October 2, 2019

NG Biotech
% Anna Klavins
Lead Performance Studies Microbiologist
Hardy Diagnostics
1430 West McCoy Lane
Santa Maria, California 93455

Re: K191889
Trade/Device Name: NG-Test CARBA 5
Regulation Number: 21 CFR 866.1640
Regulation Name: Antimicrobial Susceptibility Test Powder
Regulatory Class: Class II
Product Code: PTJ
Dated: July 11, 2019
Received: July 15, 2019

Dear Anna Klavins:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's

requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Ribhi Shawar, Ph.D. (ABMM)
Chief, General Bacteriology and Antimicrobial
Susceptibility Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

510(k) Summary

I. SUBMITTER

July 11th, 2019

Correspondent:
Anna Klavins
Lead Performance Studies Microbiologist
Hardy Diagnostics
1430 W. McCoy Lane
Santa Maria, CA 93455
Phone: 805-346-2766 x5752
E-mail: ClinicalTrials@hardydiagnostics.com

Applicant:
Milovan Stankov-Puges
CEO
NG Biotech
Z.A. Courbouton, secteur 1, Atelier relais le Tremplin
Guipry 35480
France
Phone: :+33 (0) 2 23 30 17 83
Fax: +33 (0) 9 71 70 53 10
E-mail: msp@ngbiotech.com

II. DEVICE

Name of Device: NG-Test CARBA 5
Classification Name: Antimicrobial Susceptibility Test Powder
Regulatory Class: II
Product Code: PTJ

III. PREDICATE DEVICE

RAPIDEC CARBA NP, K162385

IV. DEVICE DESCRIPTION

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 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 five carbapenemases. If the control line does not appear, the test result is invalid. The sample port, sample pad, and nitrocellulose strip are contained within a plastic cassette. After addition of colonies in extraction buffer to the sample port, a result can be read after 15 minutes.

V. 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.

VI. PERFORMANCE DATA

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 *Enterobacteriaceae* (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 *Enterobacteriaceae* 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 the Comparator Method vs. CARBA 5 for all sites combined – Analysis by Target

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	*Enterobacteriaceae* (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 *Enterobacteriaceae*, 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 *Enterobacteriaceae* 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 *Enterobacteriaceae* 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 *Enterobacteriaceae*

Enterobacteriaceae		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.

VII. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS WITH THE PREDICATE DEVICE

Attribute	Device	Predicate	Substantially Equivalent?
Name	NG-Test CARBA 5	RAPIDEC CARBA NP	
510(k) Details	Product Code PTJ 21 CFR 866.1640 "Antimicrobial Susceptibility Test Powder" Class II Panel 83 Microbiology	Product Code PTJ 21 CFR 866.1640 "Antimicrobial Susceptibility Test Powder" Class II Panel 83 Microbiology	Yes
Intended 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.	Yes
Inoculum Preparation	By touching well-isolated colonies with a loop	By touching well-isolated colonies with an applicator stick	Yes
Sample Type	Bacterial isolates/colonial growth	Bacterial isolates/colonial growth	Yes
Interpretation	Visual	Visual	Yes
Controls	Build-in procedural control in every test strip	Build-in procedural control in every test strip	

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 88/92 (95.7%) from blood agar and 90/92 (97.8%) from MacConkey agar. After the mCIM analysis, the final sensitivity for all target organisms evaluated was 41/41 (100%) from HardyCHROM™ CRE agar. Two IMP-producing *P. aeruginosa* isolates (IMP-14 and IMP-18) were negative on NG-Test CARBA 5 but positive by mCIM. On blood agar only, two *Proteus mirabilis* strains resulted in false negative results.

Table 7. Analytical Reactivity Summary for Carbapenemase Producing Organisms (CPO)

Organism Group	Number of **strains** tested on Blood/ MacConkey	**Detected Target**	Number of **targets** tested on Blood/ MacConkey agar	Number of **targets** Tested HC CRE	**Variants Tested**	**Variants Not Detected**
Enterobacteriacae	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	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	14, 18
		NDM	2		1	
		None	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).

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. Organisms tested included *Enterobacteriaceae* (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

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

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.

CONCLUSIONS

The analytical data presented in this submission demonstrates that NG-Test CARBA 5 is substantially equivalent to the predicate device.