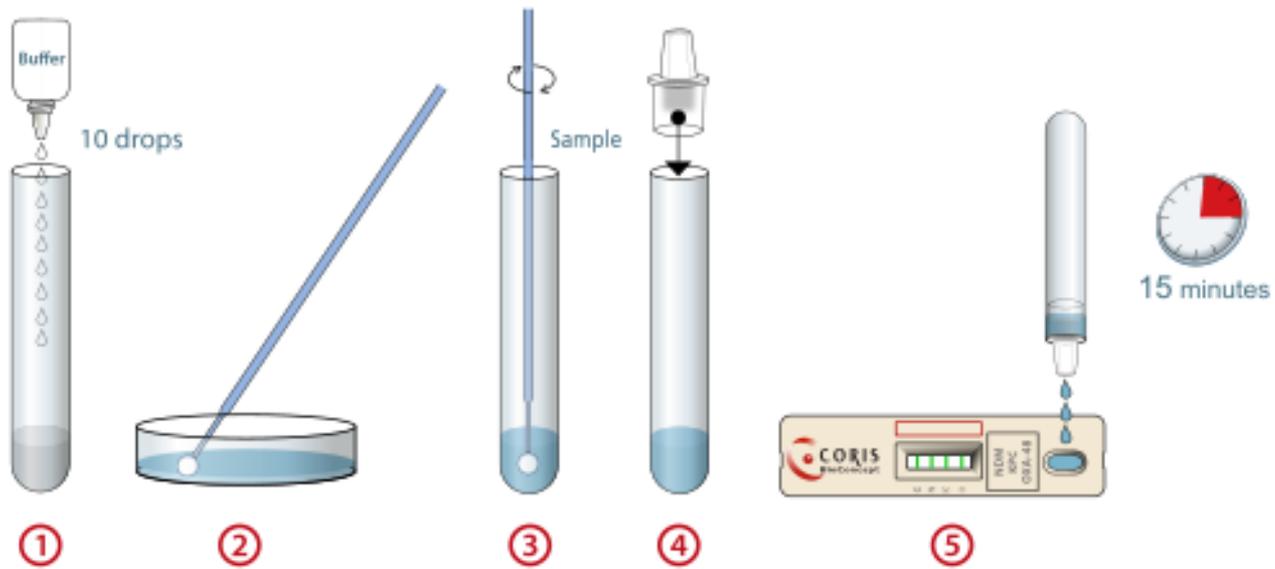


Lateral flow assays for beta-lactamase detection

- almost too good to be true

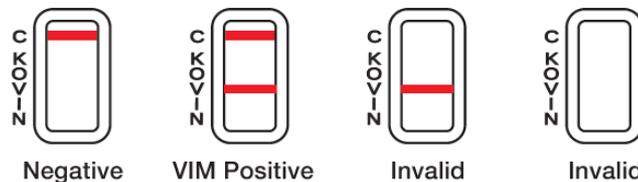
Ulrik Stenz Justesen, MD, DMSc
Department of Clinical Microbiology
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Odense, Denmark

Lateral flow assays for carbapenemase detection



C: Control
 K: KPC
 O: OXA-48 like
 V: VIM
 I: IMP
 N: NDM

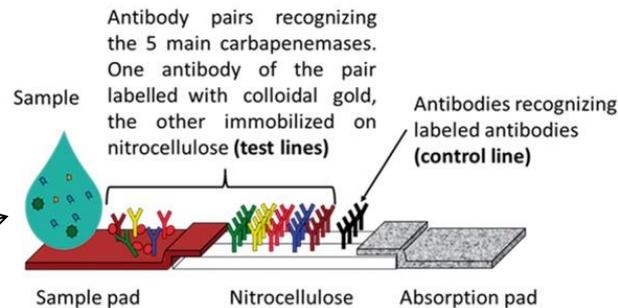
Interpretation



NOTE: Multiple lines or one line on K, O, V, I, N position must be considered as a positive result

Lateral flow assays for carbapenemase detection

1 - Structure of the strip

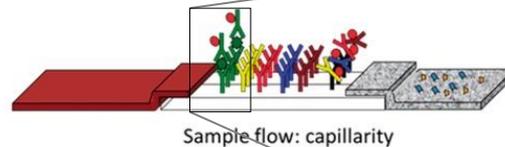


Immunochematographic assay

Round sticky green ones = dangerous beta-lactamase!

Round red ones = Gold nanoparticles!

2 - Immunological detection



3 - Result

- ✓ The control line appears: the test is correct
- ✓ One or several test lines appear: positive test for the corresponding carbapenemase(s)
- ✓ No test line appears: negative test for the 5 carbapenemases

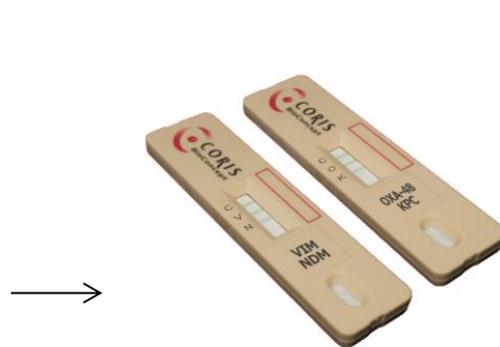


The Carba5 principle
Boutal et al. JAC. 2018.

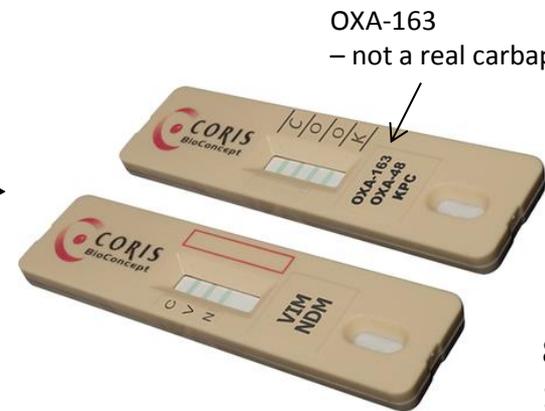
Different Lateral flow assays and editions



Resist-3 O.K.N. K-Set



Resist-4 O.K.N.V. K-Set



Resist-5 O.K.N.V. K-Set

OXA-163
– not a real carbapenemase

86 Dkr. ≈
12 €

C: Control
K: KPC
O: OXA-48 like
V: VIM
I: IMP
N: NDM



NG-Test CARBA 5

135 Dkr. ≈
18 €

OUH
Odense University Hospital
Svendborg Hospital

Lateral flow assays – package insert

Coris BioConcept Resist-3/4/5 O.K.N.V. K-Set:

Detection and characterisation of carbapenemases in *Enterobacteriaceae*.

Sensitivity: **100%** - Specificity: **100%**

NGbioTec NG-Test CARBA 5:

Detection and characterisation of carbapenemases. No limitation regarding species.

Sensitivity: **100%** - Specificity: **100%**

Lateral flow assays – detection of...

OXA-48 like	KPC	NDM	IMP	VIM
OXA-48 (n = 21)	KPC-2 (n = 19)	NDM-1 (n = 16)	IMP-1 (n = 5)	VIM-1 (n = 12)
OXA-162 (n = 1)	KPC-3 (n = 3)	NDM-3	IMP-4	VIM-2 (n = 2)
OXA-181 (n = 3)	KPC-4	NDM-4 (n = 2)	IMP-6	VIM-4 (n = 2)
OXA-204 (n = 5)	KPC-9	NDM-5 (n = 1)	IMP-7	VIM-5
OXA-232 (n = 2)		NDM-6 (n = 1)	IMP-8 (n = 5)	VIM-19 (n = 1)
OXA-244 (n = 2)		NDM-7 (n = 1)	IMP-10	VIM-26
OXA-245		NDM-8	IMP-11 (n = 1)	VIM-27
OXA-370		NDM-9 (n = 2)	IMP-22	VIM-31
OXA-436			IMP-29	VIM-46
OXA-484				VIM-51
OXA-517 (n = 1)				VIM-52
OXA-519 (n = 1)			IMP-13	VIM-54
OXA-535 (n = 1)			IMP-14	VIM-56
				VIM-58
				VIM-59

Summary from +10 studies in *Enterobacteriaceae*,
Pseudomonas spp. and *Acinetobacter* spp. (and more)
 with both assays.

Isolates from the NordicAST CPE study

Head-to-head test:

Resist-4 O.K.N.V. K-Set **versus** NG-Test CARBA 5

J Antimicrob Chemother 2018; **73**: 2738–2747
doi:10.1093/jac/dky276 Advance Access publication 24 July 2018

**Journal of
Antimicrobial
Chemotherapy**

**Performance of the EUCAST disc diffusion method and two MIC
methods in detection of Enterobacteriaceae with reduced
susceptibility to meropenem: the NordicAST CPE study**

Table 1. Bacterial strains (n = 27) used in this study and their relevant characteristics

Carbapenemase class/species	Meropenem MIC (mg/L)	Carbapenemases	Other β -lactamases	Isolate no.
Class A (serine) (n = 4)				
<i>K. pneumoniae</i>	1	KPC-2	SHV-11; TEM-1	4
<i>E. cloacae</i> complex	≥ 16	KPC-2	CTX-M-15; OXA-1; TEM-1	19
<i>E. cloacae</i> complex	8	KPC-2	ACT-6[v ^a]; OXA-9-p ^b ; TEM-1	28
<i>E. cloacae</i> complex	1	IMI-9	ACT[v]	3
Class B (MBL) (n = 9)				
<i>K. pneumoniae</i>	≥ 16	NDM-1 + OXA-181 ^d	CTX-M-15; OXA-1; SHV-11; TEM-1	2
<i>K. pneumoniae</i>	≥ 16	NDM-1	CTX-M-15; CMY-6; OXA-1; SHV-11	5
<i>P. mirabilis</i>	0.25	NDM-1	CMY-16; OXA-10	8
<i>E. coli</i>	2	NDM-5	SHV-12; TEM-1	9
<i>Citrobacter</i> sp.	4	NDM-1	CMY-4; OXA-10; SED-1	11
<i>E. coli</i>	1	VIM-29	CTX-M-15; CMY-4; OXA-1	7
<i>K. pneumoniae</i>	2	VIM-1	SHV-12	27
<i>K. pneumoniae</i>	≥ 16	VIM-27	SHV-11	29
<i>E. coli</i>	1	IMP-26	CTX-M-15; TEM-1	24
Class D (OXA-48-like) (n = 6 ^c)				
<i>K. variicola</i> ^c	0.5	OXA-48	LEN-16	6, 10, 25
<i>K. pneumoniae</i>	2	OXA-48	CTX-M-15; OXA-1; SHV-76; TEM-1	1
<i>K. pneumoniae</i>	0.5	OXA-48	SHV-11	14
<i>E. coli</i>	1	OXA-48	CTX-M-24; TEM-1	17
<i>E. coli</i>	0.5	OXA-181 ^d	CTX-M-15; CMY-2; OXA-1; TEM-1	23
<i>K. pneumoniae</i>	2	OXA-162 ^d	CTX-M-15, SHV-100, TEM-1	26
CPE-negative (n = 8)				
<i>K. pneumoniae</i>	4	—	CTX-M-15; SHV-11; OXA-1	12
<i>E. coli</i>	8	—	CMY-42; OXA-1; TEM-1	13
<i>K. pneumoniae</i>	4	—	CTX-M-15; OXA-1; SHV-27	15
<i>E. cloacae</i> complex	0.25	—	ACT-15	16
<i>E. coli</i>	8	—	CTX-M-15; OXA-1	18
<i>P. mirabilis</i>	0.5	—	—	20
<i>Enterobacter aerogenes</i>	8	—	—	21
<i>P. rettgeri</i>	2	—	PER-1	22

^av = variant gene.^bp = partial gene.^cThe *K. variicola* strain was provided in triplicate.^dOXA-181 and OXA-162 belong to the OXA-48-like group.

Not detected with the Resist-4 O.K.N.V. K-Set

Detected with the NG-Test CARBA 5 but not the Resist-4 O.K.N.V. K-Set - IMP not included

Limitations (are limited)

- False-negative results for NDM can occur (Resist-3 O.K.N. K-Set).
Recommend an inoculum of 1 that is harvested adjacent to an ertapenem or meropenem disk and the use of agars with sufficient zinc content to achieve the best performance.
(Saleh et al. JCM 2018)
- IMP-13- and IMP-14-like carbapenemases were not detected (NG-Test CARBA 5).
Problems with detecting these IMP variants were also observed during an evaluation of the Cepheid XpertVR Carba-R assay.
(Hopkins et al. JAC. 2018)
- VIM-5 variant being undetected, in one *P. aeruginosa* (Resist-4 O.K.N.V. K-Set).
(Glupczynski et al. JAC. 2019)

EUCAST guidelines for detection of resistance
mechanisms and specific resistances of clinical and/or
epidemiological importance

Version 2.0¹
July 2017

2.4 Recommended methods for detection of carbapenemases in Enterobacteriaceae

Lateral flow assays

A new immunochromatographic lateral flow assay was recently described. The test is based on immunological capture of epitopes of OXA-48, using colloidal gold nanoparticles bound to a nitrocellulose membrane within a lateral flow device. The principle of the test is that monoclonal anti-OXA-48 antibodies are selected as specific capture reagents, for direct identification OXA-48-like enzymes (42). The assay takes around four minutes, and has been evaluated both from colonies and from spiked blood culture bottles (43-46). Recently, a similar test for KPC has also been developed, but its robustness has not been evaluated by multiple single center studies or one multicenter study (46).

Colonies and directly on blood!

IDENTIFICATION PROCESS FROM BACTERIAL CULTURE OR DIRECT BLOOD CULTURE



*Validated on: TSA, Mueller Hinton, ChromID® CARBA SMART, Drigalski (DRIG) CHROMagar™ mSuperCARBA™...etc

Wash and spin – very easy

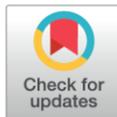
RESEARCH ARTICLE

Rapid detection of NDM, KPC and OXA-48 carbapenemases directly from positive blood cultures using a new multiplex immunochromatographic assay

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OPEN ACCESS

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files. The detailed protocols can additionally be accessed at protocols.io <http://dx.doi.org/10.17504/protocols.io.rtrd6m6>.

Abstract

Bloodstream infections caused by carbapenemase-producing Enterobacteriaceae (CPE) are associated with treatment failure and increased mortality. Detection of CPE from blood cultures (BC) by standard methods takes 16–72 hours, which can delay the initiation of appropriate antimicrobial therapy and compromise patient outcome. In the present study, we developed and evaluated a new method for the rapid detection of carbapenemases directly from positive BC using a new multiplex immunochromatographic test (ICT). The new ICT was assessed using 170 molecularly characterized *Enterobacteriaceae* clinical isolates including 126 CPE (OXA-48-like (N = 79), KPC (N = 18) and NDM (N = 29)). After spiking with bacteria and incubation in a BC system, blood from positive BC bottles was hemolyzed, bacteria concentrated by centrifugation and lysed. The lysate was transferred to the RESIST-3 O.K.N. ICT (Coris BioConcept, Gembloux, Belgium), which detects OXA-48-like, KPC and NDM carbapenemases. The final results of the ICT were read when they became positive, at the latest after 15 min. All CPE isolates (126/126) were correctly detected with the new protocol (100% sensitivity, 100% specificity). There was perfect concordance between ICT results and molecular characterization. Total time to result was 20–45 min.

Conclusions: This proof-of-principle study demonstrates that with the newly developed method, OXA-48-like, KPC and NDM carbapenemases can be reliably detected directly from positive BC bottles. The new method is more rapid than other currently available assays and can be performed in any routine microbiology laboratory. This can help to rapidly identify patients with CPE BSI and optimize the management of patients with these difficult-to-treat infections. Further studies are needed to assess the performance of the ICT in routine diagnostics.



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MICROBIOLOGY

Antimicrobial Agents
and Chemotherapy®

MECHANISMS OF RESISTANCE



NG-Test Carba 5 for Rapid Detection of Carbapenemase-Producing *Enterobacterales* from Positive Blood Cultures

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^dBacteriology-Hygiene Unit, Assistance Publique/Hôpitaux de Paris, Bicêtre Hospital, Le Kremlin-Bicêtre, France

ABSTRACT The immunochromatographic assay, NG-test Carba 5 (NG Biotech), has been evaluated for detection of carbapenemase-producing *Enterobacterales* (CPE) from spiked blood cultures ($n = 205$). It detected and discriminated in less than 30 minutes KPC, IMP, VIM, NDM, and OXA-48-like producers with a sensitivity and specificity of 97.7% and 96.1%, respectively. Thus, it might help the rapid optimization of treatment of bloodstream infections due to CPE.

Lateral flow immunochromatographic assay for rapid screening of faecal carriage of carbapenemase-producing Enterobacteriaceae

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Background: Rapid and effective screening of carbapenemase-producing Enterobacteriaceae (CPE) appears essential for adequate patient management and rapid implementation of infection control measures. Most of these screening techniques require a minimum of 24 h of culture. Molecular assays are an exception since these can be achieved within 1 h, but are expensive and usually require specialized facilities and trained personnel. In this context, lateral immunochromatography performed directly from rectal swab samples could represent a cost-effective alternative with a reduced turnaround time.

Objectives: In this study, we assessed the performance of the OKN K-SeT test (Coris BioConcept, Gembloux, Belgium) for the rapid detection of OXA-48, KPC and NDM CPE directly from rectal swab samples.

Methods: A total of 149 residual rectal swabs, routinely screened for CPE through selective culture and confirmed by PCR, were tested with a defined protocol consisting of a 2.5 h incubation of the swab in an enrichment medium containing meropenem followed by OKN K-SeT testing after centrifugation.

Results: This method displayed an overall sensitivity of 96% and a specificity of 100% with a limit of detection ranging between 10⁴ and 10⁵ cfu/mL.

Conclusions: Whereas this assay appears highly specific, it displays a reduced sensitivity compared with the standard procedure encompassing a culture step. Nonetheless, this rapid method allows an accelerated identification of most CPE carriers at a lower cost and, accordingly, the implementation of early appropriate management procedures.

Summary – lateral flow assays

Detection (and characterisation) of carbapenemases:

- A positive predictive value of “100%” on colonies, blood and faecal samples (key message)
- Sensitivity less than 100% on blood and faecal samples
- Works for *Enterobacteriaceae*, *Pseudomonas* spp. and *Acinetobacter* spp.
- A valuable supplement in the routine clinical microbiology laboratory (personal opinion)
- Does not detect ALL the different carbapenemases in the world
- Still need regular susceptibility testing
- Epidemiological information is limited