ISSN: 2588-4115; Infection Epidemiology and Microbiology. 2026;12(2):145-151 doi 10.52547/iem.12.2.145

Analytical Evaluation of KarbaDia, a Multiplex Immunochromatographic Assay for Rapid Detection of Major Carbapenemase Families in Gram-Negative Bacteria

ARTICLE INFO

Article Type
Original Article

Authors
Percevent Jeremy Ducrest, *MSc*[1]
Laurent Philippe Gilliard, *MSc*[2]
Carlos Alberto Mestriner, *PhD*[1]

[1] GaDia SA, Route de l ile-au-Bois 1A, 1870 Monthey, Switzerland
[2] Independent Researcher, 1202 Geneva, Switzerland

** Correspondence*
GaDia SA, Route de l ile-au-Bois 1A, 1870 Monthey, Switzerland.
E-mail: percevent.ducrest@gadia.ch

How to cite this article
Ducrest P.J., Gilliard L.Ph., Mestriner C.A. Analytical Evaluation of KarbaDia, a Multiplex Immunochromatographic Assay for Rapid Detection of Major Carbapenemase Families in Gram-Negative Bacteria. Infection Epidemiology and Microbiology. 2026;12(2): 145-151.

Article History
Received: February 26, 2026
Accepted: June 28, 2026
Published: July 27, 2026

ABSTRACT

Background: Rapid detection of carbapenemase-producing Gram-negative bacteria is critical for timely antimicrobial stewardship and infection control interventions. This study evaluated the analytical diagnostic performance of KarbaDia as a new multiplex lateral flow immunochromatographic assay for rapid detection of KPC, NDM, VIM, IMP, and OXA-48-like carbapenemases.

Materials & Methods: An analytical diagnostic accuracy evaluation study was conducted using 78 non-duplicate well-characterized Gram-negative bacterial isolates obtained from publicly available diversity panels. The collection included carbapenemase-producing and carbapenemase-negative isolates of *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Acinetobacter baumannii*. Whole genome sequencing (WGS) served as the reference standard. KarbaDia (GaDia SA, Switzerland) was evaluated in parallel with the Carbapenemase Detection Kit (Colloidal Gold; Macro & Micro-Test). Sensitivity, specificity, and 95% confidence intervals (CI) were calculated for each carbapenemase family and for overall assay performance.

Findings: KarbaDia demonstrated 100% sensitivity for KPC (6 of 6), NDM (13 of 13), VIM (3 of 3), and IMP (2 of 2) and 90.0% for OXA-48-like enzymes (9 of 10). The overall sensitivity was 97.1% (95% CI: 85.1–99.9), while the specificity reached 100% (95% CI: 99.0–100). No false-positive results were observed. The comparator assay demonstrated similar overall sensitivity (97%) but produced four invalid results (4.8%), mainly associated with viscous *K. pneumoniae* colonies and incomplete chromatographic migration.

Conclusion: KarbaDia demonstrated high analytical accuracy for rapid and multiplex detection of major carbapenemase families. The assay combines rapid turnaround time, multiplex capability, and ease of visual interpretation without dedicated instrumentation. These characteristics support its potential applicability in routine clinical microbiology laboratories; however, prospective multicenter clinical validation studies remain necessary before routine implementation.

Keywords: Carbapenemases, Drug Resistance, Immunochromatography, *Klebsiella pneumoniae* , *Pseudomonas aeruginosa, Acinetobacter baumannii*

CITATION LINKS

[1] Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: An evolving... [2] Queenan AM, Bush K. Carbapenemases: The versatile β-lactamases. Clin Microbiol Rev... [3] Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing... [4] van Dijk K, Voets GM, Scharringa J, et al. A disc diffusion assay for detection of class A, B and OXA-48 carbapenemases in Enterobacteriaceae... [5] Hrabak J, Chudackova E, Papagiannitsis C. Detection of carbapenemases in Entero... [6] 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... [7] Zhu Y, Jia P, Li Q, et al. Carbapenemase detection by NG-Test CARBA 5-a rapid immunochromatographic assay in carbapenem-resistant... [8] Han R, Li X, Bai Y, et al. Evaluation of the Immunochromatographic... [9] Bogaerts P, Huang TD, et al. Comparison of two multiplex immunochromatographic assays for the rapid detection of major carbapenemases in... [10] Pancotto LR, et al. Performance of rapid tests for carbapenemase detection among Brazilian Enterobacteriaceae isolates... [11] De Swardt H, Bernabeu S, Dortet L, Emeraud C. Evaluation of the Carbapenemase Detection... [12] Jenkins SL, Ledeboer NA, Westblade LF, Burnham CA, Faron ML, Bergman Y, et al. Evaluation of NG-Test CARBA 5 for rapid... [13] Del Corpo O, Senécal J, Hsu JM, Lawandi A, Lee TC. Rapid phenotypic testing for detection of carbapenemase- or extended-spectrum ß-lactamase-producing Enterobacterales directly from...

Introduction

Carbapenem-resistant Gram-negative bacteria (CR-GNB) constitute a major global public health concern because of limited therapeutic options, prolonged hospitalization, increased healthcare costs, and elevated mortality rates [1]. The rapid worldwide dissemination of carbapenemase-producing organisms among *Enterobacterales, Pseudomonas aeruginosa*, and *Acinetobacter baumannii* has substantially complicated infection prevention and antimicrobial stewardship strategies [2]. Among carbapenemase enzymes, *Klebsiella pneumoniae* carbapenemase (KPC), New Delhi metallo-β-lactamase (NDM), Verona integron-encoded metallo-β-lactamase (VIM), imipenemase (IMP), and oxacillinase-48-like (OXA-48-like) enzymes are the most clinically-relevant mechanisms of carbapenem resistance [3]. Their epidemiology differs geographically. KPC-producing isolates are predominant in North and South America, whereas OXA-48-like enzymes are particularly prevalent in the Mediterranean basin and Middle Eastern countries. NDM-producing isolates have expanded rapidly across South Asia, Europe, and Africa [4]. Therefore, rapid and accurate identification of these resistance determinants is essential for early optimization of antimicrobial therapy and implementation of infection control measures. Conventional phenotypic methods for carbapenemase detection, including modified Hodge test and inhibitor-based disk diffusion assays, are limited by prolonged turnaround times, suboptimal specificity, and interpretative variability [5]. Molecular amplification techniques such as polymerase chain reaction (PCR) remain reference methods for carbapenemase gene identification but require specialized instrumentation, trained personnel, and increased laboratory costs [6]. Lateral flow immunochromatographic assays (LFIAs) have emerged as practical alternatives capable of detecting major carbapenemase families directly from cultured colonies within approximately 15 minutes [7]. Commercial assays such as NG-Test CARBA 5 (NG-Biotech, France) and RESIST-5 O.O.K.N.V. (Coris BioConcept, Belgium) have demonstrated high sensitivity and specificity in several clinical evaluations [8-10]. Nevertheless, variability in detection performance has been reported for certain carbapenemase variants, particularly among OXA-48-like enzymes [11]. In addition, independent analytical evaluations of newly developed multiplex assays remain limited. KarbaDia (GaDia SA, Switzerland) is a newly developed multiplex LFIA based on colloidal gold immunochromatography, designed for simultaneous visual detection of KPC, NDM, VIM, IMP, and OXA-48-like carbapenemases directly from bacterial colonies within approximately 15 minutes. Compared with existing commercial assays, KarbaDia has been developed to provide simplified workflow, broad multiplex detection, and variant coverage without requiring dedicated instrumentation or external reader devices.

Objectives: The present study aimed to perform an analytical diagnostic accuracy evaluation of KarbaDia using publicly available genomically-characterized Gram-negative bacterial diversity panels and to compare its analytical performance with a commercially available colloidal gold-based comparator assay.

Materials and Methods

This study was designed as an analytical diagnostic accuracy evaluation of a multiplex immunochromatographic assay for rapid carbapenemase detection.

A total of 78 non-duplicate Gram-negative bacterial isolates were included. Non-duplicate isolates were defined as unique strains representing distinct genomic entries within publicly available diversity panels,

Table 1) Distribution of carbapenemase determinants among Gram-negative bacterial isolates included in the analytical evaluation

Carbapenemase	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Acinetobacter baumannii*	Total
NDM	5	-	4	9
KPC	5	1	-	6
IMP	-	-	1	1
VIM	1	2	-	3
OXA-48 like	7	-	-	7
OXA-48 & NDM	3	-	-	3
IMP & NDM	1	-	-	1
Negative	38	1	9	48
Total	60	4	14	78

with no repeated isolates from the same panel designation, resistance phenotype, or genomic accession.

The study collection included 30 carbapenemase-producing isolates and 48 carbapenemase-negative control isolates (Table 1). The species distribution comprised *K. pneumoniae, P. aeruginosa*, and *A. baumannii*. The panel included four isolates carrying multiple carbapenemase determinants, including three isolates co-harboring NDM and OXA-48-like enzymes and one isolate carrying both NDM and IMP. Carbapenemase-negative isolates lacking these genes but harboring other antimicrobial resistance genes were included to evaluate assay specificity. All bacterial strains were cultured on tryptic soy agar (TSA) plates (AxonLab SA, Switzerland) and incubated at 37 °C for 18 hours prior to testing to ensure adequate colony growth and purity. Bacterial isolates were obtained from well-characterized diversity panels with publicly accessible genomic data to ensure reproducibility and transparency. The *P. aeruginosa* MRSN diversity panel (NR-51829) was obtained through BEI Resources. Associated whole genome shotgun (WGS) sequences and metadata are available in the NCBI database under the title BioProject PRJNA446057. The *A. baumannii* MRSN diversity panel plate (NR-60450) was also obtained through BEI Resources. Associated genomic data are available under BioProject PRJNA545079. The *K. pneumoniae* MRSN diversity panel plate (NR-60451) was obtained through BEI Resources with associated genomic data available under BioProject PRJNA717739. These publicly available resources include multilocus sequence typing (MLST), antimicrobial susceptibility data, and genomic characterization. All isolates were supplied through BEI Resources, NIAID, and NIH, provided by the Multidrug-Resistant Organism Repository and Surveillance Network (MRSN) at the Walter Reed Army Institute of Research (WRAIR).

Whole genome sequencing served as the reference standard, providing definitive identification of carbapenemase genes and minimizing the ambiguity associated with phenotypic characterization methods.

KarbaDia (GaDia SA, Switzerland) is a multiplex lateral flow immunochromatographic assay based on colloidal gold-labelled monoclonal antibodies, designed for simultaneous detection of KPC, NDM, VIM, IMP, and OXA-48-like carbapenemases on a single test strip. The assay provides visually interpretable results within 15 minutes and does not

require a reader device or additional instrumentation. The assay was performed according to the manufacturer's instructions. Briefly, isolated bacterial colonies were suspended in the supplied extraction buffer and applied to the test cassette. Results were interpreted visually after 15 minutes. The simultaneous presence of a visible control line and the corresponding test line was interpreted as positive for the presence of the respective carbapenemase family.

The Carbapenemase Detection Kit (Colloidal Gold; Macro & Micro-Test, China) was evaluated in parallel under identical laboratory conditions according to the manufacturer's instructions and previously published evaluation studies [11]. All assays were independently interpreted by two investigators blinded to the WGS results. Invalid tests were defined by the absence of a visible control line or incomplete chromatographic migration. Invalid tests were repeated once when sufficient bacterial material was available.

Diagnostic performance estimates were calculated on a per-target basis rather than per-isolate basis because each isolate could contribute to both positive and negative determinations across the five multiplex assay targets. Sensitivity, specificity, and corresponding 95% confidence intervals (CI) were calculated using WGS as the reference standard. Invalid results were excluded from sensitivity and specificity calculations but reported separately. Descriptive statistical analyses were performed due to the analytical nature of the study and limited sample size across several carbapenemase subgroups.

Findings

KarbaDia demonstrated 100% sensitivity for KPC (6 of 6; 95% CI: 54.1–100%), NDM (13 of 13; 95% CI: 75.3–100%), VIM (3 of 3; 95% CI: 29.2–100%), and IMP (2 of 2; 95% CI: 15.8–100%) carbapenemases. Sensitivity

Table 2) Analytical performance of KarbaDia vs. colloidal gold (macro & micro)

		KarbaDia (GaDia SA)						Colloidal Gold (Macro & Micro Test)					
Target carbapenemase	No. positive isolates	TP	FN	Sensitivity (%)	TN	FP	Specificity (%)	TP	FN	Sensitivity (%)	TN	FP	Specificity (%)
KPC (all variants)	**6**	**6**	**0**	**100%**	**72**	**0**	**100%**	**5**	**0**	**100%**	**65**	**4**	**94.2%**
KPC-2	4	4	0	100%				3*	0	100%			
KPC-3	2	2	0	100%				2	0	100%			
NDM (all variants)	**13**	**13**	**0**	**100%**	**65**	**0**	**100%**	**12**	**0**	**100%**	**62**	**0**	**100%**
NDM-1	11	11	0	100%				10*	0	100%			
NDM-5	1	1	0	100%				1	0	100%			
NDM-7	1	1	0	100%				1	0	100%			
VIM (all variants)	**3**	**3**	**0**	**100%**	**75**	**0**	**100%**	**3**	**0**	**100%**	**71**	**0**	**100%**
VIM-1	1	1	0	100%				1	0	100%			
VIM-6	1	1	0	100%				1	0	100%			
VIM-11	1	1	0	100%				1	0	100%			
IMP (all variants)	**2**	**2**	**0**	**100%**	**78**	**0**	**100%**	**2**	**0**	**100%**	**72**	**0**	**100%**
IMP-15	1	1	0	100%				1	0	100%			
IMP-16	1	1	0	100%				1	0	100%			
OXA-48-like (all variants)	**9**	**9**	**1**	**90%**	**68**	**0**	**100%**	**7**	**1**	**88%**	**66**	**0**	**100%**
OXA-48	6	6	1	86%				5*	1	83%			
OXA-181	1	1	0	100%				1	0	100%			
OXA-232	2	2	0	100%				1*	0	100%			
Overall		**33**	**1**	**97.1%**	**358**	**0**	**100%**	**29**	**1**	**96.7%**	**336**	**4**	**98.8%**

* indicates invalid test results (no visible control line). Invalid results were excluded from performance calculation.

for OXA-48-like enzymes was 90.0% (9 of 10; 95% CI: 55.5–99.7%) (Table 2).

No false-positive reactions were observed across 358 evaluable negative target determinations, yielding an overall specificity of 100% (358 of 358; 95% CI: 98.7–100). Because KarbaDia is a multiplex assay, specificity calculations were performed independently for each carbapenemase target using all isolates lacking the corresponding resistance determinants as negative comparators. The overall sensitivity for KarbaDia was 97.1% (33 of 34; 95% CI: 82.9–99.8). The comparator Macro & Micro-Test demonstrated an overall sensitivity of 96.7% (29 of 30; 95% CI: 82.9–99.8) and a specificity of 98.8% (336 of 340, 95% CI: 96.8–99.6). However, four invalid results were observed among the positive samples (4 of 78; 5.1%), due to the absence of a visible control line and incomplete chromatographic migration. Invalid results occurred only with viscous *K. pneumoniae* colonies. Among the four invalid results, one was related to a KPC-2-producing isolate, another one was related to an NDM-1-producing isolate, and the other two were related to OXA-48-like-producing isolates. Therefore, only eight OXA-48-like carbapenemase-producing isolates, twelve NDM-producing isolates, and five KPC-producing isolates were considered evaluable for the comparator Macro & Micro-Test . These invalid results were excluded from sensitivity calculations in accordance with the predefined study criteria.

Discussion

Rapid carbapenemase detection remains essential for optimizing antimicrobial therapy, implementing infection prevention measures, and reducing the transmission of multidrug-resistant organisms in healthcare settings. Previous studies have demonstrated that LFIA platforms, such as NG-Test CARBA 5 and Coris RESIST assays, provide rapid and reliable phenotypic detection of major carbapenemase families with high diagnostic accuracy [7-10].

In the present study, KarbaDia demonstrated high analytical performance across multiple carbapenemase families, with an overall sensitivity and specificity of 97.1 and 100%, respectively. The assay was performed consistently across genetically diverse isolates of *K. pneumoniae*, *P. aeruginosa,* and *A. baumannii*, with no observed false-positive reactions.

An important practical advantage of KarbaDia is its rapid turnaround time of approximately 15 minutes compared with molecular methods such as PCR, which generally require specialized equipment, batch processing, and substantially longer reporting times [5, 6]. The assay also allows multiplex detection of the five most clinically relevant carbapenemase families without additional instrumentation, supporting its potential applicability in routine diagnostic laboratories and resource-limited settings. KarbaDia successfully detected clinically important variants including NDM-5, NDM-7, and OXA-232. These variants are of increasing epidemiological importance because of their association with international dissemination and multidrug-resistant outbreaks. NDM-5 and NDM-7 have been increasingly reported in Asia, Europe, and Africa, whereas OXA-232 has emerged in several Mediterranean and Middle Eastern regions [4]. Therefore, accurate recognition of these variants is clinically important for both antimicrobial stewardship and epidemiological surveillance.

The comparator Macro & Micro-Test demonstrated good overall analytical performance, consistent with previously published evaluations [11]. Nevertheless, the assay generated five invalid results associated with viscous *K. pneumoniae* colonies due to incomplete chromatographic migration. In addition,

reduced evaluability of OXA-48-like carbapenemase-producing isolates highlights previously reported analytical challenges related to structural heterogeneity within this carbapenemase family [8, 11]. Detection of OXA-48-like enzymes remains challenging for several immunochromatographic assays because structural diversity may influence antibody recognition [7]. In the present study, KarbaDia achieved 90% sensitivity for OXA-48-like variants, which remains within the range reported for established commercial LFIA assays [8, 9]. The same false negative result was observed with OXA-48 producing isolates using both assays. Importantly, no false-positive reactions were observed, reducing the risk of unnecessary therapeutic escalation or inappropriate infection control interventions.

This study has several limitations. First, the analytical design relied on characterized strain collections rather than prospective clinical specimens. Second, the number of isolates within individual carbapenemase subgroups, particularly IMP and VIM producers, was limited, resulting in wide confidence intervals and reduced statistical precision. Third, clinical performance was not evaluated using direct patient specimens or positive blood culture samples.

Future multicenter prospective studies including larger numbers of clinical isolates and blood culture specimens will be necessary to further establish the clinical utility and operational performance of KarbaDia in routine microbiology workflows [12, 13].

Conclusion

In this analytical evaluation study, KarbaDia demonstrated high analytical accuracy for rapid and multiplex detection of major carbapenemase families, including KPC, NDM, VIM, IMP, and OXA-48-like enzymes. The assay showed high overall sensitivity and specificity compared with whole genome sequencing as the reference standard, although these estimates should be interpreted within the context of limited sample size and subgroup distribution. The rapid turnaround time (approximately 15 minutes), ease of use, and instrument-free multiplex format represent important practical advantages for routine microbiology workflows and antimicrobial stewardship programs. The use of well-characterized genomic diversity panels further supports the robustness and reproducibility of the analytical evaluation. Nevertheless, prospective multicenter clinical studies using consecutive clinical isolates and direct clinical specimens remain necessary to further validate the diagnostic and clinical utility of KarbaDia before routine implementation in clinical microbiology laboratories.

Acknowledgments

The bacterial isolate panels were obtained free of charge through BEI resources, NIAID, and NIH, provided by the Multidrug-Resistant Organism Repository and Surveillance Network (MRSN) at the Walter Reed Army Institute of Research (WRAIR).

Ethical permissions: This study used publicly available, de-identified bacterial strain panels and did not involve human participants or patient data. Therefore, institutional ethics committee approval was waived according to local regulatory requirements.

Authors' contributions: PJ Ducrest performed the study and wrote the first version of the manuscript, CA Mestriner reviewed and corrected the manuscript, and LP Gilliard reviewed and corrected the manuscript.

Conflicts of Interests: PJ Ducrest is an employee of GaDia SA, the manufacturer of the KarbaDia rapid test. There are no other conflicts of interest to declare.

Funding/Supports: Not applicable.

Declaration of Artificial Intelligence (AI) Use: The authors used ChatGPT (GPT-5.5) to assist with grammar checking and improve language clarity. All content has been reviewed and revised by the authors. The authors take full responsibility for the accuracy and originality of the work.

Consent to participate: This study used publicly available, de-identified bacterial strain panels and did not involve human participants or patient data. No consent to participate was necessary.

Note: One supplementary file is available with the online version of this article.

References

1. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: An evolving crisis of global dimensions. Clin Microbiol Rev. 2012;25(4):682-707.
2. Queenan AM, Bush K. Carbapenemases: The versatile β-lactamases. Clin Microbiol Rev. 2007;20(3):440-58.
3. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. 2011;17(10):1791-8.
4. van Dijk K, Voets GM, Scharringa J, et al. A disc diffusion assay for detection of class A, B and OXA-48 carbapenemases in Enterobacteriaceae using phenyl boronic acid, dipicolinic acid and temocillin. Clin Microbiol Infect. 2014;20(4):345-9
5. Hrabak J, Chudackova E, Papagiannitsis C. Detection of carbapenemases in Enterobacteriaceae: a challenge for diagnostic microbiological laboratories. Clinical Microbiology and Infection, 2014;20: 839-853
6. 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(4):909-15.
7. Zhu Y, Jia P, Li Q, et al. Carbapenemase detection by NG-Test CARBA 5-a rapid immunochromatographic assay in carbapenem-resistant Enterobacterales diagnosis. Ann Transl Med. 2021 May;9(9):769
8. Han R, Li X, Bai Y, et al. Evaluation of the Immunochromatographic NG-Test Carba 5, RESIST-5 O.O.K.N.V., and IMP K-SeT for Rapid Detection of KPC-, NDM-, IMP-, VIM-type, and OXA-48-like Carbapenemase Among Enterobacterales. Front. Microbiol. 2021 11:609856.
9. Bogaerts P, Huang TD, et al. Comparison of two multiplex immunochromatographic assays for the rapid detection of major carbapenemases in Enterobacterales. J Antimicrob Chemother. 2020;75(6):1491-1494
10. Pancotto LR, et al. Performance of rapid tests for carbapenemase detection among Brazilian Enterobacteriaceae isolates. Braz J Microbiol. 2018;49(4):914-918
11. De Swardt H, Bernabeu S, Dortet L, Emeraud C. Evaluation of the Carbapenemase Detection Kit (Colloidal Gold; Macro & Micro Test): A new immunochromatographic assay for rapid detection of carbapenemases in Enterobacterales. J Antimicrob Chemother. 2026;81(3):dkag066.
12. Jenkins SL, Ledeboer NA, Westblade LF, Burnham CA, Faron ML, Bergman Y, et al. Evaluation of NG-Test CARBA 5 for rapid phenotypic detection and differentiation of five common carbapenemase families: Results of a multicenter clinical evaluation. J Clin Microbiol. 2020;58(7):e00344-20.
13. Del Corpo O, Senécal J, Hsu JM, Lawandi A, Lee TC. Rapid phenotypic testing for detection of carbapenemase- or extended-spectrum ß-lactamase-producing Enterobacterales directly from blood cultures: a systematic review and meta-analysis. Clin Microbiol Infect. 2023 Dec;29(12):1516-1527