NG-Test CARBA 5

Rapid test for the detection of carbapenemases KPC, OXA-48-like, VIM, IMP and NDM in a bacterial colony from culture
For professional in vitro diagnostic use only

Ref: ENO022CAR / Rev: 200128 / EN

Introduction

NG-Test CARBA 5 is an in vitro rapid and visual multiplex immunochromatographic assay for the qualitative detection and differentiation of five common carbapenemases (KPC, OXA-48-like, VIM, IMP and NDM) from carbapenem non-susceptible pure bacterial colonies. It is an in vitro diagnostic assay, for professional use only, that aids in the rapid identification and infection control in the detection of carbapenemase-producing ***Enterobacteriaceae*** (including ***Escherichia coli***, ***Klebsiella pneumoniae***) and ***Pseudomonas aeruginosa*** in healthcare settings.

Summary and principal

β-Lactams are first-line antibiotics for the treatment of infections caused by Enterobacteriaceae. Nevertheless, since the beginning of their massive use in the 1940s, their efficacy has been challenged by the production of enzymes which inactivate them: the β-lactamases. Among them are carbapenemases which hydrolyze carbapenem antibiotics. Before the 1940s, most of the resistance to antibiotics was associated with the production of Extended-Spectrum β-Lactamases (ESBLs) belonging to classes A, B, C, and D of Ambler's classification. Since then, studies have shown an increase in the production of carbapenemases among the Enterobacteriaceae family. Among those carbapenemases, the β-lactamase KPC (Class A) has spread worldwide in the 2000s. In addition, the metallo β- lactams (MBL) of IMP type, VIM and NDM (Class B) as well as OXA-48 (Class D) have also expanded[1,3]. These carbapenemases are mainly detected in hospital settings and are responsible for most of the nosocomial infections, raising major global health problems since their presence can be difficult to detect.

NG-Test CARBA 5 is an in vitro rapid and visual multiplex immunochromatographic assay that detects one or more of the five common types of carbapenemase enzymes (KPC (K), OXA-48-like (O), IMP (I), VIM (V), NDM (N)) in bacterial colonies. Liquid extraction buffer is used as a cell lysing solution when mixed with colonies. Monoclonal antibodies that individually recognize each of the five carbapenemases are immobilized on a nitrocellulose membrane. Free monoclonal antibodies are present in the conjugate pad and labelled with colloidal gold. Upon addition of colonies mixed with extraction buffer to the sample pad, the capillary action of the nitrocellulose draws the sample through the mobile antibodies and immobile antibodies on the test strip. The immobilized control antibodies capture any mobile antibodies that run through the sample pad and nitrocellulose without binding to other test lines. A positive result occurs when a red line appears on the control region (C) and one or more lines appear in the test regions (K, O, V, I, or N) and indicates that the sample contains one or more carbapenemases. A negative result occurs when only the control line is observed and indicates that the sample does not contain any of the 5 carbapenemases. If the control line does not appear, the test result is invalid.

Reagents and materials supplied

Each kit contains:

- 20 Test cassettes in aluminium pouches with desiccant
- 20 Eppendorf tubes
- 20 Disposable pipettes of 100 µL
- 1 Extraction buffer solution in a plastic bottle (4,5 mL)
- 1 Instructions for use

Materials required but not supplied

- Timer
- Single use gloves
- Loop
- Vortex

Precautions

- *In vitro* diagnostic test. For professional use only.
- All the operations must be carried out according to good laboratory practices.
- Do not use after the expiry date.
- The devices must remain in the sealed pouches until they are used.
- Handle the samples as if they were potentially infectious.
- After use, discard the device in an infectious waste container.
- Do not reuse the device.

Storage and stability

Store the devices in their sealed pouches between 4 and 30°C. Do not freeze. Kits are stable in their intact packaging (sachet with desiccant) until the expiry date indicated on every kit.

Culture and sampling

The samples to be tested shall be obtained and handled according to the standardised microbiology procedures.

Validated culture media
Luria Broth (LB) and LB agar, Trypticase soja agar (TSA), Mueller Hinton (MH) agar, UriSelect™ 4, Columbia agar + 5 % horse blood, ChromID® ESBL agar, ChromID® CARBA SMART, Drigalski (DRIG) agar, CHROMagar™ mSuperCARBA™, TSA + 5 % sheep blood, Mac Conkey, HardyCHROM™ CRE agar.

Operating procedure

1. Wear protective gloves.
2. Bring the kit components at room temperature for at least 10 minutes.

Preparing the sample

1. Dispense 5 drops (150 µL) of extraction buffer in one of the microtubes provided into the kit.
2. From a solid agar-based culture, touch 3 colonies with a loop, and then suspend it in the microtube containing 150 µL of extraction buffer.
3. Close the microtube.
4. Vortex to homogenise the mixture before use.

NOTE: Mucous colonies can lead to migration problems, due to their high viscosity. Vortex for 3 minutes a colony in extraction buffer and incubate for 10 minutes at room temperature before performing the test.

Carrying out the test

1. Open the pouch, and take out the device. Once opened, use the test immediately.
2. Using the provided pipette, add 100 µL of the prepared mixture (sample must reach the black line indicated on the pipette to accurately aspirate 100 µL) in the sample well labelled "S".
3. Read the results at 15 minutes and interpret them as indicated below**.**

NOTE: Do not interpret the test results after 15 minutes.

Result interpretation

Negative

Negative result

If only one red line appears in the control region (C): the sample does not contain any carbapenemase or a non-detectable level and must be interpreted as a negative result.

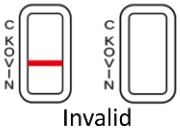
VIM
Positive

Positive result

If one red line appears in the control region (C) and one or several lines appear in the test regions K, O, V, I, N: the sample contains one or several carbapenemases and must be interpreted as a positive result.

NOTE: The intensity of the red test line(s) may vary depending on the concentration of the carbapenemases present in the sample. A weak line should be considered as a positive result.

Invalid result



Invalid

If the control line (C) does not appear, the test result is invalid. Insufficient sample volume or an incorrect procedure are the most likely reasons for control line failure. Deterioration of the test kit may have occurred. Repeat the procedure using a new test. If the problem persists, do not reuse the kit and contact your distributor.

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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.

Limitations

1. A negative result does not preclude the presence of carbapenemase producing organisms (example: SME, GES, IMI).
2. False negative results may occur with multiple subcultures of a bacterial isolate without any selective pressure.
3. Some false negative results may occur with *Proteus mirabilis* tested from blood agar only.
4. *Proteus* spp. tend to exhibit swarming growth on blood agar, thus only the surface of the swarming should be touched with a loop in 3 different places.
5. This test is a qualitative assay and will not yield any quantitative result.
6. This test should be used as an aid for the rapid identification of patients bearing a resistance to carbapenem antibiotics. The obtained results must be confirmed with alternative or complementary diagnostic procedures.
7. A positive or a negative test does not rule out the presence of other mechanisms of antibiotic resistance

Performances and characteristics

Detection limit

The detection limit was determined using purified recombinant enzymes:
KPC 600 pg/mL
OXA 300 pg/mL
VIM 300 pg/mL
IMP 200 pg/mL
NDM 150 pg/mL

Clinical Evaluation

NG-Test Carba 5 was evaluated at the NRC (National Reference Center for Antibiotic Resistance, Kremlin-Bicêtre Hospital, Paris, France). 147 strains were blind-tested with WGS-characterized β-lactamase content. Among the evaluated collection, only 2 rare D179Y KPC variants were not detected (KPC-31 and KPC-33). This D179Y impared the carbapenem hydrolytic activity of the enzyme which cannot be considered as a carbapenemase, leading also to negative result by biochemical tests. The sensitivity and specificity of the NG-Test CARBA 5 was calculated by concidering the KPC-31 and KPC-33 variants as negative results.

Table 1: Results obtained at NRC Kremlin Bicêtre[11]

		WGS		
		Positive	**Negative**	**total**
NG-Test CARBA 5	**Positive**	**119**	**0**	**119**
	Negative	**0**	**28**	**28**
	Total	**119**	**28**	**147**

Sensitivity	**100%**	CI 95% = 96.9 – 95.6%
Specificity	**100%**	CI 95% = 87.9 - 100%

Analytical specificity

Variants detected by NG-Test CARBA 5 [8;9;10;11;12;13]:
Type NDM: NDM-1 -2 -3 -4 -5 -6 -7 -8 -9 -11 -19
Type KPC: KPC-1 -2 -3 -4 -5 -6 -7 -12 -14 -23 -28 -39
Type IMP: IMP-1 -2 -4 -5 -6 -7 -8 -10 -11 -13 -14 -15 -16 -18 -19 -22 -26 -29 -31 -37 -39 -46 -47 -56 -58 -61 -63 -71 -79
Type VIM: VIM-1 -2 -4 -5 -6 -19 -23 -26 -27 -31 -39 -46 -51 -52 -54 -56 -58 -59,
OXA-48-like: OXA-48 -58 -162- 181 -204 -232 -244 -245 -370 -436 -484 -515 -517 -519 -535 -793
Non-carbapenemases (cross-reactivity) : OXA-163 and OXA-405 (OXA-48-like extended spectrum oxacillinases with very weak carbapenemase activity).

Note: the above list may not be exhaustive.

Summary of variants detected by NG-Test CARBA 5 in publications

Organism Group	Target	Variants detected in the publications [8;9;10;11;12]
*Entero-bacteriaceae**	KPC	2,3,4,5,6,7,9,12,14,23,28,39
	OXA-48-like	48,162,181,204,232,244,245,370,436,484,515,517,519,535,793 (163 & 405)
	VIM	1,2,4,5,6,19,23,26,27,31,39,46,51,52,54,56,58,59
	IMP	1,4,6,7,8,10,11,14,22,26,47,58
	NDM	1,2,3,4,5,6,7,8,9,11,19
Pseudomonas aeruginosa	KPC	1,2,5
	OXA-48-like	181
	VIM	2,5,11
	IMP	1,2,4,5,7,8,13,15,16,18,19,26,29,31,37,39,46,56,63,71,79
	NDM	1

*Including some: *Citrobacter.braakii, C. freundii, C. koseri, C. sedlakii, Enterobacter aerogenes, E. asburiae, E. cloacae, E. cloacae complex, **Escherichia coli**, E. hermanii, Klebsiella aerogenes, K. variicola, K. ozaenae, K. oxytoca, **K. pneumoniae**, Kluyvera ascorbate, Morganella morgannii, Proteus mirabilis, Providencia stuartii, P. rettgeri, Pantoea species, Providencia alcalifaciens, Raoultella ornithinolytica, Salmonella enterica, S. senftenberg, Serratia marcescens, Shigella boydii, S. sonnei, Shewanella sp, S.bicestrii, Yersinia enterolitica…*

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12. Klavins A et al. Evaluation of NG-Test CARBA 5 with Carbepenemase producing organisms and non-carbapenemase producing organisms. CPHM 991. ASM Microbe 2019-Chicago
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Symbols

Σ 20	Content for 20 assays		Expiry date
IVD	*in vitro* diagnostic medical device		Do not re-use
LOT	Batch number	REF	Catalogue reference
	Consult instructions for use	+4°C +30°C	Temperature limit
	Manufacturer	**Contains NaN3**	0.01% sodium azide

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This test was developed in collaboration with the CEA*.
*The French Alternative Energies and Atomic Energy Commission is a key player in research, development and innovation.

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