 4-snap strip is used it is necessary to modify the eUDP to a 4-snap program by opening the eUDP in "Test Editor" and selecting the corresponding 4-snap extraction strip type in the "Extraction Type" drop down.

Specimen Collection/Transport

Stool specimens should be collected, transported, and stored according to institutional and laboratory standard operating procedures.

Specimen Preparation

Direct Stool Processing

Collect a **10 µL** loopful of direct stool and add to the SBT. Do not add more than approximately 10 µL of direct stool to the SBT, excessive stool matrix can introduce extraction and/or PCR inhibitors.

Copan eSwab™, or Copan FecalSwab™ Processing

Carefully remove the collection swab from the eSwab™ collection tube or Fecal Swab™ collection tube and express the tip against the inside of the tube. Transfer **50 µL** of the ESwab™ Liquid Amies solution OR **50 µL** FecalSwab™ Cary-Blair solution to the SBT.

The end user may choose to validate a different pretreatment method or volume of sample/specimen to load other than the suggested sample processing outlined below. If different sample/specimen volumes are deemed appropriate for the existing workflow of the laboratory it will be necessary to modify Guardrail parameters accordingly. BioGX does not make claims for processing methods or sample types other than those described in this product insert.

Other Sample Types

⚠ This assay has been optimized for use with the sample types and volumes described above. Use of any other specimen type, collection method, or sample volumes may be inhibitory to the PCR or disrupt extraction without appropriate Guardrail and processing volume adjustments. BioGX does not make claims for processing methods or sample types other than those described in this product insert.

Setting up the Unitized Reagent Strip on the BD MAX™

1. Wear nitrile gloves when handling Sample-Ready™ lyophilized reagents to reduce the generation of static charges. DO NOT use latex gloves.
2. Use only BD MAX™ ExK™ DNA-2 extraction kits with the BioGX Carbapenem Resistance KNO – OSR for BD MAX™. DO NOT use BD MAX™ mastermix or the blank 0.3 mL conical tubes from the BD MAX™ ExK™ DNA-2 extraction kit.
3. Load one extraction cartridge into the extraction tray per specimen to be tested.
4. Snap one BD MAX™ ExK™ DNA-2 Extraction Tube into position 1 (Snap-in 1) of each Unitized Reagent Strip. (See Figure 1)

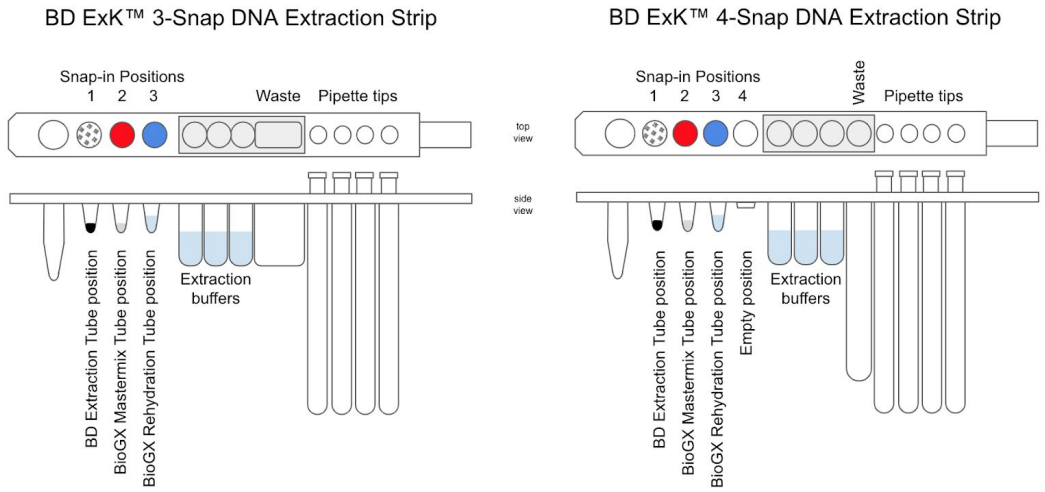

Figure 1 – Diagram of BD MAX™ ExK™ 3-snap and 4-snap Unitized Reagent Strips

5. Snap one BioGX Sample-Ready™ lyophilized PCR Master Mix reagent tube into position 2 (Snap-in 2) of each Unitized Reagent Strip. Check to make sure the Sample-Ready™ lyophilized cake is at bottom of tube prior to inserting into Unitized Reagent Strip. The funnel-shaped cake may be in any orientation (v, >, ^, <) in the ***bottom*** of the tube.
6. Snap one BioGX Rehydration Buffer tube into position 3 (Snap-in 3) of each Unitized Reagent Strip. Check to make sure the buffer is at bottom of tube prior to inserting into Unitized Reagent Strip.
7. Lift the tray and briefly examine the bottom of each Unitized Reagent Strip to ensure all reagents are at the bottom of each tube.
8. Proceed with worklist generation and sample loading per BD MAX™ operating instructions. Select the appropriate User Defined Protocol (eUDP) provided by BioGX. eUDPs are specific to extraction kit type and are programmed to be used with 3-snap strips unless otherwise indicated. If a 4-snap strip is used it is necessary to modify the eUDP to a 4-snap program by opening

the eUDP in "Test Editor" and selecting the corresponding 4-snap extraction strip type in the "Extraction Type" drop down.

9. Load the extraction tray and, if necessary, a new PCR card into the instrument, close the door, and click "Start Run."

NOTE: Always first insert all Snap 1 tubes, then all Snap 2 tubes, then all Snap 3 tubes into the Unitized Reagent Strip.

NOTE: If using a 4-snap extraction strip, snap-in position 4 will remain empty.

QUALITY CONTROL

CONTROL

Each BioGX Carbapenem Resistance KNO - OSR for BD MAX™ includes molecular primers and probes specific for the detection of the DNA sample processing control (SPC) present in the BD MAX™ ExK™ DNA-2 Extraction Kit. No external addition of SPC is required. The SPC serves as both a sample extraction control and a PCR internal amplification control (IAC).

RESULTS INTERPRETATION

Results are available on the *Results* tab in the *Results* window on the BD MAX™ System monitor. The BD MAX™ System software automatically interprets the test result when the BioGX eUDP is used. Possible results for each target are shown in Table 1. Presence of one or more of the targets is possible, and will result in multiple targets being positive at once.

Results	Interpretation
KPC POSITIVE	• The bla_{KPC} target has a Ct within the valid range and endpoint above the minimum setting.
NDM-1 POSITIVE	• The bla_{NDM-1} target has a Ct within the valid range and endpoint above the minimum setting.
OXA-48 POSITIVE	• The bla_{OXA-48} target has a Ct within the valid range and endpoint above the minimum setting.
KPC NEGATIVE, **NDM-1 NEGATIVE,** OR **OXA-48 NEGATIVE**	• The respective target did not amplify and the SPC has a Ct within the valid range and endpoint above the minimum setting.
UNR	• Unresolved Result. No target amplification; No SPC amplification.

IND	• Indeterminate due to BD MAX™ System failure (with Warning or Error Codes*)
INC	• Incomplete Run (with Warning or Error Codes*)

*Refer to the "Troubleshooting section of the BD MAX™ System User's Manual for interpretation of warning and error codes.

NOTE: In the presence of a high concentration positive result for any target, the SPC may or may not amplify. This is normal.

REPEAT TEST PROCEDURE

In case of instrument failure, repeat testing can be performed by setting up a new run using the original sample/specimen and a fresh SBT as described above in the Specimen Preparation section.

LIMITATIONS OF THE PROCEDURE

- This product is intended for use with specimens collected using specimen collection and transport devices listed in the "Equipment and Materials Required But Not Provided" section.
- This product should only be used with BD MAX™ Open System Reagents on the BD MAX™ System.
- Incorrect test results may occur from improper specimen collection, handling or storage, technical error, sample mix-up or because the number of organisms in the specimen is below the analytical sensitivity of the test. Careful compliance with the package insert instructions and the BD MAX™ System User's Manual are necessary to avoid erroneous results.
- Good laboratory technique is essential for the proper performance of this assay. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of all materials and reagents.
- A positive test result does not necessarily indicate the presence of viable infectious organisms. A positive result is indicative of the presence of target nucleic acid.
- As with all PCR-based *in vitro* diagnostic tests, extremely low levels of target below the limit of detection of the assay may be detected, but results may not be reproducible.
- False negative results may occur due to loss of nucleic acid from inadequate collection, transport or storage of specimens, or due to a inadequate cell lysis and/or extraction. The Sample Processing Control has been added to the test to aid in the identification of specimens that contain inhibitors to PCR amplification and as a control for reagent integrity and of the assay system as a whole. The Sample Processing Control does not indicate if nucleic acid has been lost due to

inadequate collection, transport or storage of specimens, or if cells have been adequately lysed.

- The BioGX Carbapenem Resistance KNO – OSR for BD MAX™ results may sometimes be Unresolved due to an invalid Sample Processing Control, or be Indeterminate or Incomplete due to instrument failure, and require retesting that can lead to a delay obtaining final results.
- Mutations or polymorphisms in primer- or probe-binding regions may affect detection of new or unknown bla_{KPC}, bla_{NDM-1}, and bla_{OXA-48} resulting in a false negative result with the BioGX Carbapenem Resistance KNO – OSR for BD MAX™
- The BioGX Carbapenem Resistance KNO – OSR for BD MAX™ requires the use of four (4) optical channels from the BD MAX™ System: 475/520 channel, 530/565 channel, 585/630 channel, and 680/715 channel.

PERFORMANCE CHARACTERISTICS

Analytical and Diagnostic Specificity

Specificity was determined by running negative sample matrix (Direct stool, eSwab, and FecalSwab) spiked with positive control template. The Carbapenem Resistance KNO - OSR for BD MAX™ was positive for KPC, NDM-1, and OXA-48.

The BioGX Carbapenem Resistance KNO - OSR for BD MAX™ was run with ATCC MSA-1002 (20 Strain Even Mix Genomic Material) which does not contain organisms containing bla_{KPC}, bla_{NDM-1}, or bla_{OXA-48}. Results were negative for KPC, NDM-1, and OXA-48.

The Carbapenem Resistance KNO - OSR for BD MAX™ was tested against the QCMD 2016 Extended Spectrum β-lactamase and Carbapenemase EQA Programme. All samples reported out were concordant with the expected result except for one sample that was suspected to be environmental contamination.

QCMD Extended Spectrum β-lactamase and Carbapenemase EQA Programme Results

Sample and Carbapenemase Types	Expected Result	Result
Klebsiella pneumoniae NDM-1 OXA-232 (OXA-48 like)	OXA-48 positive	100% concordant
Klebsiella pneumoniae OXA-48	OXA-48 positive	100% concordant
Klebsiella pneumoniae VIM-1	Negative	100% concordant
Klebsiella pneumoniae KPC-2	KPC positive	100% concordant
Escherichia coli	Negative	100% concordant
Pseudomonas aeruginosa IMP-13	Negative	100% concordant

Enterobacter cloacae OXA-48	OXA-48 positive	100% concordant
Proteus vulgaris	Negative	OXA-48 positive*

*OXA-48 amplification was recorded at Ct of 36.3. The late amplification in the OXA-48 optical channel is beyond the Limit of Detection for this target.

To determine the inclusivity of the OXA types, *in silico* analysis was performed on the primer and probe sequences for the assay against available OXA sequences in the NCBI BLAST tool. The following OXA types have reported sequences (GENbank) that are expected to be detected by the assay with 100% coverage of the full length amplicon and 97-100% identity with no primer or probe mismatches: 48, 48b, 62, 162, 163, 181, 204, 244, 245, 247, 370, 405, 416, 438, 439, 514, 517, 519, 567. The following OXA types have reported sequences are detected with less than 100% coverage (48-90% of the full length amplicon) and 97-99% identify (mismatches outside of the primer and probe binding region: 1, 48, 48b, 181, 199, 232, 244, 252, 484, 515, 547.

Analytical and Diagnostic Sensitivity

The analytical sensitivity for the BioGX Carbapenem Resistance KNO - OSR for BD MAX™ was determined as follows: Dilution series of positive synthetic DNA samples for each target were added to the SBT in duplicate. Analytical sensitivity (Limit of Detection, LOD) was defined as the lowest concentration at which 95% of all replicates tested positive.

Target	LOD (copies per SBT)
bla_{KPC}	15
bla_{NDM-1}	51
bla_{OXA-48}	22

Analytical sensitivity during co-infection was tested by challenging the BioGX Carbapenem Resistance KNO - OSR for BD MAX™ in pairs of high concentration (10,000X LOD) of one target against low concentration (3X LOD) of another for all possible pairs in the test. All low concentration targets were positive and were not outcompeted by amplification of the pair of high concentration targets.

Reproducibility

The reproducibility study was performed on bla_{NDM-1} synthetic target template by three separate technicians independently on two BD MAX™ instruments. All users obtained equivalent results.

Manufacturing Reproducibility

Two independent lots were manufactured and were found to be equivalent based on internally established QC acceptance procedures.

REFERENCES

1. Clinical and Laboratory Standards Institute. Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (Refer to the latest edition).
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5. Pfeifer Y. et. al., Emergence of OXA-48-Type Carbapenemase-Producing *Enterobacteriaceae* in German Hospitals, Antimicrob Agents Chemo. 2012 Apr; 56(4): 2125-2128.
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8. Jacobs DM, Safir MC, Huang D, Minhaj F, Parker A, and Rao GG, Triple combination antibiotic therapy for carbapenemase-producing *Klebsiella pneumoniae:* a systematic review, *Ann Clin Microbiol Antimicrob* (2017) 16:76.

REVISION HISTORY

Version	Date	Description of Change
05	01 FEB 2019	Updated storage recommendations from 2-8°C to 2-25°C.
04	09 NOV 2018	Added use of BD ExK 4-snap and additional inclusivity for OXA.
03	30 AUG 2018	Updated reagents section to reflect new packaging. Added new performance data.
02	20 JUN 2018	Updated open pouch stability.
01	02 APR 2018	Initial Release.

SYMBOLS

Symbol	Meaning
	Catalog number
	*In vitro* diagnostic medical device
	Do not reuse
	Batch code
	Caution
	Consult instructions for use
	Manufacturer
	Contains sufficient for <n> tests
	Authorized Representative in the European Community
	Temperature limitation
	Keep dry
	Keep away from sunlight
	Expiration date
	Biological Risks
	Control

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