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INTERNATIONAL SCIENTIFIC PUBLICATIONS & POSTERS
SUMMARY

Carbapenemases……………………………………………………1-14

Colistin Resistance ……………………………………………………15-17

Extended Spectrum β-Lactamases ……………………………..19-21
A multiplex lateral flow immunoassay for the rapid identification of NDM-, KPC-, IMP- and VIM-type and OXA-48-like carbapenemase-producing Enterobacteriaceae.

Hervé Boutal and all – France – Published on JAC January 2018

➢ Objectives

The global spread of carbapenemase-producing Enterobacteriaceae represents a substantial challenge in clinical practice and rapid and reliable detection of these organisms is essential. The aim of this study was to develop and validate a lateral flow immunoassay (Carba5) for the detection of the five main carbapenemases (KPC-, NDM-, VIM- and IMP-type and OXA-48-like).

➢ Methods

Carba5 was retrospectively and prospectively evaluated using 296 enterobacterial isolates from agar culture. An isolated colony was suspended in extraction buffer and then loaded on the manufactured Carba5.

➢ Results

All 185 isolates expressing a carbapenemase related to one of the Carba5 targets were correctly and unambiguously detected in 15 min. All other isolates gave negative results except those producing OXA-163 and OXA-405, which are considered low-activity carbapenemases. No cross-reaction was observed with nontargeted carbapenemases, ESBLs, AmpCs or oxacillinases (OXA-1, -2, -9 and -10). Overall, this assay reached 100% sensitivity and 95.3% (retrospectively) to 100% (prospectively) specificity.

➢ Conclusions

Carba5 is efficient, rapid and easy to implement in the routine workflow of a clinical microbiology laboratory for confirmation of the five main carbapenemases encountered in Enterobacteriaceae.
Evaluation of the NG-Test® CARBA 5 multiplex immunochromatographic assay for the detection of KPC, OXA-48-like, NDM, VIM and IMP carbapenemases.

Katie L. Hopkins and all – UK – Published on JAC September 2018

➢ Objectives

No single method currently commercialized for detecting acquired carbapenemases offers comprehensive coverage, but local testing is needed to support rapid detection and prompt action. Within the UK and globally most carbapenemase producers harbour one or more of the ‘big 5’ families: KPC, OXA-48-like, NDM, VIM and IMP. Diagnostic laboratories should therefore consider tests that reliably detect at least four or, preferably, all five of these families.

We evaluated the NG-Test CARBA 5 immunochromatographic assay (NG Biotech, Guipry, France), for detecting the ‘big 5’ carbapenemases in isolates referred to the UK’s national reference laboratory.

➢ Methods

The NG-Test CARBA 5 assay was evaluated using 197 previously characterized bacterial isolates including 177 confirmed carbapenemase producers and 20 carbapenem-resistant. One colony of overnight growth harvested from Columbia blood agar plates was tested according to the manufacturer’s instructions.

➢ Results

After the first round of testing, i.e. including the false-positives but excluding the true-negatives due to gene loss, the overall sensitivity and specificity of the NG-Test CARBA 5 were 97.31% (95% CI 93.84%–99.12%) and 99.75% (95% CI 99.12%–99.97%), respectively. Although both sensitivity and specificity increased following repeat testing, the first NG-Test CARBA 5 assay results would be recorded in a frontline laboratory, with no reason to repeat.

➢ Conclusion

The NG-Test CARBA 5 assay was easy to perform and set-up took 5min per isolate with relatively little hands-on time. Although the final result was read at 15min, positive results started to appear within 2–6min.
Phenotypic Detection of Carbapenemase-Producing Organisms from Clinical Isolates.

Pranita D. Tamma, Patricia J. Simner (J. Hopkins University of Medicine) – USA – Published on JCM Nov 2018

Lateral flow immunoassays.

LFIAs are antibody-based methods to identify the presence of carbapenemases. A number of LFIAs have been recently developed but generally enable the detection of one or a few of the most epidemiologically important carbapenemases. LFIAs have been developed for NDM production, IMP-production, OXA-48-like production, KPC and OXA-48-like production, and KPC, NDM, and OXA-48-like production. Available data suggest that LFIAs produce accurate results from cultured isolates within 15 min.

Recently, an LFIA targeting the five main carbapenemase families (i.e., KPC, NDM, VIM, IMP, and OXA-48-like carbapenemases) was evaluated. Investigators both retrospectively and prospectively evaluated this LFIA, named the NG-Test Carba 5, using 296 Enterobacteriaceae isolates. Briefly, they collected a single bacterial colony from a Mueller-Hinton agar plate and suspended it in 150 µl of extraction buffer. Subsequently, 100 µl of this extract was loaded on a cassette, and results were read within 15 min of migration, based on the presence of visible lines indicating a positive test.

In both the retrospective and prospective studies, the sensitivity of the NG-Test Carba 5 was 100% (11). The specificity was 95% for the retrospective cohort and 100% for the prospective cohort, with two noncarbapenemase OXA enzymes (an OXA-405 and two OXA-163 enzymes) misclassified as OXA-48-like enzymes.

Importantly, the Carba5 was accurately able to detect isolates producing two carbapenemases (e.g., NDM-type and OXA-48-like carbapenemases), and cross reactivity was not observed with nontargeted carbapenemases (e.g., GES, SME, etc.).

In the future, it is plausible that the NG-Test® Carba 5 or similar LFIAs could offer easy-house, accurate, rapid, and cost-efficient approaches for clinical microbiology laboratories to identify the presence of specific carbapenemases similar to molecular-based approaches.
NG-Test® CARBA 5
Validation of NG-Test® CARBA 5 with CHROMagar™ mSuperCARBA™

By CHROMagar – France – January 2018

➢ Objectives

Rapid immunoassay, ready to use, for the detection of the 5 main carbapenemases: KPC (Class A), OXA (Class D), and the metallo β-lactams of IMP type, VIM as well as NDM (Class B) in a fresh bacterial colony obtained from solid agar medium after culturing (16 hours) and processed by vortex in an extraction buffer (150 µL).

➢ Limitations

Qualitative assay (not quantitative) giving results to be confirmed with some culture in a specific agar-based medium and by assessing the minimum inhibitory concentration (MIC). A positive test does not rule out the presence of other mechanism of antibiotic resistance.

➢ Results

The 5 main carbapenemases targeted were correlated with the phenotypes of the QC strains used with CHROMagar™ mSuperCARBA™. The following carbapenemase variants were detected:
NDM-1, KPC, OXA-48, OXA-244, IMP-1 and VIM-1 from colonies growing on CHROMagar™ mSuper-CARBA™. Non-CPE strains showed negative results.

➢ Conclusion

NG-Test CARBA 5 showed 100% sensibility and 100% specificity for carbapenemase producers. The NG-test can be carried directly from colonies growing on CHROMagar™ mSuperCARBA™.
Evaluation of a multiplex immunochromatographic assay for the rapid detection of carbapenemase-producing Enterobacteriaceae from culture colonies.

Andrea Bartolini, Margherita Scapaticci, Maira Zoppelletto, G. Da Rin – Italy – SIBioC Congress November 2018

➢ Background:

An accurate and fast detection of infected patients or colonized carriers is mandatory for both therapeutic management and infection control purposes.

The aim of this study was to assess the performance of a multiplex immunochromatographic assay (NG-Test® CARBA 5, NG Biotech, Guipry, France) for the rapid detection (15 minutes) of KPC, NDM, VIM, IMP and OXA-48 CPE directly from pure colonies.

➢ Method:

A collection of 49 non-replicated Enterobacteriaceae isolates with decreased susceptibility to carbapenems, including 45 Klebsiella pneumoniae, 3 Escherichia coli and 1 Enterobacter cloacae, were analysed with NG-Test CARBA 5.

Concurrently combination disk test (CDT) was performed according to EUCAST indications, while confirmation of carbapenemase production was achieved by PCR. ATCC 700603 and NCTC 13438 were used as negative and positive control, respectively.

➢ Results:

PCR assay permitted to found 41 CPE strains, including 38 K. pneumoniae (29 producing KPC, 5 NDM, 1 VIM and 3 co-producing NDM and OXA-48) and 3 E. coli (2 NDM+OXA-48 and 1 OXA-48), while 8 isolates were found as non-carbapenemase producing: 6 K. pneumoniae, 1 E. coli, 1 E. cloacae. CDT allowed us to consider those 8 strains as ESBL or AmpC β-lactamase producers.

NG-Test CARBA 5 successfully identified 41/41 CPE (100% sensitivity, 100% specificity). In addition we report that, unlike NG-Test CARBA 5, CDT was not able to correctly identify the 5 strains co-producing NDM and OXA-48 carbapenemases.

➢ Conclusion:

NG-Test CARBA 5 is a reliable assay that can be useful in contexts requiring a rapid identification of CPE directly from culture colonies. Furthermore, this test is an easy-to-use option that permits to avoid misidentification of carbapenemases co-producers’ strains.
RAPID DETECTION OF THE 5 MAIN CARBAPENEMASES BY PHENOTYPIC METHODS.

Laura Viñuela, Lorena López-Cerero, Felipe Fernández-Cuenca, Inmaculada López, Álvaro Pascual – SEIMC – Spain 2018

Introduction/ Objectives

Currently in our environment, the carbapenemase producing isolates behave epidemiologically different according to the enzyme group. In addition, recently it began to detect isolated producers of more than one carbapenemase which makes interpretation and phenotypic characterization of these isolates difficult. For both reasons, it is necessary to quickly identify the determinant of resistance in the clinical laboratory. The study of the carbapenemase group can be carried out by molecular techniques, which are expensive, or by immunochromatography. Our objective has been to evaluate a new rapid multiplex technique of immunochromatography that allows to detect NDM, OXA-4, KPC, VIM and IMP in 15 minutes.

Material and methods

Thirty gram-negative isolates resistant to carbapenems were selected (K. pneumoniae 43%, E. cloacae 17%, E. coli and C. freundii 10%, K. oxytoca and P. aeruginosa 7%, A. baumanii and L. adercaboxylata 3%) of the Andalusian Molecular Typing Reference Laboratory (PIRASOA) in which the genes coding for the main carbapenemase groups (KPC, VIM, IMP, NDM and OXA-48) were characterized by GeneXpert® (Cepheid), PCR conventional and sequencing.

The isolates producing the OXA-48 group were also characterized by the immunochromatography of Coris BioConcept (LT). We included 21 (70%) strains with a single type of carbapenemase, 4 (13%) strains with two types of carbapenemase, 3 (10%) strains with plasmid AmpC (pAmpC) and 2 (7%) non-producers of carbapenemase.

The readings were made at 1, 5, 10 and 15 minutes. These isolates were studied with the commercial method NG-Test CARBA 5® (NG Biotech) from colony after 18 h of incubation, according to manufacturer’s instructions.

Results

The expected carbapenemases were detected in 23 (88.1%) isolates. The 4 isolates with 2 carbapenemases were all positive (2 OXA-48 + VIM, NDM + VIM, NDM + OXA-48). The isolates with KPC-2 and KPC-3 variants were positive, but the KPC-31 producing isolate was negative. An isolate, producer of IMP-16 (25% of positive IMPs), gave a positive result by NG5 and negative by GeneXpert. An OXA-48 producing isolate (11% of OXA-48) was negative by NG5 and positive by LT. The rest of the OXA-48 producers were positive, including the OXA-245 variant. No positive reaction was observed in the 5 isolates no carbapenemases producers.

48% of the positive results were obtained in 1 minute and 92% in 5 minutes.

Conclusions

1) This technique allows an identification of the main carbapenemase groups detected in our environment, even in isolated producers of more than one carbapenemase.
2) It is a rapid technique, since the results can be obtained in most cases in less than 5 minutes, easy to perform in a clinical laboratory and with less cost than commercial molecular techniques.
3) The concordance of this rapid technique is good, with both genotypic and phenotypic methods, except for some OXA-48 producing isolates and rare variants of KPC.
Evaluation of NG-Test CARBA5 for the detection of carbapenemase-producing Gram-negative bacilli.

Sayaka Ando, Ryuichi Nakano, Ayako Tanouchi, Tomoki Mizuno, Akiyo Nakano, Yuki Suzuki, Naoki Kakuta, Takashi Masui, Hisakazu Yano – Japan – ECCMID April 2019

➢ Background
Various carbapenemases have been reported, with KPC, IMP, NDM, VIM and OXA-48 detected globally. Their rapid detection is important to prevent further transmission and for appropriate antimicrobial therapies. An immunochromatographic assay NG-Test CARBA5 can readily and rapidly detect these ‘big 5’ carbapenemase-producers. Here, we evaluated the NG-Test CARBA5 for carbapenemase-producing Gram-negative bacilli isolated in Japan.

➢ Materials/Methods
Gram-negative bacilli including clinical isolates and stock in our laboratory (n=157) were used. One hundred five carbapenemase-producers contained class A (KPC, NMC-A, IMI, GES), class B (IMP, NDM, VIM), and class D (OXA-23-like, OXA-48-like, OXA-51-like). Fifty-two non-carbapenemase-producers contained ESBL and AmpC β-lactamases. Antimicrobial susceptibility was tested by microdilution according to the CLSI guideline. NG-Test CARBA5 was performed according to the manufacturer’s protocol.

➢ Results
The NG-Test CARBA5 was positive for 98 of 99 strains (98.9%) producing KPC, IMP, NDM, VIM, or OXA-48-like strains, regardless of carbapenem MIC (Table 1). A false-negative strain was producing IMP-66. Of the six strains co-expressing carbapenemases, each carbapenemase could be also identified simultaneously. Another six carbapenemase (NMC-A, IMI, and others) producers and 52 non-carbapenemase-producers were negative, even though when they were resistant to carbapenem.

➢ Conclusions
NG-Test CARBA5 is able to detect and identify the ‘big 5’ carbapenemases with high specificity, including strains with low carbapenem MIC. This test is rapid and easy and is expected to be used in any clinical site globally.

Table 1. Results of antimicrobial susceptibility testing and NG-Test

<table>
<thead>
<tr>
<th>Carbapenemase (number of variants)</th>
<th>Strains</th>
<th>NG-Test positive</th>
<th>MIC range (mg/L)</th>
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<tr>
<td></td>
<td></td>
<td>Imipenem</td>
<td>Meropenem</td>
</tr>
<tr>
<td>KPC-2</td>
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<td>22</td>
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</tr>
<tr>
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<td>24</td>
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</tr>
<tr>
<td>NDM (3)</td>
<td>23</td>
<td>23</td>
<td>8 - &gt;8</td>
</tr>
<tr>
<td>VIM-2</td>
<td>10</td>
<td>10</td>
<td>≤0.5 - &gt;8</td>
</tr>
<tr>
<td>OXA-48-like (4)</td>
<td>13</td>
<td>13</td>
<td>≤0.5 - 4</td>
</tr>
<tr>
<td>co-expressing strains</td>
<td>6</td>
<td>6</td>
<td>1-&gt;8</td>
</tr>
<tr>
<td>NMC-A, IMI, GES-5, OXA-51-like, or OXA-23-like</td>
<td>6</td>
<td>0</td>
<td>2 - &gt;8</td>
</tr>
<tr>
<td>None</td>
<td>52</td>
<td>0</td>
<td>≤0.5 - &gt;8</td>
</tr>
</tbody>
</table>
Verification of the NG-Test CARBA 5 Immunochromatographic Assay to Simultaneously Detect KPC, NDM, OXA48-like, VIM and IMP Enzymes in Species-Diverse Carbapenem-resistant Gram-Negative Bacilli.

Bryn Hazlett, Yaroslav Sokolskyy, Pauline Lo, T. Mazzulli, A. McGeer, S M. Poutanen - Canada - ECCMID April 2019

➢ Objectives

Rapid detection of carbapenemase-producing organism (CPO) is essential for containment and patient management. We evaluated the ability of the NG-Test CARBA 5 immunochromatographic assay (NG Biotech, France) to detect five common carbapenemases using well-characterized Gram-negative bacilli (GNB).

➢ Methods

297 GNB including 257 CPO (250 targeted-CPO: 127 KPC, 69 NDM, 32 OXA, 11 NDM+OXA, 8 VIM, 3 IMP; 8 non-targeted CPO (3 GES, 3 SME, 1 NMC), and 40 non-CPO were tested comprising 289 Enterobacteriaceaeae (115 Klebsiella pneumoniae, 79 Escherichia coli, 51 Enterobacter cloacae, 44 other) and 8 non-Enterobactericeae GNB (2 Acinetobacter baumannii, 3 Pseudomonas species, 2 Aeromonas hydrophila, and 1 Shewanella putida).

Isolates were recovered from -80OC under selective pressure (MacConkey with ertapenem disc) and plated to Oxoid MacConkey-cefpodoxime/MacConkey-meropenem CPO screening bi-plates. As directed, a fresh 18h colony of each isolate was collected with a loop and suspended in extraction buffer; 100μL of each was added to the sample well with results read at 15min. The reader was blinded and discrepancies were repeated.

➢ Results

Of 297 tests, results were easy to interpret and all but three (2 OXA, 1 mucoid KPC) were available within 5 minutes.
CARBA 5 initially identified 257/261 (98.5%; 96.0-99.5) targeted CPO-proteins [125/127 (98.4%; 94.1-99.9) KPC; 79/80 (98.7%; 92.6-99.9) KPC; 42/43 (97.7%; 86.8-99.9) OXA, 8/8 (100%; 62.8-100) VIM; 3/3 IMP (100%; 38.0-100%)].
Four (1.5%) targeted-CPO that were initially missed were positive on repeat testing suggesting too low an inoculum was possibly initially used.
All 48 (100%; 91.1-100) non-targeted-CPO/non-CPO were negative.

Final CPO-detection sensitivities/specificities were 100% for all targets; respective 95%CI were: KPC 96.5-100/96.6-100; NDM 94.5-100/97.5-100; OXA48-like 90.2-100/ 96.6-100; VIM 62.8-100/97.9-100; IMP 38.0-100/98.2-100.

➢ Conclusion

The NG-Test CARBA 5 was easy to use and provided highly-accurate (100% sensitive/specific) rapid detection for KPC, NDM, OXA48-like, VIM and IMP CPO.
Comparison of five methods for detection of carbapenemases in Enterobacterales with proposal of a new algorithm.

Luis Lucena Baeza, Niels Pfennigwerth, Christopher Greissl, Stephan Göttig, Ahmad Saleh, Yvonne Stelzer, Sören G. Gatermann, Axel Hamprecht – Germany – Published on CMI April 2019

➢ Objectives

The aim of this study was to evaluate the performance of five different carbapenemase tests and to develop an algorithm which will permit the detection of most common and rare carbapenemases in routine microbiology laboratories.

➢ Materials and Methods

The immunochromatographic tests CARBA-5 (NG), RESIST-4 O.K.N.V. (Coris), the colorimetric β-CARBA (BioRad), a newly developed carbapenem-inactivation method (CIM) supplemented with zinc (zCIM) and the Xpert Carba-R (Cepheid) were challenged with a collection of 189 molecularly characterized Enterobacterales isolates, including 146 carbapenemase producers (CPE): VIM (n=48), 41 OXA-48-like (n=40), NDM (n=29), KPC (n=13), IMI (n=9), IMP (n=9), OXA-58 (n=2) and GES (n=2).

➢ Results

The overall sensitivity/specificity values for the five carbapenemase detection tests were 84.2% (CI 77.6%-89.2%) /100% (CI 91.8%-100%) for RESIST-4, 88.2% (CI 82.1%-92.4%) /100% (CI 91.8%-100%) for CARBA-5, 88.2% (CI 82.1%-92.4%) /100% (CI 91.8%-100%) for Xpert Carba-R, 73.7% (CI 66.2%-80.0%) /100% (CI 93.4%-99.0%) for β-CARBA and 97.4% (CI 87.9%-99.6%) /97.7% (CI 87.9%-99.6%) for zCIM. The four common carbapenemases (KPC, OXA-48-like, NDM and VIM) were detected with ≥97.6% sensitivity by all tests except for β-CARBA (76.6% [CI 68.4%-83.2%]). IMI and GES were only detected by zCIM (sensitivity 90.9% [CI 62.3%-98.4%]). Based on these results a new algorithm was developed, consisting of an immunochromatographic assay as the first test followed by zCIM, which allows detection of 99.3% of all carbapenemases assessed.

➢ Conclusion

Most evaluated assays showed good sensitivity and specificity, at least for the common carbapenemase families OXA-48-like, KPC, VIM and NDM and are suitable for the routine microbiology lab, thereby decreasing the time to detection and the need to refer these isolates to reference laboratories. However, the detection of rare carbapenemases (e.g. IMI, GES, IMP or OXA-58) is still problematic with most of the commercially available tests. The combination of two tests, e.g. CARBA-5+zCIM, will enable most laboratories to detect these rare variants at low costs. This can help to optimize patient treatment and to limit the further spread of CPE.

Elias Bodendoerfer, Peter M. Keller and Stefano Mancini – Switzerland – Published on JAC March 2019

➢ Objectives

Bloodstream infections (BSIs) caused by carbapenemase-producing Enterobacteriales (CPE) represent one of the major causes of infectious disease mortality worldwide. Phenotypic detection of CPE from blood cultures by conventional methods usually takes 16–72h, which can delay the initiation of appropriate therapy and affect the outcome for the patient. Rapid detection and characterization of the carbapenemase class are equally important for optimizing the management of patients with CPE BSI.

➢ Materials and Methods

Hamprecht et al. have recently established a protocol for rapid identification of OXA-48-like, KPC and NDM carbapenemases directly from positive blood cultures. This method is based on SDS-based lysis of human blood cells followed by concentration and lysis of bacterial cells. We assessed a this protocol for rapid identification of OXA-48-like, KPC, NDM, VIM and IMP carbapenemases directly from positive blood cultures using Carba-5.

➢ Results

A collection of 158 carbapenem-resistant CRE, including 31 carbapenemase negative isolates, 26 Ambler class A producers (KPC), 43 class B producers (29 NDM, 11 VIM and 3 IMP), 57 class D OXA-48-like producers and 1 isolate containing both an NDM and an OXA-48-like enzyme, were used in this evaluation.

Carba-5 allowed detection of all 127 CPE (100% sensitivity) and produced no band with the 31 carbapenemase-negative isolates (100% specificity).

Of note, all tested NDM, KPC, IMP, VIM and OXA-like variants were detected, and this study added the carbapenemase variants VIM-5, NDM-19 and KPC-14 to the range previously found. Time to positivity was 5min for all enzymes.

➢ Conclusion

Using the proposed bacterial preparatory method and the Carba-5 test, detection and characterization of the ‘big five’ carbapenemases in CPE causing BSIs can be achieved with minimal technical expertise in 20–40min. This protocol is faster than any currently available assay and can be implemented in any microbiology laboratory for the detection of CPE BSIs.
Introduction

Reliable detection of carbapenemases is required in the microbiological laboratory. A novel test principle is the detection of carbapenemases by immunochromatographic test strips, e.g. the NG-Test Carba5 (detecting KPC, VIM, IMP, NDM and OXA-48).

Method

The Carba5 test was evaluated at the German National Reference Center for Multidrug-resistant Gram-negative bacteria using a challenging strain collection of 99 Gram-negative clinical isolates with 63 strains producing 44 different variants or combinations of VIM, NDM, OXA-48-like, KPC and IMP carbapenemases that were previously characterized by phenotypic and genotypic tests. Isolates producing DIM, FIM and GIM were also tested to analyse the potential of false-positive results.

Results and Discussion

The Carba5 test was able to detect 55/63 carbapenemase producing isolates (Table 1). No false-positive results were observed. Three of the non-detected carbapenemases were DIM-1, FIM-1 and GIM-1, which are not covered by the test and thus were expected to be non-detectable. These isolates were counted as correct negative results. The Carba5 test was not able to detect the IMP variants IMP-13, IMP-14, IMP-31 and IMP-50 according to the manual.

A total of 14 isolates produced more than one carbapenemase. All these carbapenemases were correctly detected by the Carba5 test.

Conclusions:

We evaluated the NG-Test Carba5 strip for detection of the five most important carbapenemases in Enterobacteriales with a challenging strain collection. The NG-Test Carba5 showed a very good performance with a sensitivity of 91.7 % and an excellent specificity of 100 %. The positive and negative predictive values (PPV/NPV) were 1 and 0.89, respectively. The Carba5 test was not able to detect several IMP variants. One NDM-producing isolate was not detected.

Table 1: Results of the Carba5 test evaluation

<table>
<thead>
<tr>
<th>carbapenemase</th>
<th>number of isolates</th>
<th>test results</th>
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<td>DIM-1</td>
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</tr>
<tr>
<td>FIM-1</td>
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<td>negative</td>
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<td>GIM-1</td>
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<td>NDM, OXA-48-like</td>
</tr>
<tr>
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<td>OXA-306</td>
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<td>VIM-31</td>
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<td>VIM</td>
</tr>
</tbody>
</table>

*not covered by the Carba5 test
Evaluation of the NG-Biotech Carba 5 and CORIS BioConcept RESIST-4 O.K.N.V. immunochromatographic lateral flow assays for the detection of carbapenemase enzymes in Enterobacterales.

Bronwyn D Davison, Medical Laboratory Scientist-New Zealand-Published on NZIMLS journal 2019

➢ Objective:

To evaluate and compare the NG-Biotech Carba 5 and CORIS BioConcept RESIST-4 O.K.N.V. immunochromatographic lateral flow assays for the detection of carbapenemase enzymes in Enterobacterales. Both tests having the ability to detect the main four carbapenemases; OXA-48-like, NDM, KPC, VIM, and additionally the Carba 5 being able to detect IMP types. The importance of these tests in diagnostic microbiology laboratories is increasing due to the emergence of Carbapenemase-producing Enterobacterales (CPE), resulting in limited treatment options and associated issues relating to patient management and infection control.

➢ Methods:

Carba 5 and RESIST-4 O.K.N.V. were performed with 58 Enterobacterales isolates with reduced susceptibility to meropenem; 45 CPE and 13 non-CPE.

➢ Results:

Both assays produced 100% specificity for the detection of all carbapenemase classes tested. The sensitivity results for the Carba 5 and RESIST-4 O.K.N.V. are as follows respectively: OXA-48-like – 95% (18/19) and 90% (17/19), NDM – 95% (20/21) and 91% (19/21), KPC – 100% (4/4) and 80% (3/4), VIM – 100% (2/2) and 100% (2/2). The Carba 5 additionally detects IMP types with a sensitivity of 83% (5/6).

➢ Conclusion:

Carba 5 and RESIST-4 O.K.N.V. are both highly sensitive and specific assays to detect the main carbapenemase classes in Enterobacterales, they are robust, user-friendly kits that provide rapid results.
Evaluation of the NG-Test CARBA 5 multiplex lateral flow immunochromatographic assay for the detection of carbapenemases.

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➢ Introduction:

The global rise of carbapenemase-producing Enterobacteriaceae (CPE) is alarming and represents an increasing threat to healthcare delivery and patient safety.

While Gram-negative organisms have developed several mechanisms to avert the bactericidal effects of commonly prescribed antibiotic agents, the increasing prevalence of CPE is particularly concerning given the rapid spread of mobile genetic elements containing carbapenemase genes, the limited treatment options for infections caused by these organisms, and the high mortality rates associated with CPE infections. Detection of infected patients and carriers with carbapenemase producers is necessary to take correct infection control measures and prevent of their spread.

➢ Methods:

NG-Test CARBA 5 was evaluated using 238 isolates from CHROMagar™, mSuperCARBA™ or Mueller Hinton agar. 154 CPE isolates with characterized β-lactamase genes (blaKPC n=47, blaNDM n= 25, blaOXA-48-LIKE n=40, blaVIM n=19, blaIMP n=1, blaIMI n=3, blaSME n=1, combination of bla genes n=18), non-carbapenemase-producing carbapenem-resistant (n=27) and carbapenem-susceptible (n=19) enterobacterial isolates were tested. Carbapenem-resistant A. baumannii (CRAB, n=39) and E. coli (n=2) were added to evaluate the cross reactivity of the OXA-type enzyme (blaOXA-1 n=2, blaOXA-65 n=6, blaOXA-66 n=9, blaOXA-69 n=2, blaOXA-71 n=11, blaOXA-248 n=8, blaOXA-70 combined with blaNDM n=1).

The results from the NG-Test CARBA 5 assay were compared to results of the PCR method.

➢ Results:

All isolates expressing a carbapenemase related to one of the NG-Test CARBA 5 targets were correctly detected in <15 minutes. All other isolates gave negative results except an OXA-48-false positive (ME) result recorded with a S. marcescens isolate expressing blaSME. No cross-reactivity was observed with other OXA-type variants. The NG-Test CARBA 5 assay reached 100.0% sensitivity and 99.0% specificity.

➢ Conclusions:

NG-Test CARBA 5 assay is efficient, rapid and easy to implement in routine workflow of a clinical microbiology laboratory for the detection of main carbapenemases.
Evaluation of two immunochromatographic kits for the detection of Carbapenemase-producing enterobacterial strains.

RICA-Paris- December 2018

➢ Conclusion

These rapid tests are simple, effective and rapid with a low cost for detecting CPEs. The panel of targeted antigens for these two tests is adapted to the epidemiology of CPE circulating in the Indian Ocean region. The CARBA 5 test was finally selected for its 5th valence IMP (IMP-10 already detected in Mauritius). These tests could also be an interesting alternative for detection of Gram-non-fermenting bacilli.

➢ Results

Sensitivity & specificity of CARBA 5: 97.8% / 100%
Sensitivity & specificity of Resist- 4: 97.7% / 100%


RICA- Paris- December 2018

➢ Conclusion

The RESIST-4 O.K.V.N and NG-Test CARBA 5 tests are rapid (15minutes) and high-performance tools for the detection and characterization of EPCs. Their ease of use and interpretation make them the tools of choice for routine diagnostics in laboratories. Molecular biology remains necessary for the characterization of the different enzymatic variants.

➢ Results

Sensitivity & specificity for the 2 tests: 100% / 100%

S S Miah, M H F Abdul Momin, T Naas, D W Wareham - Ireland - ECCMID 2019

➢ Background

Mobilisable colistin resistance (MCR) in Enterobacteriaceae due to plasmid encoded phosphoethanola- mine transferase (mcr-like) enzymes threatens the efficacy of polymyxins as treatments for multi-drug resistant infections. A rapid, cheap and sensitive diagnostic test, able to inform on therapy and resistance surveillance is desirable. We assessed the performance of the NG-Test MCR-1 lateral flow assay for detection of MCR-1 producing isolates in polymyxin resistant Enterobacteriaceae from human and animal samples.

➢ Material/Methods

36 colistin resistant Escherichia coli (human and poultry) and 14 human faecal swabs were tested directly. All samples were obtained during an AMR surveillance study in Brunei Darussalam. Pure isolates were recovered using CHROMagar COL-APSE media. Colistin susceptibility was determined by broth microtiter dilution with multiplex PCR for detection of mcr-1/2/3/4/5. NG-Test MCR-1 lateral flow cards were loaded with 100 µl of a single bacterial colony, emulsified in 5 drops of extraction buffer (EB). Faecal swabs were inoculated in 3mls of TSB broth + colistin (1 mg/ml) and incubated for 2 hrs at 37 °C. Pellets obtained by centrifugation were re-suspended in EB and analysed directly on flow cards.

➢ Results

All thirty-six MCR-1 producing E. coli isolates were positive within 3 min of loading onto cards. Negative results were observed for 9 type strain control organisms (Proteus mirabilis, Serratia marcescens, Enterococcus faecalis, Enterococcus gallinarum, Candida albicans, Enterobacter cloacae, Stenotrophomonas maltophilia, Pseudomonas aeruginosa, Escherichia coli) indicating 100% sensitivity and specificity using pure isolates. Sensitivity was reduced with faecal swabs (14%). Only 2/14 swabs were positive, despite 14/16 (87 %) found to contain MCR-1 producing E. coli following culture on COL-APSE media.

➢ Conclusions

NG-Test MCR-1 flow cards were highly specific and sensitive in the detection of MCR-1 producing strains amongst colistin resistant strains of E. coli. The assay requires no investment in any special equipment and has a rapid turnaround time of <10 mins. Direct detection in faecal samples, although possible, was poorly sensitive despite a colistin enrichment step. This assay could be useful in rapid detection of colistin resistance in cultured samples, but requires further protocol development for use as a point of care test directly with clinical specimens.
Development and multicentric validation of a lateral flow immunoassay for the rapid detection of MCR-1-producing Enterobacteriaceae.


➢ Background:

Colistin has become a last resort antibiotic for the treatment of highly drug resistant Gram negatives. Moreover, it has been widely used in the livestock sector. As a consequence, colistin resistance is emerging worldwide. Among colistin resistance mechanism, the spread of the plasmid-encoded colistin resistance gene mcr-1 (mostly in E. coli) is of particular concern due to its increased transfer abilities compared to chromosome-encoded resistance. Early detection of MCR-1-producing bacteria is essential to prevent further spread and provide appropriate antimicrobial therapy.

➢ Methods:

Lateral flow immunoassays (LFIs) were manufactured with selected monoclonal antibodies. A collection of 177 human and 121 animal enterobacterial isolates were tested in a multicentric study. One bacterial colony grown on agar plates was suspended in extraction buffer and dispensed on the cassette. Migration was allowed for 15 minutes and the results were monitored by the appearance of a specific band.

➢ Results:

The positive results showed a pink line resulting in an unambiguous interpretation. All MCR-1-producing isolates were found to be positive by the LFIA test and no false-negative results were observed. Three out of four MCR-2-producing isolates were also found to be positive. Our test does not detect MCR-3, -4, -5-producing isolates.

➢ Conclusions:

LFIA allows the detection of MCR-1 with 100% sensitivity and 98% specificity. This test is fast, sensitive, specific, easy to use, and cost-effective and can therefore be implemented in any microbiology laboratory worldwide. The LFIA test is a major tool for rapid diagnosis and monitoring of MCR-1 producers in humans and animals.
A multicentric validation of a rapid detection test for MCR-1 producing bacteria

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REVIEW ABSTRACT

Background
The emergence and spread of Enterobacteriaceae resistant to critically important antibiotics such as carbapenems is a matter of great public health concern. Accordingly, colistin, a cationic polypeptide introduced in the 1950s, has become a last-resort molecule that has been increasingly prescribed in clinics in the last years. Of note, colistin is also widely used in the food animal sector. Late 2015, a plasmid-borne colistin resistant gene (mcr-2) was reported, and a rapid detection of MCR-1 producing bacteria is essential to prevent further spread and provide appropriate antimicrobial therapy. Here, we provide validation data of a Lateral Flow Immunoassay (LFA) to detect MCR-1 producers within 15 minutes.

Methods
LFA (strip + cassette) were manufactured using monoclonal antibodies previously produced and selected. A retrospective collection of mcr-1 positive enterobacterial isolates of human and animal origin was tested in a multicentric way. The isolates were grown on agar and one colony was suspended in extraction buffer and then dispensed on the cassette. Migration was allowed for 15 minutes and results were monitored by the appearance of a specific band.

Results
Positive results showed a dark pink colored band leading to no ambiguous interpretation. All mcr-1 positive isolates were detected as positive by the LFA test and no false negative result was observed. Three out of four strains producing MCR-1 were detected positively. Our test does not detect MCR-2, MCR-4 or MCR-5 producing strains.

Conclusions
Our LFA is able to detect MCR-1 with 100% sensitivity and 100% specificity. It also appears usable for the detection of MCR-2 (4%) but further studies are needed to better assess the performances of MCR-2-detection. It is compatible with samples handled in laboratories. This test is rapid, sensitive, specific, easy to use, cost-effective and could thus be implemented in any microbiology laboratory around the world. Considering the relevance of colistin resistance in humans and animals, and the possible transfers of MCR producers between them, the LFA test could be of major help for diagnostic and monitoring purposes in the two sectors.
Extended Spectrum β-Lactamases

BACTERIAL IDENTIFICATION AND DETECTION OF ESBL DIRECTLY FROM URINE AND BLOOD CULTURE SAMPLES

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1 Hospital Clínic de Barcelona, Barcelona, 2 Hospital Clínic de Barcelona. ISGlobal. Universidad de Barcelona, Barcelona, 3 Hospital Clínic de Barcelona. Universidad de Barcelona, Barcelona

Objectives of the study:

Early identification of the pathogen responsible for bacteremia, infections of body fluids and pyelonephritis.
Rapid determination of Gram Negative bacteria producing ESBL for early treatment and efficacy.

Material and methods:

Liquids in Blood culture vials: (blood (10), bile(2), joint fluid(7), ascetic fluid(6) = total of 25 samples)
=> 10 retrospective ESBL positive samples
15 prospective samples

Selection of positive samples after analysis with BD Bactec™ and Gram stain.

AND

Urine (65 samples)

=> 40 retrospective ESBL positive samples
25 prospective samples

Selection of positive samples for which Nitrite and/or leukocyte are Positive from the reacting strip for which the Flow Cytometer (Sysmex®) detects a bacillus type morphology and a bacterial content > 5000 bacteria/μl.

Then, 10 ml of the selected sample is centrifuged at low speed, the supernatant is then centrifuged at high speed and washed 2x.
From the resulting pellet, microbiological identification is performed by mass spectrometry MALDI-TOF Bruker®.

A LFIA test is performed on the resulting pellet in order to determine the presence of CTX-M Group 1 with NG Biotech® NG-Test CTX-M G1 test.
Strains with negative answer to this CTX-M test but ESBL-positive on the culture, were also tested with NG Biotech® NG-Test CTX-M MULTI (including Groups 1, 2, 8, 9, 25).

On the negative strains, a PCR is also performed in order to confirm that they do not belong to any of the tested groups.
Results:

1 hour instead of 48 hours in routine, this procedure enables faster treatment and efficacy.

Concordance between MALDITOF bacteria identification realized from direct sample and realized after culture of the same strain from the same sample:

100% on the Blood Culture samples and 93% on the urine samples.

Duration of the operation:

Directly on the sample = 1 hour

Routine method = 48 hours

<table>
<thead>
<tr>
<th>Identified Microorganisms</th>
<th>Production of ESBL in cultured sample</th>
<th>Detection of ESBL per Rapid Test (RT)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RT on direct sample</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CTX M Group 1</td>
</tr>
<tr>
<td>40 Escherichia coli</td>
<td>ESBL postive = 45 (56.25%)</td>
<td>36 positive (80%)</td>
</tr>
<tr>
<td>27 Klebsiella pneumoniae</td>
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<td></td>
</tr>
<tr>
<td>13 other Enterobacteriae</td>
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</tr>
<tr>
<td>(80 strains)</td>
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<tr>
<td>ESBL negative = 35 (43.75%)</td>
<td>9 negative (20%)</td>
<td></td>
</tr>
<tr>
<td>24 E. Coli</td>
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<td></td>
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<tr>
<td>17 K. Pseudomonia</td>
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</tr>
<tr>
<td>4 other Enterobacteriae</td>
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<td></td>
</tr>
<tr>
<td>ESBL negative = 35 (43.75%)</td>
<td>35 negative</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions:

This protocol demonstrates a gain of 48h in the detection of ESBL in case of bacteremia, biological fluid infections and pyelonephritis for an effective and early treatment.

This study clearly demonstrates that the use of NG-Test CTX-M and NG-Test CTX-M Multi RAPID Tests in combination to bacteria identification by MALDI-TOF provides an extremely rapid information allowing effective and early treatment in cases of bacteremia, infections in body fluids and pyelonephritis.
Validation of lateral flow immunoassays for the rapid detection of CTX-M group 1, NDMs, OXA-48-like producers.

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2 EAT361, UPSUD, Hôpital de Bièvre, APHP, Le Kremlin-Bicêtre, France

**Background:** Enterobacteriaceae have a major role as causes of nosocomial infections (and, for E. coli, also of community-acquired infections), and expanded-spectrum cephalosporins and carbapenems are essential in the treatment of these infections. The dissemination of broad-spectrum β-lactamases (extended spectrum β-lactamases ESBls and carbapenemases) among Enterobacteriaceae is undoubtedly a matter of great public health concern. Detection of those multidrug-resistant bacteria is primarily based on indirect detection of antimicrobial resistances. The strategies involve fast identification of the resistance mechanisms, followed by strict hygiene and contact precautions of the patients. Here, we validate Lateral Flow Immuno Assays (LFIA) to detect NDMs, OXA-48-like and CTX-Ms group 1 producers within 15 minutes.

**Materials and methods:** LFIA (strip + cassette) were manufactured using monoclonal antibodies previously produced and selected. 180 enterobacterial isolates with characterized β-lactamases content, referred to the French National Reference Centre (NRC) for antibiotic resistance between 2008 and 2014, were grown on agar plates. One colony was suspended in extraction buffer (lysis step) and then dispensed on the cassette. Migration was allowed for 15 minutes and results read by naked eye. A prospective evaluation was also done with clinical isolates showing a decreased susceptibility to at least one carbapenem and referred to the NRC during the validation period. CTX-M 1G LFIA was used in routine with clinical laboratory samples showing an extended spectrum β-lactamase activity.

**Results:** LFIA validation with 180 isolates (characterized β-lactamase content by PCR), corresponding to 55 non-carbapenemase producers and 125 carbapenemase producers (22 NDMs, 6 NDM-1/OXA-48 like, 37 OXA-48-like, 17 VIMs, 11 IMPs, 22 KPCs, 10 others

**NDM LFIA validation (27 NDMs producers)**
- 22 NDM-1, 2 NDM-4, 1 NDM-5, 1 NDM-7 and 1 NDM-9
- 27 strains detected
- All non-NDM producers gave negative results
- Prospective evaluation (74 strains)
- decreased susceptibility to at least one carbapenem
- 33 carbapenemase producers (Carba NP test and PCR)
- 2 NDM-1 and 5 NDM-5 detected with LFIA
- 100% sensitivity and specificity for the NDM LFIA

**CTX-M G1 validation (70 CTX-M G1 producers)**

**OXA-48-like LFIA validation**
- 21 OXA-48, 9 OXA-181, 5 OXA-204, 2 OXA-232, 2 OXA-244
- Each of OXA-162, 60, 51, 195
- 2 OXA-163 and OXA-405 (no carbapenemase activity)
- 45 strains detected
- 1 OXA-181 gave negative result (also negative with selective media)
- All non-OXA-48-like producers gave negative results

**Prospective evaluation (41 strains)**
- 22 carbapenemase producers (Carba NP test and PCR)
- 14 OXA-48 and 2 OXA-181 detected
- 98% sensitivity and 100% specificity for the OXA-48-like LFIA

**Routine use of CTX-M G1 LFIA (100 strains)**

**Perspectives:** A multiplex LFIA (NDMs, KPCs, VIMs, IMPs and OXA-48-like) will soon be evaluated with the 180 NRC strains from agar plates.

A CTX-Ms LFIA (G1, G2, G9) development is in progress with new antibodies produced and selected (G2 and G9).

This CTX-Ms LFIA will soon be evaluated for direct detection of CTX-Ms expressing strains grown in urine or blood culture.
A MULTIPLEX LATERAL FLOW IMMUNOASSAY FOR THE RAPID IDENTIFICATION OF CTX-M PRODUCING ENTEROBACTERIACEAE

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REVISED ABSTRACT

Background: The dissemination of broad-spectrum beta-lactamases (ESBLs) such as CTX-Ms among Enterobacteriaceae is a matter of great concern given the major role these pathogens play as causes of nosocomial infections and of community-acquired infections. Detection of these multidrug-resistant clones is primarily based on indirect detection of antimicrobial resistances. The strategies involve fast identification of the resistance mechanisms, followed by strict hygiene and contact precautions of the patients. Here, we have validated a Lateral Flow Immunoblotting (LFI), called CTX-M MULTI, to detect CTX-Ms producers from agar plates. One version (V1) gives results with a profile depending on the CTX-M group, while a second version (V2) does not allow this discrimination.

Methods: LFI (strip - cassette) were manufactured using our monoclonal antibodies by Biotech (Moscow, France). The CTX-M MULTI was validated with 101 enterobacterial isolates well characterized in terms of ESBLs content. For this study, a colony from agar culture was suspended in extraction buffer and added to the device. Prospectively, 101 clinical isolates with diverse susceptibility to broad spectrum cephalosporines were also tested. This validation was performed at the hospital of Fréjus (France). In each case, the result was read 35 minutes after the sample deposition. Positive results were confirmed by PCR and sequencing to determine the CTX-M variants detected.

Results: In this study, with CTX-M MULTI (V1) no false positive were observed. Few invalid results were obtained with muscular strains necessitating a protocol adjustment. The false negative results obtained turned positive when using the CTX-M MULTI (V2). The variants CTX-M-1, 2, 3, 4, 5, 10 - 15, 17, 18, 27, 32, 37, 55, 57, 58, 67, 82, 92, 94, 100, 102 and 108 were detected. Moreover, the CTX-M MULTI identification with CTX-M MULTI (V2) gave some ambiguous results avoided by the use of the CTX-M MULTI (V2).

Conclusions: Overall, the CTX-M MULTI showed good performances. Its first version (V1) showed 100% sensitivity and specificity on agar media growth strains. Also for practical concerns, this first version will be preferred for commercial purposes. This test would be able to detect 99% of the ESBL producing Enterobacteriaceae identified in clinical settings.

Illustration of obtained results

For the retrospective and prospective validations, CTX-M MULTI (V2) reached 96.5% and 99.3% specificity respectively, while 100% specificity for both.

Overall, CTX-M MULTI reached 99.2% of correct CTX-M group identification in the second version (V2) for an easier interpretation.