NG-Test CARBA 5 IVD ℞ only

Distributed in the United States by Hardy Diagnostics.

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Indications for Use

NG-Test 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 Enterobacterales 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*).

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

Summary and Principles

β-Lactams are first-line antibiotics for the treatment of infections caused by Enterobacterales. Nevertheless, since the beginning of their massive use in the 1940s, their efficacity has been challenged by the production of enzymes which inactivate them: the β-lactamases. Among them are carbapenemases which hydrolyze carbapenem antibiotics. Before the 1940s, most of the resistance to antibiotics was associated with the production of Extended-Spectrum β-Lactamases (ESBLs) belonging to classes A, B, C, and D of Ambler's classification. Since then, studies have shown an increase in the production of carbapenemases among the Enterobacterales. Among those carbapenemases, the β-lactamase KPC (Class A) has spread worldwide in the 2000s[2]. In addition, the metallo β-lactams (MBL) of IMP type, VIM and NDM (Class B) as well as OXA-48 (Class D) have also expanded[1,3]. These carbapenemases are mainly detected in hospital settings and are responsible for most of the nosocomial infections, raising major global health problems since their presence can be difficult to detect.

NG-Test CARBA 5 is an *in vitro* rapid and visual multiplex immunochromatographic assay that detects one or more of the five common types of carbapenemase enzymes (KPC (K), OXA-48-like (O), IMP (I), VIM (V), NDM (N)) in bacterial colonies. Liquid extraction buffer is used as a cell lysing solution when mixed with colonies. Monoclonal antibodies that individually recognize each of the five carbapenemases are immobilized on a nitrocellulose membrane. Free monoclonal antibodies are present in the sample pad and labelled with colloidal gold. Upon addition of colonies mixed with extraction buffer to the sample pad, the capillary action of the nitrocellulose draws the sample through the mobile antibodies and immobile antibodies on the test strip. The immobilized control antibodies capture any mobile antibodies that run through the sample pad and nitrocellulose without binding to other test lines. A positive result occurs when a red line appears on the control region (C) and one or more lines appear in the test regions (K, O, V, I, or N) and indicates that the sample contains one or more carbapenemases. A negative result occurs when only the control line is observed and indicates that the sample does not contain any of the 5 carbapenemases. If the control line does not appear, the test result is invalid.

Reagents and materials supplied

Each kit contains:

- 20 Test cassettes in aluminum pouches with desiccant
- 20 Eppendorf tubes
- 20 Disposable pipettes of 100 µL
- 1 Extraction buffer solution in a plastic bottle (4.5 mL)
- 1 Instructions for Use

Materials required but not supplied

- Timer
- Single use gloves
- Loop
- Vortex

Precautions

- *In vitro* diagnostic test. For professional use only.
- All the operations must be carried out according to good laboratory practices.
- The devices must remain in the sealed pouches until they are used.
- Handle the samples as if they were potentially infectious.
- After use, discard the device in an infectious waste container.
- Do not reuse the device.

Storage and stability

Store the devices in their sealed pouches between 4 and 30°C. Do not freeze. Kits are stable in their intact packaging (sachet with desiccant) until the expiry date indicated on every kit. Do not use after the expiry date.

Culture and sampling

The samples to be tested shall be obtained and handled according to standardised microbiology procedures.

Operating procedure

1. Wear protective gloves and standard personal protective equipment.
2. Bring the kit components to room temperature for at least 10 minutes.

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, and then suspend it in the microtube containing 150 µL of extraction buffer.
3. Close the microtube.
4. Vortex to homogenise the mixture before use.

Carrying out the test

1. Open the pouch, and take out the device. Once opened, use the test immediately.
2. Using the provided pipette, add 100 µL of the prepared mixture (sample must reach the black line indicated on the pipette to accurately aspirate 100 µL) in the sample well labelled "S".
3. Read the results at 15 minutes and interpret them as indicated below[1].

[1]Do not interpret the test results after 20 minutes.

Limitations

1. The performance of NG-Test CARBA 5 was established with colonies from blood agar, MacConkey agar, and HardyCHROM™ CRE agar. Performance with other culture media has not been evaluated and is therefore unknown.
2. A negative result does not preclude the presence of carbapenemase producing organisms (example: SME, GES, IMI).
3. False negative results may occur with multiple subcultures of a bacterial isolate without any selective pressure.
4. Two out of seven *Proteus mirabilis* tested in-house for Analytical Reactivity resulted in a false negative from blood agar only.
5. *Proteus* spp. tend to exhibit swarming growth on blood agar, thus only the surface of the swarming should be touched with a loop in 3 different places.
6. This test is a qualitative assay and will not yield any quantitative result.
7. This test should be used as an aid for the rapid identification of patients bearing a resistance to carbapenem antibiotics.
8. A positive or a negative test does not rule out the presence of other mechanisms of antibiotic resistance.
9. The performance of NG-Test CARBA 5 with bacteria other than Enterobacterales and *Pseudomonas aeruginosa* has not been evaluated.
10. Organism identification and elevated carbapenem MICs should be determined prior to testing with NG-Test CARBA 5.

Result interpretation

Negative

Negative result

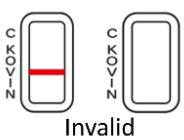
If only one red line appears in the control region (C): the sample does not contain any carbapenemase or contains carbapenemase(s) at a non-detectable level and must be interpreted as a negative result.

VIM Positive

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

Invalid result

If the control line (C) does not appear, or if it is above or below the letter (C) on the cartridge, then it is considered invalid.

Insufficient sample volume or incorrect sample processing are the two most likely reasons for control line failure.

Deterioration of the test kit may have occurred. Repeat the procedure using a new test. If the problem persists, do not use the kit and contact your distributor.

Quality control

An internal quality control is included in the test. When the control line develops, it confirms the sample volume was sufficient and the procedure was correct.

The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Expected Results
Klebsiella pneumoniae ATCC® BAA-1705*	Positive KPC Line
Klebsiella pneumoniae NCTC 13442	Positive OXA-48-like Line
Klebsiella pneumoniae NCTC 13439	Positive VIM Line
Escherichia coli NCTC 13476	Positive IMP Line
Klebsiella pneumoniae ATCC® BAA-2146	Positive NDM Line
Klebsiella pneumoniae ATCC® BAA-1706	No Positive Test Lines

*According to CLSI document M100, *Klebsiella pneumoniae* ATCC® BAA-1705 may undergo a spontaneous loss of the plasmid encoding the carbapenemase, leading to false-negative QC results[10]. To avoid false-negative QC results, *K. pneumoniae* ATCC® BAA-1705 as well as other carbapenemase-producing organisms, should be maintained on a carbapenem-containing medium or with a selective antimicrobial disk on non-selective agar prior to testing QC.

Performances and characteristics

Clinical Evaluation

Performance of NG-Test CARBA 5 was evaluated at three geographically diverse hospitals with prospectively-collected and stock bacterial isolates. The identification of carbapenemase production on NG-Test CARBA 5 was compared to another FDA-cleared device, Xpert Carba-R by Cepheid (PCR for KPC, OXA-48 or 181, IMP, VIM, NDM), modified carbapenem inactivation method (mCIM) and EDTA carbapenemase inactivation method (eCIM) as described by CLSI M100, S29, and antibiotic susceptibility testing results to ertapenem, imipenem, and meropenem. Identity and susceptibility of organisms were confirmed using FDA-cleared ID and AST systems. NG-Test CARBA 5 quality control was performed in parallel every day of testing.

A total of 310 organisms were tested against PCR (Xpert Carba-R, Cepheid) and phenotypic tests (mCIM, eCIM, and disk diffusion). One organism did not meet enrollment criteria because it was a species of *Pseudomonas* (Cornell 50) other than *P. aeruginosa* and was therefore excluded from the analysis. Of the remaining 309 organisms tested, a total of 240 Enterobacterales (which provided 244 results since four isolates co-produced two carbapenemases) and 69 *P. aeruginosa* isolates were tested on NG-Test CARBA 5 with concordant results obtained by phenotypic testing paired with Xpert Carba-R results.

Performance was equivalent between blood and MacConkey agar. Table 1 indicates the PPA and NPA for each individual target separated out by organism group. The overall PPA for Enterobacterales was 100.0% (97.6% - 100.0%) and the overall NPA was 95.5% (88.9% - 98.2%) (Table 2). The overall PPA for *P. aeruginosa* was 100.0% (77.2% - 100.0%) and the overall NPA was 94.6% (85.4% - 98.2%) (Table 3). *P. aeruginosa* with NDM (n=2) were evaluated analytically in the bench testing.

Table 1. Performance of NG-Test CARBA 5 vs. the comparator method for all sites combined

Plate	Organism Group[6]	Total Targets	Target	TP[1]	FP[3]	FN	TN	PPA	low 95%[2]	high 95%	NPA	low 95%	high 95%
5% Sheep Blood or MacConkey agar	Enterobacterales (ENT)	244	KPC	84	0	0	160	**100.0**	95.6	100.0	**100.0**	97.7	100.0
			OXA-48-like	20	0	0	224	**100.0**	83.9	100.0	**100.0**	98.3	100.0
			VIM	11	0	0	233	**100.0**	74.1	100.0	**100.0**	98.4	100.0
			IMP	4	3[4]	0	237	**100.0**	51.0	100.0	**98.8**	96.4	99.6
			NDM	37	1[4]	0	206	**100.0**	90.6	100.0	**99.5**	97.3	99.9
	Pseudomonas aeruginosa	69	KPC	2	0	0	67	**100.0**	34.2	100.0	**100.0**	94.6	100.0
			OXA-48-like	0	0	0	69	**n/a**	n/a	n/a	**100.0**	94.7	100.0
			VIM	9	0	0	60	**100.0**	70.1	100.0	**100.0**	94.0	100.0
			IMP	2	3[5]	0	64	**100.0**	34.2	100.0	**95.5**	87.6	98.5
			NDM	0	0	0	69	**n/a**	n/a	n/a	**100.0**	94.7	100.0

[1]No True Positive results for OXA-48-like and NDM for the *P. aeruginosa* organism group in multicentric clinical testing.

[2]Lower bounds are below 90% due to the low prevalence of the OXA, IMP, and VIM carbapenemases. The claim of NG-Test CARBA 5 detection of OXA, IMP, and VIM carbapenemases is supported by analytical reactivity data.

[3]For Enterobacterales, three isolates were false positive for IMP on NG-Test CARBA 5 (positive IMP on NG-Test CARBA 5, positive mCIM, and negative Xpert Carba-R result). One isolate was a false positive for NDM on NG-Test CARBA 5 (positive NDM on NG-Test CARBA 5, positive mCIM, negative Xpert Carba-R result). For *P. aeruginosa*, three isolates were false positive for IMP on NG-Test CARBA 5 (positive IMP on NG-Test CARBA 5, positive mCIM, and negative Xpert Carba-R result).

[4]All three isolates were confirmed to have the IMP-8 gene, making these true positives for IMP after discrepant analysis. (IMP-8 is predicted to be detected by Xpert Carba-R based on *in silico* analysis but has not been demonstrated analytically.) One isolate was confirmed to have an NDM-1 gene making this isolate true positive for NDM after discrepant analysis. (NDM-1 is predicted to be detected by Xpert Carba-R based on *in silico* analysis and has been tested analytically.) After discrepant analysis, the Enterobacterales overall PPA increased to 100.0% (97.7% - 100.0%) and the overall NPA increased to 100.0% (95.6% - 100.0%).

[5]All three isolates were confirmed to have an IMP gene (IMP-7, IMP-15, and IMP-19) making these true positives for IMP after discrepant analysis. (IMP-7 is a known limitation of Xpert Carba-R. IMP-19 is predicted to be detected by Xpert Carba-R based on *in silico* analysis but has not been tested analytically. The ability of Xpert Carba-R to detect IMP-15 is unknown.) After discrepant analysis, the *P. aeruginosa* overall PPA increased to 100% (80.6% - 100%) and the overall NPA increased to 100% (93.2% - 100%).

[6]Ertapenem disks were routinely used to maintain selective pressure for isolated colonies of retrospective Enterobacterales isolates. No selective pressure was used for isolated colonies of retrospective *P. aeruginosa* isolates.

Table 2. Agreement of NG-Test CARBA 5 with the composite reference method when testing Enterobacterales

Enterobacterales		Composite Reference Method		
		Positive	Negative	Total
NG-Test CARBA 5	Positive	156	4[1,2]	160
	Negative	0	84	84
	Total	156	88	244
Positive Percent Agreement (PPA)		156/156 = 100% (95% CI: 97.6-100%)		
Negative Percent Agreement (NPA)		84/88 = 95.5% (95% CI: 88.9-98.2%)		

[1]An alternative PCR assay showed that the NDM false positive isolate harbored a bla_{NDM}-*1* variant. Isolate was positive by mCIM.

[2]An alternative PCR assay and bidirectional sequencing showed that the three IMP false positive isolates harbored bla_{IMP} -8/-47 variant that is predicted by *in silico* analysis but not analytically demonstrated to be detected by the assay. Isolates were positive by mCIM

Table 3. Agreement of NG-Test CARBA 5 with the composite reference method when testing *P. aeruginosa*

P. aeruginosa		Composite Reference Method		
		Positive	Negative	Total
NG-Test CARBA 5	Positive	13	3[1]	16
	Negative	0	53	53
	Total	13	56	69
Positive Percent Agreement (PPA)		13/13 = 100% (95% CI: 77.2-100%)		
Negative Percent Agreement (NPA)		53/56 = 94.6% (95% CI: 85.4-98.2%)		

[1]An alternative PCR assay and bidirectional sequencing showed that the three IMP false positive isolates harbored bla_{IMP} variants that (i) are not detected by the FDA-cleared PCR assay (bla_{IMP} variant -7), (ii) are predicted by *in silico* analysis but not analytically demonstrated to be detected by the assay (bla_{IMP} -19), or (iii) the reactivity of the assay is unknown (bla_{IMP} variant -15). Isolates were positive by mCIM.

The bacterial isolates used to evaluate NG-Test CARBA 5 from blood and MacConkey agar were also used internally to evaluate the performance of NG-Test CARBA 5 from HardyCHROM™ CRE agar. These results were compared to Xpert Carba-R, mCIM, and eCIM as described by CLSI M100, S29, and antibiotic susceptibility testing results to ertapenem, imipenem, and meropenem. Identity and susceptibility of organisms were confirmed using FDA-cleared ID and AST systems. NG-Test CARBA 5 quality control was performed in parallel every day of testing.

Of the 186 organisms enrolled, one organism was not available for testing and was excluded from the analysis. Of the 185 organisms that fell under HardyCHROM™ CRE claims, 180/185 (97.3%) organisms (184 target results) were recovered from Raw stool, and 178/185 (96.2%) organisms (182 target results) were recovered from C&S Cary Blair stool onto HardyCHROM™ CRE. Table 4 indicates the PPA and NPA for each individual target separated out by organism group. The overall PPA from raw stool specimen inoculated to HardyCHROM™ CRE was 100.0% (97.4% - 100.0%) and the overall NPA was 90.2% (77.5% - 96.1%) (Table 5). The overall PPA from C&S Cary Blair stool specimen inoculated to HardyCHROM™ CRE was 100.0% (97.3% - 100.0%) (Table 6) and the overall NPA was the same as the raw stool specimen.

Table 4. Performance of NG-Test CARBA 5 vs. the comparator method - Analysis by Target

Plate	Specimen Type	Organism Group	Target	TP	FP[2]	FN	TN	PPA	low 95%[1]	high 95%	NPA	low 95%	high 95%
HardyCHROM™ CRE agar	**Raw Stool**	*E. coli*, KES	KPC	76	0	0	108	**100.0**	95.2	100.0	**100.0**	96.6	100.0
			OXA-48-like	18	0	0	166	**100.0**	82.4	100.0	**100.0**	97.7	100.0
			VIM	9	0	0	175	**100.0**	70.1	100.0	**100.0**	97.9	100.0
			IMP	4	3	0	177	**100.0**	51.0	100.0	**98.3**	95.2	99.4
			NDM	36	1	0	147	**100.0**	90.4	100.0	**99.3**	96.3	99.9
	C&S Cary Blair Stool	*E. coli*, KES	KPC	75	0	0	107	**100.0**	95.1	100.0	**100.0**	96.5	100.0
			OXA-48-like	18	0	0	164	**100.0**	82.4	100.0	**100.0**	97.7	100.0
			VIM	8	0	0	174	**100.0**	67.6	100.0	**100.0**	97.8	100.0
			IMP	4	3	0	175	**100.0**	51.0	100.0	**98.3**	95.2	99.4
			NDM	36	1	0	145	**100.0**	90.4	100.0	**99.3**	96.2	99.9

[1]Lower bounds are below 90% due to the low prevalence of the OXA, IMP, and VIM carbapenemases. The claim of NG-Test CARBA 5 detection of OXA, IMP, and VIM carbapenemases is supported by analytical reactivity data.
[2]Three isolates were false positive for IMP on NG-Test CARBA 5 (positive IMP on NG-Test CARBA 5, positive mCIM, and negative Xpert Carba-R result). All three isolates were confirmed to have the IMP-8 gene, making these true positives for IMP after discrepant analysis. (IMP-8 is predicted to be detected by Xpert Carba-R based on *in silico* analysis but has not been demonstrated analytically.) One isolate was a false positive for NDM on NG-Test CARBA 5 (positive NDM on NG-Test CARBA 5, positive mCIM, negative Xpert Carba-R result). This isolate was confirmed to have an NDM-1 gene making this isolate true positive for NDM after discrepant analysis. (NDM-1 is predicted to be detected by Xpert Carba-R based on *in silico* analysis and has been tested analytically.) After discrepant analysis, the overall PPA increased to 100.0% (97.5% - 100.0%) and the overall NPA increased to 100.0% (90.6% - 100.0%) for raw stool specimen. The overall PPA increased to 100.0% (97.4% - 100.0%) and the overall NPA increased to 100.0% (90.6% - 100.0%) for C&S Cary Blair stool specimen.

Table 5. Agreement of NG-Test CARBA 5 with the composite reference method when testing bacterial growth on HardyCHROM™ CRE agar after seeded in Raw Stool

Raw Stool		Composite Reference Method		
		Positive	Negative	Total
NG-Test CARBA 5	Positive	143	4[1,2]	147
	Negative	0	37	37
	Total	143	41	184
Positive Percent Agreement (PPA)		143/143 = 100% (95% CI: 97.4-100%)		
Negative Percent Agreement (NPA)		37/41 = 90.2% (95% CI: 77.5-96.1%)		

[1]An alternative PCR assay showed that the NDM false positive isolate harbored a bla_{NDM} -1 variant. Isolate was positive by mCIM.
[2]An alternative PCR assay and bidirectional sequencing showed that the three IMP false positive isolates harbored bla_{IMP} -8/-47 variant that is predicted by *in silico* analysis but not analytically demonstrated to be detected by the assay. Isolates were positive by mCIM.

Table 6. Agreement of NG-Test CARBA 5 with the composite reference method when testing bacterial growth on HardyCHROM™ CRE agar after seeded in C&S Cary Blair Stool

C&S Cary Blair Stool		Composite Reference Method		
		Positive	Negative	Total
NG-Test CARBA 5	Positive	141	4[1,2]	145
	Negative	0	37	37
	Total	141	41	182
Positive Percent Agreement (PPA)		141/141 = 100% (95% CI: 97.3-100%)		
Negative Percent Agreement (NPA)		37/41 = 90.2% (95% CI: 77.5-96.1%)		

[1]An alternative PCR assay showed that the NDM false positive isolate harbored a bla_{NDM} -1 variant. Isolate was positive by mCIM.
[2]An alternative PCR assay and bidirectional sequencing showed that the three IMP false positive isolates harbored bla_{IMP} -8/-47 variant that is predicted by *in silico* analysis but not analytically demonstrated to be detected by the assay. Isolates were positive by mCIM.

ANALYTICAL REACTIVITY
NG-Test CARBA 5 was evaluated with ninety-two strains characterized to have a target carbapenemase. Each organism was incubated aerobically for 16 hours on sheep's blood agar and MacConkey agar at 35°C or 18 hours on HardyCHROM™ CRE agar at 35°C. Each test was performed in triplicate from each type of media. NG-Test CARBA 5 test result was read 15 minutes after inoculating the buffer mixed with bacteria into the sample port. The operator was blinded to the expected result while setting up and interpreting the test. All organisms that yielded a negative NG-Test CARBA 5 result were further analyzed by modified carbapenemase inactivation method (mCIM, CLSI M100, S29). After the mCIM analysis, the final sensitivity for all target organisms evaluated was 90/92 (97.8%) from blood agar and 92/92 (100%) from MacConkey agar. After the mCIM analysis, the final sensitivity for all target organisms evaluated was 41/41 (100%) from HardyCHROM™ CRE agar. On blood agar only, two *Proteus mirabilis* strains resulted in false negative results.

Table 7. Analytical Reactivity Summary

Organism Group	Number of **strains** tested on Blood/ MacConkey	**Target**	Number of **targets** tested on Blood/ MacConkey agar	Number of **targets** Tested HC CRE	**Variants Tested**
Enterobacterales	66	KPC	17	8	2, 3, 4, 6, 12
		OXA-48-like	12	7	48, 181, 163, 232 (48 type)
		VIM	11	9	1, 4, 5, 6, 23, 27, 31
		IMP	8	7	4, 8/47[2], 26[1]
		NDM	15	11	1[1], 5, 6, 7
		None[3]	5	2	
		Total	68	44	
Pseudomonas aeruginosa	26	KPC	5		2, 5
		OXA-48-like	0		
		VIM	13		2, 11
		IMP	6		1, 7, 14, 18, 19, 26
		NDM	2		1
		None[3]	0		
		Total	26		

[1]NDM-1 and IMP-26 not detected in *P. mirabilis* growth from blood agar, but yielded positive results from MacConkey agar.
[2]IMP-8 and IMP-47 were determined to be the same protein based on sequence analysis by the Beta-Lactamase Database (http://www.bldb.eu/BLDB.php?prot=B1#IMP).
[3]Isolates have targeted carbapenemase resistance genes but were negative by mCIM making them true negatives for carbapenemase production. They were also negative by NG-Test CARBA 5.

ANALYTICAL SPECIFICTY
81 organisms that exhibit antibiotic resistance mechanisms other than the targets NG-Test CARBA 5 lateral flow assay detects, are carbapenem-susceptible, or are carbapenem non-susceptible were tested on NG-Test CARBA 5 from blood agar and MacConkey agar (Table 8). Organisms tested included Enterobacterales (n=54) and *P. aeruginosa* (n=20), as well as other phylogenetically related organisms (n=7). HardyCHROM™ CRE was inoculated with 16 cross reactive organisms that were tested on NG-Test CARBA 5 and showed no cross reactivity. These organisms were included in the list of claimed organisms for HardyCHROM™ CRE but which do not produce one of the 5 target carbapenemases. Each organism was incubated aerobically at 35°C on sheep's blood agar and MacConkey agar for 16 hours. HardyCHROM™ CRE was incubated for 18 hours prior to testing. NG-Test CARBA 5 test result was read 15 minutes after inoculating the buffer mixed with bacteria into the sample port. The operator was blinded to the expected result while setting up and interpreting the test. 81/81 (100%) of organisms tested from blood and MacConkey agar produced a negative NG-Test CARBA 5 result. 16/16 (100%) of organisms tested from HardyCHROM™ CRE produced a negative NG-Test CARBA 5 result. Organisms with NMC-A, FRI, and GIM mechanisms were tested by the National Reference Center in France and did not cross react.

Table 8. Resistance Mechanisms Evaluated with NG-Test CARBA 5 for Specificity

	Resistant mechanisms evaluated	
Organism Group	**Blood & MacConkey agar**	**HardyCHROM™ CRE agar**
Enterobacterales	ACT-type, ACT-2, AmpC, CTX-M [1, 3, 8, 9, 14, 15, 22, 24, 30, 40, 55, 74, 75, 79, 124], DHA-1, ESBL, IMI, mrc-1, OmpK35, OmpK37, OXA [1, 2, 30], SHV [11(2b), 12(2be), 18, 28, 31, 89(2b), 108(u), 154, 179(u), 180(u), 182(u), OSBL(2b)], SME, SME-2, TEM [1, 1(2b), 11(2be), 63(2be), 93(2be), 210(u), OSBL(2b)], tet(A), tet(B)	ACT-2, AmpC, CTX-M [9, 14, 30], DHA-1, IMI, MIR-8, OXA, SME, TEM-129(2be), tet(A)
Pseudomonas aeruginosa	aadA6, aadB, aph(3')-IIb, catB7, GES-1, GES-5(c), OXA [10, 50], PAO, PDC [1, 5, 19, 35], PER-1, strA, strB, sull, tet(c), VEB-1, inducible AmpC	N/A
Other	VanA	N/A

INCUBATION STUDY
In order to confirm that NG-Test CARBA 5 delivered consistent results over a range of incubation time, twenty-two strains were tested from blood and MacConkey agar every two hours from 16 to 24 hours of incubation. Fifteen of the twenty-two organisms were also tested from HardyCHROM™ CRE every two hours from 18 to 24 hours. All organisms tested produced the expected result on NG-Test CARBA 5 at every time point tested. NG-Test CARBA 5 test result was read 15 minutes after inoculating the buffer mixed with bacteria into the sample port. The operator was blinded to the expected result while setting up and interpreting the test.

REFRIGERATION STORAGE STUDY
In order to determine if agar media that has been stored in the refrigerator can be used with NG-Test CARBA 5, twelve strains were cultured and evaluated over time from refrigerated storage. Blood and MacConkey agar plates were inoculated directly with organism (colonies) for the fresh culture, streaking for isolation. HardyCHROM™ CRE was inoculated with organisms at $3x10^4$ CFU/mL in raw stool and stool in C&S Cary Blair Transport Media, streaking for isolation with a 1µL loop. Each strain was incubated aerobically on sheep's blood agar and MacConkey agar at 35°C and tests were performed after 16 to 24 hours of incubation (Day 0). Ten of the twelve organisms were also tested from HardyCHROM™ CRE after 18 to 24 hours of incubation. All organisms tested produced the expected result on NG-Test CARBA 5 for each day of refrigeration for up to 3 days. The operator was blinded to the expected result while setting up and interpreting the test.

REPRODUCIBILITY
Prior to initiating the clinical study, a panel of 20 blinded isolates provided by Hardy Diagnostics was tested at three distinct study sites on five work days to demonstrate reproducibility and to document proficiency in the performance of the test. Agreement of >95% with known test results was required before proceeding with the study. The testing was done with at least one operator and two readers, blinded to each other's results, per site. All target carbapenemase positive isolates tested (100%) were detected by NG-Test CARBA 5 on all days of the reproducibility study.

Variants Detected by NG-Test CARBA 5
Table 9 below shows which variants were evaluated from bacterial isolates in analytical and clinical testing. Variants that have been reported to be detected in publications are also listed. This list may not be exhaustive of all enzyme variants that may be detected by NG-Test CARBA 5.

Table 9. Summary of Variants Detected by NG-Test CARBA 5

		Variants Detected Analytically	Variants Detected in US clinical trial (Table 1)	Variants Detected in publications[8,9,11,13,14,15]
Enterobacterales	KPC	2, 3, 4, 6, 12	2, 3, 4, 12	2, 3, 4, 5, 6, 7, 9, 14, 23, 28, 31, 33, 39
	OXA-48-like	48, 181, 163, 232	48, 181, 232	48, 162, 163, 181, 204, 232, 244, 245, 370, 405, 436, 484, 505, 517, 519, 793
	VIM	1, 4, 5, 6, 11, 23, 27, 31	1, 4, 5, 6, 23, 27, 31	1, 2, 4, 5, 19, 26, 27, 31, 39, 46, 51, 52, 54, 56, 58, 59
	IMP	4, 8/47[2], 26	1, 4, 8/47[2]	1, 4, 6, 7, 8/47[2], 10, 11, 22, 29,58
	NDM	1, 5, 6, 7	1, 5, 6, 7	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 19
Pseudomonas aeruginosa	KPC	2, 5	Variants unknown	2
	OXA-48-like			181
	VIM	2, 11	2	1, 2, 4, 5, 6, 11, 28, 30
	IMP	1, 7, 13, 14, 15, 18, 19, 26, 56	7, 19, 26	1, 2, 4, 5, 6, 7, 8/47[2], 10, 13, 15, 16, 18, 19, 26, 29, 31, 37, 39, 46, 56, 63, 71, 79
	NDM	1		1

[1]Publications and Analytical Testing
[2]IMP-8 and IMP-47 were determined to be the same protein based on sequence analysis by the Beta-Lactamase Database (http://www.bldb.eu/BLDB.php?prot=B1#IMP).

Bibliography

1. Yohei D et al. Carbapenemase-Producing Enterobacteriaceae. *Semin Respir Crit Care Med*. 2015; 36(1): 74–84.
2. Nordmann P et al. Rapid Detection of Carbapenemase-producing Enterobacteriaceae. *Emerging Infectious Diseases*. 2012; 18(9).
3. Bush K et al. Updated Functional Classification of β-Lactamases. *Antimicrobial Agents and Chemotherapy*. 2010; 54(3):969-976.
4. Nordmann P et al. Carbapenemases in enterobacteriaceae. *Archives de Pédiatrie*. 2010;17:S154-S162.
5. Demir Y et al. Investigation of VIM, IMP, NDM-1, KPC AND OXA-48 enzymes in Enterobacteriaceae strains. *Pak J Pharm Sci*. 2015; May;28(3 Suppl):1127-33.
6. Oteo J et al. Evolution of carbapenemase-producing Enterobacteriaceae at the global and national level: what should be expected in the future? *Enferm Infecc Microbiol Clin*. 2014; 32 Suppl 4:17-23.
7. Kulkarni MV et al. Use of imipenem to detect KPC, NDM, OXA, IMP, and VIM carbapenemase activity from gram-negative rods in 75 minutes using liquid chromatography-tandem mass spectrometry. *J Clin Microbiol*. 2014; 52(7):2500-5.
8. Boutal H 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.
9. Hopkins KL, Meunier D, Naas T, Volland H, Woodford N. Evaluation of the NG-Test CARBA 5 multiplex immunochromatographic assay for the detection of KPC, OXA-48-like, NDM, VIM and IMP carbapenemases. *J Antimicrob Chemother* 2018
10. CLSI. Performance Standards for Antimicrobial Disk Susceptibility Testing; Twenty-Second Informational Supplement. CLSI document M100. Wayne, PA: Clinical and Laboratory Standards Institute.
11. Baeza LL, Pfennigwerth N, Greissl C, Göttig S, Saleh A, Stelzer Y, Gatermann SG, Hamprecht A, Comparison of five methods for detection of carbapenemases in *Enterobacterales* with proposal of a new algorithm, *Clinical Microbiology and Infection*, https://doi.org/10.1016/j.cmi.2019.03.003
12. Dortet L, Fusaro M, Naas T, Improvement of Xpert Carba-R Kit for the Detection of Carbapenemase-Producing Enterobacteriaceae, *Antimicrobial Agents and Chemotherapy*. 2016.
13. Volland H et al. Improvement of the Immunochromatographic NG-Test Carba 5 Assay for the Detection of IMP Variants Previously Undetected. *Antimicrob Agents Chemother*. 2019 Dec; 64(1).
14. Yoon J et al. Application of a multiplex immunochromatographic assay for rapid identification of carbapenemases in a clinical microbiology laboratory: performance and turn-around-time evaluation of NG-test Carba 5. *BMC Microbiol*. 2021; 21:260.
15. Potron A et al. Evaluation of the Immunochromatographic NG-Test Carba 5 for Rapid Identification of Carbapenemase in Nonfermenters. *Antimicrob Agents Chemother.* 2019 Sep; 63(9).

Symbols

Σ 20	Content for 20 assays		Expiry date
IVD	*in vitro* diagnostic medical device		Do not re-use
LOT	Batch number	REF	Catalogue reference
	Consult instructions for use	+4°C +30°C	Temperature limit
	Manufacturer	**Contains NaN3**	0.01% sodium azide
℞	For prescription use		

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