GaDia Diagnostics IVD REF KAR-025

KarbaDia

Instruction for Use – English

Please read this user manual carefully before using the test

INTENDED USE

KarbaDia is a rapid, non-automated immunochromatographic assay intended for the qualitative detection of KPC, NDM, IMP, VIM, and OXA-48 carbapenemases from bacterial colonies. This device is intended for professional use in laboratory settings and may aid in the identification of carbapenem-resistant bacterial strains associated with these carbapenemases. Results should be interpreted in conjunction with other clinical and laboratory findings, including complementary methods such as molecular testing, antimicrobial susceptibility testing, and other microbiological analyses.

SUMMARY

Carbapenem-resistant Enterobacteriaceae (CRE) represent a major public health concern due to their resistance to broad-spectrum antibiotics and the limited therapeutic options available. This resistance is primarily mediated by carbapenemases, β-lactamases capable of hydrolyzing carbapenems, classified according to Ambler into classes A, B, and D. Class B carbapenemases (metallo-β-lactamases, MBL), including IMP, VIM, and NDM, as well as class A (e.g., KPC) and class D (e.g., OXA-48) enzymes, are associated with Enterobacteriaceae and other clinically relevant Gram-negative bacteria. Rapid detection of these enzymes is essential for early identification of resistance mechanisms and to support therapeutic decision-making and infection control measures.

DETECTION PRINCIPLE

KarbaDia is a sandwich immunochromatographic assay for the qualitative detection of KPC, NDM, IMP, VIM, and OXA-48 carbapenemases. The device contains five test lines (K, N, I, V, O) and one control line (C) on a nitrocellulose membrane. If a carbapenemase is present in the sample, it binds to specific antibodies conjugated to gold particles. The complex migrates by capillary action and is captured by immobilized antibodies at the corresponding test lines, producing one or more colored lines. The control line (C) contains anti-mouse antibodies and must appear in all cases, confirming test validity (sufficient sample volume, proper migration, and reagent integrity). A positive result is indicated by the appearance of one or more test lines (K/N/I/V/O) in addition to the control line. A negative result is indicated by the presence of the control line only.

KIT COMPONENTS

Components	Quantity per kit
Carbapenem-resistant K.N.I.V.O rapid test	25
Sample treatment solution in dropper bottle	10.0 mL
Dropper tubes for sample preparation	25
Instructions for use	1

Note: The components of kits cannot be exchanged

Materials Required but Not Supplied

1. Timer
2. Bacterial Inoculation loop (1 µl)
3. Optional: Disposable sterile micro-centrifuge tubes (1.5 ml)

STORAGE CONDITIONS AND SHELF LIFE

1. Store at 5-30°C for 24 months, store in a dry and cool place.
2. The rapid test should be used within 1 hour after opening the aluminum foil bag. The sample treatment solution should be stored at 5-30°C after opening and is stable until expiry date.
3. The expiry date is printed on the labels.

SAMPLE PREPARATION

1. Sample type: Freshly cultured Bacterial colonies
   Validated Culture media: Luria Broth (LB) agar, Trypticase soja agar (TSA), Mueller Hinton (MH) agar, Columbia agar + 5 % horse blood, ChromID® ESBL agar, ChromID® CARBA SMART, CHROMagar™ mSuperCARBA™, TSA + 5 % sheep blood, Mac Conkey, Hardy CHROM™ CRE agar.
2. Specimen collection: Specimens to be tested should be obtained and handled by standard microbiological methods.
3. Avoid contamination during sample collection, transportation, and preservation.

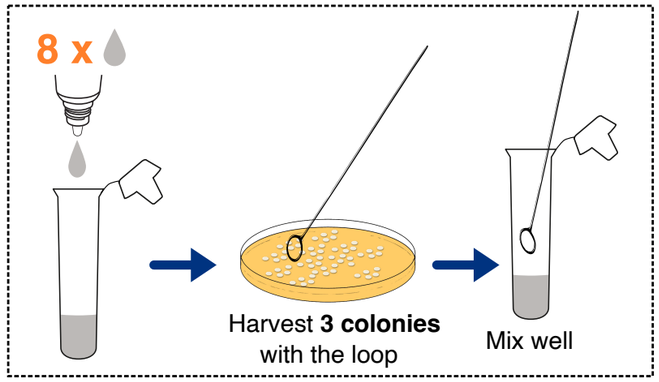
TEST PROCEDURE

Make the temperature of the kit and bacterial colonies samples reach room temperature (15-30°C). Open the package and take out the test cassette. Clearly identify the test and sample ID on the test cassette and tube.

1. Sample Pre-treatment

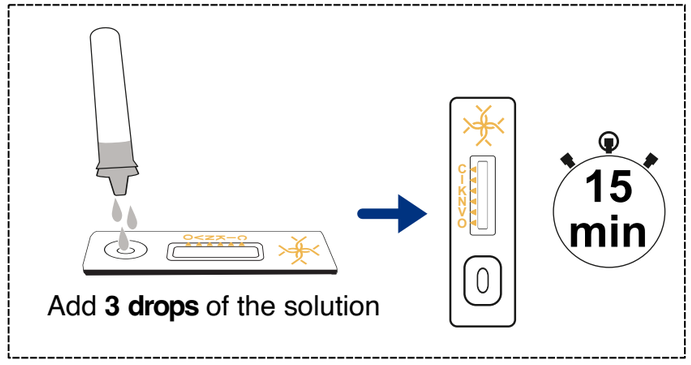
- Add **8 drops (200 µl)** of sample treatment solution in a dropper tube for sample or disposable sterile micro-centrifuge tube (not provided).
- Harvest bacteria by taking **3 colonies** with a disposable bacteriological loop (*equivalent to 3 loops of 1-µl capacity*) and resuspend it well in the tube containing the sample treatment solution. Use the same bacteriological loop to collect the 3 colonies.
- Mix well using the bacteriological loop and make sure that the bacterial colonies are well homogenized.

Mucous colonies: Use 10 drops of Sample treatment solution, take 3 bacterial colonies and resuspend it in the solution, mix 3 minutes using a vortex and incubate for 10 minutes at room temperature before performing the test.



2. Detection

- Take out the test device from aluminum foil pouch and place it on a clean horizontal bench.
- Add **3 drops (70 µl)** of sample mixture in test well (S).
- Wait **15 minutes** and read the result. Do not move the test during incubation. Do not interpret the result **after 20 minutes.**

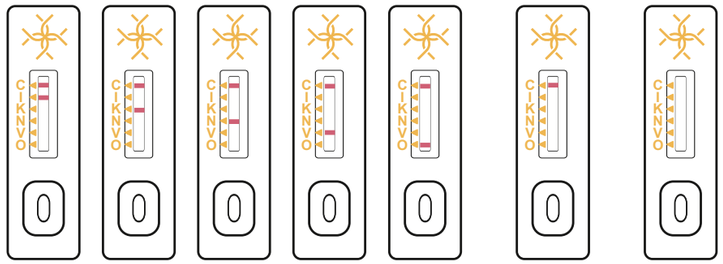


RESULTS INTERPRETATION

+ The presence of one or more red line(s) in test area, regardless of the intensity of the test line, indicates a positive result of its corresponding carbapenemase type (K N I O V).
- A single control line (C) indicates a negative result.
• If the control line (C) does not appear, the result is invalid and the test should be repeated.

1. A negative result does not preclude the presence of other carbapenemase producing organisms.
2. A positive or a negative test does not rule out the presence of other mechanisms of antibiotic resistance.
3. The color intensity of the test lines cannot be used as the basis for determining the total content of Carbapenemase (qualitative only).

REF KAR-025



QUALITY CONTROL

An internal quality control is included in the test. When the control line develops, it confirms the sample volume was sufficient and the procedure was correct. Reference strains can be used as external positive control for the tests. If needed, contact the manufacturer for more information.

LIMITATIONS

1. The product is only used to test freshly cultured bacterial strains. The assay performance characteristics have not been established for non-bacteria-strain samples. The presence or absence of carbapenemase is related to bacteria, not to patients.
2. This test is a qualitative assay and will not give a quantitative result.
3. The test results of this kit should be used as an aid for the rapid identification of bacteria producing carbapenemase. The results must be confirmed with alternative or complementary diagnostic procedures. The clinical management of patients should be comprehensively considered in conjunction with their symptoms, medical history, other laboratory tests and treatment responses.

PERFORMANCES

1. Limit of detection (LOD)

The Limit of Detections (LODs), determined with recombinant proteins, of KPC, NDM, IMP, VIM and OXA-48 are 0.50 ng/mL; 0.20 ng/mL; 0.20 ng/mL; 0.30 ng/mL and 0.10 ng/mL, respectively.

2. Hook effect

No Hook effect has been observed with concentration up to 1 µg/mL of carbapenemase.

3. Interfering substances and cross-reactions

No interfering substances or cross-reaction have been observed with this test kit. No cross-reaction with specific bacteria has been detected (*Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii*). No cross-reaction observed with other antimicrobial resistances (ESBLs, MCR-1, TEM, SHV and OXA-1 enzymes). A study of variants detected has been conducted and the list of variants is presented in the following table. Variants not included in this table have not been evaluated or may not be detected.

Carbapenemase type	Variant detected
KPC	KPC-1, KPC-2, KPC-3, KPC-4, KPC-27, KPC-74
NDM	NDM-1, NDM-2, NDM-5, NDM-6, NDM-7, NDM-9, NDM-24
VIM	VIM-1, VIM-2, VIM-4, VIM-5, VIM-9, VIM-10, VIM-19, VIM-53
IMP	IMP-1, IMP-3, IMP-4, IMP-5, IMP-6, IMP-10, IMP-11, IMP-15, IMP-25, IMP-26, IMP-29, IMP-30, IMP-34, IMP-38, IMP-40, IMP-42
OXA	OXA-48, OXA-162, OXA-163, OXA-181, OXA-204, OXA-232, OXA-244

4. Repeatability and reproducibility

Reproducibility and reproducibility of the test have been evaluated internally with three different lots and a coefficient of variation (CV) of less than 10% was observed.

5. Clinical performances

A total of 212 clinical isolates were collected from European hospitals (81%) and American hospitals (15%) in 2019 and 2020, including 19 carbapenemase-negative isolates (9%) and 193 (91%) carbapenem-resistant Enterobacterales (CRE) with various carbapenemase types. These were collected from various infection sources including respiratory, urinary tract, intra-abdominal and chorionic villus sampling. Molecular analysis was performed on all isolates and MIC determination on discordant results. The isolates were cultivated on blood agar for 24h at 37°C and analyzed with KarbaDia rapid test [6].

KPC	**+**	**-**	**Sensitivity**	**100%**	(CI95%: 87-100%)
+	32	0	**Specificity**	**100%**	(CI95%: 97-100%)
-	0	180	**PPV**	**100%**	(CI95%: 87-100%)
			NPV	**100%**	(CI95%: 97-100%)
OXA	**+**	**-**	**Sensitivity**	**98%**	(CI95%: 90-100%)
+	58	2	**Specificity**	**99%**	(CI95%: 95-100%)
-	1	149	**PPV**	**97%**	(CI95%: 87-99%)
			NPV	**99%**	(CI95%: 96-100%)
NDM	**+**	**-**	**Sensitivity**	**97%**	(CI95%: 89-99%)
+	65	1	**Specificity**	**99%**	(CI95%: 95-100%)
-	2	139	**PPV**	**98%**	(CI95%: 91-100%)
			NPV	**99%**	(CI95%: 94-100%)
IMP	**+**	**-**	**Sensitivity**	**93%**	(CI95%: 66-100%)
+	14	0	**Specificity**	**100%**	(CI95%: 98-100%)
-	1	190	**PPV**	**100%**	(CI95%: 73-100%)
			NPV	**99%**	(CI95%: 97-100%)
VIM	**+**	**-**	**Sensitivity**	**100%**	(CI95%: 85-100%)
+	29	0	**Specificity**	**100%**	(CI95%: 97-100%)
-	0	183	**PPV**	**100%**	(CI95%: 85-100%)
			NPV	**100%**	(CI95%: 97-100%)

An external evaluation using 126 *Klebsiella pneumoniae* isolates (including 66 carbapenemase-producing strains) at a clinical microbiology laboratory (Novara, Italy) showed an overall concordance of 95.5% for carbapenemase detection compared with the molecular reference method (Xpert® Carba-R assay) [7]. Internal evaluation conducted on 84 Gram-negative bacterial isolates from ATCC demonstrated 100% sensitivity for KPC, NDM, VIM and IMP targets, and 90% sensitivity for OXA-48 variants (9/10) with 100% specificity.

WARNING AND PRECAUTIONS

1. Read the instruction for use carefully before using the test.
2. Clearly identify the sample ID on the test cassettes and tubes.
3. This product is for *in vitro* diagnostic and professional use only.
4. Do not reuse the test
5. Do not use the test after expiry date
6. Read the test results within the specific time to avoid wrong interpretation.
7. Do not use the components from different batches or different types of reagents.
8. Properly dispose the specimen and used materials following the local biohazardous disposal regulation.
9. Use protective equipment when using the test and handling samples as they may contain infectious agents, human or animal components.
10. The sample treatment solution contains Tris-buffer solution with a detergent and preservative. Dispose material according to relevant local regulations and avoid contact with eyes and skin.
11. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

REFERENCES

1. Nordmann et al. Rapid Detection of Carbapenemase-producing Enterobacteriaceae. Emerging Infectious Diseases. 2012; 18(9).
2. Bush et al. Updated Functional Classification of β-Lactamases. Antimicrobial Agents and Chemotherapy. 2010; 54(3):969-976.
3. Oteo et al. Evolution of carbapenemase-producing Enterobacteriaceae at the global and national level: what should be expected in the future? Enferm. Infecc. Microbiol. Clin. 2014; 32 Suppl 4:17-23.
4. Nordmann et al. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. Clin. Microbiol. Infect. 2014; 20:821–830.
5. Sadek et al. Evaluation of novel immunological rapid test for rapid detection of carbapenemase producers in multidrug-resistant gram negatives, Diagnostic Microbiology and Infectious Disease (2022)
6. Hawser et al. Preliminary evaluation of KarbaDiag, a new rapid test for the detection of carbapenemase in bacterial colonies, ECCMID 2022 Poster, 05084
7. Camaggi et al. Rilevazione rapida di Klebsiella pneumoniae produttrice di carbapenemasi: confronto tra test molecolare, MALDI-TOF MS e test immunocromatografici a flusso laterale, AMCLI 2026, Poster P247

SYMBOLS

	Manufacturer		Expiry Date
	Do not reuse	LOT	Lot Number
	Manufacturing date	EU REP	European Authorized Representative
	Consult instructions for use	IVD	In vitro diagnostic medical device
	Temperature limitation	REF	Catalog number
	Sufficient for <n> Test	CE	CE Marking
	Not for near-patient testing		Not for self-testing

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SWISS MADE

CE IVD

Ref. KAR-004 Rev.1-26S-EN