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 immunochromatographic test intended to be used for the qualitative detection of KPC-type, NDM-type, IMP-type, VIM-type and OXA-48-type carbapenemase in bacterial colonies. The assay is for professional use only and can aid in the diagnosis of KPC-type, NDM-type, IMP-type, VIM-type and OXA-48-type carbapenemase resistant strains. The test should be used in conjunction with other diagnostic procedures, such as genetic analysis, susceptibility testing and other microbial analysis.

SUMMARY

Carbapenem-resistant Enterobacter (CRE) have become a global public health issue due to their broad-spectrum drug resistance, and treatment options for patients are very limited. Carbapenemase refers to a type of β-lactamase enzyme that significantly hydrolyze imipenem or meropenem and by Ambler molecular structure. Class B are metallo-β-lactamases (MBLs), including carbapenemases such as IMP, VIM and NDM, mainly found in *Pseudomonas aeruginosa* and *Enterobacteriaceae* bacteria. Class A and D are serinase. Class A, such as KPC-type carbapenemase, have been detected primarily in Enterobacteriaceae bacteria, and Class D, such as OXA-type carbapenemase, were frequently detected in Acinetobacteria. KPC has become one of the most important contemporary pathogens. Infections due to KPCs are associated with high therapeutic failure and mortality rates of at least 50%. To develop rapid carbapenemase diagnostic products is of great significance for the early typing of drug-resistant strains, the guidance of medication, and the improvement of human's medical and health standards.

DETECTION PRINCIPLE

KarbaDia is a sandwich immunochromatographic assay. The test has 5 pre-coated test lines (K N I V O) on nitrocellulose membrane and one Control line (C) per test. If KPC-type, NDM-type, IMP-type, VIM-type or OXA-48-type carbapenemase are present in the specimen, it will bind to the gold-conjugated anti-KPC, anti-NDM, anti-IMP, anti-VIM or anti-OXA-48 antibodies, respectively, pre-dried on conjugate pad. The gold-conjugated antibody-antigen complex moves upward on the membrane by capillary action where it will react with the test lines. The immobilized anti-KPC, anti-NDM, anti-IMP, anti-VIM or anti-OXA-48 monoclonal antibodies on test lines will capture the gold-conjugated antibody-antigen complex and form red line(s). Whether the sample is positive or not, the nanoparticle complex continues to move across the membrane where immobilized goat anti-mouse antibodies (control line) will bind the gold-conjugated and form a visible red control line. Positive test results form one or more red line(s) in test area (K/N/I/V/O). Negative test results form only a control line (C line). The quality control line (C line) is an internal quality control that serve as (1) verification that sufficient volume is added, (2) that proper flow is obtained and (3) an internal control for the reagents. The control line must always appear.

KIT COMPONENTS

Components	Quantity per kit
Carbapenem-resistant K.N.I.V.O duo test	25
Sample treatment solution	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. Inoculation loop (5 μl)
3. Optional: Pipettes and sterile tips
4. Optional: Disposable sterile micro-centrifuge tubes (1.5 ml)

STORAGE CONDITIONS AND SHELF LIFE

1. Store at 2-30°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 2-8°C after opening.
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

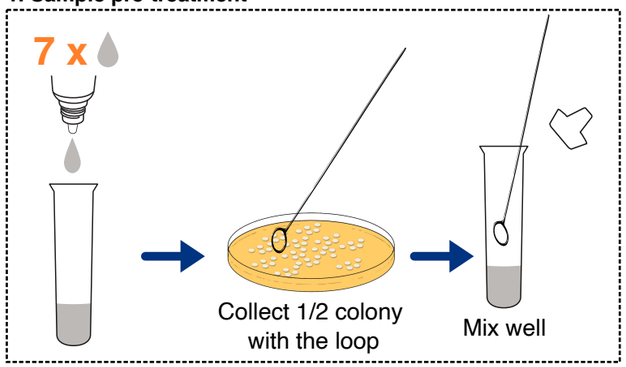
Make the temperature of the kit and bacterial colonies samples reach room temperature (15-30°C). Open the package and take out the test cassette. Clearly identify the test and sample ID on the test cassettes.

1. Sample Pre-treatment

- Add 7 drops (200 μl) of sample treatment solution in a dropper tube for samples or disposable sterile micro-centrifuge tube (not provided).
- Take half of a pure colony on an agar plate with a loop and resuspend it well in the tube containing the sample treatment solution.
- Mix well.

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

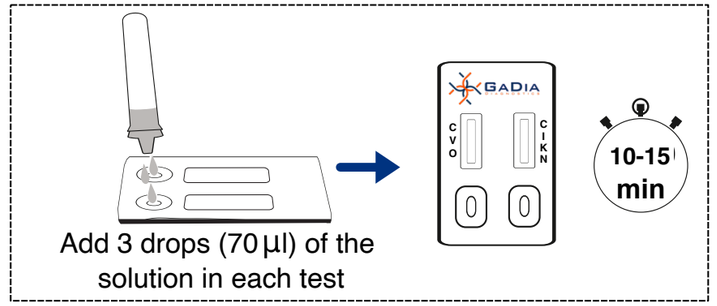
1. Sample pre-treatment



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 on each test device.
- Wait **10-15 minutes** and read the result. Do not move the test during incubation. Do not interpret the result **after 20 minutes.**

2. Detection

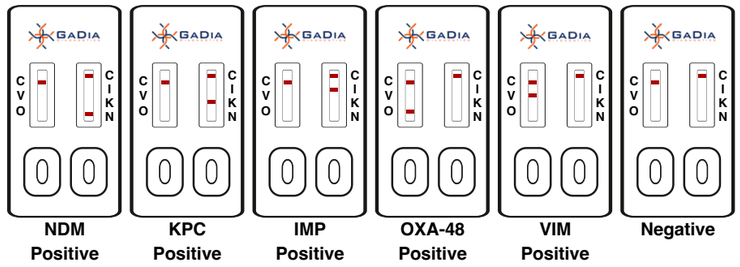


RESULTS INTERPRETATION

+ 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 cultured 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 with a resistance to carbapenem antibiotics. 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; 0.15 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*). A study of variants detected has been conducted and the list of variants is presented in the following table.

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 analysed with KarbaDia rapid test [6].

KarbaDiag vs genetic testing and MIC

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%)

A second study in a Swiss reference center using a collection of 252 well-characterized carbapenemase and non-carbapenemase producing strains was conducted. The bacteria were cultivated 20h on Muller Hinton agar plates at 37°C before the evaluation. The overall sensitivity of KarbaDia was 96.8 % (CI95: 93.6%–98.4%) and the specificity was 100 % (CI95: 79.6%–100%).

WARNING AND PRECAUTIONS

1. Read the instruction for use carefully before using the test.
2. Clearly identify the sample ID on the test cassettes.
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. Sodium azide is used as preservative in the sample treatment solution. 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

SYMBOLS

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

GaDia SA
Route de l'Ile-au-Bois 1A
1870 Monthey (Switzerland)
www.gadia.net
info@gadia.net

SWISS QUALITY

CE IVD

EC REP **ER Egészségügyi, Kereskedelmi és Szolgáltató Kft.**
Budafoki út 57/b
1111 Budapest (Hungary)
ujszaszi.istvan@erkft.hu