510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

K162385

B. Purpose for Submission:

To obtain a substantial equivalence determination for the RAPIDEC CARBA NP test for qualitative detection of carbapenemase enzymes in pure colonies of *Enterobacteriaceae* and *Pseudomonas aeruginosa* that have elevated MIC values to any Carbapenem.

C. Measurand:

Carbapenemase enzymes

D. Type of Test:

Qualitative phenotypic (colorimetric) assay

E. Applicant:

bioMérieux SA

F. Proprietary and Established Names:

RAPIDEC CARBA NP

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.1640 (Antimicrobial susceptibility test powder)

2. Classification:

   Class II

3. Product code:

   PTJ

4. Panel:

83- Microbiology

H. Intended Use:

1. Intended use(s):

   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.

2. Indication(s) for use:

   Same as the Intended Use.

3. Special conditions for use statement(s):

   For prescription use only.

   Limitations of the RAPIDEC CARBA NP assay:
   1) The performance of the RAPIDEC CARBA NP assay for detection of carbapenemase enzymes encoded by genetic markers other than KPC, NDM, OXA-48, VIM and/or IMP has not been established. In addition, RAPIDEC CARBA NP results may be influenced by the local epidemiology regarding genetic markers of resistance, i.e., depending on the local distribution/prevalence of different carbapenemase genetic markers, and more false negative results may occur. Conduct alternative testing if negative results are obtained and carbapenemase enzyme production is suspected based on local epidemiology.
   2) RAPIDEC CARBA NP testing should be used as an adjunct to other laboratory test(s) such as antimicrobial susceptibility testing.
   3) The performance of the RAPIDEC CARBA NP test with bacteria other than *Enterobacteriaceae* and *Pseudomonas aeruginosa* has not been evaluated. Organism identification and elevated carbapenem MICs should be determined prior to testing on the RAPIDEC CARBA NP.
   4) *Proteus* species, *Providencia* species, *Morganella* species may have elevated

imipenem MICs due to intrinsic resistance mechanisms. *Pseudomonas aeruginosa* has been shown to exhibit resistance to ertapenem due to intrinsic resistance mechanisms.

5) The detection of OXA variants other than OXA-48 has not been evaluated sufficiently in the study.
6) Hyper-mucoid colonies may lead to false positive or false negative results and should not be tested by the RAPIDEC CARBA NP test. A hyper-mucoid colony tends to stretch itself to form a continuous viscous filament > 5 mm in length when picked up from an agar plate using a bacteriology loop/needle.[1]
7) Agar media containing pH indicator for colony color differentiation (e.g., Bromocresol Purple, MacConkey, Cysteine Lactose Electrolyte-Deficient, etc.) are not compatible with the RAPIDEC CARBA NP and require subculturing growth/biomass on a sheep blood agar for testing.
8) The performance of RAPIDEC CARBA NP has been evaluated for subculturing growth on 5% sheep blood agar incubated for 18- 24 hours (Routine procedure) and 4-5 hours (Short Incubation procedure) only. The performance with other culture media has not been evaluated and is therefore unknown.
9) The performance of the RAPIDEC CARBA NP test when testing *Enterobacteriaceae* and *Pseudomonas aeruginosa* containing OXA-181, OXA-232, SME, GIM, SPM, and IMI carbapenemase enzymes has not been established due to the low number of positive isolates available using the Composite Reference Method.

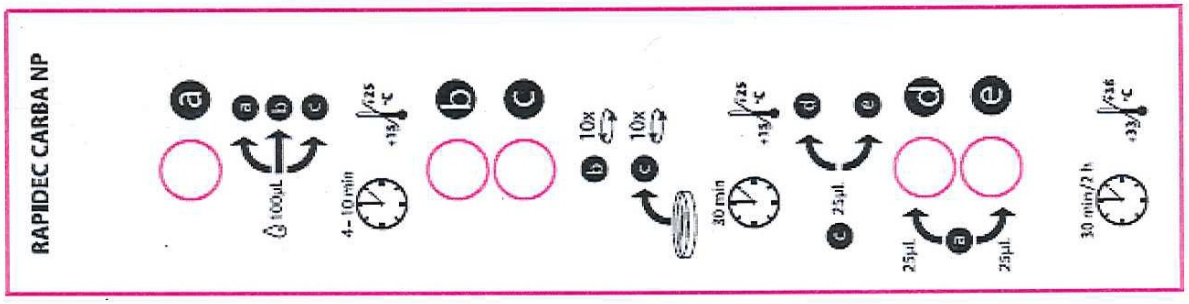
4. Special instrument requirements:

   Not Applicable; results are read manually

I. Device Description:

The RAPIDEC CARBA NP strip (Figure 1) is composed of 5 wells prepared with premeasured portions of the necessary substrates for the reactions. In addition, the RAPIDEC CARBA NP kit contains the necessary accessories for performing the test.

Figure 1: RAPIDEC CARBA NP Strip



[1] Shon AS, Bajwa RPS, Russot A. Hyper-virulent (hyper-mucoviscous) *Klebsiella pneumonia*. A new and dangerous breed. *Land Bioscience*, 2013, February 15, virulence 4:2, 107-118.

In order to rehydrate the dry reagents and initiate the reactions, wells (**a)**, (**b)** and (**c)** are filled with 100 µL of API Suspension Medium (purified water). The strip is left at room temperature for 4-10 minutes to allow the dry reagents to reconstitute in the wells. The bacterial inoculum suspension is prepared in well (**c)** until the turbidity equals well (**b)**. Well (**c)** contains the lysis buffer. The lysis of the inoculum suspension enables the extraction of the enzyme; the strip is left at room temperature for additionally 30 minutes.

Transfer 25 µL of the lysed inoculum suspension is to wells (**d)** and (**e**) and 25 µL from well (**a)** [phenol red solution] is also transferred to wells (**d)** and (**e**). The strip is incubated for 30-40 minutes at 33-38°C to allow the hydrolysis to occur and change in color of the phenol red solution in the presence of a carbapenemase enzyme. The hydrolysis acidifies the medium which results in the change in color of the pH indicator. The function of each well of the RAPIDEC CARBA NP strip is shown in Table 1:

Table 1: Function of the RAPIDEC CARBA NP Strip Wells

Well	Reagent
(a)	Phenol red solution
(b)	Turbidity Control
(c)	Lysis buffer
(d)	Control well without imipenem
(e)	Reaction well containing imipenem

Place the strip on the two-colored (black and white) support. Position wells (**d)** and (**e**) on the white background to facilitate reading. Reading is performed by comparing the colors in wells (**d)** and (**e)**, ensuring that the strip is firmly pressed against the support. A test is positive when a significant variation in color is observed between the two wells. For example, the control well is red and the test well has changed to yellow/orange. Result interpretation is shown in Table 2.

Table 2: Interpretation of Results

Control well (d)	Test well (e)	Interpretation
Red	Red	Negative (Absence of carbapenemase)
Orange	Orange	
Red	Yellow, light orange, orange, dark orange	Positive (Presence of carbapenemase)
Orange	Yellow	
Any color other than red or orange	Not applicable	*Uninterpretable
Orange	Red	

*An uninterpretable result should be retested. If the retest yields an uninterpretable result, consider testing with an alternate method to determine carbapenemase status of the isolate.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Clearview Exact PBP2a Test

2. Predicate 510(k) number(s):

K091766

3. Comparison with predicate:

Table 3: Comparison with the Predicate Device

Item	Device RAPIDEC CARBA NP	Predicate Clearview Exact PBP2a Test Clearview Exact PBP2a Test (K091766)
Similarities		
Intended Use	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.	The Clearview Exact PBP2a Test is a qualitative, *in vitro*, immunochromatographic assay for the detection of penicillin binding protein 2a (PBP2a) in isolates identified as *Staphylococcus aureus*, as an aid in detecting methicillin-resistant *Staphylococcus aureus* (MRSA). The Clearview Exact PBP2a Test is not intended to diagnose MRSA nor to guide or monitor treatment for MRSA infections.

Item	Device RAPIDEC CARBA NP	Predicate Clearview Exact PBP2a Test Clearview Exact PBP2a Test (K091766)
Detection Capability	Marker of antimicrobial resistance	Same
Sample Type	Pure colonial growth	Same
Reading	Visual based on color change	Same

Differences		
Mode of Detection	Carbapenemase enzymes encoded by genetic markers KPC, NDM, OXA-48, VIM and/or IMP	Penicillin-binding protein 2a (PBP2a)
Technology	Imipenem hydrolysis by carbapenemase producers (i.e. *Enterobacteriaceae* or *Pseudomonas aeruginosa*) resulting a change in color of the pH indicator	Immunochromatographic membrane assay
Bacterial Isolate Type	Gram negative colonies identified as *Enterobacteriaceae* or *Pseudomonas aeruginosa* that have any elevated carbapenem MIC values	Gram positive cocci identified as *Staphylococcus aureus*
Culture Media	Non-selective sheep blood agar (Columbia agar + 5% sheep blood, trypticase soy agar + 5% sheep blood)	Non-selective sheep blood agar (Columbia agar + 5% sheep blood, trypticase soy agar + 5% sheep blood) and Mueller Hinton agar

K. Standard/Guidance Document Referenced (if applicable):

CLSI M100-S25: Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement

L. Test Principle:

The RAPIDEC CARBA NP test is based on the detection of carbapenem hydrolysis by carbapenemase-producing bacteria. Hydrolysis acidifies the medium which results in the change in color of the pH indicator. The bacteria are first lysed to release the carbapenemase enzyme. The lysate is added to a detection solution containing:

- A carbapenem- imipenem (carbapenemase substrate)
- Phenol red (pH indicator)
- Zinc- required for the detection of metallo-dependent carbapenemase-producing strains.

After incubating for a maximum of 2 hours, reading is performed visually by comparing the

color in the control well (without imipenem) to the color of reaction/test well (with imipenem) as described in Figure 2 above.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The reproducibility panel of 10 strains (six carbapenemase producers and four non-carbapenemase producers) was tested in triplicates for five days at three sites interpreted by two operators with results blinded to each other. The carbapenemase producers included VIM producing *Pseudomonas aeruginosa* (1), NDM (1), IMP (1), OXA-48 (1), and KPC (2) producing *Enterobacteriaceae.* Reproducibility isolates information is shown in Table 4.

Table 4: Reproducibility Strains

Organism	Resistance Mechanism		Expected Result
	Carbapenemase	Other	
R001 *E. coli*	---	AmpC	Neg
R002 *K. pneumoniae*	---	Porin loss	Neg
R007 *K. pneumoniae*	---	ESBL	Neg
R008 *E. aerogenes*	---	Porin loss	Neg
R003 *E. aerogenes*	KPC-3	---	Pos
R004 *E. coli*	KPC	---	Pos
R005 *K. pneumoniae*	NDM	---	Pos
R010 *P. aeruginosa*	VIM	---	Pos
R011 *K. pneumoniae*	IMP	---	Pos
R012 *E. coli*	OXA-48	---	Pos

Two separate lots of RAPIDEC CARBA NP were used in the study to include 327 and 573 results from Lot #1 and Lot #2 respectively:

10 strains x triplicates x 5 days x 3 sites x 2 operators = 900

Two subculture/incubation procedures were assessed in the reproducibility studies:

- In the Routine Subculture/Incubation procedure, the RAPIDEC CARBA NP test was performed on colonial growth on sheep blood agar medium that has been incubated for 18-24 hours.
- In the Short Subculture/Incubation procedure, organisms were sub-cultured onto MacConkey agar and incubated for 18- 24 hours. The organisms from the MacConkey then were subcultured again onto blood agar and incubated for 4-5 hours to obtain sufficient growth/biomass to perform the RAPIDEC CARBA NP.

Reproducibility for Routine and Short Subculture/Incubation is shown in Tables 5 and 6 respectively with acceptable results >95%.

Table 5: Reproducibility for Routine Subculture/Incubation (18-24 hours)

RAPIDEC CARBA NP - Reproducibility (Agreement with the expected result)												
	Site 1				Site 2				Site 3			
	Operator 1		Operator 2		Operator 1		Operator 2		Operator 1		Operator 2	
Organism ID	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
R001	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%
R002	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%
R003	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%
R004	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%
R005	15/15	100.0%	15/15	100.0%	13/15	86.7%	13/15	86.7%	13/15	60.0%	13/15	60.0%
R007	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%
R008	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%
R010	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%
R011	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%
R012	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%
Within-Site Reproducibility	**N**			**%**	**N**			**%**	**N**			**%**
	300/300			100.0%	296/300			98.7%	288/300			96.0%
Between-site Reproducibility	98.2% (884/900)											

Table 6: Reproducibility for Short Subculture/Incubation (4-5 hours)

RAPIDEC CARBA NP - Reproducibility (Agreement with the expected result)												
	Site 1				Site 2				Site 3			
	Operator 1		Operator 2		Operator 1		Operator 2		Operator 1		Operator 2	
Organism ID	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
R001	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%
R002	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%
R003	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%
R004	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%
R005	15/15	100.0%	15/15	100.0%	13/15	86.7%	13/15	86.7%	13/15	86.7%	13/15	86.7%
R007	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%
R008	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%
R010	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%
R011	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%
R012	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%	15/15	100.0%
Within-Site Reproducibility	**N**			**%**	**N**			**%**	**N**			**%**
	300/300			100.0%	296/300			98.7%	296/300			98.7%
Between-site Reproducibility	99.1% (892/900)											

b. Linearity/assay reportable range:

Not applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Quality Control (QC)

The QC panel consists of one positive and one negative control organism. The performance is summarized in Tables 7 and 8 for Routine and Short Incubation respectively with acceptable results.

Table 7: QC Summary for Routine Subculture/Incubation (18-24 hours)

QC Strain	Expected Results	RAPIDEC CARBA NP Result
Klebsiella pneumoniae ATCC BAA-1705	Positive	100% (159/159) 95% CI [97.6; 100.0]%
Klebsiella pneumoniae ATCC BAA-1706	Negative	100% (159/159), 95% CI [97.6; 100.0]%

Table 8: QC Summary for Short Subculture/Incubation (4-5 hours)

QC Strain	Expected Results	RAPIDEC CARBA NP Result
Klebsiella pneumoniae ATCC BAA-1705	Positive	100% (168/168), 95% CI [97.8; 100.0]%
Klebsiella pneumoniae ATCC BAA-1706	Negative	100% (165/165), 95% CI [97.8; 100.0]%

d. Detection limit:

Not applicable

Analytical Reactivity

A total of 43 carbapenemase producers as determined by the reference composite method were tested by the RAPIDEC CARBA NP. There were two VIM producing *Pseudomonas aeruginosa* and 41 carbapenemase producing *Enterobactericeae* consisting of those encoded by genetic markers as follows: KPC (16), NDM (10), OXA-48 (7), VIM (5) and IMP (3).

The analytical sensitivity is based on an initial reading performed at 30 minutes. If negative, a final reading was performed at a total of 2 hours of 33-38°C incubation. Considering any positive result, the analytical sensitivity was 100%; however, four negative results at the initial 30-minute read became positive at the final 2–hour read while eight initial positive results at 30 minutes read became negatives when read again at 2 hours incubation. This information is provided as a footnote in the labeling:

> *"There were four negative results at the initial 30-minute read but became positive at the final 2–hour read; there were eight initial positive results that changed back to negatives when read again at 2 hours incubation."*

In addition, a Caution statement is included in the Reading and Interpretation section of the labeling:

> *"Positive tests results are frequently obtained at 30 minutes of incubation. Re-incubate if negative or doubtful. After 30 minutes, there is a risk that a positive result may change back to a negative. Therefore, it is imperative to perform the initial reading at 30 minutes of incubation."*

Analytical Agar Culture Media Compatibility Studies

The RAPIDEC CARBA NP result tested with strains on Columbia agar + 5% sheep blood (COS) were compared with strains on Trypticase Soy agar + 5% sheep blood (TSS). A total of 106 strains (92 carbapenemase enzyme producers and 14 non-carbapenemase enzyme producers) were tested in the compatibility study. The carbapenemase producers included those encoded by genetic markers as follows: IMP

(19), KPC (20), VIM (20), NDM (17), and OXA-48 (16).

The positive rate for the carbapenemase producers was 94.6% (87/92) and 92.4% (85/92) for TSS and COS respectively at the initial 30-minute read. There were five (i.e., four OXA-48 producing *K. pneumoniae* and one KPC producing *K. pneumoniae*) that were negative for both media and two NDM producing *Providencia* spp. (i.e., *P. rettgeri* and *P. stuartii*) for COS at the 30-minute read. At the final 2-hour read, the positive rate was the same for both COS and TSS at 98.9% (91/92) due to one negative (i.e., KPC producing *K. pneumoniae*) result.

The negative rate for non-carbapenemase producers was 100% for both media at the 30-minute and 2-hour reads.

The results indicate no significant differences between TSS and COS at 30-minute and 2-hour reads.

A limitation is included in the limitation section of the labeling:

> *"The performance of RAPIDEC CARBA NP has been evaluated for subculturing growth on 5% sheep blood agar incubated for 18- 24 hours (Routine procedure) and 4-5 hours (Short Incubation procedure) only. The performance with other culture media has not been evaluated and is therefore unknown."*

e. Analytical specificity:

A total of 93 non-carbapenemase producing strains with elevated carbapenem MICs were tested by RAPIDEC CARBA NP. The resistance mechanisms included Gram negative organisms harboring AmpC, high level AmpC, porin loss, ESBL, porin loss/ESBL, porin loss/AmpC, in addition to resistant Gram positive organisms (MRSA, and VRE)

The set included 67 strains related to the intended use: *Enterobacteriaceae* (59), *P. aeruginosa* (8), and 26 other organisms comprised of non-fermenting gram negative rods, gram positive organisms and yeast. All strains were well-characterized. An initial reading was performed at 30 minutes and if the result was negative a final reading was performed at a total of 2 hours of 33-38°C incubation.

The analytical specificity was 97.9% (91/93) at 30 minutes of incubation due to two intrinsically carbapenem-resistant *Stenotrophomonas maltophilia*. The analytical specificity was 93.6% (87/93) at 2 hours incubation caused by six positive at 2 hours incubation. The false positive results were caused by five non-fermenting gram negative rods (three intrinsic resistant *Stenotrophomonas maltophilia*, one each for *Burkholderia cepaciae* and *Sphingomonas paucimobilis*) and one *Morganella morganii* with an AmpC resistance.

For evaluation of intended *Enterobacteriaceae* and *P. aeruginosa*, the analytical specificity is 100% (67/67) at 30 minutes and 98.5% (66/67) at 2 hours of incubation.

The false positive was caused by one AmpC producing *Morganella morganii*.

Two limitations are included in the limitation section of the labeling:

- *The performance of the RAPIDEC CARBA NP test with bacteria other than Enterobacteriaceae and Pseudomonas aeruginosa has not been evaluated. Organism identification and elevated carbapenem MICs should be determined prior to testing on the RAPIDEC CARBA NP.*

- *Proteus species, Providencia species, Morganella species may have elevated imipenem MICs due to intrinsic resistance mechanisms. Pseudomonas aeruginosa has been shown to exhibit resistance to ertapenem due to intrinsic resistance mechanisms.*

f. Assay cut-off:

Not Applicable

2. Comparison studies:

a. Method comparison with predicate device:

Not Applicable

b. Matrix comparison:

Not Applicable

3. Clinical studies:

a. Clinical Sensitivity:

In the multi-center study, each isolate was tested by the RAPIDEC CARBA NP and the composite reference method which includes carbapenem (Imipenem, Meropenem, Ertapenem, and/or Doripenem) MIC, CLSI Carba NP, and carbapenemases by validated PCR. Results from those three different tests were used to determine the carbapenemase positive/negative status of an isolate, with the final composite reference result based on agreement of at least two of the three tests.

Agreement between RAPIDEC CARBA NP and composite reference method was assessed. When a RAPIDEC CARBA NP result was not in agreement with the composite reference result, it was evaluated for false negative and false positive. A negative RAPIDEC CARBA NP result was considered as a false negative when the composite reference result was determined to be positive. A positive RAPIDEC CARBA NP result was considered as a false positive when the composite reference result was determined to be negative, indicating a false carbapenemase producer. The comparative performance is

shown in Table 9.1 below.

Table 9.1: Comparative Performance of RAPIDEC CARBA NP (*Enterobacteriaceae + Pseudomonas aeruginosa*)

Incubation	Total #	Agreement* #	Agreement* %	Negative #	Positive #	False Positive[a] # (%)	False Negative[b] # (%)
Routine Subculture							
Clinical	306	301	98.4	155	151	5 (3.2)	0
Challenge	151	150	99.3	37	114	0	1 (0.9)
Combined	**457**	**451**	**98.7**	**192**	**265**	**5 (2.6)**	**1 (0.4)**
Short Subculture							
Clinical	300	294	98.0	152	148	5 (3.3)	1 (0.7)
Challenge	149	146	98.0	36	113	0	3 (2.7)
Combined	**449**	**440**	**98.0**	**188**	**261**	**5 (2.7)**	**4 (1.5)**

[a] False positive for carbapenemase; RAPIDEC CARBA NP positive result for a non-carbapenemase producing *Enterobacteriaceae* or *P. aeruginosa*

[b] False negative for carbapenemase; RAPIDEC CARBA NP negative result for a carbapenemase producing *Enterobacteriaceae* or *P. aeruginosa*

* Composite reference method is composed of three results (i.e., carbapenem MIC, carbapenemase by PCR and CLSI Carba NP); a two-out-of-three approach is used to determine isolate positivity status

Routine Subculture/Incubation

The RAPIDEC CARBA NP test was evaluated with *Enterobacteriaceae* and *Pseudomonas aeruginosa* colonial growth on sheep blood agar medium incubated for 18-24 hours (routine subculture/incubation). An initial RAPIDEC CARBA NP reading was performed at 30 minutes and if negative a final reading was performed at a total of 2 hours of 33-38°C incubation.

The routine subculture/incubation procedure included 457 samples in which 306 were clinical samples provided by the investigational sites and 151 were well-characterized challenge samples. It included 394 *Enterobacteriaceae* and 63 *Pseudomonas aeruginosa* with carbapenemases described below:

- 265 isolates were positive for carbapenemase enzyme encoded by genetic markers as follows: KPC (147), NDM (52), VIM (26), IMP (17), and OXA-48 (23).
- 192 isolates were carbapenemase enzymes negative.

The Routine Subculture/Incubation performance in 2x2 tabular format is presented in Table 9.2.

Table 9.2: RAPIDEC CARBA NP Performance (Routine Subculture/Incubation)

		Composite Reference Method		
		Positive	Negative	Total
RAPIDEC CARBA NP	Positive	264	5[a]	269
	Negative	1[b]	187	188
	Total	265	192	457

[a] False carbapenemase producing *Enterobacteriaceae* or *P. aeruginosa*: 2.6% (5/192)
[b] False non-carbapenemase producing *Enterobacteriaceae* or *P. aeruginosa*: 0.4% (1/265)

Short Subculture/Incubation

In the Short Subculture/Incubation procedure, organisms were sub-cultured onto MacConkey agar and incubated for 18- 24 hours. The organisms from the MacConkey then were subcultured again onto blood agar and incubated for 4-5 hours to obtain sufficient growth/biomass to perform the RAPIDEC CARBA NP. An initial RAPIDEC CARBA NP reading was performed at 30 minutes and if negative a final reading was performed at a total of 2 hours of 33-38°C incubation.

The short subculture/incubation procedure included 449 samples in which 300 were clinical samples provided by the investigational sites and 149 were well-characterized challenge samples. It included 392 *Enterobacteriaceae* and 57 *Pseudomonas aeruginosa* with carbapenemase enzymes described below:

- 261 isolates were positive for carbapenemase enzymes: KPC (146), NDM (52), VIM (23), IMP (17), and OXA-48 (23).
- 188 isolates were carbapenemase enzyme negative.

The Short Subculture/Incubation performance in 2x2 tabular format is presented in Table 9.3.

Table 9.3: RAPIDEC CARBA NP Performance (Short Subculture/Incubation)

		Composite Reference Method		
		Positive	Negative	Total
RAPIDEC CARBA NP	Positive	257	5[a]	262
	Negative	4[b]	183	187
	Total	261	188	449

[a] False carbapenemase producing *Enterobacteriaceae* or *P. aeruginosa*: 2.7% (5/188)
[b] False non-carbapenemase enzyme producing *Enterobacteriaceae* or *P. aeruginosa*: 1.5% (4/261)

The Routine and Short Incubation performance of the RAPIDEC CARBA NP test with *Enterobacteriaceae* and *Pseudomonas aeruginosa* and target carbapenemase enzymes is demonstrated in Tables 10.1 and 10.2 respectively.

Table 10.1: Overall Performance of RAPIDEC CARBA NP with *Enterobacteriaceae* and *Pseudomonas aeruginosa* expressing the indicated Carbapenemase Genetic Markers

	RAPIDEC CARBA NP Performance			
	Routine Subculture/Incubation		Short Subculture/Incubation	
	Number	Agreement	Number	Agreement
Enterobacteriaceae	388/394	98.5%	383/392	97.7%
Pseudomonas aeruginosa	63/63	100%	57/57	100%
All Samples	**451/457**	**98.7%**	**440/449***	**98.0%**

* Insufficient growth/biomass for six *P. aeruginosa* and two *K. pneumoniae* in the short subculture procedure. They were three VIM producing *P. aeruginosa*, and one KPC producing *K. pneumoniae*; four negative samples including three *P. aeruginosa* and one *K. pneumoniae.*

Table 10.2: Performance of RAPIDEC CARBA NP Evaluated by Carbapenemase Genetic Markers

Carbapenemase determination by composite reference method		RAPIDEC CARBA NP Performance			
		Routine Subculture/Incubation		Short Subculture/Incubation	
		Number	Agreement	Number	Agreement
Enterobacteriaceae	KPC	143/144[a]	99.3%	142/143[c]	99.3%
	NDM	51/51	100%	50/51[c]	98.0%
	VIM	15/15	100%	14/15[c]	93.3%
	IMP	12/12	100%	12/12	100%
	OXA-48	23/23	100%	22/23[c]	95.7%
	Total	244/245	99.6%	240/244	98.4%
Pseudomonas aeruginosa	KPC	3/3	100%	3/3	100%
	NDM	1/1	100%	1/1	100%
	VIM	11/11	100%	8/8	100%
	IMP	5/5	100%	5/5	100%
	Total	20/20	100%	17/17	100%
Positive Samples		**264/265[a]**	**99.6%**	**257/261[c]**	**98.5%**
Enterobacteriaceae		144/149[b]	96.6%	143/148[d]	96.6%
Pseudomonas aeruginosa		43/43	100%	40/40	100%
Negative Samples		**187/192[b]**	**97.4%**	**183/188[d]**	**97.3%**
All Samples		**451/457**	**98.7%**	**440/449***	**98.0%**

[a] Routine subculture false negative (false non-carbapenemase producer) rate was 0.4% (1/265) for claimed carbapenemase enzymes; the false negative was KPC-producing *Enterobacteriaceae*

[b] Routine subculture false positive (false carbapenemase producer) rate was 2.6% (5/192) for *P. aeruginosa* and *Enterobacteriaceae*; the five false positives were from *Enterobactericeae*

[c] Short subculture false negative (false non-carbapenemase producer) rate was 1.5% (4/257) for claimed carbapenemase enzymes; one false negative each for KPC, NDM, VIM, and OXA-48 from *Enterobacteriaceae*

[d] Short subculture false positive (false carbapenemase-producer) rate was 2.7% (5/188); the five false positives were from *Enterobacteriaceae*

* Insufficient growth/biomass for six *P. aeruginosa* and two *K. pneumoniae* in the short subculture procedure. They were three VIM producing *P. aeruginosa*, and one KPC producing *K. pneumoniae*; four negative samples including three *P. aeruginosa* and one *K. pneumoniae.*

In addition, 21 isolates of *Enterobacteriaceae* and *Pseudomonas aeruginosa* expressing carbapenemases other than KPC, NDM, VIM, OXA-48 and IMP were tested. The results are shown in Table 11. The performance of RAPIDEC CARBA NP with *Enterobacteriaceae* and *Pseudomonas aeruginosa* containing OXA-181, OXA-232, SME, GIM, SPM, and IMI has not been established. A limitation is included in the limitation section of the labeling:

"The performance of the RAPIDEC CARBA NP test when testing Enterobacteriaceae and Pseudomonas aeruginosa containing OXA-181, OXA-232, SME, GIM, SPM, and IMI carbapenemase enzymes has not been established due to the low number of positive isolates available using the Composite Reference Method."

Table 11: RAPIDEC CARBA NP Performance with *Enterobacteriaceae* and *P. aeruginosa* Expressing Carbapenemases Other Than KPC, NDM, VIM, OXA-48, and IMP

Carbapenem MIC[a]	Carbapenemase Gene (#Tested)	CLSI Carba NP[e]	Carbapenemase Determination by Composite Reference Method[d]	RAPIDEC CARBA NP Result[b]	
				Positive	Negative
Pseudomonas aeruginosa					
NS	SPM (1)	ND	Positive	1	0
NS	GIM (1)	Positive	Positive	1	0
Enterobacteriaceae					
NS	IMI (2)	ND	Positive	2	0
NS	OXA-181 (5)	ND	Positive	0	5
NS	OXA-232 (2)	ND	Positive	0	2
NS	SME (8)	ND	Positive	5	3
NS	Negative[c] (2)	Positive	Positive	2	0
Total				**11**	**10**

[a] NS (Non-susceptible) indicates elevated MIC to at least one of the carbapenems tested.
[b] RAPIDEC CARBA NP results shown for the Routine Incubation procedure. For the Short Incubation, there were 10 positive RAPIDEC results and 11 negative RAPIDEC results with the same isolates.
[c] Negative for carbapenemase KPC, NDM, VIM, IMP, GIM, SPM, and OXA-48 like; negative for beta-lactamases TEM, SHV, VEB, PER, and GES
[d] Composite reference method is composed of three (i.e., carbapenem MIC, carbapenemase by PCR and CLSI Carba NP) results; a two-out-of-three approach is used to determine isolate positivity status
[e] ND (Not Determined)

*b. Clinical specificity**:***

See comments in 3a above.

c. Other clinical supportive data (when a. and b. are not applicable):

Not Applicable

4. Clinical cut-off**:**

Not Applicable

5. Expected values/Reference range:

The Routine Subculture/Incubation procedure included 457 isolates. Of 457 isolates tested, 265 were positive for targeted carbapenemase enzymes and 192 were negative for carbapenemase enzymes. There were 449 isolates for the Short Incubation in which 261 were carbapenemases positive and 188 were negative for carbapenemases.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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