STANDARD M10 CARBA

STANDARD™ M10 CARBA

REF M10-CAR-01

INSTRUCTIONS FOR USE

For use with STANDARD™ M10 system

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SD BIOSENSOR

Contents

1. Intended Purpose

STANDARD M10 CARBA is a multiplex real-time PCR test intended for use with the automated STANDARD M10 system for the qualitative detection and differentiation of the carbapenemase genes *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{OXA-48}$ and *bla*$_{GES}$ in rectal swab specimens from patients at risk of intestinal colonization with carbapenem-non-susceptible bacteria.

This test is designed to aid in the diagnosis of colonization or infection with carbapenem-non-susceptible bacteria in patients. It is not intended to guide or monitor treatment for such infections. The positive results are indicative of the presence of the carbapenemase gene sequence and negative result does not preclude the presence of other resistance mechanisms.

This test is intended for use in conjunction with clinical presentation, laboratory findings, and epidemiological information. Results of this test should not be used as the sole basis for patient management decisions. Concomitant cultures are necessary to recover organisms for epidemiological typing, antimicrobial susceptibility testing, and for further confirmatory bacterial identification. This test is intended for use by trained professionals within hospital laboratories or clinical diagnostic laboratories, and not for near-patient testing environments.

2. Summary and Explanation

Carbapenem-resistant organisms (CROs), including carbapenem-resistant *Enterobacteriaceae* (CRE), *Pseudomonas aeruginosa*, and *Acinetobacter* species, are a group of bacteria that are resistant to at least one carbapenem antibiotics, such as doripenem, imipenem, meropenem, or ertapenem. These bacteria are difficult to treat and can cause healthcare-associated infections. In case bacteria have acquired resistance to carbapenem antibiotics, CROs often display multidrug resistance across several other classes, severely limiting treatment options and leading to increased patient morbidity and mortality. Since this is directly related to the patient's recovery rate, management of CRO infection is very important. The global spread of carbapenem resistance is facilitated by mobile genetic elements such as plasmids that carry carbapenemase genes, allowing horizontal transfer between bacterial species. Certain carbapenemase-producing strains, like *K. pneumoniae* ST258 carrying *bla*$_{KPC}$, have caused widespread outbreaks in the United States and Israel. Similarly, *bla*$_{NDM}$-producing organisms have emerged in South Asia and spread to Europe and beyond. Additional resistance mechanisms involving *bla*$_{VIM}$ and *bla*$_{IMP}$ have been concerns in Europe and Asia, while *bla*$_{OXA-48}$-mediated resistance is rapidly expanding across Europe.

β-lactamase are classified into four classes A-D by Ambler. Among them, class A, B and D are defined as carbapenemases : class A (serine carbapenemases), class B (metallo-β-lactamases), and class D (oxacillinase carbapenemases). Class A include *Klebsiella pneumoniae* carbapenemase (KPC) and Guiana extended-spectrum β-lactamase (GES), class B include Imipenemase (IMP), Verona integrin-encoded metallo-β-lactamase (VIM), New Delhi metallo-β-lactamase (NDM), and class D include oxacillinase-48 (OXA-48).

Currently the standard method for detecting patients who are colonized with carbapenem-non-susceptible organisms is to use culture-based disk diffusion or broth microdilution. However, these methods are laborious and may require several days to generate a final results. Moreover, when culture results indicate carbapenem resistance, further molecular testing such as PCR is recommended to determine the specific genotype of the carbapenemase gene responsible for resistance. This step is critical to distinguish between different resistance mechanisms and to guide appropriate infection control and treatment strategies. STANDARD M10 CARBA targets six clinically important carbapenemase genes : *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{OXA-48}$ and *bla*$_{GES}$. Rapid and accurate detection of carbapenemase genes directly is needed for several reasons. First, it helps early identification of colonized patients, which may lead to better infection control interventions, including patient isolations and contact precautions. Second, it helps in therapeutic decision-making by distinguishing between carbapenemase-mediated resistance and other mechanisms, enabling appropriate antimicrobial selection. Third, it supports epidemiological tracking and containment during outbreak investigations.

[Cartridge Description]

STANDARD M10 CARBA is a molecular *in vitro* diagnostic assay that aids in the detection of carbapenemase genes *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{OXA-48}$ and *bla*$_{GES}$ based on nucleic acid amplification technology, Real-Time PCR. STANDARD M10 CARBA cartridge contains bacterial DNA extraction buffers and Real-Time PCR reagents for the *in vitro* qualitative detection of carbapenemase genes in rectal swab specimens.

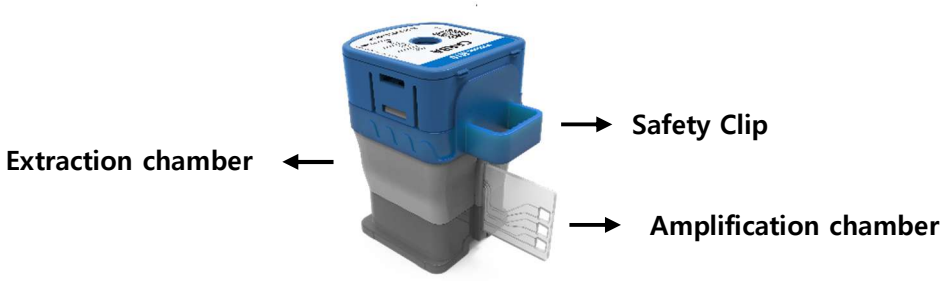

Figure 1. Layout of STANDARD™ M10 CARBA cartridge

3. Principle of the Procedure

STANDARD M10 CARBA test is an automated *in vitro* diagnostic molecular test for qualitative detection of nucleic acid from patients at risk of intestinal colonization with carbapenem-non-susceptible bacteria. STANDARD M10 CARBA test is performed on STANDARD M10 system.

STANDARD M10 system automates and integrates sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in various specimens using molecular diagnostic assays. The system consists of STANDARD M10 Module and STANDARD M10 Console with preloaded software for running tests and viewing the results. The system requires the use of single-use disposable cartridges that hold the Real-Time PCR reagents and host the Real-Time PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, see STANDARD M10 User Manual.

STANDARD M10 CARBA test includes reagents for the detection of carbapenemase genes bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{OXA-48} and bla_{GES} in rectal swab specimens. The cartridge is present to control for adequate processing of the sample and Real-Time PCR reaction.

The table below shows the target that each channel is designed to detect.

Table 1. Fluorescent channel of each target gene

Target	Channel
VIM	FAM
KPC	HEX
NDM	FAM
OXA-48	HEX
IMP	FAM
GES	HEX
Internal control (IC)	Cy5

4. Active Ingredients

- Guanidine Thiocyanate 0.47g
- Taq DNA Polymerase 110 U/bead
- Target primers and probes 2-30 pmol

5. Materials Provided

STANDARD M10 CARBA contains sufficient reagents to process 10 specimens or quality control samples.

Table 2. Contents of STANDARD M10 CARBA

No.	Contents	Quantity	Usage in each reaction
1	Cartridge	10	1
2	STANDARD™ M10 CARBA Sample Buffer	10	1
3	Nozzle Cap	10	1
4	STANDARD™ Fixed volume dropper (300㎕)	10	1
5	Quick Reference Instructions	1	-

6. Storage and Handling

Store the STANDARD M10 CARBA at a temperature range of 2-28 °C (36-82 °F). If the components of the kit have been stored under refrigerated conditions, perform the test after stabilizing all components at 15-30 °C (59-86 °F) for at least 30 minutes. Do not remove the Safety Clip of the cartridge and do not press the cartridge until actual use. Do not use a cartridge that has leaked or is wet. Under these conditions, cartridges can be stored until the expiration date printed on the packaging.

7. Materials Required but Not Provided

- STANDARD M10 system with User Manual
  At least one STANDARD M10 Console (Cat. No. 11M1011) and one STANDARD M10 Module (Cat. No. 11M1012)
- Commercially available sterilized transport medium for collecting rectal swab
  - Copan ESwab® (Copan Italia S.p.A, 480CE)
  - Transport Medium (ASAN Pharmaceutical, AM608-01)
  - Copan Transystem™ (Copan Italia S.p.A, 134C)
- Sample transfer pipette
  - Micropipette with filter tips
- PPE (Personal Protective Equipment)
- Biohazard container

8. Warnings and Precautions

1) This kit is only for *in vitro diagnosis* use.
2) For professionals use only.
3) Please read the Instructions for Use carefully before testing.
4) Improper specimen collection, transfer, storage, and processing may cause erroneous test results.
5) Keep the cartridge away from UV/sunlight and keep it in a dry condition with the silver foil pouch closed.
6) Do not remove the Safety Clip of the cartridge before use.
7) Do not press the cartridge until actual use.
8) Press (punch) the cartridge and ensure that the cartridge lid (Top cover) is fully engaged with the cartridge body before dispensing the sample.
9) Insert the tip or the supplied dropper deeply into the sample port to ensure that the sample is dispensed accurately.
10) Do not use a cartridge that has leaked or is wet.
11) Do not use the kit after its expiration date.
12) Do not shake, tilt, or invert the cartridge, especially after pressing the cartridge to punch the seal. It may yield invalid or false test results.
13) Do not use a cartridge with a damaged barcode label.
14) Do not reuse processed cartridges.
15) All patient samples should be handled as if these samples are infectious.
16) All materials should be considered potentially infectious and should be handled with precautions.
17) As this test involves extraction of bacterial DNA and PCR amplification, care should be taken to avoid contamination.

Regular monitoring of laboratory contamination is recommended.

18) When using this kit, it should be operated strictly in accordance with the instructions and follow the technical requirements of the clinical gene amplification laboratory.
19) Follow your institution's environmental waste procedures for proper disposal of used cartridges.
20) Results of this test cannot be diagnosed as the sole basis for patient management decisions, and the confirmed diagnosis must be made by a specialist considering further tests or clinical results.
21) Any serious incident that has occurred in relation to this kit shall be reported to the manufacturer or its authorized representative, and to the competent authority of the Member State in which the user and/or the patient is established, in accordance with the requirements of Regulation (EU) 2017/746.
22) The Summary of Safety and Performance is available on EUDAMED database.

9. Chemical Hazards

This kit contains components classified as follows in accordance with the regulation (EC) No. 1272/2008:

*** Hazard pictogram:** 

* **Signal word:** Danger

*** Hazard statements:**

- H314 Causes severe skin burns and eye damage
- H318 Causes serious eye damage

*** Precautionary statements:**

1) Prevention

- P260 Do not breathe dust/fume/gas/mist/vapours/spray.
- P264 Wash hands thoroughly after handling.
- P280 Wear protective gloves/protective clothing/eye protection/face protection/hearing protection.

2) Response

- P301+P330+P331 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
- P303+P361+P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
- P304+P340 IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
- P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- P310 Immediately call a POISON CENTER or doctor/physician.
- P321 Specific treatment
- P363 Wash contaminated clothing before reuse.

3) Storage

- P405 Store locked up.

4) Disposal

- P501 Dispose of contents/container in accordance with local/regional/national/international regulation

10. Specimen Collection, Transport, and Storage

Proper specimen collection, transportation, and storage are critical to the performance of the test. Improper specimen collection, inappropriate handling and/or transportation can lead to false results. In order to obtain an adequate specimen, follow your institution's guidelines of collecting rectal swab specimens for STANDARD M10 CARBA testing.

10.1 Specimen collection

Collect and transfer the rectal swab into the commercially available sterilized transport medium.

Use a sterile, commercially available swab designed for rectal specimen collection. Carefully insert the swab about 3 to 5 cm (1-2 inches) into the anal canal. Gently rotate the swab clockwise for 5-10 seconds and withdraw the swab carefully.

- Specimen type: Rectal swab
- Required specimen volume: 300 µL (Liquid transport medium), 1 swab (Solid transport medium)

10.2 Specimen Storage and Transport

- Refrigerated (2–8 °C / 36–46 °F): Specimens may be stored and transported under refrigeration for up to 6 days (liquid medium) and 1 day (solid medium).
- Room temperature (15–25°C / 59–77°F): Specimens may be stored within the temperature of 15–25 °C for up to

24 hours.

- Frozen storage (−20 °C or lower): Specimens are stable up to 6 months when stored at -20℃ or lower. For long-term storage, specimens should be frozen immediately after collection. Avoid repeated freeze-thaw cycles.

11. Procedure

11.1 Preparing the sample

1) Stabilize cartridge and Sample Buffer at 15-30 °C for 30 minutes before testing if the components of the kit have been stored under refrigerated conditions.
2) Rectal swab specimens must be processed by following method.

A. **Solid transport medium**

(1) Remove one swab from the specimen transport medium tube.
(2) Insert the swab into the Sample Buffer, and stir the swab more than 10 times.
(3) Remove the swab while squeezing the sides of the tube to extract the specimen collected from the swab.
(4) Firmly press the Nozzle Cap onto the Sample Buffer tube.
(5) The prepared specimen is used in the process of 11.3.

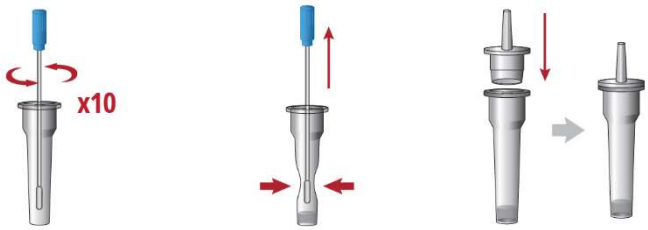

Figure 2. Preparation of the solid transport media

B. **Liquid transport medium**

(1) Ensure that the specimen transport medium tube is securely closed, then invert it 5 times to mix the specimen.
(2) Take 300 µL of the specimen from the transport medium tube using a Fixed volume dropper or micropipette and transfer it into the Sample Buffer tube.
(3) Firmly press the Nozzle Cap onto the Sample Buffer tube.
(4) The prepared specimen is used in the process of 11.3.

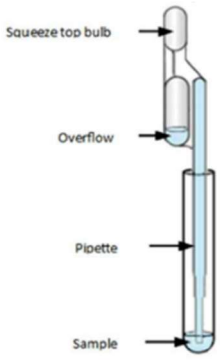

Figure 3. STANDARD™ Fixed volume dropper (300㎕)

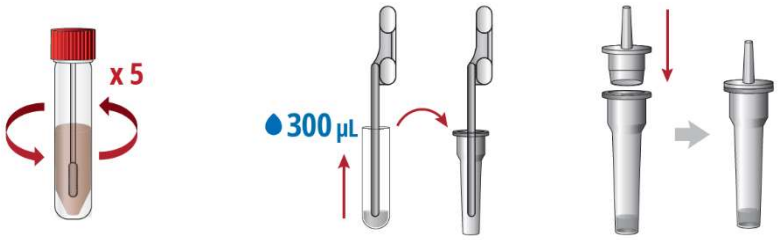

Figure 4. Preparation of the liquid transport media

11.2 Starting STANDARD M10 system

Note	For detailed instructions, refer to STANDARD M10 User Manual. If you have scanned the cartridge barcode in the STANDARD M10 and the software version is not compatible, a 'Not Supported Device' error message appears. Update the software before proceeding the test.

1) Turn on STANDARD M10 system.
2) Check STANDARD M10 Console and the STANDARD M10 Module is connected and working.

Figure 5. Power connection

3) Enter the User ID and Password on the Log In screen of STANDARD M10 Console and click the Log In button.
4) Touch STANDARD M10 Module to run on the Home screen.
(The door of the selected STANDARD M10 Module will automatically open for cartridge loading.)

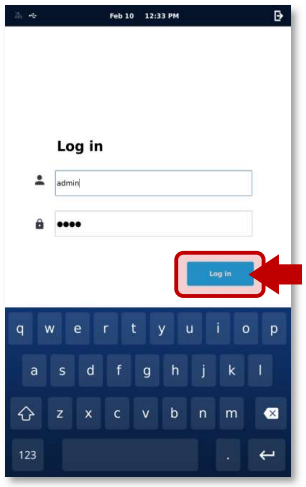

Figure 6. Log In screen

Figure 7. Home screen, Status of M10 module

5) Enter a Patient ID and Sample ID by scanning the barcode or using virtual keyboard on the M10 Console screen.
(Patient ID is optional. You can turn off the Patient ID option from the 'Settings'.)

6) Enter a Sample ID by scanning the barcode of the specimen or using virtual keyboard on the M10 Console screen. Make sure that the specimen tube cap is firmly closed when scanning the ID barcode printed on the specimen tube.
(For quality control test, tick the QC check box.)

Figure 8. Entering Sample ID

Figure 9. Scanning a cartridge

7) Scan STANDARD M10 CARBA cartridge to be used. The STANDARD M10 Console will automatically recognize the assay to be run based on the cartridge barcode.

Note	If you have scanned the cartridge barcode in the STANDARD M10 and the expiration date has expired, an 'Expired Device' error message appears. Check validity period and test with unexpired cartridges.

11.3 Loading a sample into STANDARD M10 CARBA cartridge

⚠ Caution	If the cartridge has been refrigerated, perform the test after stabilizing it for at least 30 minutes at 15-30 °C. Once the sample has been loaded into the cartridge, start the test within 30 minutes.
Note	False negative results may occur if insufficient sample is added into the cartridge.

1) Remove the Safety Clip located underneath the lid of the cartridge.
2) Press down the cartridge to pierce the seal until fully engaged into the cartridge groove.
3) Open the lid and check that the seal is completely punctured before loading a sample.
4) Insert the Nozzle of the Sample Buffer tube into the sample loading hole on the cartridge (upper right corner), then squeeze the tube to load the entire specimen sample into the cartridge. (Sample prepared by the method described in 11.1)

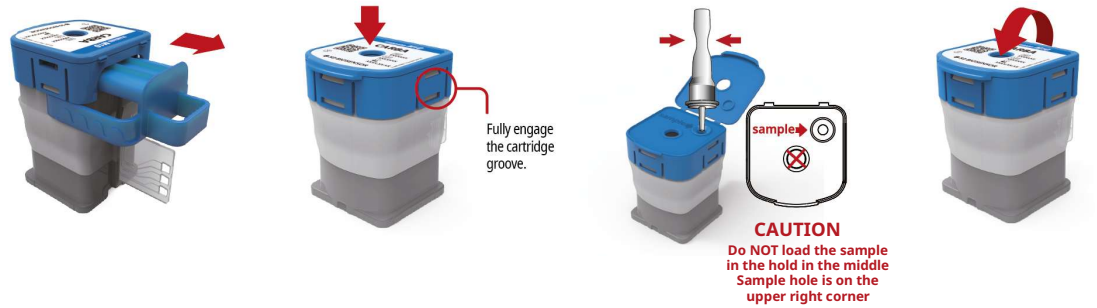

Figure 10. Loading a sample

5) After a few seconds, Sample Guide screen will automatically change to the Insert Cartridge screen. Touch the Sample Guide screen if you want to skip the guide.
6) Close the lid.

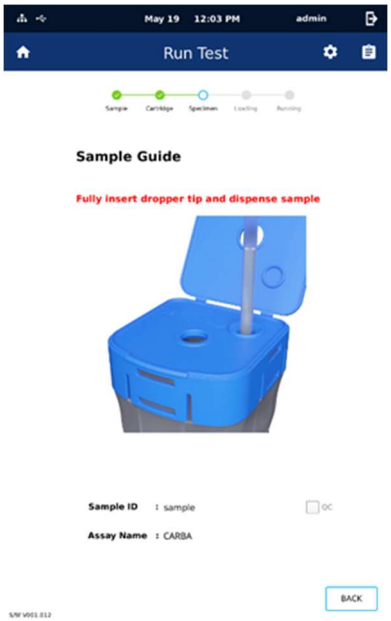

Figure 11. Sample Guide Screen

Figure 12. Insert Cartridge screen

11.4 Running a test

1) Load the cartridge on the selected STANDARD M10 Module with the Amplification chamber facing the inside of the module (The status indicator of the selected module will blink green).
2) Close the door completely.
3) After confirming the sample and cartridge information, touch the OK button on the screen (Touch the Reset button to re-input the information).
4) Assay starts automatically, and remaining time will appear on the screen.

Figure 13. Confirm the test screen

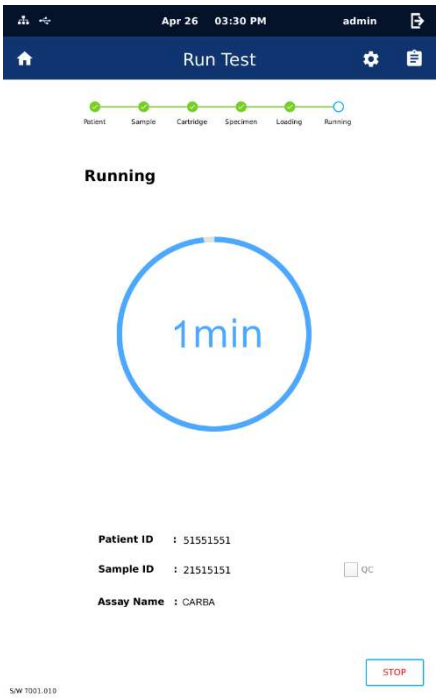

Figure 14. Running screen

5) When the run is finished, it switches to the Review screen and the result is displayed.
6) Dispose the used cartridges in the appropriate biohazard waste container according to your institution's standard practices.
7) To run another test, touch the Home icon and repeat the process (If another STANDARD M10 Module connected to the STANDARD M10 Console is available, you can start a new test while another test is running).

12. Interpretation of Results

The results are interpreted automatically by STANDARD M10 Console and are clearly shown in the Review screen. STANDARD M10 CARBA test provides test results based on the detection of carbapenemase genes; *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{OXA-48}$ and *bla*$_{GES}$ according to the algorithms shown in Table 3-6.

Table 3. Summary of results

Interpretation	VIM	KPC	IC	NDM	OXA-48	IC	IMP	GES	IC
VIM detected	+	-	+/-	-	-	+	-	-	+
KPC detected	-	+	+/-	-	-	+	-	-	+
NDM detected	-	-	+	+	-	+/-	-	-	+
OXA-48 detected	-	-	+	-	+	+/-	-	-	+
IMP detected	-	-	+	-	-	+	+	-	+/-
GES detected	-	-	+	-	-	+	-	+	+/-
Not detected	-	-	+	-	-	+	-	-	+
Invalid	-	-	-	-	-	-	-	-	-
Error	**No result**								

Table 4. Description of results

Outcome (Home screen)	Result (Review screen)	Description
Positive		Target gene is detected.
Negative		Target gene is not detected.
Invalid		All target genes in a same well are not detected and IC signal does not have a Ct value within the valid range. One or more wells exhibit an invalid result, the entire test is considered as invalid.
Error		The test failed because either an error occurred or the test was canceled by the user.

Table 5. Description of IC results

Outcome (Summary screen)	Result (Summary screen)	Description
Valid		IC has a Ct within the valid range. The test was completed. Report positive/negative results of target according to the interpretation shown in **Table 6**. (If the target is detected, it is displayed as 'Valid' regardless of the IC Ct value.)
Invalid		All target genes in a same well are not detected and the IC signal does not have a Ct value within the valid range.
Error		The test failed because either an error occurred or the test was canceled by the user. Repeat the test.

Table 6. Interpretation of results

Result	Interpretation
VIM detected	*bla*$_{VIM}$ target gene is detected. • VIM signal has a Ct value within the valid range. • IC: N/A (not applicable); IC is ignored because VIM amplification occurred.
KPC detected	*bla*$_{KPC}$ target gene is detected. • KPC signal has a Ct value within the valid range. • IC: N/A (not applicable); IC is ignored because KPC amplification occurred.
NDM detected	*bla*$_{NDM}$ target gene is detected. • NDM signal has a Ct value within the valid range. • IC: N/A (not applicable); IC is ignored because NDM amplification occurred.
OXA-48 detected	*bla*$_{OXA-48}$ target gene is detected. • OXA-48 signal has a Ct value within the valid range. • IC: N/A (not applicable); IC is ignored because OXA-48 amplification occurred.
IMP detected	*bla*$_{IMP}$ target gene is detected. • IMP signal has a Ct value within the valid range. • IC: N/A (not applicable); IC is ignored because IMP amplification occurred.
GES detected	*bla*$_{GES}$ target gene is detected. • GES signal has a Ct value within the valid range. • IC: N/A (not applicable); IC is ignored because GES amplification occurred.
Not detected	• *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{OXA-48}$ and *bla*$_{GES}$ target genes are not detected. • IC: Valid; IC has a Ct within the valid range.

Invalid	All target genes in a same well are not detected and the IC does not meet the acceptance criteria. If one or more wells exhibit an invalid result, the entire test must be interpreted as invalid.-Repeat test. • IC: Invalid; IC and DNA signals do not have a Ct within valid range.
Error	The test failed because either an error occurred or the test was canceled by the user. Presence or absence of target nucleic acids cannot be determined. Repeat the test.

Note	- If the IC of the well is negative but any other target in the same well is positive, the results for all targets are considered valid. A high copy number of target-specific gene can lead to reduced or absent IC. - If one or more wells exhibit an invalid result, the entire test must be interpreted as invalid. Please perform a re-test - Multiple carbapenemase gene targets may be detected in a single specimen: VIM, KPC, NDM, OXA-48, IMP, GES. - Detection of GES alone does not distinguish between carbapenemase-producing and non-carbapenemase-producing GES variants. If GES is detected, additional characterization may be required.

13. Quality Control

Quality Control procedures are intended to monitor cartridge and assay performance. If the controls are not valid, the patient results cannot be interpreted.

Internal control(IC): Ensures that a proper sample has been applied, the reagents in the cartridge are well functioning, there are no other interfering factors in the sample, and the procedure has been performed correctly. In clinical samples showing positive signal for VIM, KPC, NDM, OXA-48, IMP and/or GES gene, the IC is reluctant and is ignored. If the IC fails and no 6 type of carbapenemase genes is detected, the result is considered invalid.

External Controls: External controls are available but not provided and may be used in accordance with local, state, and federal accrediting organizations, as applicable.

- Positive control : Carbapenemase-producing bacteria *K. pneumoniae* KPC-2 (catalog number ATCC BAA-1705); *K. pneumoniae* NDM-1 (catalog number ZeptoMetrix 0801906); *K. pneumoniae* VIM-1 (catalog number ZeptoMetrix 0801908); *K. pneumoniae* OXA-48 (catalog number ZeptoMetrix 0801907); *Escherichia coli* IMP-1 (catalog number Microbiologics 01136P); and *P. aeruginosa* GES (catalog number ATCC BAA-3248)
- Negative control : *E.coli* (catalog number ATCC 4157)

Always use external controls as follows: Spike 5uL of external controls into the Sample Buffer and firmly press the Nozzle Cap onto the Sample Buffer tube. After then, insert the Nozzle of the Sample Buffer tube into the sample loading hole on the cartridge (upper right corner), then squeeze the tube to load the entire specimen sample into the cartridge.

14. Performance

14.1 Limit of Detection Test

The analytical sensitivity was evaluated for the detection of carbapenemase genes (blaKPC, blaNDM, blaVIM, blaIMP, blaOXA-48, and blaGES) using 12 types of positive reference materials. The reference materials were serially diluted in negative rectal swab specimens and tested at 5 concentration levels over 3 days using 2 lots, with 20 replicates per concentration level. Based on the experimental results, the Limit of Detection (LoD) was determined using Probit analysis, and the results are presented in Table 7.

Comparison of LoD values obtained using liquid transport medium (Copan eSwab) and solid transport medium (AM608-01) demonstrated differences within approximately 2-fold across all targets. Analytical equivalency between the solid and liquid transport media was confirmed in a separate transport media equivalency study, demonstrating suitability of both transport media for use with the product.

Table 7. Limit of Detection(LoD) of STANDARD M10 CARBA

Target	Strain	LoD (CFU/test) (Solid Medium: AM608-01)	LoD (CFU/test) (Solid Medium: Copan eSwab 480CE)
KPC-2	*E. coli*	128.0	234.0
KPC-19	*K. pneumoniae*	128.01	131.36
NDM-1	*K. pneumoniae*	30.8	35.4
NDM-1	*E. coli*	543.4	719.3
OXA-48	*K. pneumoniae*	519.5	660.3
OXA-232	*E. coli*	27.8	45.0
VIM	*C. freundii*	68.9	86.9
VIM-2	*P. aeruginosa*	647.3	742.4
IMP-6	*P. aeruginosa*	42.2	55.8
IMP-13	*K. pneumoniae*	1023.5	1321.6
GES-5	*K. pneumoniae*	121.6	260.0
GES-24	*P. aeruginosa*	18.6	27.3

14.2 Inclusivity

To evaluate the inclusivity of the product, total of 48 bacterial strains were tested representing 53 carbapenemase gene variants, including 6 strains for KPC, 10 strains for NDM, 19 strains for the OXA-48 family, 9 strains for VIM, 7 strains for IMP, and 2 strains for GES.

Each strain was evaluated at three concentration levels based on the established 1xLoD for the respective target, with five replicates tested at each concentration level. The results demonstrated that the LoD established using representative strains for each target was applicable to diverse gene variants and strains within the same target group. These confirmed the inclusivity of the product across the carbapenemase gene variants evaluated in this study.

Table 8. Strains tested in Inclusivity test

No.	Target	Strain	Manufacturer	Cat. No.
1	KPC-3	*Escherichia coli*	NCCP	16214
2	KPC-2	*Klebsiella pneumoniae*	ATCC	BAA-1705
3	KPC-2	*Klebsiella pneumoniae*	Zeptomatrix	0801886
4	KPC-3	*Klebsiella pneumoniae*	Zeptomatrix	0804295
5	KPC-3	*Klebsiella pneumoniae*	Microbiologics	01117P
6	KPC-4, NDM-1	*Klebsiella pneumoniae*	NCCP	16700
7	NDM-1	*Providencia sp.*	Zeptomatrix	0801906
8	NDM-1	*Pseudomonas aeruginosa*	NCCP	17542
9	NDM-4	*Escherichia coli*	NCCP	16801
10	NDM-5	*Klebsiella pneumoniae*	NCCP	16702
11	NDM-6	*Klebsiella pneumoniae*	NCCP	16704
12	NDM-7	*Escherichia coli*	NCCP	16802
13	OXA-232	*Klebsiella pneumoniae*	NCCP	15864
14	OXA-232	*Klebsiella pneumoniae*	NCCP	16125
15	OXA-232, NDM-1	*Klebsiella pneumoniae*	NCCP	16208
16	OXA-48	*Klebsiella pneumoniae*	NCCP	16126
17	OXA-48	*Klebsiella pneumoniae*	Zeptomatrix	0801907
18	OXA-48	*Escherichia coli*	NCCP	16805
19	OXA-48	*Klebsiella pneumoniae*	NCCP	17324
20	OXA-48	*Klebsiella pneumoniae*	NCCP	17325
21	NDM-1, OXA-181	*Klebsiella pneumoniae*	NCCP	16212
22	OXA-181	*Escherichia coli*	NCCP	16803
23	OXA-181	*Klebsiella pneumoniae*	NCCP	16705
24	NDM-5, OXA-181	*Escherichia coli*	NCCP	NM133
25	OXA-232	*Klebsiella pneumoniae*	NCCP	16053
26	OXA-232	*Escherichia coli*	NCCP	16804
27	OXA-232	*Klebsiella pneumoniae*	NCCP	NMS1118
28	OXA-232	*Klebsiella pneumoniae*	NCCP	NMS1121
29	OXA-48	*Escherichia coli*	ATCC	BAA-2523
30	OXA-48, VIM-4	*Enterobacter cloacae*	ATCC	BAA-3274
31	OXA-232	*Escherichia coli*	ATCC	BAA-3292

32	VIM-2	*Pseudomonas monteilii*	NCCP	16310
33	VIM-1	*Pseudomonas aeruginosa*	Zeptomatrix	0801908
34	VIM-2	*Pseudomonas aeruginosa*	NCCP	16081
35	VIM-3	*Pseudomonas aeruginosa*	NCCP	16105
36	VIM-1	*Klebsiella pneumoniae*	Microbiologics	01112P
37	VIM-1	*Klebsiella pneumoniae*	Microbiologics	01245P
38	VIM-2	*Escherichia coli*	NCCP	16215
39	VIM-1	*Citrobacter freundii*	NCCP	16683
40	IMP-6	*Pseudomonas aeruginosa*	NCCP	16080
41	IMP-6	*Pseudomonas aeruginosa*	NCCP	16084
42	IMP-6	*Pseudomonas aeruginosa*	NCCP	16087
43	IMP-26	*Pseudomonas aeruginosa*	NCCP	16104
44	IMP-10	*Pseudomonas aeruginosa*	NCCP	16331
45	IMP	*Escherichia coli*	Microbiologics	01136P
46	IMP-1	*Pseudomonas aeruginosa*	NCCP	14571
47	GES-5	*Pseudomonas aeruginosa*	ATCC	BAA-3248
48	GES-5	*Pseudomonas aeruginosa*	ATCC	BAA-3312

14.3 Cross reactivity & Microbial Interference

Cross-reactivity and microbial interference studies were performed using 30 microorganisms, including organisms genetically related to the target analytes and organisms associated with similar clinical symptoms. These substances were diluted in negative rectal swab samples to the test concentration and evaluated in three replicates at both negative sample levels and 3X LoD concentrations for each target. No cross-reactivity was observed with non-target microorganisms, and no interference affecting target detection was observed in the presence of the microorganisms tested.

Table 9. Substances tested in Cross-reactivity & Microbial Interference test

No.	Cross-reactive Substances (Microbial interference)	Manufacturer	Cat No.	Test concentration
1	*Escherichia coli*	ATCC	4157	$1x10^6$ CFU/test
2	*Enterococcus faecalis*	ATCC	51299	$1x10^6$ CFU/test
3	*Klebsiella pneumoniae*	ATCC	13883	$1x10^6$ CFU/test
4	*Staphylococcus aureus*	ATCC	12598	$1x10^6$ CFU/test
5	*Pseudomonas aeruginosa*	ATCC	10145	$1x10^6$ CFU/test
6	*Enterobacter cloacae*	ATCC	13047	$1x10^6$ CFU/test
7	*Enterococcus faecium*	ATCC	19434	$1x10^6$ CFU/test
8	*Klebsiella oxytoca*	ATCC	49131	$1x10^6$ CFU/test
9	*Acinetobacter baumannii*	ATCC	19606	$1x10^6$ CFU/test
10	*Citrobacter freundii*	ATCC	8090	$1x10^6$ CFU/test
11	*Morganella morganii*	NCCP	14482	$1x10^6$ CFU/test
12	*Stenotrophomonas maltophilia*	NCCP	17084	$1x10^6$ CFU/test
13	*Citrobacter koseri*	ZeptoMetrix	0801745	$1x10^6$ CFU/test
14	*Providencia stuartii*	ZeptoMetrix	0804013	$1x10^6$ CFU/test
15	*Klebsiella aerogenes*	ATCC	15038	$1x10^6$ CFU/test
16	*Proteus mirabilis*	ATCC	25933	$1x10^6$ CFU/test
17	*Streptococcus agalactiae*	ATCC	13813	$1x10^6$ CFU/test
18	*Campylobacter jejuni*	ATCC	49943	$1x10^6$ CFU/test
19	*Enterococcus casseliflavus*	NCCP	16486	$1x10^6$ CFU/test
20	*Helicobacter pylori*	ATCC	43504	$1x10^6$ CFU/test
21	*Serratia marcescens*	ATCC	13880	$1x10^6$ CFU/test
22	*Escherichiacoli*	NCCP	1457	$1x10^6$ CFU/test
23	*Klebsiellapneumoniae*	NCCP	15861	$1x10^6$ CFU/test
24	*Proteusmirabilis*	NCCP	16064	$1x10^6$ CFU/test
25	*Pseudomonasaeruginosa*	NCCP	16089	$1x10^6$ CFU/test
26	*Escherichiacoli*	NCCP	14576	$1x10^6$ CFU/test
27	*Escherichiacoli*	NCCP	15860	$1x10^6$ CFU/test
28	*Klebsiellapneumoniae*	NCCP	NMS11056	$1x10^6$ CFU/test

29	*Klebsiellapneumoniae*	NCCP	NMS112	$1x10^6$ CFU/test
30	*Escherichia coli* Z136; CTX-M-15, titered (1 mL)	ZeptoMetrix	0801905	$1x10^6$ CFU/test

14.4 Interfering Substances

A total of 15 endogenous and exogenous interfering substances that may be present in rectal swab specimens were evaluated. Testing was performed using negative and 3×LoD target-positive specimens in the presence and absence of the interfering substances. No interference affecting target detection was observed at the concentrations tested for the 15 substances listed below.

Table 10. Interfering substances tested in Interfering Substance test

No.	Interfering Substance	Active Ingredient	Test Concentration
1	Lipid	Stearic acid	0.25% w/v (2.5 mg/mL)
2		Palmitic acid	0.25% w/v (2.5 mg/mL)
3	Anti-fungal/anti-itch	Benzocaine	0.25% w/v (2.5 mg/mL)
4	Anti-hemorrhoid creams/ointments	Anti-hemorrhoid creams/ointments	0.25% w/v (2.5 mg/mL)
5	Antibiotic (oral)	Cephalexin	0.25% w/v (2.5 mg/mL)
6		Ciprofloxacin	0.25% w/v (2.5 mg/mL)
7	Enemas	Mineral oil	0.25% w/v (2.5 mg/mL)
8	Topicalcream (K-Y Jelly)	Topicalcream (K-Y Jelly)	0.25% w/v (2.5 mg/mL)
9	Imaging compound	Barium sulfat	0.25% w/v (2.5 mg/mL)
10	Anti-diarrheal medication	Loperamide Hydrochloride	0.25% w/v (2.5 mg/mL)
11		bismuth subsalicylate	0.25% w/v (2.5 mg/mL)
12	Creams/ointment/suppositories	Hydrocortisone	0.25% w/v (2.5 mg/mL)
13	Antacids	Calcium carbonate	0.25% w/v (2.5 mg/mL)
14	Moist towelettes	Moist towelettes	0.25% w/v (2.5 mg/mL)
15	Laxative	Sennosides	0.25% w/v (2.5 mg/mL)

14.5 Precision (Repeatability & Reproducibility)

[Repeatability]

The positive reference materials for carbapenamase genes; *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{OXA-48}$ and *bla*$_{GES}$ were diluted in negative rectal swab samples to 4 test concentrations (0, 0.1X, 1X, and 5X LoD). Testing was performed with 1 lot, 4 repetitions per day, over 20 days. Based on the test results, within-run, between-run, between-day, and within-laboratory precision were evaluated, confirming the repeatability of the product with an SD < 2.0 Ct and CV < 5%.

[Reproducibility]

The positive reference materials for carbapenamase genes; *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{OXA-48}$ and *bla*$_{GES}$ were diluted in negative rectal swab samples to 4 test concentrations (0, 0.1X, 1X and 3X LoD). Testing was performed with between-operator, between-lots, between-sites, and between-operators five repetitions per day over five days. Based on the test results, the product demonstrated reproducibility with SD < 2.0 Ct and CV < 5%.

14.6 Carry-over Contamination

High-concentration positive reference materials for *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{OXA-48}$ and *bla*$_{GES}$ were tested in cross validation using negative rectal swab samples with one lot of the product, repeating the test five times. All high-concentration positive samples were positive, and all negative samples remained negative. No carry-over contamination was observed on the STANDARD™ M10 System.

14.7 Clinical performance study

The clinical performance of STANDARD M10 CARBA was evaluated using a total of 300 rectal swab specimens in Copan Transystem™ transport medium (Copan Italia S.p.A., 134C). The study population consisted of archived residual clinical specimens and contrived specimens prepared by spiking carbapenemase-producing strains into negative rectal swab specimens to supplement low-prevalence carbapenemase gene variants.

Clinical performance was established by comparison with a composite reference method consisting of culture testing, antimicrobial susceptibility testing (AST), carbapenemase genotyping of CRE isolates. Sequencing was used for discrepant result analysis when applicable.

Clinical sensitivity was 100% for KPC, NDM, VIM, OXA-48, and GES, and 98.0% for IMP. Clinical specificity was 98.79% for KPC, 98.8% for NDM, 100% for VIM, 99.19% for IMP, 100% for OXA-48, and 99.6% for GES.

All evaluated targets met the pre-defined acceptance criteria for clinical sensitivity and clinical specificity.

Table 11. Summary of the clinical sensitivity and specificity test results

1) KPC

- Clinical sensitivity = 100% (52/52) [95% CI: 96.51%–100.00%]
- Clinical specificity = 98.79% (245/248) [95% CI: 96.51% to 99.75%]

KPC		Reference method		Total
		Positive	Negative	
STANDARD M10 CARBA	Positive	52	3	55
	Negative	0	245	245
Total		52	248	300

2) NDM

- Clinical sensitivity = 100% (50/50) [95% CI: 92.89% to 100.00%]
- Clinical specificity = 98.80% (247/250) [95% CI: 96.53% to 99.75%]

NDM		Reference method		Total
		Positive	Negative	
STANDARD M10 CARBA	Positive	50	3	53
	Negative	0	247	247
Total		50	250	300

3) VIM

- Clinical sensitivity = 100% (50/50) [95% CI: 92.89%-100.00%]
- Clinical specificity = 100% (250/250) [95% CI: 98.54%-100.00%]

VIM		Reference method		Total
		Positive	Negative	
STANDARD M10 CARBA	Positive	50	0	50
	Negative	0	250	250
Total		50	250	300

4) OXA-48

- Clinical sensitivity = 100% (50/50) [95% CI: 92.89%-100.00%]
- Clinical specificity = 100% (250/250) [95% CI: 98.54%-100.00%]

OXA-48		Reference method		Total
		Positive	Negative	
STANDARD M10 CARBA	Positive	50	0	50
	Negative	0	250	250
Total		50	250	300

5) IMP

- Clinical sensitivity = 98.08% (51/52) [95% CI: 89.74% to 99.95%]
- Clinical specificity = 99.19% (246/248) [95% CI: 97.12% to 99.90%]

IMP		Reference method		Total
		Positive	Negative	
STANDARD M10 CARBA	Positive	51	2	53
	Negative	1	246	247
Total		52	248	300

6) GES

- Clinical sensitivity = 100% (50/50) [95% CI: 92.89% to 100%]
- Clinical specificity = 99.6% (249/250) [95% CI: 97.79% to 99.99%]

GES		Reference method		Total
		Positive	Negative	
STANDARD M10 CARBA	Positive	50	1	50
	Negative	0	249	250
Total		50	250	300

15. Limitations

1) STANDARD M10 CARBA detects the carbapenemase genes *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{OXA-48}$ and *bla*$_{GES}$ from patients at risk of intestinal colonization with carbapenem-non-susceptible bacteria.
2) This product must be used with human rectal swab specimens.
3) Performance characteristics of this test have been established with the specimen types listed in the **Intended Purpose** Section only. The performance of this assay with other specimen types or samples has not been evaluated.
4) Modifications to the procedures provided in the IFU (Instructions for Use) within the package may after the performance of the test.
5) A false negative result may occur if :
- Sample concentrations are near or below the limit of detection of the test.
- A specimen is improperly collected, transported or handled.
- Inadequate number of organisms are present in the specimen.
- Cartridges are exposed to improper environmental factors.

6) False positive results may happen from cross-contamination between patient samples, specimen mix-up and/or DNA contamination during product handling.
7) Qualitative detection of positive results in this kit does not indicate the presence of live bacteria. It is recommended to use other methods for confirmation at the same time.
8) This kit only classifies and identifies the *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{OXA-48}$ and *bla*$_{GES}$ genes. The test results are for clinical reference only. The clinical diagnosis and treatment of patients should be combined with their symptoms / signs, medical history, other laboratory tests and treatment responses considering.
9) Detection of GES alone does not distinguish between carbapenemase-producing and non-carbapenemase-producing GES variants. If GES is detected, additional characterization may be required.

16. References

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7) Korea Disease Control and Prevention Agency (KDCA). Analysis of resistance trends and characteristics of carbapenem-resistant Enterobacteriaceae (CRE) isolated in Korea, 2017–2020.
8) Korea Disease Control and Prevention Agency (KDCA). Analysis of resistance trends and characteristics of carbapenem-resistant Enterobacteriaceae (CRE) isolated in Korea, 2021.
9) Kyung-Hwa Lee, The significance and clinical application of surveillance cultures for carbapenem-resistant Enterobacterales. Korean Society for Healthcare-associated Infection Control and Prevention. 2023.28.2.185.
10) Kim NH, Hong KH, Park JS, Roh KH, Shin S. Labotratory Diagnosis of Carbapenemase-producing Enterobacterales. Korean Society for Healthcare-associated infection Control and Preventio. 2023.28.1.42
11) Korea Disease Control and Prevention Agency (KDCA), 2022 Guidelines for the Management of Healthcare-Associated Infections and Integrated Guidelines for the Diagnosis of Notifiable Infectious Diseases, 3rd edition, Part 1.
12) CDC (https://www.cdc.gov/drugresistance/index.html)

17. Symbols

	Reference number		Batch code
	*In vitro* diagnostics medical device		Manufacturer
	Consult Instructions for Use		Date of manufacture
	Contains Sufficient for <n> Tests		Keep dry
	Caution		Keep away from sunlight
	Temperature limit		Do not use if packaging is damaged
	Do not re-use.		Use-by date
	Indicates the UK Responsible Person		This product fulfills the requirements of UK MDR 2002
	Authorized representative in the European Community		CE marking – European Conformity
	Not for Near-Patient Testing		Catalog number
	Contents		Manufacturing site
	Cartridge		Sample Buffer
	Nozzle Cap		Quick Reference Instructions

For further information on

STANDARD M10
CARBA

Please contact your
SD BIOSENSOR representative

L28MCAREN2R0 / 2026.07

SD Biosensor, Inc.
C-4th&5th, 16, Deogyeong-daero 1556beon-gil, Yeongtong-gu,
Suwon-si, Gyeonggi-do, 16690, REPUBLIC OF KOREA

Manufacturing site
14, Jeungpyeongsandan-ro, Jeungpyeong-eup,
Jeungpyeong-gun, Chungcheongbuk-do, 27915, REPUBLIC OF KOREA

UK REP **MT Promedt Consulting Ltd.**
c/o Critchleys LLP, First Floor, Park Central, 40-41 Park End
Street, Oxford OX1 1JD, United Kingdom
Phone | +44 1865 679004

EU REP MT Promedt Consulting GmbH
Ernst-Heckel-Straße 7 66386 St. Ingbert Germany

IVD **For *In Vitro* Diagnostic Use Only**

CE 0123 UKCA

Any inquiries regarding instructions provided should be addressed to: ts@sdbiosensor.com
or you can also contact us through www.sdbiosensor.comsensor.com