

Direct Detection of Carbapenemases from Clinical Isolates of *Enterobacteriaceae* and *Pseudomonas aeruginosa* Using the Hardy NG-Test CARBA 5

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Introduction

NG-Test CARBA 5 (CARBA 5) is an *in vitro* rapid and visual multiplex immunochromatographic assay for the qualitative detection and differentiation of five common carbapenemases (KPC, OXA-48-like, VIM, IMP and NDM) from carbapenem non-susceptible pure bacterial colonies when grown on the following media: 5% sheep blood agar or MacConkey agar (16-24 hours) for testing *Enterobacteriaceae* and *Pseudomonas aeruginosa*, HardyCHROM™ CRE agar (18-24 hours) for testing *E. coli* and KES (*Klebsiella aerogenes*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Enterobacter cloacae* complex and *Serratia marcescens*).

CARBA 5 is intended as an aid for infection control in the detection of carbapenemase-producing Enterobacteriaceae and Pseudomonas aeruginosa in healthcare settings. CARBA 5 is not intended to guide or monitor treatment for carbapenem non-susceptible bacterial infections. A positive or negative CARBA 5 test result does not rule out the presence of other mechanisms of antibiotic resistance. CARBA 5 should be used in conjunction with other laboratory tests including phenotypic antimicrobial susceptibility testing.

Study Objectives

We verified isolates from our Michigan Medicine (MM) CRE collection and isolates from the CDC AR Isolate Bank. The MM collection have been previously characterized by Verigene GN and/or Michigan Department of Health and Human Services (MDHHS) PCR. Isolates tested include: *Escherichia coli*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Providencia stuartii*, *Morganella morganii*, *Enterobacter cloacae* and *Pseudomonas aeruginosa*. Two of the isolates carried two carbapenemase markers, both *Klebsiella pneumoniae*, with OXA and NDM, one of which was our MM patient and the other from the CDC AR Isolate Bank.

NG-Test CARBA 5 IVD  only

• Distributed in the United States by Hardy Diagnostics.

Methods

Preparing the sample

1. Dispense 5 drops (150 µL) of extraction buffer in one of the microtubes provided into the kit.
2. From the agar culture, touch 3 colonies with a loop, suspend it in the microtube with buffer.
3. Vortex to homogenize the mixture before use.
4. Open the pouch, and take out the device. Once opened, use the test immediately.
5. Using the provided pipette, add 100 µL of the prepared mixture in the sample well labelled "S".
6. Read the results after 15 minutes and interpret them as indicated below.

Result interpretation

Negative result

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

Positive result

If one red line appears in the control region (C) and one or several lines appear in the test regions K (KPC), O (OXA-48-like), V (VIM), I (IMP), N (NDM): the sample contains one or several carbapenemases and must be interpreted as a positive result.

The intensity of the red test line(s) may vary. A weak line is a positive result.

Invalid result

If the control line (C) does not appear, the test result is invalid. Insufficient sample volume or incorrect sample processing are the two most likely reasons for control line failure.



Negative



Positive

C Control
 K KPC
 O OXA
 V VIM
 I IMP
 N NDM



Conclusions

1. CARBA 5 test performance is equal to the Verigene (LDT) which we currently use for direct isolate detection of carbapenemases.
2. CARBA 5 test is **easy to perform**, takes 15 minutes, does not require the genus species for detection.
3. The cost of the Vergiene is 3-4 times **higher** than CARBA 5.
4. CARBA 5 test allows us to move to an FDA cleared test.

Results (n=49)

KPC

Verigene/PCR

		POS	NEG
CARBA 5	POS	11	0
	NEG	0	38

OXA

Verigene/PCR

		POS	NEG
CARBA 5	POS	12	0
	NEG	0	37

VIM

Verigene/PCR

		POS	NEG
CARBA 5	POS	6	0
	NEG	0	43

IMP

Verigene/PCR

		POS	NEG
CARBA 5	POS	4	0
	NEG	1	44

There was one discrepant isolate for IMP
 - *Pseudomonas aeruginosa* IMP 14 from the CDC AR Isolate Bank (#92)
 - CARBA 5 package insert states that for this organism there are variants which the may not be detected

NDM

Verigene/PCR

		POS	NEG
CARBA 5	POS	8	0
	NEG	0	41