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Evaluation of novel immunological rapid test (K.N.I.V.O. Detection K-Set) for rapid detection of carbapenemase producers in multidrug-resistant gram negatives

Mustafa Sadek[a,b,c], Maxime Bouvier[a,d], Auriane Kerbol[a,d], Laurent Poirel[a,c,d], Patrice Nordmann[a,c,d,e,*]

[a] Medical and Molecular Microbiology, Faculty of Science and Medicine, University of Fribourg, Fribourg, Switzerland
[b] Department of Food Hygiene and Control, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt
[c] INSERM European Unit (IAME), University of Fribourg, Fribourg, Switzerland
[d] Swiss National Reference Center for Emerging Antibiotic Resistance (NARA), University of Fribourg, Fribourg, Switzerland
[e] Institute for Microbiology, University of Lausanne and University Hospital Centre, Lausanne, Switzerland

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ABSTRACT

The K.N.I.V.O Detection K-Set is a novel immunochromatographic test for detection of the 5 major carbapenemases (KPC, NDM, IMP, VIM, and OXA-48-like). This test is rapid, easy to perform, and shows a good specificity and sensitivity (96.8% and 100%, respectively), being suitable for microbiology laboratories together with biochemical rapid tests.

Infections caused by carbapenemase-producing Gram-negative bacteria are considered in the top list of the most difficult-to-treat bacterial infections, representing a great concern for public health. A variety of acquired carbapenemases has been reported in strains of Enterobacterales, *Pseudomonas aeruginosa, Acinetobacter baumannii*, and other Gram-negatives [1]**.** The most clinically important types of carbapenemases are grouped into 3 classes according to their amino acid identity (Ambler classification): KPC (Ambler class A), NDM, VIM and IMP (Ambler class B), and OXA-48-types enzymes (Ambler class D) [2]. Among the different types of carbapenemases, class B metallo-β-lactamases confer resistance to almost all ß-lactams, and are resistant to the action of all clinically-available inhibitors, therefore leaving very limited therapeutic options [3]. The rapid and accurate identification of carbapenemases in patients being infected or colonized is of critical importance for both treating infected patients and infection control purposes. The biochemical detection of the carbapenem hydrolysis [4–6] or an immunological detection of those ß-lactamases [7] are widely used testing methods for rapid detection of carbapenemases. Molecular based-detection of carbapenemase encoding genes is an interesting alternative but remains costly, time consuming, not easily implementable worldwide, and only detect the most common carbapenemase-encoding genes, missing the so-called "minor" or even unknown ones.

Very recently, a novel immunochromatographic detection test, namely carbapenem-resistant K.N.I.V.O Detection K-Set (Goldstream, Beijing Gold Mountainriver Tech Development Co., Ltd.), has been launched onto the European market. This lateral flow assay is intended for detection of the five major carbapenemase types, namely KPC-type, NDM-type, IMP-type, VIM-type, and OXA-48-type carbapenemases. The present study was aimed to evaluate this new immunochromatographic test using a representative collection of well-characterized carbapenemase and noncarbapenemase producers of the various clinical Gram-negative species (n = 252) from the National Reference Center for Emerging Antibiotic Resistance for Switzerland (Table 1). This collection included 189 of 252 Enterobacterales (*Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Klebsiella aerogenes, Serratia marcescens, Providencia stuartii, Enterobacter spp., Citrobacter freundii, Hafnia alvei, Proteus mirabilis*), 1 of 252 *Empedobacter falsenii*, 33 of 252 *Acinetobacter baumannii*, and 29 of 252 *Pseudomonas aeruginosa*. The carbapenemase types were as follows: KPC (n = 17), IMI (n = 2), SME (n = 1), NDM (n = 75), VIM (n = 26), IMP (n = 16), OXA-48-type (n = 70; OXA-48, OXA-181, OXA-162, OXA-204, and OXA-244), and OXA-23 (n = 16). Noteworthy, 3

* Corresponding author. Tel.: +41 263009581.
E-mail address: patrice.nordmann@unifr.ch (P. Nordmann).

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Table 1
Results of the K.N.I.V.O. Detection K-Set assay.

Species (n)	Resistance determinant(s) (n)	K.N.I.V.O Assay
KPC -producers (17)		
Escherichia coli	KPC-2 (1)	Positive
Klebsiella pneumoniae	KPC-2 (6)	Positive
Pseudomonas aeruginosa	KPC-2 (3)	Positive
Escherichia coli	KPC-3 (1)	Positive
Klebsiella pneumoniae	KPC-3 (4)	Positive
Citrobacter freundii	KPC-3 (2)	Positive
IMI -producers (2)		
Enterobacter cloacae	IMI-1 (2)	**Negative**
NDM-producers (75)		
Escherichia coli	NDM-1 (5)	Positive
Klebsiella pneumoniae	NDM-1 (27)	Positive
Klebsiella oxytoca	NDM-1 (2)	Positive
Enterobacter cloacae	NDM-1 (2)	Positive
Citrobacter freundii	NDM-1 (2)	Positive
Proteus mirabilis	NDM-1 (2)	Positive
Providencia stuartii	NDM-1 (1)	Positive
Acinetobacter baumannii	NDM-1 (12)	Positive
Pseudomonas aeruginosa	NDM-1 (5)	Positive
Escherichia coli	NDM-5 (9)	Positive
Klebsiella pneumoniae	NDM-5 (1)	Positive
Enterobacter hormaechei	NDM-5 (1)	Positive
Citrobacter freundii	NDM-5 (1)	Positive
Escherichia coli	NDM-6 (2)	Positive
Klebsiella pneumoniae	NDM-7 (1)	Positive
Enterobacter cloacae	NDM-7 (1)	Positive
Enterobacter cloacae	NDM-9 (1)	Positive
Escherichia coli	NDM-24 (1)	Positive
VIM-producers (26)		
Escherichia coli	VIM-1 (1)	Positive
Klebsiella pneumoniae	VIM-1 (1)	Positive
Klebsiella oxytoca	VIM-1 (1)	Positive
Enterobacter cloacae	VIM-1 (3)	Positive
Citrobacter freundii	VIM-1 (1)	Positive
Proteus mirabilis	VIM-1 (1)	Positive
Providencia stuartii	VIM-1 (1)	Positive
Pseudomonas aeruginosa	VIM-1 (1)	Positive
Pseudomonas aeruginosa	VIM-2 (3)	Positive
Citrobacter freundii	VIM-4 (1)	Positive
Proteus mirabilis	VIM-4 (2)	Positive
Pseudomonas aeruginosa	VIM-4 (3)	Positive
Escherichia coli	VIM-19 (1)	Positive
Klebsiella pneumoniae	VIM-19 (1)	Positive
Pseudomonas aeruginosa	VIM-53 (5)	Positive
IMP-producers (16)		
Klebsiella pneumoniae	IMP-1 (2)	Positive
Pseudomonas aeruginosa	IMP-2 (1)	**Negative**
Klebsiella pneumoniae	IMP-4 (1)	Positive
Acinetobacter baumannii	IMP-4 (1)	Positive
Acinetobacter baumannii	IMP-5 (1)	Positive
Escherichia coli	IMP-8 (1)	**Negative**
Klebsiella pneumoniae	IMP-8 (1)	**Negative**
Enterobacter cloacae	IMP-8 (2)	**Negative**
Pseudomonas aeruginosa	IMP-10 (1)	Positive
Serratia marcescens	IMP-11 (1)	Positive
Pseudomonas aeruginosa	IMP-13 (1)	**Negative**
Pseudomonas aeruginosa	IMP-15 (1)	Positive
Pseudomonas aeruginosa	IMP-19 (1)	**Negative**
Pseudomonas aeruginosa	IMP-29 (1)	Positive
SME-producers (1)		
Serratia marcescens	SME-2 (1)	**Negative**
OXA-48-type producers `(70)		
Escherichia coli	OXA-48 (12)	Positive
Klebsiella pneumoniae	OXA-48 (22)	Positive
Klebsiella aerogenes	OXA-48 (1)	Positive
Klebsiella oxytoca	OXA-48 (1)	Positive
Enterobacter cloacae	OXA-48 (1)	Positive
Citrobacter freundii	OXA-48 (5)	Positive
Citrobacter koseri	OXA-48 (1)	Positive
Hafnia alvei	OXA-48 (1)	Positive
Escherichia coli	OXA-181 (6)	Positive
Klebsiella pneumoniae	OXA-181 (9)	Positive
Klebsiella pneumoniae	OXA-162 (2)	Positive
Klebsiella pneumoniae	OXA-204 (2)	Positive
Escherichia coli	OXA-244 (7)	Positive
OXA-23-producers (16)		
Acinetobacter baumannii	OXA-23 (16)	**Negative**
Double carbapenamase producers (17)		
Klebsiella pneumoniae	NDM-1 + OXA-48 (3)	Positive
Providencia stuartii	NDM-1 + OXA-48 (1)	Positive
Klebsiella pneumoniae	NDM-1 + OXA-181 (2)	Positive
Citrobacter freundii	NDM-1 + OXA-181 (1)	Positive
Klebsiella pneumoniae	NDM-5 + OXA-181 (2)	Positive
Enterobacter cloacae	VIM-1 + OXA-48 (2)	Positive
Klebsiella oxytoca	VIM-1 + OXA-48 (3)	Positive
Citrobacter freundii	VIM-1 + OXA-181 (1)	Positive
Klebsiella pneumoniae	KPC-2 + VIM-1 (1)	Positive
Klebsiella pneumoniae	KPC-3 + NDM-1 (1)	Positive
Noncarbapenemae producers (12)		
Escherichia coli	Wild type (1)	**Negative**
Escherichia coli	CTX-M-1 (1)	**Negative**
Enterobacter cloacae	SHV12 (1)	**Negative**
Enterobacter spp.	Overexpressed cephalosporinase + impermeability (1)	**Negative**
Klebsiella aerogenes	Overexpressed cephalosporinase + impermeability (1)	**Negative**
Empedobacter falsenii	Natural carbapenemase (1)	**Negative**
Acinetobacter baumannii	Wild type (2)	**Negative**
Acinetobacter baumannii	Overexpressed OXA-51 (1)	**Negative**
Pseudomonas aeruginosa	Overexpressed cephalosporinase + impermeability (2)	**Negative**
Pseudomonas aeruginosa	Carbapenemase GES-5 (1)	**Negative**

isolates in our assays coproduced two carbapenemases (NDM-1 + OXA-48, NDM-1 + OXA-181, NDM-5 + OXA-181, VIM-1 + OXA-48, VIM-1 + OXA-181, KPC-2 + VIM-1, and KPC-3 + NDM-1) considering that double carbapenemase producers are increasingly identified worldwide (Table 1). All carbapenemase genes were identified by PCR experiments followed by sequencing. All isolates were grown on URISelect™ 4 agar plates or Muller Hinton agar plates (Bio-Rad; Cressier, Switzerland) for 16 h at 37 °C. The K.N.I.V.O Detection K-Set assay was performed according to the manufacturer's recommendations. Briefly, one calibrated 1 μL-loopful of the strain to be tested was resuspended well in five drops of sample treatment solution included in the kit. Then, 50 μL of the lysis mixture was added into the sample well of the K.N.I. Detection Cassette A (for detection of KPC, NDM, IMP) and the V.O. Detection Cassette B (for detection of VIM and OXA-48). The results were obtained within 10 to 15 minutes of incubation at room temperature. The K.N.I.V.O Detection K-Set allowed the detection of all KPC, OXA-48-types, NDM, VIM and IMP-producers (except IMP-2, -8, -13,-19) (Table 1). Interestingly, those variants (IMP-2, -8, -13,-19) were well detected using the NG-Test Carba 5 version, but not the IMP-13 and IMP-15 variants. In the NG-test Carba 5 version 2, all those variants including IMP-13 and IMP-15 were well detected [8]. The significant diversity of the IMP-type MBLs therefore makes their detection difficult (Figure S1). Currently, more than 80 natural IMP variants, belonging to various sublineages, have been described as the result of the accumulation of more than 54 amino acid substitutions distributed in different regions of the protein [9], unlike NDM-type MBLs for which the number of substitutions is lower, three main amino-acid changes being most commonly identified [10]. Substantial kinetic differences identified among IMP variants respect to the different ß-lactam substrates show that allotypic diversity might have structural and functional implications [1]. IMP-1 is the most prevalent IMP-type MBLs in *Pseudomonas aeruginosa* worldwide especially in Asia, while they are rarely detected in Europe [11]. Noteworthy, this test was able to detect double carbapenemase producing isolates such as NDM-1 and OXA-48-types, KPC-2 and VIM-1. No false positive was observed among the isolates tested

(Table 1). The overall sensitivity and specificity were found to be 96.8 % (CI95 93.6%–98.4%) and 100 % (CI95 79.6%–100%), respectively. However, this test cannot detect the most commonly encountered carbapenemases in *A. baumannii* such as OXA-23 but it was not designed for such detection.

Besides its simplicity of use, the K.N.I.V.O Detection K test gives results of the presence of the five major carbapenemases identified in Enterobacterales, *A. baumannii* and *P. aeruginosa* isolates within 15 minutes. However, that test cannot detect several less frequently encountered carbapenemases, such as the GES, NMC-A, SME, FRI, and IMI enzymes.

A recent multiplex immunochromatographic test has been developed, namely NG-test Carba 5, that can detect carbapenemases (KPC, IMP, VIM, NDM and OXA-48 like enzymes [7]. The NG-test Carba 5 test incorporates specific antibodies against KPC, IMP, VIM, NDM and OXA-48 in a single test cassette. Another immunochromatographic test has been developed, namely the O.K.N.V k-set test (Coris BioConcept; Belgium), which allows the identification of only four enzymes (KPC, OXA-48-like, NDM and VIM) and lack the detection of the IMP carbapenemases [12]. Furthermore, the O.K.N.V k-set test failed to detect some chromosomally encoded carbapenemases such as NDM in *P. mirabilis*.

One limitation of our study with the K.N.I.V.O Detection K test is that it has been done only with cultured strains and has not been established yet for clinical samples. An additional limitation point of all immunochromatographic tests is their ability to detect only known major enzymes compared to biochemical tests, previously proven to be sensitive for rare or unknown carbapenemase detection such as the Rapidec Carba NP® and more recently the Nitrospeed Carba NP test that can detect any type of carbapenemase activity, including potential emerging enzymes, with excellent sensitivity and specificity [4–6].

In conclusion, the K.N.I.V.O Detection K test is a rapid, easy to perform, and showed an overall good specificity for detecting different variants of the five most common carbapenemases identified in Gram-negative bacteria. As already observed for other immunochromatography based tests, it may fail to detect a few IMP producers mostly identified in *P. aeruginosa* and in Asia (Japan) [1]. Given its user friendliness, simplicity, and short time-to-result, the K.N.I.V.O Detection K assay is suitable for microbiology laboratories as well as the broad-spectrum carbapenemase screening rapid tests based on biochemistry.

Authors statement

MS, PN, designed the study. MS, MB and AK performed the experiments. MS and PN drafted the manuscript. LP, MS and PN wrote the final version of the manuscript. All authors agreed on the final version.

Ethical approval

Not required.

Declaration of competing interest

PN and LP are inventors of the Rapid Carba NP test aimed to identify biochemically carbapenemase production in Gram negatives

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.diagmicrobio.2022.115761.

References

[1] Cornaglia G, Giamarellou H, Rossolini GM. Metallo-β-lactamases: a last frontier for β-lactams? Lancet Infect Dis 2011;11:381–93. doi: 10.1016/S1473-3099(11)70056-1.
[2] Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis 2011;17:1791–8. doi: 10.3201/eid1710.110655.
[3] Mojica MF, Rossi MA, Vila AJ, Bonomo RA. The urgent need for metallo-β-lactamase inhibitors: an unattended global threat. Lancet Infect Dis 2022;22:e28–34. doi: 10.1016/S1473-3099(20)30868-9.
[4] Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis 2012;18:1503–7. doi: 10.3201/eid1809.120355.
[5] Bernabeu S, Dortet L, Naas T. Evaluation of the β-CARBA^{TM} test, a colorimetric test for the rapid detection of carbapenemase activity in Gram-negative bacilli. J Antimicrob Chemother 2017;72:1646–58. doi: 10.1093/jac/dkx061.
[6] Nordmann P, Sadek M, Demord A, Poirel L. NitroSpeed-Carba NP test for rapid detection and differentiation between different classes of carbapenemases in enterobacterales. J Clin Microbiol 2020;58:e00932-20. doi: 10.1128/JCM.00932-20.
[7] Boutal H, Vogel A, Bernabeu S, Devilliers K, Creton E, Cotellon G, et al. A multiplex lateral flow immunoassay for the rapid identification of NDM-, KPC-, IMP- and VIM-type and OXA-48-like carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother 2018;73:909–15. doi: 10.1093/jac/dkx521.
[8] Volland H, Girlich D, Laguide M, Gonzalez C, Paris V, Laroche M, et al. Improvement of the immunochromatographic NG-Test Carba 5 assay for the detection of IMP variants previously undetected. Antimicrob Agents Chemother 2020;64 e01940-19. doi: 10.1128/AAC.01940-19.
[9] López C, Delmonti J, Bonomo RA, Vila AJ. Deciphering the evolution of metallo-β-lactamases: a journey from the test tube to the bacterial periplasm. J Biol Chem 2022;298:101665. doi: 10.1016/j.jbc.2022.101665.
[10] Naas T, Oueslati S, Bonnin RA, Dabos ML, Zavala A, Dortet L, et al. Beta-lactamase database (BLDB)-structure and function. J Enzyme Inhib Med Chem 2017;32:917–9. doi: 10.1080/14756366.2017.1344235.
[11] Hickey C, Nguyen S, Anes J, Hurley D, Donoghue O, Fanning S, et al. Differences in antimicrobial susceptibility testing complicating management of IMP carbapenemase-producing Enterobacterales infection. J Glob Antimicrob Resist 2021;27:284–8. doi: 10.1016/j.jgar.2021.09.010.
[12] Kolenda C, Benoit R, Carricajo A, Bonnet R, Dauwalder O, Laurent F. Evaluation of the new multiplex immunochromatographic O.K.N.V. K-SeT assay for rapid detection of OXA-48-like, KPC, NDM, and VIM carbapenemases. J Clin Microbiol 2018;56:e01247-18. doi: 10.1128/JCM.01247-18.