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

A. 510(k) Number:

K181092

B. Purpose for Submission:

To obtain a substantial equivalence determination for the CHROMID CARBA Agar

C. Measurand:

Colonies of carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae*

D. Type of Test:

Selective and differential culture medium

E. Applicant:

bioMérieux SA

F. Proprietary and Established Names:

CHROMID CARBA agar (CARB)

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.1700: Culture medium for antimicrobial susceptibility tests

2. Classification:

   Class II

3. Product code:

   JSO: Culture Media, Antimicrobial Susceptibility Test, Excluding Mueller Hinton Agar

4. Panel:

   83: Microbiology

H. Intended Use:

1. Intended use(s):

   CHROMID CARBA agar is a selective and differential chromogenic medium that is intended for the qualitative detection and presumptive identification of carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in rectal swab specimens from patients at risk of colonization. CHROMID CARBA agar is intended as an aid in the detection, identification of colonization and control of these bacteria in a healthcare setting.

   Rectal swabs are inoculated directly onto CHROMID CARBA agar without enrichment and results can be interpreted after incubation for 18-24 hours. Presumptive carbapenemase-producing colonies of *E. coli* appear pink to burgundy and those of *K. pneumoniae* appear blue-green or blue-grey.

   Other organisms besides carbapenemase-producing *E. coli* and *K. pneumoniae* can also grow on CHROMID CARBA agar with colonies that appear pink to burgundy or blue-green to blue-grey. Sub-culture to non-selective medium is required to confirm organism identity, for antimicrobial susceptibility testing, confirmation of carbapenemase production and epidemiological typing.

   A lack of growth or the absence of pink to burgundy or blue-green to blue-grey colonies does not preclude the carriage of carbapenemase producing organisms.

2. Indication(s) for use:

   Same as Intended Use.

3. Special conditions for use statement(s):

   For prescription use only.

   A lack of growth or the absence of pink-burgundy or blue-green/blue-grey colonies does not preclude the carriage of carbapenemase producing organisms.

   Based on analytical studies, high concentrations ($\geq 10^8$ CFU/mL) of carbapenemase-producing *E. cloacae*, *M. morganii* and *P. aeruginosa* may inhibit or mask the growth of low levels of carbapenemase-producing *E. coli*. In addition, high concentrations ($\geq 10^8$ CFU/mL) of carbapenemase-producing *E. coli* and *M. morganii* may inhibit or mask the growth of low levels of carbapenemase-producing *K. pneumoniae*.

   Additional testing by an alternative method (e.g., enrichment broth culture) is required if a negative result is obtained with CHROMID CARBA agar and carriage of carbapenemase-producing *E. coli* or *K. pneumoniae* is suspected.

   Performance of CHROMID CARBA agar was established for carbapenemase-producing *E. coli* and *K. pneumoniae* that carry *K. pneumoniae* Carbapenemase (KPC).

Performance with Carbapenemase-producing *Enterobacteriaceae* (CPE) other than *E. coli* and *K. pneumoniae* and other types of carbapenemase enzyme has not been established.

Organisms producing the following non-KPC carbapenemase enzymes may grow and produce pink to burgundy or blue-green to blue-grey pigmented colonies after 18 to 24 hours on CHROMID CARBA agar: IMP, NDM, OXA-48, SME and VIM.

Some carbapenem non-susceptible organisms that do not produce carbapenemase but which exhibit other resistance mechanisms may grow on CHROMID CARBA agar and produce blue-green to blue-grey or pink to burgundy pigmented colonies after 18 to 24 hours.

Sub-culture to non-selective medium is required to for organism identification, antimicrobial susceptibility testing and confirmation of carbapenemase production.

4. Special instrument requirements:

   None

I. Device Description:

CHROMID CARBA agar is a selective and differential culture medium that contains a combination of antimicrobial agents and chromogenic substrates. The medium is intended for use in screening of rectal swab specimens for the presence of carbapenemase-producing *E. coli* and *K. pneumoniae*. Presumptive carbapenemase-producing colonies of *E. coli* appear pink-burgundy whereas those of *K. pneumoniae* appear blue-green/blue-grey.

All isolates must be sub-cultured for identification, antimicrobial susceptibility testing and confirmation of carbapenemase production, as well as epidemiological typing (if required).

J. Substantial Equivalence Information:

1. Predicate device name(s):

   bioMérieux CHROMID VRE agar

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

   K091025

3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
	CHROMID CARBA agar (K181092)	**CHROMID VRE agar (K091025)**
Intended Use	CHROMID CARBA agar is a selective and differential chromogenic medium that is intended for the qualitative detection and presumptive identification of carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in rectal swab specimens from patients at risk of colonization. CHROMID CARBA agar is intended as an aid in the detection, identification of colonization and control of these bacteria in a healthcare setting. Rectal swabs are inoculated directly onto CHROMID CARBA agar without enrichment and results can be interpreted after incubation for 18-24 hours. Presumptive carbapenemase-producing colonies of *E. coli* appear pink to burgundy and those of *K. pneumoniae* appear blue-green or blue-grey. Other organisms besides carbapenemase-producing *E. coli* and *K. pneumoniae* can also grow on CHROMID CARBA agar with colonies that appear pink to burgundy or blue-green to blue-grey. Sub-	CHROMID VRE agar is a selective and differential chromogenic medium containing 8 µg/mL vancomycin, for the qualitative detection of *Enterococcus faecium and E. faecalis* showing acquired vancomycin resistance (VRE) in stool specimens. CHROMlD VRE agar can be used as an aid to identify, prevent and control VRE colonization in healthcare settings. CHROMlD VRE agar is not intended to diagnose VRE infection nor to guide or monitor treatment for infections. Subculture to non-selective media (e.g. trypticase soy agar with 5% sheep blood) is needed for further identification, susceptibility testing and epidemiological typing.

	culture to non-selective medium is required to confirm organism identity, for antimicrobial susceptibility testing, confirmation of carbapenemase production and epidemiological typing. A lack of growth or the absence of pink to burgundy or blue-green to blue-grey colonies does not preclude the carriage of carbapenemase producing organisms.	
Technology	Selective, differential chromogenic culture medium	Same
Inoculation	Direct specimen	Same
Interpretation	Manual, visual	Same
Results	Presumptive	Same
Subculture	Required for confirmation of organism identity and antimicrobial susceptibility testing	Same

Differences		
Item	**Device**	**Predicate**
	CHROMID CARBA agar (K181092)	**CHROMID VRE agar (K091025)**
Specimen Type	Rectal swab	Stool
Target Species, Resistance Mechanisms and Associated Colony Colors	Carbapenemase-producing *E. coli* (pink-burgundy) and *K. pneumoniae* (blue-green/blue-grey)	Vancomycin-resistant *E. faecium* (violet) and *E. faecalis* (blue-green)
Incubation Time	18-24 hours	24-48 hours

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

Guidance for Industry and FDA: Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems; August 28, 2009.

CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 27th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.

L. Test Principle:

CHROMID CARBA agar consists of nutritive medium containing peptones and a mixture of antimicrobial agents that select for growth of carbapenemase-producing *Enterobacteriaceae* (CPE), and chromogenic substrates that enable presumptive identification of the most common carbapenemase-producing species. Colonies of carbapenemase-producing *E. coli* appear pink-burgundy due to the production of β-glucuronidase or β-galactosidase, while colonies of *K. pneumoniae* produce β-glucosidase and appear blue-green/blue-grey. The performance of CHROMID CARBA agar was established with strains of *E coli* and *K. pneumoniae* that produce *K. pneumoniae* Carbapenemase (KPC). Performance with other species or other types of carbapenemase has not been evaluated.

Testing is performed by direct inoculation of a rectal swab onto the surface of culture medium. The plates are incubated for 18-24 hours at 35±2°C in an aerobic atmosphere. All presumptive isolates of carbapenemase-producing *E. coli* and *K. pneumoniae* must be sub-cultured for identification, antimicrobial susceptibility testing and confirmation of carbapenemase production according to established methods.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   The reproducibility of results obtained with CHROMID CARBA agar was evaluated in a study conducted at three sites using blinded panels of samples comprised of 10 confirmed KPC-positive, carbapenem non-susceptible strains of *E. coli* (4) and *K. pneumoniae* (6). The samples were prepared in saline at approximately the Limit of Detection (LoD) (**Section M(1)(d)**) and tested in triplicate on each of five days. Testing of bacterial suspensions in saline was deemed acceptable because the LoD was shown to be similar with organisms suspended in saline and rectal swab matrix (**Section M(2)(b)**). On each day at each site, one operator prepared three independent suspensions of each strain and used these to inoculate duplicate CHROMID CARBA agar plates (50µL per plate) that were then incubated for 24 hours and read independently by two different operators (3 sites x 5 days x 3 suspensions x 1 plate x 2 operators = 90 results per strain). A summary of the results of the study is presented in **Table 1**. The day-to-day, site-to-site, operator-to-operator and overall reproducibility of the CHROMID CARBA agar was determined to be acceptable.

Table 1. Results from the CHROMID CARBA agar Reproducibility Study, stratified by site

#	Species	Resistance Marker(s)	Site 1	Site 2	Site 3	Total
1	*K. pneumoniae*	KPC	100% (30/30)	100% (30/30)	100% (30/30)	**100% (90/90)**
2	*E. coli*	KPC-3, TEM-1	100% (30/30)	80% [1] (24/30)	100% (30/30)	**93.3% (84/90)**
3	*K. pneumoniae*	KPC	100% (30/30)	100% (30/30)	100% (30/30)	**100% (90/90)**
4	*E. coli*	KPC-3	100% (30/30)	100% (30/30)	100% (30/30)	**100% (90/90)**
5	*K. pneumoniae*	KPC-2	100% (30/30)	100% (30/30)	100% (30/30)	**100% (90/90)**
6	*K. pneumoniae*	KPC-2	100% (30/30)	100% (30/30)	100% (30/30)	**100% (90/90)**
7	*K. pneumoniae*	KPC-3	100% (30/30)	100% (30/30)	100% (30/30)	**100% (90/90)**
8	*E. coli*	KPC	100% (30/30)	100% (30/30)	100% (30/30)	**100% (90/90)**
9	*E. coli*	KPC	100% (30/30)	100% (30/30)	100% (30/30)	**100% (90/90)**
10	*K. pneumoniae*	KPC-2	100% (30/30))	100% (30/30)	100% (30/30)	**100% (90/90)**
Overall			**100% (300/300)**	**98.0% (294/300)**	**100% (300/300)**	**99.3% (894/900)**

[1] Both operators at Site 2 on Day 4 of testing observed no growth with Sample #2

b. Linearity/assay reportable range:

Not applicable.

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

Quality Control
Quality Control (QC) testing must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and the testing laboratory's internal procedures. Recommended strains for use as Positive and Negative External Controls are included in the device labeling.

QC was performed on each day of testing during the Clinical Studies (Prospective, Contrived and Challenge) described in **Section M(3)(a)** by preparing a 1:100 dilution of a 0.5 McFarland suspension of each recommended QC strain, streaking for isolation using a 10µL loop and incubating for 18-24 hours at 35±2°C. A summary of the results from QC testing at the four external sites involved in the Clinical Studies is shown in **Table 2**.

Table 2. Summary of Quality Control test results using CHROMID CARBA agar

Species	Strain	Carbapenemase	Expected Result	Agreement (%)
K. pneumoniae	ATCC BAA-1705	*bla*$_{KPC}$ Positive	Blue-green/blue-grey colonies	165/165 (100)
E. coli	ATCC BAA-2340	*bla*$_{KPC}$ Positive	Pink-burgundy colonies	165/165 (100)
K. pneumoniae	ATCC 700603	Negative	No growth	164/165 (99.4)

Reagent Stability
CHROMID CARBA agar should be stored at 2-8°C until the expiration date shown on the outer packaging.

Specimen Stability
Rectal swab specimens should be collected using the Copan Liquid Amies Elution (Eswab) Collection and Transport System (K061301), or equivalent, and may be stored for up to 24 hours at 2-8°C prior to culture on CHROMID CARBA agar.

Performance with other specimen collection and transport devices has not been demonstrated.

d. *Detection limit:*

Limit of Detection
The analytical sensitivity of CHROMID CARBA agar was determined for one strain each of KPC-producing *E. coli* (ATCC BAA-2340) and *K. pneumoniae* (ATCC BAA-1705). To estimate the Limit of Detection (LoD), a standardized 0.5 McFarland suspension of each strain was prepared and diluted in simulated rectal swab matrix (stool in saline solution) prior to plating on CHROMID CARBA agar (50µL per plate). Trypticase Soy Agar (TSA) was also inoculated in parallel as a growth control. All the plates were incubated for 18-24 hours at 35±2°C and the LoD was estimated as the lowest concentration at which ≥10 CFU exhibiting the expected colony color were obtained on each CHROMID CARBA agar plate. The estimated LoD for each strain was 1.5 x 10^3 CFU/mL and this value was confirmed by observation of appropriate colony counts on three additional plates that were inoculated at the LoD target level.

Analytical Reactivity/Inclusivity
A study was performed using a panel of well-characterized isolates to evaluate the ability of CHROMID CARBA agar to recover carbapenemase-producing strains of *E. coli* and *K. pneumoniae*. Strains were characterized as carbapenemase producing based the presence of *bla*$_{KPC}$ and non-susceptibility to at least one carbapenem (ertapenem, imipenem or meropenem). Included in the panel were strains with known *bla*$_{KPC}$ variants: KPC-2 (7), KPC-3 (3), KPC-4 (1).

Of 11 strains of *bla*$_{KPC}$ positive *E. coli* that were included in the study, nine (9) grew

on CHROMID CARBA agar with the expected pink-burgundy colony color and all were carbapenem non-susceptible. Of the two strains that failed to grow, one (KPC-4) had an MIC in the susceptible range for ertapenem and was intermediate for imipenem and meropenem, and the other was susceptible to all three carbapenems and its failure to grow on CHROMID CARBA agar was therefore expected.

All (100%) of 41 *bla*$_{KPC}$-positive, carbapenem non-susceptible strains of *K. pneumoniae* grew on CHROMID CARBA agar with the expected blue-green/blue-grey colony color.

These results are acceptable. The absence of data to demonstrate reliable detection of carbapenemase-producing strains of *E. coli* and *K. pneumoniae* that carry other types of carbapenemase enzyme besides KPC is noted in the Intended Use and as a Limitation in the device labeling.

Incubation Time
A study was performed to evaluate the effect of incubation time on the ability to recover KPC carbapenemase-producing strains of *E. coli* and *K. pneumoniae* on CHROMID CARBA agar. Ten *bla*$_{KPC}$-positive, carbapenemase non-susceptible strains were included in the study (four (4) *E. coli* and six (6) *K. pneumoniae*). Each strain was diluted in saline to 4.5 x 10^3 CFU/mL (3X LoD) and inoculated onto CHROMID CARBA agar (50µL per plate). The plates were incubated aerobically at 35±2°C. Colonies were counted and their color noted after 16 hours and every two (2) hours thereafter up to 28 hours. Colony counts remained stable over the course of the study and each of the strains exhibited their characteristic colony color. The results of this study support the ability to recover carbapenemase-producing *E. coli* and *K. pneumoniae* on CHROMID CARBA agar after incubation for 18-24 hours as stipulated in the device labeling.

e. *Analytical specificity:*

A study was performed to evaluate the analytical specificity of CHROMID CARBA agar for carbapenemase-producing strains of *E. coli* and *K. pneumoniae* by inoculating the medium with high concentrations of target and non-target organisms that may be present in stool specimens. Included in the study were CPE other than *E. coli* and *K. pneumoniae* as well as strains of these two species that produce other carbapenemases besides KPC type enzyme. Fifty microliters of a 0.5 McFarland suspension of each non-target organism (approximately 1-2 x 10^8 CFU/mL for bacteria and 1-5 x 10^6 CFU/mL for yeast) was used to inoculate each plate of CHROMID CARBA agar. The plates were incubated for 18-24 hours at 35±2°C in aerobic conditions after which the presence/absence of growth was recorded together with the characteristics of any colonies present. A list of the organisms tested and the associated results is shown in **Table 3**.

Table 3. Organisms used to evaluate the analytical specificity of CHROMID CARBA agar

Species [1]	CHROMID CARBA		Known Resistance Marker	Carbapenem Susceptibility [2]
	Growth	Colony Color		
Acinetobacter baumannii	Yes	Colorless	NDM	
Aeromonas hydrophila	No			
Bacillus cereus	No			
Bacillus subtilis	No			
Candida albicans	No			
Candida glabrata	No			
Candida krusei	No			
Candida tropicalis	No			
Citrobacter freundii (3)	Yes	Blue-grey	KPC	Non-susceptible
Corynebacterium diphtheriae	No			
Corynebacterium jeikium	No			
Klebsiella (Enterobacter) aerogenes	No		CASE	
Klebsiella (Enterobacter) aerogenes	Yes	Blue-green	KPC	Non-susceptible
Enterobacter cloacae	Yes	Blue-green	KPC	Non-susceptible
Enterococcus durans	No			
Enterococcus faecalis (2)	No			
Enterococcus faecium (2)	No			
Escherichia coli	No			
Escherichia coli	Yes	Pink-burgundy	NDM	Non-susceptible
Escherichia coli	Yes	Pink-burgundy	VIM	Non-susceptible
Escherichia coli	Yes	Pink-burgundy	IMP	Non-susceptible
Escherichia coli	No		ESBL	
Hafnia alvei	No			
Klebsiella pneumoniae	No			
Klebsiella pneumoniae (2)	No		ESBL	
Klebsiella pneumoniae	Yes	Blue-green	NDM	Non-susceptible
Klebsiella pneumoniae	Yes	Blue-green	VIM	Non-susceptible
Klebsiella pneumoniae	Yes	Blue-green	IMP	Non-susceptible
Klebsiella pneumoniae	Yes	Blue-green	OXA-48	Non-susceptible
Lactobacillus acidophilus	No			
Leuconostoc mesenteroides	No			
Morganella morganii	Yes	Blue-green	NDM	Susceptible
Morganella morganii	Yes	Blue-green	NDM	Non-susceptible
Proteus mirabilis	No			
Proteus mirabilis	No		CASE	
Proteus vulgaris	No			
Providencia rettgerii	Yes	Blue-green	KPC	Non-susceptible
Pseudomonas aeruginosa	No			
Pseudomonas aeruginosa	Yes	Colorless	VIM	Non-susceptible

Species [1]	CHROMID CARBA		Known Resistance Marker	Carbapenem Susceptibility [2]
	Growth	Colony Color		
Pseudomonas putida	Yes	Colorless		No breakpoints [3]
Saccharomyces cerevisiae	No			
Salmonella enteritidis	No			
Serratia marcescens	No			
Serratia marcescens	Yes	Blue-green	SME	Non-susceptible
Shigella sonnei	No			
Staphylococcus aureus	No			
Staphylococcus aureus	No		*mec*A	
Staphylococcus epidermidis (2)	No			
Staphylococcus haemolyticus	No			
Stenotrophomonas maltophilia	Yes	Colorless	VIM	No breakpoints [2]
Streptococcus agalactiae	No			
Streptococcus group C	No			
Streptococcus group O	No			

Shaded cells: Not applicable

CASE: Cephalosporinase; ESBL: Extended Spectrum β-Lactamase; IMP: Imipenem hydrolyzing β-lactamase; KPC: *Klebsiella pneumoniae* Carbapenemase; NDM: New Delhi Metallo-β-lactamase; SME: *Serratia marcescens* enzyme; VIM: Verona Integron-encoded Metallo-β-lactamase

[1] The number of strains is noted in parenthesis if >1

[2] Carbepenem susceptibility using ertapenem, imipenem and meropenem was only determined for those organisms that grew on CHROMID CARBA agar

[3] No recognized breakpoints exist for carbapenems with *P. putida* or *S. maltophilia* (intrinsically resistant)

Of the 59 organisms tested, 20 grew of CHROMID CARBA agar after 18-24 hours. Of these, four (4) non-lactose fermenting species (*A. baumannii*, *P. aeruginosa*, *P. putida* and *S. maltophilia*) exhibited colorless colonies that could readily be distinguished from the characteristic pink-burgundy and blue-green/blue-grey colonies of carbapenemase-producing *E. coli* and *K. pneumoniae*. Three (3) of these strains were known to harbor a carbapenem resistance marker (bla_{VIM} or bla_{NDM}). The remaining 16 organisms that grew on CHROMID CARBA agar included three (3) strains of *E. coli* (pink-burgundy colonies) and four (4) strains of *K. pneumoniae* (blue-green/blue-grey colonies), all of which carried a carbapenemase resistance marker other than bla_{KPC} (bla_{IMP}, bla_{NDM}, bla_{OXA-48} or bla_{VIM}) and were phenotypically carbapenem non-susceptible. In addition, there were nine (9) other strains of *Enterobacteriaceae* (*C. freundii* (3), *M. morganii* (2), *K. aerogenes* [*E. aerogenes*] (1), *E. cloacae* (1), *P. rettgerii* (1) and *S. marcescens* (1)) that exhibited blue-green/blue-grey colonies and which carried a carbapenemase gene (bla_{KPC}, bla_{NDM} or bla_{SME}), eight (8) of which were non-susceptible to carbapenems.

The results of the Analytical Specificity Study demonstrate that *E. coli* and *K. pneumoniae* with carbapenemases other than KPC-type carbapenemase may exhibit pink-burgundy or blue-green/blue-grey colonies on CHROMID CARBA agar, although performance with such isolates has not been established. Similarly, CPE other than *E. coli* and *K. pneumoniae* may also grow on CHROMID CARBA agar

with pink-burgundy or blue-green/blue-grey colonies, although insufficient isolates were tested to demonstrate performance. These Limitations are noted in the device labeling.

f. Assay cut-off:

Not applicable.

g. Assay interference:

Microbial Interference
A study was conducted to evaluate the ability to recover the targeted carbapenemase-producing *E. coli* and *K. pneumoniae* in the presence of other carbapenem susceptible and non-susceptible organisms. Testing was performed by plating four (4) representative KPC-producing target organisms at low levels (3X LoD) in the presence of 10^4-10^8 CFU/mL of seven (7) carbapenem non-susceptible competitor organisms that exhibited different resistance mechanisms. In addition, one (1) carbapenem susceptible strain of *K. pneumoniae* was also included as a competitor and each competitor strain was also inoculated onto CHROMID CARBA agar in the absence of the target species in order evaluate its growth characteristics.

As observed in the Analytical Specificity Study (**Section M(1)(e)**), both carbapenemase-producing *Enterobacter cloacae* and *Providencia rettgeri* exhibited blue-green colonies on CHROMID CARBA agar, while carbapenemase-producing *M. morganii* and *P. aeruginosa* produced blue and colorless colonies, respectively. In all cases, when the competitive species was present at $<10^8$ CFU/mL, colonies of the target organisms grew with the characteristic color (either pink-burgundy for *E. coli* or blue-green/blue-grey for *K. pneumoniae*). However, when the competitive species were present at 10^8 CFU/mL, in some cases, characteristic colonies of the target organisms were not observed, either due to no growth or the inability to discern the characteristic colored colonies of the target species from colonies of the competing organism (i.e., masking) (**Table 4**). The potential for high concentrations of carbapenemase-producing non-target organisms to interfere with recovery of carbapenemase-producing *E. coli* and *K. pneumoniae* is noted as a Limitation in the device labeling.

Table 4. Summary of results with obtained with mixtures of organisms on CHROMID CARBA agar

Competitor Organism					Carbapenemase-producing Target Organism [1]		
Species	**Strain**	**Carbapenem Phenotype**	**Resistance Mechanism**	**CHROMID CARBA agar**	**Species**	**Strain [2]**	**CHROMID CARBA agar**
E. cloacae	bMx 1012298	CPE	KPC	Blue-green	*E. coli*	bMx 0911025	No growth/Masked
K. pneumoniae	bMx 0911175	Resistant	Impermeability	No Growth		ATCC BAA-2340	Pink-burgundy
						bMx 0911025	Pink-burgundy
K. pneumoniae	ATCC 13883	Non-CPE (susceptible)	Not applicable	No Growth		ATCC BAA-2340	Pink-burgundy
M. morganii	bMx 1411132	CPE	NDM	Blue		bMx 0911025	No growth/Masked
P. aeruginosa	bMx 1008028	CPE	VIM	Colorless		ATCC BAA-2340	No growth/Masked
						bMx 0911025	Pink-burgundy
P. mirabilis	ATCC BAA-856	Non-CPE (susceptible)	ESBL	No Growth		ATCC BAA-2340	Pink-burgundy
E. coli	bMx 1011190	CPE	NDM	Pink-burgundy	*K. pneumoniae*	ATCC BAA-1705	No growth/Masked
						bMx 0505034	No growth/Masked
M. morganii	bMx 1411132	CPE	NDM	Blue		bMx 0505034	No growth/Masked
P. aeruginosa	bMx 1008028	CPE	VIM	Colorless		ATCC BAA-1705	Blue-green/Blue-grey
						bMx 0505034	Blue-green/Blue-grey
P. mirabilis	ATCC BAA-856	Non-CPE (susceptible)	ESBL	No Growth		ATCC BAA-1705	Blue-green/Blue-grey
P. rettgeri	bMx 1411134	CPE	KPC	Blue-green		ATCC BAA-1705	Blue-green/Blue-grey

CPE: Carbapenemase-producing *Enterobacteriaceae*; ESBL: Extended Spectrum β-Lactamase; KPC: *Klebsiella pneumoniae*-carbapenemase; NDM: New Delhi Metallo-β-lactamase; VIM: Verona Integron-encoded β-lactamase; ATCC: American Type Culture collection; bMx: bioMérieux

[1] Results shown are in the presence of 10^8 CFU/mL of the Competitor Organism. In all cases when the Competitor Organism was present at $<10^8$ CFU/mL, growth and appearance of colonies of the Carbapenemase-producing Target Organism were as expected.

[2] Both strains of each of the two Carbapenemase-producing target species were positive for bla_{KPC}

Interfering Substances

In addition to investigating the potential for microbial interference, a study was also conducted to evaluate the potential for interference with growth on CHROMID CARBA agar from endogenous and exogenous substances that may be present in rectal swab specimens. Testing was performed with each substance in the presence of two (2) representative carbapenemase-producing target organisms: *E. coli* strain ATCC BAA-2340 and *K. pneumoniae* ATCC BAA-1705 (both bla_{KPC}-positive). Each target organism was tested at the LoD target level (1.5×10^3 CFU/mL) in the presence of 22 potentially interfering substances at the highest concentration likely to be encountered in a clinical specimen. The number of colonies of characteristic appearance obtained on CHROMID CARBA agar was compared to the number obtained on MacConkey agar that was inoculated in parallel. For there to be no evidence of interference, the number of colonies present on the CHROMID CARBA agar after incubation for 18-24 hours had to equal or exceed the number observed on the MacConkey agar. No interference on CHROMID CARBA agar was observed in the presence of any of the substances tested (**Table 5**). These results are acceptable.

Table 5. Substances evaluated for potential interference with growth on CHROMID CARBA agar

Category	Substance	Concentration or Quantity Tested
Antidiarrhea Medications	Loperamide hydrochloride (Loperamide)	1 capsule/mL
	Diosmectite (Smecta)	50% v/v [1]
Dietary Supplement	lspaghula husk (Transilane)	50% v/v [2]
Diluent	Physiological saline water	50% v/v
Endogenous Substance	Human Blood	50% v/v
Personal Hygiene Products	Playtex Personal wipes	50% v/v [3]
	Talc	4.5% w/w
	Trojan Enz condoms	50% v/v [3]
Laxatives	Glycerol (Bebegel)	50% w/w
	Bisacodyl (Dulcolax)	1 tablet/mL
Lubricants	K-Y Jelly	0.5% w/w
	K-Y Liquibeads vaginal moisturizer	0.5 ovules/mL
	Vaseline	50% w/w
Topical Medications	Zinc Oxide (Oxyplastine)	50% w/w
	Pramocaine hydrochloride (Tronothane)	33% w/w
	Miconazole nitrate (Daktarin)	2.4% w/w
	Witch Hazel (Tucks Pads)	50% v/v [3]
	Phenylephrine HCI (Preparation H Ointment)	50% w/w
	Benzocaine + Resorcinol (Vagisil anti-itch creme)	33% w/w
	Ruscoside + Lidocaine hydrochloride + Prednacinolone acetonide (Cirkan Suppositories)	50% v/v [4]
	Hydrocortisone (CortiSedermyl)	8.3% w/w

[1] 1 sachet in 100mL water mixed 1:1 with bacterial suspension
[2] 1 sachet in 200mL water mixed 1:1 with bacterial suspension
[3] 1 pad, wipe or condom in 10mL water mixed 1:1 with bacterial suspension
[4] 1 suppository in 5mL water mixed 1:1 with bacterial suspension

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable.

b. Matrix comparison:

A study was conducted to compare the analytical sensitivity of CHROMID CARBA agar for the recovery of carbapenemase-producing strains of *E. coli* and *K. pneumoniae* from simulated rectal swab matrix (stool diluted in saline) and saline solution alone. Testing was performed by diluting standardized suspensions of *E. coli* ATCC BAA-2340 and *K. pneumoniae* ATCC BAA-1705 (both bla_{KPC}-positive) in the appropriate matrix to different concentrations close to the claimed LoD of CHROMID CARBA agar. Each suspension was used to inoculate multiple CHROMID CARBA agar plates that were incubated for 18-24 hours at 35±2°C, after which colonies exhibiting the characteristic pink-burgundy of *E. coli* or blue-green/blue-grey of *K. pneumoniae* were counted. The mean colony count was calculated using data form all inoculated plates. Similar colony counts were obtained with suspensions prepared in saline or simulated rectal swab matrix (**Table 6**). Use of organisms diluted in saline for selected Analytical Studies was therefore deemed acceptable.

Table 6. Comparison of colony counts obtained on CHROMID CARBA agar from culture of bacterial suspensions prepared in simulated rectal swab matrix or saline

Target Species/Strain	Level			Mean Colony Count (CFU/plate) [1]	
	Multiple of LoD	CFU/mL	Number of plates	Rectal Swab Matrix [2]	Saline
E. coli ATCC-2340	5X	7.5×10^3	10	>200	>200
	3X	4.5×10^3	30	86	94
	1X	1.5×10^3	10	31	31
	Negative	0	10	0	0
K. pneumoniae ATCC-1705	5X	7.5×10^3	10	>300	>300
	3X	4.5×10^3	30	>200	>200
	1X	1.5×10^3	10	74	71
	Negative	0	10	0	0

[1] Only colonies exhibiting the characteristic colony color associated with the carbapenemase-producing target species were counted

[2] Simulated rectal swab matrix prepared by diluting liquid stool in saline

3. Clinical studies:

a. Clinical Sensitivity:

Prospective Clinical Study
The performance of CHROMID CARBA agar was evaluated in a prospective Clinical

Study that was performed at three sites (two US and one ex-US). Recovery of presumptive carbapenemase-producing colonies of *E. coli* and *K. pneumoniae* from rectal swabs on CHROMID CARBA agar was compared to enrichment broth Reference Culture, followed by subculture to MacConkey agar and phenotypic and genetic characterization of isolated lactose fermenting colonies. Isolates of *E. coli* and *K. pneumoniae* obtained by the Reference Culture method and on CHROMID CARBA agar were identified biochemically and tested for susceptibility to ertapenem, imipenem and meropenem using an FDA-cleared method. In addition, each isolate was also evaluated for carbapenem susceptibility using an FDA-cleared Carba NP test [1] and for the presence of carbapenemase resistance markers (bla_{IMP}, bla_{KPC}, bla_{NDM}, bla_{OXA-48} and bla_{VIM}) using a validated multiplex PCR assay. Isolates were defined as carbapenemase-producing using the composite algorithm depicted in **Table 7**.

Table 7. Composite algorithm for determination of carbapenemase status of isolates obtained by the Reference Culture method or recovered on CHROMID CARBA agar

Carbapenem MIC [1]	Carba NP Test	Carbapenemase PCR [2]	Carbapenemase Status
Non-susceptible	Positive	Positive	Positive
Non-susceptible	Positive	Negative	Negative
Non-susceptible	Negative	Positive	Positive
Non-susceptible	Negative	Negative	Negative
Susceptible	Positive	Positive	Positive
Susceptible	Positive	Negative	Negative
Susceptible	Negative	Positive	Negative [3]
Susceptible	Negative	Negative	Negative

[1] Non-susceptible: Intermediate or Resistant to one or more of the carbapenem antimicrobial agents tested (ertapenem, imipenem and meropenem) based on FDA recognized breakpoints

[2] Multiplex PCR for bla_{IMP}, bla_{KPC}, bla_{NDM}, bla_{OXA-48} or bla_{VIM}

[3] Phenotypic evidence of non-susceptibility to carbapenems in addition to a positive PCR result was required to establish the Carbapenemase Status as positive

A total of 1099 residual rectal swab specimens from unique individuals that were leftover following standard of care testing were initially enrolled in the study. All the specimens were collected using ESwabs (flocked swab in liquid Amies transport medium). Three hundred and ninety (390) specimens were excluded from the analysis of performance for the following reasons: Quality Control failure with either the Reference Culture (318) or both the Reference Culture and the CHROMID CARBA agar (25); missing data (42); delay in data entry resulting in questionable data reliability (5). Isolates from a total of 709 rectal swabs specimens were therefore included in the analysis of performance. The results of the Prospective Clinical Study are summarized in **Tables 8 (a) & (b)** and **9**.

Reference Culture yielded no Carbapenemase Status positive strains of *E. coli* over the course of the study, precluding estimation of Positive Percent Agreement (PPA),

[1] Nordmann *et al*., Emerg Infect Dis 2012 18 (9): 1503-1507

although the Negative Percent Agreement (NPA) was 99.7% (95% confidence interval: 99.0-99.9%).

For Carbapenemase Status-positive *K. pneumoniae*, PPA was 84.3% (95% CI 72.0-91.8%) and NPA was 97.7% (95% CI 96.3-98.6%). All the specimens from which Carbapenemase Status-positive *K. pneumoniae* was recovered by Reference Culture were obtained from the ex-US clinical site (**Table 9**). Each of the strains of *K. pneumoniae* that was recovered by Reference Culture but which failed to grow when the rectal swabs were inoculated directly on CHROMID CARBA agar during the prospective study was shown to grow with the characteristic blue-green/blue-grey colony color when tested analytically in stool matrix at the LoD target level (**Section M(1)(d)**). Failure to recover these strains on CHROMID CARBA agar directly from rectal swabs in the prospective study may have been due to their presence at levels below the LoD of the device. A lack of growth on CHROMID CARBA agar or the absence of pink to burgundy or blue-green to blue-grey colonies does not preclude the carriage of carbapenemase producing organisms. This is noted in the device labeling.

Tables 8 (a) & (b). Prospective Clinical Study with rectal swab specimens: CHROMID CARBA agar Colony Color vs Reference Culture (Confirmed Organism Identity and Carbapenemase Status)

Table 8(a): Carbapenemase-producing *E. coli*				
		Reference Culture		
		Positive	**Negative**	**Total**
CHROMID CARBA agar	**Positive** [1]	0	2 [3]	**2**
	Negative [2]	0	707	**707**
	Total	**0**	**709**	**709**
Positive Percent Agreement		Not applicable		
Negative Percent Agreement		99.7% (707/709); 95% CI 99.0-99.9%		
Positive Predictive Value		Not applicable		
Negative Predictive Value		100% (707/707)		
Prevalence		0.0% (0/709)		

95% CI: 95% score confidence interval

[1] Pink-burgundy colonies

[2] No growth or colonies not pink-burgundy

[3] Colonies from 1/2 specimens confirmed as carbapenem non-susceptible, carbapenemase negative *K. pneumoniae*; colonies from 1/2 specimens identified as *Enterococcus faecalis*

Table 8(b): Carbapenemase-producing *K. pneumoniae*				
		Reference Culture		
		Positive	**Negative**	**Total**
CHROMID CARBA agar	**Positive** [1]	43	15 [5, 6]	**58**
	Negative [2]	8 [3, 4]	643	**651**
	Total	**51**	**658**	**709**
Positive Percent Agreement		84.3% (43/51); 95% CI 72.0-91.8%		
Negative Percent Agreement		97.7% (643/658); 95% CI 96.3-98.6%		
Positive Predictive Value		74.1% (43/58)		
Negative Predictive Value		98.8% (643/651)		
Prevalence		7.2% (51/709)		

95% CI: 95% score confidence interval

[1] Blue-green/blue-grey colonies

[2] No growth or colonies not blue-green/blue-grey

[3] Includes 1 specimen from which carbapenemase-positive *K. pneumoniae* was recovered by the Reference Method but which yielded blue-green/blue-grey colonies on CHROMID CARBA agar that were identified biochemically as *P. aeruginosa*

[4] 8/8 isolates grew on CHROMID CARBA agar when inoculated at approximately the LoD target level (10^3 CFU/mL in stool); all 8 carried bla_{KPC}, were Carba NP Test positive and carbapenem non-susceptible (resistant to ertapenem, imipenem and meropenem)

[5] 5/15 isolates on CHROMID CARBA agar were confirmed as carbapenem non-susceptible *K. pneumoniae* that carried: 4/5 isolates were Carbapenemase Status-positive and carried bla_{KPC} and 1/5 isolates was Carbapenemase Status-negative

[6] 10/15 specimens produced blue-green/blue-grey colonies that were identified as *Enterobacteriaceae* other than *K. pneumoniae* (*Serratia marcescens* (6), *Citrobacter freundii* (2) and *Enterobacter cloacae* (2))

Table 9. Prospective Clinical Study: agreement between CHROMID CARBA agar and Reference Culture, stratified by study site

Target Organism	Study Site	Percent Agreement [1]	
		Positive	Negative
Carbapenemase Status-positive *E. coli*	1	Not applicable	100%; 98.0-100% (187/187)
	2	Not applicable	99.5%; 98.1-99.9% (379/381)
	3 [2]	Not applicable	100%; 97.3-100% (141/141)
	Total	**Not applicable**	**99.7; 99.0-99.9% (707/709)**
Carbapenemase Status-positive *K. pneumoniae*	1	Not applicable	99.5%; 97.0-99.9% (186/187)
	2	Not applicable	97.6%; 95.6-98.8% (372/381)
	3 [2]	84.3%; 72.0-91.8% (43/51)	94.4%; 87.6-97.6% (85/90)
	Total	**84.3%; 72.0-91.8% (43/51)**	**97.7%; 96.3-98.6% (643/658)**

[1] With 95% score confidence interval
[2] Ex-US site

Contrived Specimen Study

Because of the anticipated low prevalence of Carbapenemase Status-positive specimens in the Prospective Clinical Study, additional testing was also performed with contrived specimens. These were prepared by spiking simulated rectal swab matrix (stool diluted in saline) with isolates of *E. coli*, *K. pneumoniae* and other *Enterobacteriaceae* of known Carbapenemase Status as defined using the algorithm depicted in **Table 7**. A summary of the isolates tested is shown in **Table 10**. Each contrived specimen was spiked with a unique bacterial strain at a concentration equivalent to 1-10X the analytical LoD of the CHROMID CARBA agar (**Section M(1)(d)**). Testing was performed in a blinded fashion at four different sites. A summary of the results obtained on CHROMID CARBA agar in comparison to the expected organism identity and Carbapenemase Status is provided in **Tables 11 (a) & (b)**.

Table 10. Isolates included in the Contrived Specimen Study

Species	Number of Isolates Tested	Carbapenemase Status	
		Positive	Negative
Citrobacter freundii	1	1	0
Enterobacter cloacae	4	4	0
Escherichia coli	38	20 [1]	18
Klebsiella oxytoca	1	1	0
Klebsiella ozaenae	1	1	0
Klebsiella pneumoniae	160	88 [2]	72
Kluyvera ascorbata	1	1	0
Morganella morganii	1	1	0
Proteus mirabilis	2	2	0
Raoultella ornithinolytica	1	1	0
Total	**210**	**120** [3]	**90**

[1] bla_{KPC}: 11 (KPC-2: 9, KPC-3: 2); bla_{NDM}: 4; bla_{OXA-48}: 2; bla_{VIM}: 2; bla_{IMP}: 1

[2] bla_{KPC}: 77 (45 KPC-3, 30 KPC-2, 2 other variants); bla_{VIM}: 4; bla_{NDM}: 3; bla_{IMP}: 2; bla_{OXA-48}: 2

[3] Overall, 110/120 isolates (92%) were positive for $bla_{KPC,}$ including 12/12 isolates (100%) of species other than *E. coli* and *K. pneumoniae*

Tables 11(a) & (b). Contrived Specimen Study: CHROMID CARBA agar Colony Color vs Expected Organism Identity and Carbapenemase Status

Table 11(a): Carbapenemase-producing *E. coli*				
		Organism Identity & Carbapenemase Status		
		Positive	**Negative**	**Total**
CHROMID CARBA agar	**Positive** [1]	16	2 [3]	**18**
	Negative [2]	4 [4]	188	**192**
	Total	**20**	**190**	**210**
Positive Percent Agreement		80.0% (16/20); 58.4-91.9%		
Negative Percent Agreement		98.9% (188/190); 95% CI 96.2-99.7%		

95% CI: 95% score confidence interval

[1] Pink-burgundy colonies

[2] No growth or colonies not pink-burgundy

[3] 2/2 isolates were carbapenem non-susceptible *E. coli* (resistant to meropenem and ertapenem and an intermediate MIC for imipenem); both isolates were positive for Extended Spectrum β-Lactamase (ESBL)

[4] False negative results were obtained with 4 strains of carbapenem non-susceptible *E. coli* that harbored $bla_{\text{OXA-48}}$ (2), bla_{NDM} (1) and bla_{VIM} (1) and which were spiked at target levels ranging from 1X to 10X LoD; 2/4 strains had an intermediate MIC to at least one carbapenem including 1 strain that was also susceptible to ertapenem

Table 11(b): Carbapenemase-producing *K. pneumoniae*				
		Organism Identity & Carbapenemase Status		
		Positive	**Negative**	**Total**
CHROMID CARBA agar	**Positive** [1]	84	11 [3, 4]	**95**
	Negative [2]	3 [5]	112 [6]	**115**
	Total	**87**	**123**	**210**
Positive Percent Agreement		96.6% (84/87); 95% CI % 90.3-98.8%		
Negative Percent Agreement		91.1% (112/123); 95% CI 84.7-94.9%		

95% CI: 95% score confidence interval

[1] Blue-green/blue-grey colonies

[2] No growth or colonies not blue-green/blue-grey

[3] 5/11 false positive results were obtained with carbapenem non-susceptible *K. pneumoniae*, all of which were positive for ESBL (3) or ESBL and AmpC (2)

[4] 6/11 false positive results were due to Carbapenemase Status-positive species of *Enterobacteriaceae* other than *K. pneumoniae* that harbored bla_{KPC}: *E. cloacae* (4), *K. oxytoca* (1) and *K. ozaenae* (1)

[5] 3/3 false negative results were obtained with Carbapenemase Status positive isolates at the LoD target level; each strain carried a different carbapenemase resistance marker (bla_{IMP} (1), bla_{KPC} (1) or $bla_{\text{OXA-48}}$ (1)); 2/3 strains had a susceptible or intermediate MIC to at least one of the three carbapenems tested (ertapenem, imipenem or meropenem)

[6] 18/112 samples contained carbapenem non-susceptible strains of *K. pneumoniae* of which 17 were phenotypically carbapenemase negative; 11/18 were positive for ESBL and 6/18 were positive for AmpC

Challenge Study

To supplement the results of the Prospective Clinical Study and Contrived Specimen Study, additional testing was performed using well characterized Carbapenemase Status-positive and -negative isolates of *E. coli* and *K. pneumoniae* that were suspended in saline at 3 x 10^3 CFU/mL (equivalent to 2X LoD [**Section M(1)(d)**]). Included in the study were strains with known bla_{KPC} variants (KPC-2 [12], KPC-3

[18] and KPC-4 [1]). Samples were blinded and tested at one study site. A summary of the results is provided in **Tables 12 (a) & (b)**.

Table 12 (a) & (b). Challenge Study: CHROMID CARBA agar Colony Color vs Expected Organism Identity and Carbapenemase Status

Table 12(a): Carbapenemase-producing *E. coli*				
		Organism Identity & Carbapenemase Status		
		Positive	**Negative**	**Total**
CHROMID CARBA agar	**Positive** [1]	12 [3]	0	**12**
	Negative [2]	0	38	**38**
	Total	**12**	**38**	**50**
Positive Percent Agreement		100% (12/12); 75.8-100%		
Negative Percent Agreement		100% (38/38); 95% CI 90.8-100%		

95% CI: 95% score confidence interval
[1] Pink-burgundy colonies
[2] No growth or colonies not pink-burgundy
[3] Includes strains of *E. coli* known to carry the following bla_{KPC} variants: KPC-2: 1; KPC-3: 3; KPC-4: 1

Table 12(b): Carbapenemase-producing *K. pneumoniae*				
		Organism Identity & Carbapenemase Status		
		Positive	**Negative**	**Total**
CHROMID CARBA agar	**Positive** [1]	27 [3]	0	**27**
	Negative [2]	0	23	**23**
	Total	**27**	**23**	**50**
Positive Percent Agreement		100% (27/27); 95% CI % 87.5-100%		
Negative Percent Agreement		100% (23/23); 95% CI 85.7-100%		

95% CI: 95% score confidence interval
[1] Blue-green/blue-grey colonies
[2] No growth or colonies not blue-green/blue-grey
[3] Includes strains of *K. pneumoniae* known to carry the following bla_{KPC} variants: KPC-2: 11; KPC-3: 15

b. Clinical specificity:

Refer to **Section M(3)(a)**, 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:

In the Prospective Clinical Study described in **Section M(3)(a)**, the prevalence of

carbapenemase-producing *E. coli* and *K. pneumoniae* based on the reference enrichment culture method was 0.0% (0/709) and 7.2% (51/709), respectively. In contrast, the prevalence of presumptive carbapenemase-producing isolates of *E. coli* and *K. pneumoniae* observed on CHROMID CARBA agar was 0.3% (2/709) and 8.2% (58/709), respectively.

N. Proposed Labeling:

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

O. Conclusion:

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