Food and Drug Administration
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510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K191889

B Applicant

NG Biotech

C Proprietary and Established Names

NG-Test CARBA 5

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PTJ	Class II	21 CFR 866.1640 - Antimicrobial Susceptibility Test Powder	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:
To obtain a substantial equivalence determination for the qualitative detection of carbapenemase enzymes in carbapenem non-susceptible pure colonies of *Enterobacteriaceae* and *Pseudomonas aeruginosa*

B Measurand:
Carbapenemase enzymes (KPC, OXA-48-like, VIM, IMP, NDM)

C Type of Test:
Qualitative multiplex immunochromatographic assay (lateral flow)

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) 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 *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*).

The NG-Test CARBA 5 is intended as an aid for infection control in the detection of carbapenemase-producing *Enterobacteriaceae* 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.

C Special Conditions for Use Statement(s):

- Rx - For Prescription Use Only
- 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.
- The performance of NG-Test CARBA 5 with bacteria other than *Enterobacteriaceae* and *Pseudomonas aeruginosa* has not been evaluated.
- Organism identification and elevated carbapenem MICs should be determined prior to testing with NG-Test CARBA 5.

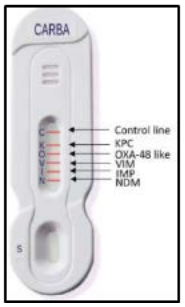
D Special Instrument Requirements:

None (results are read manually)

IV Device/System Characteristics:

A Device Description:

Figure 1. NG-Test CABA 5



NG-Test CARBA 5 (**Figure 1**) is an *in vitro* rapid and visual multiplex immunochromatographic assay that detects one or more of the five common types of carbapenemase enzymes (KPC, OXA-48-like, IMP, VIM, NDM) in bacterial colonies of species usually associated with harboring these resistance markers. The assay consists of a sample port, sample pad and nitrocellulose test strip which are contained within a plastic cassette, in addition to reagents for liquid extraction.

B Principle of Operation:

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. Colonies are mixed with extraction buffer to lyse the bacteria then added to the sample pad. 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. After addition of the bacterial suspension to the sample port, a result can be read after 15 minutes. Possible results include Positive (for one or more target), Negative or Invalid. A positive result occurs when a red line appears on the control region (marked “C”) and one or more lines appear in the test regions (marked “K”, “O”, “V”, “I”, or “N”). This result 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.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Rapidec Carba Np

B Predicate 510(k) Number(s):

K162385

C Comparison with Predicate(s):

Device & Predicate Device(s):	Device: **K191889**	Predicate: **K162385**
Device Trade Name	NG-Test CARBA 5	RAPIDEC CARBA NP
General Device Characteristic Similarities		
Intended Use/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 *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*). The NG-Test CARBA 5 is intended as an aid for infection control in the detection of carbapenemase-producing *Enterobacteriaceae* 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.	RAPIDEC CARBA NP is a phenotypic (colorimetric) in vitro diagnostic test for the qualitative detection of carbapenemase enzymes in *Enterobacteriaceae* and *Pseudomonas aeruginosa* colonies that have elevated MIC values to any carbapenem. RAPIDEC CARBA NP is performed on pure colonies grown on non-selective sheep blood agar culture media. RAPIDEC CARBA NP is intended as an aid in the prevention and control of infection caused by carbapenemase-producing *Enterobacteriaceae* and *Pseudomonas aeruginosa*. RAPIDEC CARBA NP is not intended to guide or monitor the treatment for these bacterial infections. A negative result does not preclude the presence of carbapenemase enzymes. The ability of RAPIDEC CARBA NP to detect carbapenemase enzymes encoded by genetic markers other than KPC, NDM, OXA-48, VIM, and IMP has not been established. RAPIDEC CARBA NP testing should be used in conjunction with other laboratory tests including antimicrobial susceptibility testing.

Inoculum Preparation	Touching three well-isolated colonies with a loop	Touching well-isolated colonies with an applicator stick
Sample Type	Bacterial colonies	same
Interpretation	Visual (manual)	same
Controls	Built-in procedural control in every test strip	same
General Device Characteristic Differences		
Intended Culture Media	5% sheep blood agar, MacConkey agar, HardyCHROM CRE agar	Non-selective sheep blood agar

VI Standards/Guidance Documents Referenced:

- CLSI M100, 28th ed., "Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Eighth Informational Supplement, January 2018".

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

The reproducibility of NG-Test CARBA 5 was evaluated in a study conducted with four lots of tests at three sites. The reproducibility panel of organisms comprised eight well-characterized strains of *Enterobacteriaceae* and *P. aeruginosa* that produce at least one of the targeted carbapenemases (**Table 1.1**) and two negative control organisms (**Table 1.2**).

Two of the eight carbapenemase-positive organisms co-produced two carbapenemases so that a total of ten positive test results (two for each of the five carbapenemase targets) was expected per panel. Each panel member was sub-cultured, in duplicate, onto blood agar or MacConkey agar and incubated at 35°C for 16 to 24 hours. After growth, colonies from each plate were tested each day by two operators over five days at each site (3 sites x 2 operators x 5 days x 2 targets x 2 replicates = 120 positive samples per target carbapenemase per medium type).

Table 1.1. Strains Used in Reproducibility Study: Carbapenemase-positive Organisms

Organism	Carbapenemase	Carbapenem Phenotype			Expected Positive Result	Expected # of Negative Results
		ETP	IPM	MEM		
Pseudomonas aeruginosa CDC 0441	KPC	R[1]	R	R	KPC [K]	4
Escherichia coli CDC 0150	NDM-5	R	R	R	NDM [N]	4
Klebsiella pneumoniae IHMA 1035778	IMP-4	I	R	R	IMP [I]	4
Enterobacter cloacae IHMA 889980	OXA-48, VIM-31	I	R	S	OXA [O], VIM [V]	3
Enterobacter cloacae JMI 325859	KPC-4	R	R	R	KPC [K]	4
Pseudomonas aeruginosa CDC 0444	VIM	R[1]	R	R	VIM [V]	4
Klebsiella pneumoniae CDC 0153	NDM, OXA-232	R	R	I	NDM [N], OXA [O]	3
Pseudomonas aeruginosa IHMA 855945	IMP-26	R[1]	R	R	IMP [I]	4

ETP: ertapenem, IPM: imipenem, MEM: meropenem
S: susceptible, I: intermediate, R: resistant
[1] Intrinsically resistant to ertapenem

The design of the strip also allows for interpretation of negative results, resulting in multiple expected negative results per strip. Both carbapenemase-negative organisms were expected to generate five negative results per test strip (**Table 1.2**). The six carbapenemase-positive organisms that produced a single carbapenemase were expected to produce four negative results (**Table 1.1**). The two carbapenemase-positive organisms that co-produced two carbapenemases were expected to generate three negative results (**Table 1.1**). Therefore, a total of 2400 negative results were expected from all organisms combined:
(2 carbapenemase-negative organisms x 5 negative tests) + (6 carbapenemase-positive organisms x 4 negative results) + (2 carbapenemase-positive organisms x 3 negative results)] x 3 sites x 2 operators x 5 days x 2 replicates = 2400 negative results.

Table 1.2. Strains Used in Reproducibility Study: Carbapenemase-negative Organisms

Organism	Carbapenemase	Carbapenem Phenotype			Expected # of Negative Results
		ETP	IPM	MEM	
Escherichia coli ATCC 25922	None	S	S	S	5
Pseudomonas aeruginosa CDC 0353	GES-1 [2]	R[1]	R	R	5

ETP: ertapenem, IPM: imipenem, MEM: meropenem
S: susceptible, I: intermediate, R: resistant
[1] Intrinsically resistant to ertapenem
[2] The organism harbors GES-1, which is not a target and is not detected with the NG-Test CARBA 5

All positive and negative results for each targeted carbapenemase were as expected (i.e., 120/120 [100%] positive results for each carbapenemase target and 2400/2400 [100%] negative results). The reproducibility of NG-Test CARBA 5 was determined to be acceptable.

2. Linearity:
Not applicable

3. Analytical Specificity/Cross Reactivity:
The analytical specificity of the NG-Test CARBA 5 was evaluated using organisms that were carbapenem-susceptible or carbapenem non-susceptible with antibiotic resistance mechanisms other than those targeted by the NG-Test CARBA 5. A panel of 81 isolates were tested after growth on blood agar and MacConkey agar (54 *Enterobacteriaceae*, 20 *Pseudomonas aeruginosa* and 7 phylogenetically related organisms). A panel of 16 select *Enterobacteriaceae* (i.e., *E. coli* and KES [*Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Enterobacter cloacae complex* and *Serratia marcescens*]) isolates were tested after being seeded in raw stool or stool in C&S Cary Blair Transport Media, followed by sub-culture and growth on HardyCHROM CRE agar.

The organism groups and their relevant molecular characteristics used in the analytical cross-reactivity study is provided in **Table 2**. All organisms tested from blood and MacConkey agar (81/81, 100%) and from HardyCHROM CRE agar (16/16, 100%) yielded a negative NG-Test CARBA 5 result. The cross-reactivity of the NG-Test CARBA 5 was determined to be acceptable.

Table 2. Resistant mechanisms evaluated for Cross-Reactivity

Organism Group	**Resistant mechanisms evaluated**	
	Blood & MacConkey agar	**HardyCHROM CRE agar**
Enterobacteriaceae	**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, sulI, tet(c),* VEB-1, inducible AmpC**	N/A
Other	**VanA**	N/A

N/A: not applicable, HardyCHROM CRE agar is only intended for use with *E. coli* and KES organisms.

4. Assay Reportable Range:
Not applicable

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

a) Agar Incubation Study

The ability of the NG-Test CARBA 5 to consistently provide correct results over the range of recommended incubation times was evaluated using carbapenem non-susceptible isolates previously characterized to have a target carbapenemase. Twenty-two strains were tested after growth on blood and MacConkey agar every two hours from 16 – 24 hours of incubation. Fifteen of the 22 organisms were also tested after growth on HardyCHROM CRE every two hours from 18 – 24 hours of incubation. All organisms tested produced the expected result on NG-Test CARBA 5 at each time point tested.

b) Refrigerated Storage Study

To evaluate whether organism cultures (plated on agar media) stored in the refrigerator could be used with NG-Test CARBA 5, strains were evaluated each day for three days after refrigeration. Twelve strains were first tested after growth on blood and MacConkey agar. Ten of the 12 strains were also tested after growth on HardyCHROM CRE agar. All organisms tested produced the expected result on NG-Test CARBA 5 for each day of refrigeration.

c) Quality Control

Quality control testing was performed each day of the clinical study. The QC panel consisted of one positive control organism for each target carbapenemase and one negative control organism (**Table 3**). The QC testing gave the expected results each day of testing.

Table 3. QC Strains used for NG-Test CARBA 5 evaluation

QC Strain	Expected Results [1]
Klebsiella pneumoniae ATCC BAA-1705 [2]	Positive KPC (K) Line
Klebsiella pneumoniae NCTC 13442	Positive OXA-48 (O) Line
Klebsiella pneumoniae NCTC 13439	Positive VIM (V) Line
Escherichia coli NCTC 13476	Positive IMP (I) Line
Klebsiella pneumoniae ATCC BAA-2146	Positive NDM (N) Line
Klebsiella pneumoniae ATCC BAA-1706	No Positive Test Lines

[1] Positive Control Line (C) is expected for results to be considered valid.

[2] According to CLSI document M100, *Klebsiella pneumoniae* ATCC BAA-1705 may undergo spontaneous loss of the plasmid encoding the carbapenemase, leading to false-negative QC results. This strain should be maintained on a carbapenem-containing medium or with a selective antimicrobial disk on non-selective agar prior to QC testing.

6. Detection Limit:

Analytical Reactivity/Inclusivity

The analytical reactivity (inclusivity) of NG-Test CARBA 5 was evaluated using a panel of 92 isolates previously characterized to have a targeted carbapenemase (**Table 4**): 66 *Enterobacteriaceae* isolates (expected to provide 68 positive results) and 26 *P. aeruginosa* isolates (expected to provide 26 positive results). All 92 isolates were tested in triplicate after growth on blood agar and MacConkey agar. Forty-two select *Enterobacteriaceae* (i.e., *E. coli* and KES) isolates of the panel (expected to provide 44 positive results) were also tested in triplicate after being seeded in raw stool or stool in C&S Cary Blair Transport Media and cultured on HardyCHROM CRE agar. Organisms that yielded a negative NG-Test CARBA 5

result were further analyzed by modified Carbapenemase Inactivation Method (mCIM, as described in CLSI M100, 29th Edition). Negative NG-Test CARBA 5 results that yielded negative mCIM results were determined to be true negatives. Negative NG-Test CARBA 5 results that yielded positive mCIM results were determined to be false negatives.

Table 4. Targets and variants evaluated in Analytical Reactivity Study

Organism Group	Target Detected	Number of targets tested on Blood & MacConkey	Number of targets tested on Hardy CHROM CRE	Variants tested	Variants not detected
Enterobacteriaceae	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[1], 26[2],	
	NDM	15	11	1[2], 5, 6, 7	
	none [3]	5	2		
	Total	**68**	**44**		
Pseudomonas aeruginosa	KPC	5		2, 5	
	OXA-48-like	0			
	VIM	13		2, 11	
	IMP	6		1, 7, 14, 18, 19, 26	14, 18
	NDM	2		1	
	none [3]	0			
	Total	**26**			

[1] IMP-8 and IMP-47 determined to be the same protein based on sequence analysis by the Beta-Lactamase Database.

[2] *Proteus mirabilis* targets not detected from blood agar but detected from MacConkey agar.

[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.

A summary of the analytical reactivity data is provided in **Table 5**. NDM-1 and IMP-26 from two *P. mirabilis* isolates were detected after bacterial growth on MacConkey agar but not on blood agar. The following statement is included as a footnote to the performance table in the device labeling:

> *NDM-1 and IMP-26 not detected in P. mirabilis growth from blood agar, but yielded positive results from MacConkey agar.*

Of the variants tested, *P. aeruginosa* harboring IMP-14 and IMP-18 were not detected by the NG-Test CARBA 5. The following statement is included as a limitation in the device labeling:

> *False negative results have been observed with IMP-14 and IMP-18.*

The analytical reactivity of the NG-Test CARBA 5 for detection of carbapenemases in *Enterobacteriaceae* and *P. aeruginosa* was determined to be acceptable.

Table 5. Summary of Analytical Reactivity of the NG-Test CARBA 5[1]

Organism Group	Media	Agreement (%)[2]
Enterobacteriaceae	Blood Agar	64/66 (97.0)[3]
	MacConkey Agar	66/66 (100)
	HardyCHROM CRE agar	41/41 (100)
Pseudomonas aeruginosa	Blood Agar	24/26 (92.3)[4]
	MacConkey Agar	24/26 (92.3)[4]

[1] Triplicate results were all identical. Data from a single representative replicate.
[2] Percent Agreement calculated from number of organisms evaluated after mCIM analysis
[3] *Proteus mirabilis*; NDM and IMP targets not detected from blood agar but detected form MacConkey agar.
[4] *P. aeruginosa* false negative isolates were characterized as IMP-14 and IMP-18 variants.

7. Assay Cut-Off:
   Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:
   Not applicable

2. Matrix Comparison:
   Not applicable

C Clinical Studies:

1. Clinical Sensitivity:
   The performance of the NG-Test CARBA 5 was initially evaluated in a Clinical Study that was conducted at three U.S. sites using 150 prospectively collected isolates (recovered fresh or less than 6 months prior to testing) and 149 retrospectively collected isolates (stock, recovered more than 6 months prior to testing) of *Enterobacteriaceae* and *Pseudomonas aeruginosa*. These isolates were used to evaluate performance of NG-Test CARBA 5 using bacterial colonies grown on blood and MacConkey agar. A subset of these isolates was selected based on the intended use of the HardyCHROM CRE agar and used in an internal study to evaluate performance of the NG-Test CARBA 5 after bacterial growth on this medium.

   To be enrolled in the study and included in the analysis of performance, retrospective isolates must have been (i) identified to the species level and (ii) determined to be non-susceptible to at least one carbapenem antimicrobial agent by an FDA-cleared susceptibility test method or harbor any carbapenem-resistance marker. All prospective isolates were enrolled regardless of carbapenem susceptibility.

   Ertapenem disks were routinely used to maintain selective pressure for isolated colonies of retrospective *Enterobacteriaceae* isolates while no selective pressure was used for isolated colonies of retrospective *P. aeruginosa* isolates. Carbapenem susceptibility of each isolate was verified by disk diffusion using ertapenem (*Enterobacteriaceae* only), imipenem and meropenem while carbapenemase production was evaluated using the modified

Carbapenemase Inactivation Method (mCIM), as described in CLSI M100. In addition, the carbapenemase genetic marker of each isolate was determined using an FDA-cleared PCR assay for the *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-48}$, *bla*$_{IMP}$ and *bla*$_{VIM}$ markers. The NG-Test CARBA 5 results for each isolate were interpreted using the composite reference algorithm as depicted in **Table 6**.

Table 6. Composite reference algorithm for interpretation of NG Test CARBA 5 test results

PCR	mCIM	Composite Result	NG-Test CARBA 5	NG-Test CARBA 5 Interpretation
Positive	Positive	Positive	Positive	True positive [1]
Positive	Positive	Positive	Negative	False Negative
Positive	Negative	Negative	Positive	False positive
Negative	Positive	Negative	Positive	False positive
Positive	Negative	Negative	Negative	True Negative [2]
Negative	Positive	Negative	Negative	True Negative [3]
Negative	Negative	Negative	Positive	False Positive
Negative	Negative	Negative	Negative	True Negative

mCIM: modified Carbapenemase Inactivation Method

1 The carbapenemase(s) identified by NG-Test CARBA 5 and resistance marker(s) identified by PCR must correspond (e.g., positive for KPC by NG-Test CARBA 5 and *bla*$_{KPC}$ positive by PCR)

2 Gene for a carbapenemase targeted by NG-Test CARBA 5 present but not expressed (or expressed at levels below the limit of detection of NG-Test CARBA 5)

3 Isolate positive for a carbapenemase that is not targeted by the NG-Test CARBA 5

Performance of NG-Test CARBA 5 after bacterial growth on blood and MacConkey agar with the 240 *Enterobacteriaceae* isolates that provided 244 results (four isolates co-produced two carbapenemase targets) is summarized in **Table 7.1**. Performance after bacterial growth on blood and MacConkey agar with the 69 *P. aeruginosa* isolates that provided 69 results is summarized in **Table 7.2**. One isolate that was initially enrolled was excluded from the analysis because it was a species of *Pseudomonas* other than *P. aeruginosa*. There were no NDM or OXA-48-like *P. aeruginosa* isolates enrolled in the study. This is addressed in the following statement that is included in the performance section in the device labeling:

> *For P. aeruginosa, there were no OXA-48-like or NDM enrolled. However, P. aeruginosa with NDM (n=2) were evaluated analytically in the bench testing.*

Table 7.1. Agreement of NG-Test CARBA 5 with the composite reference when testing *Enterobacteriaceae*

Enterobacteriaceae		**Composite Reference Method**		
		Positive [1]	Negative [2]	Total
NG-Test CARBA 5	Positive	156	4 [3,4]	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] Defined as positive by mCIM *and* an FDA-cleared PCR assay for *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-48}$, *bla*$_{IMP}$ and *bla*$_{VIM}$

[2] Defined as negative by mCIM *and/or* an FDA-cleared PCR assay for *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-48}$, *bla*$_{IMP}$ and *bla*$_{VIM}$

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

[4] 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 7.2. Agreement of NG-Test CARBA 5 with the composite reference when testing *P. aeruginosa*

P. aeruginosa		**Composite Reference Method**		
		Positive [1]	Negative [2]	Total
NG-Test CARBA 5	Positive	13	3 [3]	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] Defined as positive by mCIM *and* an FDA-cleared PCR assay for *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-48}$, *bla*$_{IMP}$ and *bla*$_{VIM}$

[2] Defined as negative by mCIM *and/or* an FDA-cleared PCR assay for *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-48}$, *bla*$_{IMP}$ and *bla*$_{VIM}$

[3] 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.

Performance of NG-Test CARBA 5 with carbapenemase-positive isolates stratified by carbapenemase target is shown in **Table 8**. The same results were obtained regardless of whether testing colonies grown on blood agar or from colonies grown on MacConkey agar. For isolates positive by the composite reference method, the results obtained with the NG-Test CARBA 5 after growth on blood or MacConkey agar were in agreement [*Enterobacteriaceae*: 156/156 (100%); *P. aeruginosa*: 13/13 (100%)]. For isolates negative by the composite reference method, the agreement obtained with the NG-Test CARBA 5 after growth on blood and MacConkey agar was: *Enterobacteriaceae*: 84/88 (95.5%); *P. aeruginosa*: 53/56 (94.6%).

Table 8. Performance of NG-Test CARBA 5 vs Composite Reference with Carbapenemase-positive isolates stratified by each target

Comparator PCR Result		NG-Test CARBA 5 Result				Agreement % (95% CI)	
Organism Group	**Target**	**TP**	**FP**	**TN**	**FN**	**Positive**	**Negative**
Enterobacteriaceae [5] [n = 244]	**KPC**	84	0	160	0	100 (95.6-100)	100 (97.7-100)
	NDM	37	1 [1]	206	0	100 (90.6-100)	99.5 (97.3-99.9)
	OXA	20	0	224	0	100 (83.9-100)	100 (98.3-100)
	IMP	4	3 [2]	237	0	100 (51.0-100)	98.8 (96.4-99.6)
	VIM	11	0	233	0	100 (74.1-100)	100 (98.4-100)
P. aeruginosa [n = 69]	**KPC**	2	0	67	0	100 (34.2-100)	100 (94.6-100)
	NDM [4]	0	0	69	0	n/a	100 (94.7-100)
	OXA [4]	0	0	69	0	n/a	100 (94.7-100)
	IMP	2	3 [3]	64	0	100 (34.2-100)	95.5 (87.6-98.5)
	VIM	9	0	60	0	100 (70.1-100)	100 (94.0-100)

n/a: Not applicable

[1] An alternative PCR assay showed that this 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.

[3] 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.

[4] For *P. aeruginosa*, there were no NDM and OXA-48-like isolates enrolled in the study. Two isolates carrying the NDM target were evaluated in the Analytical Reactivity Study.

[5] *Enterobacteriaceae* isolates included: *C. freundii* (4), *C. koseri* (2), *E. asburiae* (2), *E. cloacae* (28), *E. cloacae complex* (4), *E. coli* (72), *K. aerogenes* (8), *K. oxytoca* (11), *K. ozaenae* (1), *K. pneumoniae* (90), *P. mirabilis* (9), *P. rettgeri* (1), *S. marcescens* (8)

To evaluate the performance of NG-Test CARBA 5 after bacterial growth on HardyCHROM CRE agar, was evaluated using a subset of 186 isolates that were eligible in accordance with the intended use of this agar medium (i.e., *E. coli* and KES isolates). One isolate was excluded because it was unavailable for testing. Six isolates were excluded from the raw stool analysis due to the lack of or poor growth on HardyCHROM CRE agar (post-seeding into raw stool). Two additional isolates (8 total) were excluded from the C&S Cary Blair stool analysis due to the lack of or poor growth on HardyCHROM CRE agar (post-seeding into C&S Cary Blair stool). In total, 180 isolates (184 results, shown in **Table 9.1**) were reported in the raw stool analysis and 179 isolates (182 results, shown in **Table 9.2**) were reported in the C&S Cary Blair stool analysis. Results stratified by carbapenemase target are shown in **Table 10**).

Table 9.1. Agreement of NG-Test CARBA 5 with the composite reference after bacterial growth on HardyCHROM CRE agar (post-seeding into Raw Stool)

Raw Stool		**Composite Reference Method**		
		Positive [1]	Negative [2]	Total
NG-Test CARBA 5	Positive	143	4 [3,4]	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 Defined as positive by mCIM ***and*** an FDA-cleared PCR assay for *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-48}$, *bla*$_{IMP}$ and *bla*$_{VIM}$

2 Defined as negative by mCIM ***and/or*** an FDA-cleared PCR assay for *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-48}$, *bla*$_{IMP}$ and *bla*$_{VIM}$

3 An alternative PCR assay showed that the NDM false positive isolate harbored a *bla*$_{NDM}$ -1 variant. Isolate was positive by mCIM.

4 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 9.2. Agreement of NG-Test CARBA 5 with the composite reference after bacterial growth on HardyCHROM CRE agar (post-seeding into C&S Cary Blair Stool)

C&S Cary Blair Stool		**Composite Reference Method**		
		Positive [1]	Negative [2]	Total
NG-Test CARBA 5	Positive	141	4 [3,4]	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 Defined as positive by mCIM ***and*** an FDA-cleared PCR assay for *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-48}$, *bla*$_{IMP}$ and *bla*$_{VIM}$

2 Defined as negative by mCIM ***and/or*** an FDA-cleared PCR assay for *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-48}$, *bla*$_{IMP}$ and *bla*$_{VIM}$

3 An alternative PCR assay showed that the NDM false positive isolate harbored a *bla*$_{NDM}$ -1 variant. Isolate was positive by mCIM.

4 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.

For isolates positive by the composite reference method, the results obtained with the NG-Test CARBA 5 after growth on HardyCHROM CRE agar were in agreement [Raw Stool: 143/143 (100%); C&S Cary Blair Stool: 141/141 (100%), **Table 10**]. For isolates negative by the composite reference method, the agreement obtained with the NG-Test CARBA 5 after growth on HardyCHROM CRE agar was the same for Raw Stool and C&S Cary Blair Stool: 37/41 (90.2%).

Table 10. Overall performance of NG-Test CARBA 5 with Carbapenemase-positive *E. coli* and KES isolates[1] grown on HardyCHROM CRE agar

Comparator PCR Result		NG-Test CARBA 5 Result				Agreement % (95% CI)	
Organism Group	**Target**	**TP**	**FP**	**TN**	**FN**	**Positive**	**Negative**
Raw Stool [n = 184 results]	**KPC**	76	0	108	0	100 (95.2-100)	100 (96.6-100)
	NDM	36	1 [2]	147	0	100 (90.4-100)	(99.3 (96.3-99.9)
	OXA	18	0	166	0	100 (82.4-100)	100 (98.3-100)
	IMP	4	3 [3]	177	0	100 (51.0-100)	98.3 (95.2-99.4)
	VIM	9	0	175	0	100 (70.1-100)	100 (97.9-100)
C&S Cary Blair Stool [n = 182 results]	**KPC**	75	0	107	0	100 (95.1-100)	100 (96.5-100)
	NDM	36	1 [2]	145	0	100 (90.4-100)	99.3 (96.2-99.9)
	OXA	18	0	164	0	100 (82.4-100)	100 (97.7-100)
	IMP	4	3 [3]	175	0	100 (51.0-100)	98.3 (95.2-99.4)
	VIM	8	0	174	0	100 (67.6-100)	100 (97.8-100)

[1] Isolates included: *E. asburiae* (2), *E. cloacae* (24), *E. cloacae complex* (3), *E. coli* (45), *K. aerogenes* (8), *K. oxytoca* (11), *K. pneumoniae* (80), *S. marcescens* (7)

[2] An alternative PCR assay showed that this isolate harbored a bla_{NDM} -1 variant.

[3] 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.

One *K. oxytoca* isolate was positive for NDM by NG-Test CARBA 5 but negative by the comparator PCR assay. Investigation of the discordant false positive result using an alternative PCR method confirmed the presence of bla_{NDM} -1 variant. Six isolates (3 *Enterobacteriaceae* and 3 *P. aeruginosa*) were positive for IMP by NG-Test CARBA 5 but negative by the comparator PCR assay. Investigation of the discordant false positive results using an alternative PCR assay and bidirectional sequencing showed that these isolates harbored bla_{IMP} variants that (i) are not detected by the FDA-cleared PCR assay (bla_{IMP} variant -7, n=1), (ii) are predicted by *in silico* analysis but not analytically demonstrated to be detected by the assay (bla_{IMP} variant -8/-47, n=3 and bla_{IMP} variant -19, n=1), or (iii) the reactivity of the assay is unknown (bla_{IMP} variant -15, n=1).

The results were determined to be acceptable.

The address the indications for use, the following statements are included in the Limitations section of the device labeling:

> *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.*

The performance of NG-Test CARBA 5 with bacteria other than Enterobacteriaceae and Pseudomonas aeruginosa has not been evaluated.

Organism identification and elevated carbapenem MICs should be determined prior to testing with NG-Test CARBA 5.

2. Clinical Specificity:
   Refer to **Section VII C(1)** above.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):
   Not applicable

D Clinical Cut-Off:
Not applicable

E Expected Values/Reference Range:
The performance of the NG-Test CARBA 5 was evaluated using a collection of prospectively collected and archived isolates of carbapenem non-susceptible bacteria. When testing for the presence of target carbapenemases, the 309 isolates of *Enterobacteriaceae* (240) and *P. aeruginosa* (69) tested in the study generated 169 positive results using a composite reference method (FDA-cleared PCR assay and mCIM method); 176 positive results were generated using the NG-Test CARBA 5. A summary of the carbapenemase targets detected by the composite reference method and by the NG-Test CARBA 5 is shown in **Table 11**.

Table 11. Summary of carbapenemase targets identified in the Clinical Study for the NG-Test CARBA 5

Organism Family	Carbapenemase Target	Number of Targets Detected (% of combined)	
		Composite Reference	NG-Test CARBA 5
Enterobacteriaceae	KPC	84 (53.8)	84 (52.5)
	NDM	37 (23.7)	38 (23.8) [1]
	OXA	20 (12.8)	20 (12.5)
	IMP	4 (2.6)	7 (4.4) [2]
	VIM	11 (7.1)	11 (6.9)
	Combined	156	160
P. aeruginosa	KPC	2 (15.4)	2 (12.5)
	NDM	n/a	n/a
	OXA	n/a	n/a
	IMP	2 (15.4)	5 (31.3) [3]
	VIM	9 (69.2)	9 (56.3)
	Combined	13	16

[1] An alternative PCR assay showed that this 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.

[3] 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.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.