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

A. 510(k) Number:

K190553

B. Purpose for Submission:

To obtain a substantial equivalence determination for the HardyCHROM CRE.

C. Measurand:

Carbapenems non-susceptible strains of *Escherichia coli* and KES *(Klebsiella oxytoca, Klebsiella pneumoniae, Klebsiella aerogenes, Enterobacter cloacae complex,* and *Serratia marcescens).*

D. Type of Test:

Selective and differential culture medium

E. Applicant:

Hardy Diagnostics

F. Proprietary and Established Names:

HardyCHROM CRE

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):

   HardyCHROM CRE is a selective and differential chromogenic agar medium intended for the qualitative and presumptive detection from stool specimens of *Escherichia coli* that are non-susceptible to carbapenems as pink colonies and KES (*Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Enterobacter cloacae* complex, and *Serratia marcescens*) that are non-susceptible to carbapenems as blue colonies.

   HardyCHROM CRE is intended as an aid in the detection, identification of colonization and control of these bacteria in a healthcare setting. HardyCHROMCRE is not intended to diagnose infection or guide therapy. Results can be interpreted after incubation for 18-24 hours.

   Subculture to non-selective medium is required for confirming identification, antimicrobial susceptibility testing and epidemiological typing.

   A lack of growth or the absence of pink or blue colonies on HardyCHROM CRE does not preclude the presence of *Escherichia coli* and KES that are non-susceptible to carbapenems.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only.

   The following limitations have been added to the device labeling:

   - *"Do not incubate plates in a CO_2 atmosphere"*
   - *"Cultures on HardyCHROM CRE should be incubated at 35-37°C for 18-24 hours. Analytical and clinical studies showed reduced specificity with cultures incubated longer than 24 hours. Do not incubate more than 24 hours."*
   - *"The clinical performance of HardyCHROM CRE was established with fresh stool samples. Compatibility with stool in C&S Medium Transport (Cary Blair Formula) was evaluated in the contrived study but not in the prospective study."*

- *"Colonies that are colorless, off-white, yellow or green, or have fuzzy/mold appearance should not be considered carbapenem non-susceptible Eschrichia coli or KES (Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Enterobacter cloacae complex, and Serriatia marcescens).*

- *"A lack of growth or the absence of pink or blue colonies on HardyCHROM™ CRE does not preclude the presence of Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Enterobacter cloacae complex, or Serratia marcescens that are non-susceptible to carbapenems. False negative results may occur due to variations in sampling, slow development or failure to develop the expected colony color, or the presence of organisms that are susceptible to the antimicrobial agents included in the HardyCHROM™ CRE medium but which are non-susceptible to other carbapenems".*

- *"A small percentage of E. coli lacking the enzyme beta-D-glucuronidase may appear as white to off-white colonies on HardyCHROM CRE and would be considered as false-negative"*

- *"Analytical and clinical testing of HardyCHROM CRE has shown that carbapenem non-susceptible Citrobacter spp. may appear as pink or blue colonies at 18 and 24 hours*

- *"Analytical and Clinical testing of HardyCHROM CRE has shown that gram positive cocci and gram positive rods may be recovered as tiny, blue colonies at 18 and 24 hours".*

- *"Analytical testing of HardyCHROM CRE has shown that Aspergillus brasiliensis may appear as blue and fuzzy at 22 to 24 hours."*

- *"The colony size of all target carbapenem non-susceptible organisms (except Serratia marcescens) may appear smaller in the presence of high concentrations of Acinetobacter baumanii, Aeromonas hydrophila, Pseudomonas fluorescens, and Stenotrophomonas maltophilia. The effect of non-target organisms on Klebsiella aerogenes colony size and color is unknown. In the presence of Stenotrophomonas maltophilia, colonies of Enterobacter cloacae complex may appear purple and small after incubation for 24 hours".*

- *"In the analytical mixed infection study, the non-target organisms were tested at a concentration of 1.5 x 108 CFU/mL. The accuracy of this device for detecting carbapenem non-susceptible Escherichia coli or KES in the presence of the non-target organisms at higher concentrations is unknown."*

- "*Some Enterobacter cloacae complex isolates may appear as pink colonies on HardyCHROM CRE and be falsely considered as E. coli.*"

- *"If no growth of carbapenem non-susceptible strains of E. coli and KES (K. aerogenes, K. oxytoca, K. pneumoniae, E. cloacae complex, and S. marcescens) is observed on HardyCHROM CRE after 18 hours of incuabation, plates should be incubated at 35 to 37°C for up to 24 hours".*

- *"Analytical testing of HardyCHROM CRE has shown that areas of heavy growth (1st quadrant) of Stenotrophomonas maltophilia may appear light blue at 24 hours and produce false positive results (blue color for carbapenem non-susceptible KES). Only well isolated colonies should be evaluated for color morphology."*

4. Special instrument requirements:

   None

I. Device Description:

HardyCHROM CRE is a selective and differential chromogenic culture medium intended for screening of stool specimen for carbapenem non-susceptible *Escherichia coli, and KES (Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Enterobacter cloacae* complex, and *Serratia marcescens)*. The selective components in HardyCHROM CRE inhibit the growth of yeast, Gram-positive bacteria, and Gram negative bacteria that are sensitive to ertapenem. HardyCHROM CRE differentiates *Escherichia coli* (pink colonies) from KES (blue colonies).
All isolates must be subcultured for confirmatory identification, antimicrobial susceptibility testing and, if required, epidemiological typing. Further biochemical analysis is required to confirm the production of carbapenemase or any other mechanism of resistance.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Bio-Rad VRESelect

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

   K122187

3. Comparison with predicate:

Table 1: Comparison with the Predicate Device

Similarities		
Item	**Device** **HardyCHROM CRE** **K190553**	**Predicate** **Bio-Rad VRESelect** **K122187**
Intended Use	HardyCHROM CRE is a selective and differential chromogenic agar medium intended for the qualitative and presumptive detection from stool specimens of *Escherichia coli* that are non-susceptible to carbapenems as pink colonies and KES (*Klebsiella aerogenes*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Enterobacter cloacae* complex, and *Serratia marcescens*) that are non-susceptible to carbapenems as blue colonies. HardyCHROM CRE is intended as an aid in the detection, identification of colonization and control of these bacteria in a healthcare setting. HardyCHROM CRE is not intended to diagnose infection or guide therapy. Results can be interpreted after incubation for 18-24 hours. Subculture to non-selective medium is required for confirming identification, antimicrobial susceptibility testing and epidemiological typing. A lack of growth or the absence of pink or blue colonies on HardyCHROM CRE does not preclude the presence of *Escherichia coli* and KES that are non-susceptible to carbapenems.	VRESelect is a selective and differential chromogenic medium, containing 8 µg/mL of vancomycin, for the qualitative detection of gastrointestinal colonization of vancomycin-resistant *Enterococcus faecium* (VREfm) and vancomycin-resistant *Enterococcus faecalis* (VREfs) and to aid in the prevention and control of vancomycin-resistant *Enterococcus* (VRE) in healthcare settings. The test is performed on rectal swabs, or fecal specimens from patients to be screened for VRE colonization. VRESelect is not intended to diagnose VRE infection nor to guide or monitor treatment of infection. Results can be interpreted after 24 to 28 hours incubation. Subculture to non-selective media (*e.g.*, Trypticase Soy Agar with 5% sheep blood) is needed for further identification, susceptibility testing and epidemiological typing.
Technology	Bacterial growth with enzymatic metabolism of chromogenic substrate	Same
Sample	Stool	Rectal swab or stool
Inoculation	Direct	Same
Interpretation	Manual, visual	Same
Subculture	Required for confirmation of identity and antimicrobial susceptibility testing	Same

Differences		
Item	**Device HardyCHROM ESBL K190553**	**Predicate Bio-Rad VRESelect K122718**
Organisms Detected	carbapenem non-susceptible *Escherichia coli*, and KES *(Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Enterobacter cloacae complex, and Serratia marcescens)*	Vancomycin resistant *E. faecalis* and *E. faecium*
Reference Method	Broth-enrichment based screening method	Growth on Bile Esculin Azide Agar with 6µg/mL Vancomycin followed by biochemical identification and vancomycin MIC

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

CLSI M100 S27"Performance Standards for Antimicrobial Susceptibility Testing". 27th ed. January 2017

L. Test Principle:

HardyCHROM CRE is a selective and differential culture medium for the detection from stool specimens of *Escherichia coli* that are non-susceptible to carbapenems as pink colonies and KES (*Klebsiella aerogenes*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Enterobacter cloacae* complex, and *Serratia marcescens*) that are non-susceptible to carbapenems as blue colonies. Phenotypic confirmation of non-susceptibility (Intermediate and/or resistant) to carbapenems is done by demonstrating non-susceptibility to one of the selected agents using MIC or disk diffusion.

The selective components in HardyCHROM CRE culture medium are designed to inhibit the growth of yeasts and gram-positive bacteria, as well as gram-negative carbapenem-sensitive bacteria. Organisms that grow on HardyCHROM CRE are differentiated based on the metabolism of chromogenic substrates. The presence of chromogens allows the differentiation of gram-negative bacteria that produce carbapenemase or that inactivate carbapenems by mechanisms other than production of carbapenemase, e.g. cephalosporinase production combined with porin loss. The colonies of those bacteria that release products when they use the chromogens as a substrate source appear colored.
Testing is performed by direct inoculation of the stool onto the culture medium. Cultures are read after incubation of the plates for 18-24 hours at 35-37°C in an aerobic atmosphere. All presumptive carbapenem non-susceptible isolates that appear pink or blue on HardyCHROM CRE must be sub-cultured to non-selective medium for identification and antimicrobial susceptibility testing according to established methods.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

a. Precision/Reproducibility:

Reproducibility testing was conducted at three sites (two external and one internal site) using a set of blinded panel of 10 Gram negative organisms in transport medium (Cary Blair). Each isolate was tested in duplicate over five days (2 replicates x 5 days x 2 operators x 3 sites = 60 results per species). The reproducibility panel included f 5 CRE-positive organisms [*Enterobacter cloacae* complex (one isolate), *Escherichia coli* (one isolate) and *Klebsiella pneumoniae* (three isolates) at 10^3 CFU/mL] , and 5 CRE negative organisms [*Shigella flexneri* (one isolate), *Shigella sonnei* (one isolate), *Staphylococcus aureus* (one isolate), *Staphylococcus epidermidis* (one isolate) and *Escherichia coli* (one isolate) at 10^7 CFU/ml]. Quality control organisms (*K. pneumoniae* ATCC BAA 1705, *K. pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922) were included in the study. Two operators and two readers, who were blinded to each other's results, participated in the study at each site. The bacterial suspensions were prepared and shipped overnight to the test sites on ice prior to each day of testing. At the test sites, 10µl loop of each bacterial suspension was streaked for isolation onto HardyCHROM CRE plate, which was then incubated for 24 hours at 35°C prior to being read by each operator. At 24h incubation, HardyCHROM CRE plates were evaluated for growth and chromogenic performance. All carbapenem non-susceptible species tested were detected by HardyCHROM CRE with the correct chromogenic reaction on all days of the reproducibility study (**Table 2**). The reproducibility results of this study stratified by organism (expected color) is summarized in **Table 2A**.

Table 2: Reproducibility Study for detection of non-susceptible CRE stratified by day

	Positive Agreement			**Negative Agreement**		
	N	**HardyCHROM Positive**	**%**	***N***	**HardyCHROM Negative**	**%**
Day 1	60	60	100	60	60	100
Day 2	60	60	100	60	60	100
Day 3	60	60	100	60	60	100
Day 4	60	60	100	60	60	100
Day 5	60	60	100	60	60	100
Overall	**300**	**300**	100	**300**	**300**	**100**

Table 2A: Reproducibility data stratified by organism (expected color)

	Positive Percent Agreement					**Negative Percent Agreement**				
	N	**HC CRE Color**	**PPA %**	**low 95%**	**high 95%**	**N**	**HC CRE Color**	**NPA %**	**low 95%**	**high 95%**
EC Pink	60	60	100	94.0	100.0	540	540	100	99.3	100.0
KES Blue	240	240	100	98.4	100.0	360	360	100	98.9	100.0
Overall	300	300	100	98.7	100.0	900	900	100	99.6	100.0

The testing demonstrated overall reproducibility of 100%. The results of the reproducibility study were acceptable.

b. Linearity/assay reportable range:

Not applicable.

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

External Controls
QC testing was performed daily during the analytical and clinical studies using organisms that are either expected to grow (CRE Positive, carbapenem non-susceptible) or expected to be inhibited (CRE Negative, carbapenem susceptible) on HardyCHROM CRE.

Testing of QC organisms on HardyCHROM CRE yielded 100% agreement with the expected results.

A list of appropriate strains for use as External controls is provided in **Table 3**. Instructions for preparation of external controls are included in the labeling.

Table 3. Recommended strains for use as External Controls and expected results

Species	Phenotype	ATCC No.	Expected Result
Escherichia coli	CRE Positive	BAA-2469	Pink colonies
Klebsiella pneumoniae[1]	CRE Positive	BAA-1705	Blue colonies
Escherichia coli	CRE Negative	25922	No growth
Klebsiella pneumoniae	CRE Negative	700603	No growth

ATCC: American Type Culture Collection
[1]According to CLSI document M100, this QC organism may undergo a spontaneous loss of plasmid encoding carbapenemase genes, leading to false-negative QC results. To avoid false-negative QC results, this QC organism should be subcultured to or maintained in a carbapenem-containing medium prior to inoculating onto HardyCHROM CRE Agar.

Specimen Stability
A study was conducted to determine the acceptable duration of storage of stool specimens that are either raw (no preservative) or preserved in transport media prior to inoculation on HardyCHROM CRE plates. One strain from each of the following carbapenem non-susceptible species: *Escherichia coli, K. pneumoniae, K. oxytoca, Enterobacter cloacae complex, Serratia marcescens* was seeded into verified CRE negative stool specimens in duplicate at a final concentration of $1.5x10^2$ CFU/mL and immediately 100µl of the stool was spread-plated onto a HardyCHROM CRE plate (0h timepoint). One set of seeded samples was stored at room temperature (22-25°C) for 24h, and the other set was stored at refrigerated temperature (2-8°C) for 168h prior to inoculation on HardyCHROM CRE. One hundred microliters of each seeded stool was spread-plated onto HardyCHROM CRE at determined timepoints: (1, 4, 8, 12 and 24h for room temperature storage) and at (1, 4, 12, 24, 48, 72, 96, 148 and 168h for refrigerated storage). The plates were incubated at 35°C and evaluated for colony count and colony morphology at 18h and 24 hours.
All species tested produced the expected color reaction at 18 and 24h with and were successfully recovered from samples held at room temperature (23-24°C) for up to 24h or at 2-8°C for up to 168h.

Appropriate storage conditions for stool specimens prior to plating on HardyCHROM CRE are included in the labeling.

Incubation Study
A study was conducted to determine the recommended range of incubation times for recovery of carbapenem non-susceptible target organisms on HardyCHROM CRE. Two well characterized strains of representative targeted species were serially diluted in Tryptic Soy Broth (TSB) to a final concentration of 1.5×10^2 CFU/mL and 100µl was inoculated to HardyCHROM CRE agar plates that were incubated at 35°C. Plates were examined for growth and color every 2 hours from 18 to 26 hours, and from 42-50 hours of incubation. The expected colony color for each target species tested was observed after 18 hours except that pigmentation darkened over time.

As a result of this study, the following limitation was added to the labeling:

> *"Cultures on HardyCHROM CRE should be incubated at 35-37°C for 18-24 hours. Analytical and clinical studies showed reduced specificity with cultures incubated longer than 24 hours. Do not incubate more than 24 hours"*.

d. Detection limit:

Limit of Detection
The analytical sensitivity of HardyCHROM CRE with raw stool was determined for carbapenem non-susceptible target organisms that exhibited various β-lactamase genotypes (**Table 4**). Each target organism was diluted in TSB/saline then inoculated into CRE-negative pooled stool specimen. Aliquots of 100ul were spread-plated onto HardyCHROM CRE plates in duplicate. Parallel dilutions of each organism were also prepared in TSB/saline and plated on blood agar (growth controls). All culture plates were incubated aerobically at 35°C and checked for colony counts after 24 hours. The Limit of detection (LoD) was estimated at 1.5×10^2 CFU/mL based on the lowest dilution at which growth with the expected color was visible on HardyCHROM CRE plates.

Confirmation of LoD
A fresh versus frozen study was performed and it was demonstrated that using frozen stool matrix is equivalent to using fresh matrix.

Because the LoD study was conducted using a homogenous pool of previously frozen stool matrix, an additional confirmation study was performed to demonstrate recovery of the targeted carbapenem non-susceptible organisms at LoD target levels from different fresh stool matrices.

One of the two strains of each genus used in the LoD study was seeded into five different stool matrices at a final concentration at 1.5×10^2 CFU/mL. Each stool specimen was tested "raw" (unpreserved) as well as diluted in C&S Medium Transport Cary Blair Formula. One hundred microliter aliquots of each stool

specimen were then used to inoculate HardyCHROM CRE Agar Plates were incubated at 35°C and checked for colony counts and colony morphology at 18 and 24 hours.

As a medium that allows the growth of discernible colonies, the LoD is dependent on the number of organisms in the suspension used to spike the specimens and the volume of the inoculum used for plating. For each strain the LOD was confirmed to be $1.5x10^2$ CFU/mL stool with 100ul inoculum in distinct stool samples (equivalent to 15 CFU/plate). The List of the targeted species used in the LoD study is shown in **Table 4**.

Klebsiella aerogenes was not evaluated in the LoD study. However, seven strains of this organism were tested in the contrived study at 3×10^4 CFU/mL with 1µl inoculum (equivalent to 30 CFU/plate) and were successfully recovered.

Table 4. Bacterial strains used to Determine the Limit of Detection of HardyCHROM CRE

Species[2]	IHMA Strain	Known β-lactamase Resistance Markers	MIC (µg/mL) [1]		
			ETP	IPM	MEM
Enterobacter cloacae complex (E. asburiae, E. cloacae)	1058880	TEM-OSBL(2b); VEB-2; NDM-1	1	8	8
	872263	ACT-2; IMP-14	1	8	8
	893165	SHV-12(2be); KPC-2	1	1	0.25
	850592	CTX-M-15; ACT-16; NDM-1	1	8	8
Escherichia coli	872855	TEM-1(2b); CTX-M-14; OXA-48(c)	1	2	0.5
	951011	TEM-OSBL(2b); CMY-42; NDM-5	1	8	8
Klebsiella oxytoca	849849	VIM-1	1	4	8
	949841	TEM-OSBL(2b); ACC-1; NDM-1	1	8	8
Klebsiella pneumoniae	868125	SHV-11(2b); KPC-3	1	8	8
	874656	SHV-11(2b); TEM-1(2b); OXA-48(c)	1	8	8
Serratia marcescens	894356	TEM-1(2b); IMP-47	1	8	4
	896907	KPC-2	1	8	8

Abbreviations- IHMA: International Health Management Associates; MIC: Minimum Inhibitory Concentration; ETP: Ertapenem; IPM: Imipenem; MEM: Meropenem

[1] Non-susceptible (intermediate and resistant) (µg/mL): ETP, ≥1; IPM: ≥2; MEM, ≥2

[2] *Klebsiella aerogenes* was not evaluated in the LoD study.

Analytical Reactivity/Inclusivity

Inclusivity of HardyCHROM CRE Agar was evaluated by testing 77 well characterized carbapenem non-susceptible strains of *Enterobacteriaceae* (*E. coli, Enterobacter cloacae, Klebsiella oxytoca, Klebsiella ozaenae, Klebsiella pneumoniae,* and *Serratia marcescens*) with various molecular and susceptibility profiles. Each strain was diluted in TSB to a target level of 1.5×10^2 CFU/mL and 100ul was spread-plated onto HardyCHROM CRE agar plate. The plates were incubated at 35°C and read for growth and colony morphology. If no growth was observed, at the determined LoD, higher target levels were tested until organism was recovered. The HardyCHROM CRE was able to recover 72 of 77 (93.5%) of the carbapenem non-susceptible strains tested at 1.5×10^2 CFU/mL (100ul inoculum) after 24 h incubation. The remaining five strains were detected at either 10^3 or 10^4 CFU/mL. *Klebsiella aerogenes* was not evaluated in the analytical reactivity study.

Variations in the colony color of KES were observed (e.g, blue, blue with pink halo). Information on potential color and morphology variations is provided in the labeling

e. Analytical specificity/Cross-Reactivity Challenge:
The analytical specificity of HardyCHROM CRE was evaluated by testing a panel of carbapenem-susceptible target species as well as non-target species that are likely to be found in stool specimens. Included were isolates of the target organisms that exhibited resistance mechanisms other than carbapenemase (e.g., Extended Spectrum β-lactamase). Testing was performed by inoculating 10µL of a 1.5 x 10^8 CFU/mL suspension of each organism on HardyCHROM CRE and incubating at 35°C for 24 hours. A non-selective culture medium was also inoculated with the same suspension of organisms as a control to demonstrate viability of the cell stock. The organisms tested are listed in **Tables 5.** The majority of organisms tested did not exhibit pink, or blue colonies or were inhibited on HardyCHROM CRE at 24 hours with the following exceptions:

- Three organisms *Pediococcus*, *E. faecalis* (vanB$^+$) and *E. faecium* (vanA$^+$) produced pinpoint blue colonies at 48 hours of incubation

- *Aspergillus brasiliensis* produced blue colonies at 22 to 24 hours but could readily be distinguished morphologically as a fungus.

- *Stenotrophomonas maltophila* produced light blue colonies in areas of high inoculum concentration at 24 hours.

- Carbapenem non-susceptible *Citrobacter* species produced pink, or pink with blue halo and/or blue or blue with pink halo colonies.

As a result of this study, the following limitations were included in the Labeling:

- *"Analytical and Clinical testing of HardyCHROM CRE has shown that gram positive cocci and gram positive rods may be recovered as tiny, blue colonies at 18 and 24 hours."*

- *Analytical testing of HardyCHROM™ CRE has shown that areas of heavy growth (1st quadrant) of Stenotrophomonas maltophilia may appear light blue at 24 hours and produce false positive results (blue color for carbapenem non-susceptible KES). Only well isolated colonies should be evaluated for color morphology.*

- *"Analytical testing of HardyCHROM CRE has shown that Aspergillus brasiliensis may appear as blue and fuzzy at 22 to 24 hours."*

- *"Analytical and clinical testing of HardyCHROM CRE has shown that carbapenem non-susceptible Citrobacter spp. may appear as pink or blue*

colonies at 18 and 24 hours."

Table 5. Organisms evaluated in the Cross-Reactivity Challenge

Bacteria	
Acinetobacter baumannii	*Listeria monocytogenes*
Aeromonas hydrophila	*Micrococcus luteus*
Bacillus cereus	*Morganella morganii*
Campylobacter coli	*Pantoea agglomerans*
Campylobacter jejuni subsp. *jejuni*	*Pediococcus acidilactici* [1]
Citrobacter braakii	*Plesiomonas shigelloides*
Citrobacter freundii	*Proteus mirabilis*
Citrobacter koseri	*Proteus vulgaris*
Corynebacterium jeikeium	*Providencia alcalifaciens*
Corynebacterium pseudodiptherium	*Providencia rettgeri*
Enterobacter aerogenes	*Providencia stuartii*
Enterobacter cloacae (AmpC)	*Pseudomonas aeruginosa*
Enterococcus casseliflavus (vanC^{+})	*Pseudomonas fluorescens*
Enterococcus durans	*Salmonella enterica*
Enterococcus faecalis (vanB^{+}) [1]	*Serratia marcescens*
Enterococcus faecium (vanA^{+}) [1]	*Shigella boydii*
Enterococcus hirae	*Shigella flexneri*
Enterococcus raffinosus	*Shigella sonnei*
Enterococcus saccharolyticus	*Staphylococcus aureus*
Escherichia coli	*Staphylococcus epidermidis*
Hafnia alvei	*Stenotrophomonas maltophilia* [2]
Klebsiella oxytoca	*Streptococcus agalactiae*
Klebsiella pneumoniae [3]	*Streptococcus mitis*
Lactobacillus acidophilus	*Streptococcus pyogenes*
Lactobacillus gasseri	*Vibrio cholerae*
Lactococcus lactis	*Weisella confusa*
Listeria grayi	*Yersinia enterocolitica*
Yeasts & Fungi	
Aspergillus brasiliensis [4]	*Geotrichum candidum*
Candida glabrata	*Penicillium aurantiogriseum*
Candida albicans	*Penicillium chrysogenum*
Candida guilliermondii	*Penicillium rubens*
Candida tropicalis	*Saccharomyces cerevisiae*

[1] Produces pin point blue colonies
[2] Produces light blue colonies in areas of high inoculum
[3] Including a strain characterized as an ESBL (Extended Spectrum β-Lactamase) producer
[4] Produces blue colonies that are distinguishable as mold

f. Assay cut-off:

Not applicable.

g. Assay Interference:

Interfering Substances
A study was conducted to evaluate the potential for interference with the growth and color morphology of colonies on HardyCHROM CRE in the presence of potentially interfering substances that might be present in stool specimen. Testing was performed in a stool matrix in the presence and absence of each interfering substance with representative target organisms (*E. coli*, *Enterobacter cloacae complex, Klebsiella oxytoca, Klebsiella pneumoniae* and *Serratia marcescens*), at a final concentration of 1.5×10^3 CFU/mL. Both 10µl (1x LoD) and 100µL (10x LoD) inoculum volumes were used. Plates were incubated at 35°C and examined for colony count and colony morphology after 24h. A list of the potentially interfering substances tested and their concentrations is provided in **Table 6**. None of the substances was found to interfere at the concentrations tested with recovery of the target organisms with the expected colony color.

Table 6. Substances tested for potential interference with growth on HardyCHROM CRE

Category	Substance	Concentration (%) [1]
Antifungal	Nystop (Nystatin)	5%
	Lotrimin (Clotrimazole)	5%
	Lotrimin Ultra (Butenafine Hydrochloride)	5%
	Lamisil (Terbinafine Hydrochloride 1%)	5%
Antiseptic	Bactine (Benzalkonium Chloride)	1%
	Ethanol	1%
Biologic	Whole blood	5%
Contraceptive	Nonoxynol-9	5%
Gastrointestinal Medication	Pepto-Bismol (Bismuth Subsalicylate)	5%
	Prilosec OTC (Omeprazole)	5%
	Alka-Seltzer (Sodium carbonate/potassium carbonate)	5%
	Mylanta ($Al(OH)_3$)	5%
	Tums ($CaCO_3$)	5%
	Rolaids ($Mg(OH)_2$)	5%
	Milk of Magnesia ($Mg(OH)_2$)	5%
	Dulcolax (Sodium picosulfate solution)	5%
	Immodium AD (Loperamide)	5%
Lubricant	Mineral oil	10%
	Petroleum jelly	10%

Category	Substance	Concentration (%) [1]
	Fleet (Glycerin)	10%
	KY Jelly	10%
Other	C&S Medium Transport	75%
	Physiological Saline	10%
	Tween 80 (Polysorbate 80)	10%
Topical Medication	Preparation H (Hemorrhoid Cream)	10%
	Cortizone 10 (Hydrocortizone)	10%

[1] w/v or v/v, as appropriate

Interfering Microorganisms

A study was performed to demonstrate the ability to discriminate the recovery of carbapenem non-susceptible target organisms in the presence of non-target species that grew on HardyCHROM CRE in the Analytical Specificity Study. Testing was performed with organism suspensions prepared in TSB. The representative target organisms were at a concentration of 7.5×10^3 CFU/mL, and the non-target species were at high concentrations of 1.5×10^8 CFU/mL. Ten microliters of each suspension were inoculated onto HardyCHROM CRE which was incubated at 35°C. Plates were read at 24 hours. A list of the potentially interfering microorganisms tested is provided in **Table 7**.

Table 7. Species of potentially interfering microorganisms included in the Interference Study

Species Name	
Acinetobacter baumanii	*Enterococcus faecium*
Aeromonas hydrophila	*Geotrichum candidum*
Apsergillus brasiliensis	*Pediococcus acidilactici*
Candida albicans	*Penicillium rubens*
Candida glabrate	*Pseudomonas aeruginosa*
Candida guilliermondii	*Pseudomonas fluorescens*
Candida tropicalis	*Stenotrophomonas maltophilia*
Enterococcus faecalis	

All the target species were recovered in the presence of non-target organisms with the expected colony color after 24 hours with the following exceptions:

- *E. cloacae* in the presence of *Stenotrophomonas maltophilia*, produced small, Dark Purple colonies.

- The colony size of all target organisms (except *S. marcescens*) was reduced in the presence of the following non-target species, although the colony color was as expected: *A. baumannii*, *A. hydrophila*, *P. fluorescens*, *P. aeruginosa* and *S. maltophilia*.

- *Pseudomonas aeruginosa* had an effect on colony size of all target strains tested except *Serratia marcescens* and *Enterobacter cloacae complex (E. asburia).*

- *K. aerogenes* was not evaluated in the interfering microorganisms study in the presence of non-targeted organisms. Therefore, the effect of the non-target organisms on the size and color of this organism colonies is unknown.

As a result of this study, the following Limitations were included in their labeling:

- *"In the presence of Stenotrophomonas maltophilia, colonies of Enterobacter cloacae complex may appear purple and small after incubation for 24 hours."*

- *"The colony size of all target carbapenem non-susceptible organisms (except Serratia marcescens) may appear smaller in the presence of high concentrations of Acinetobacter baumanii, Aeromonas hydrophila, Pseudomonas fluorescens, and Stenotrophomonas maltophilia. The effect of non-target organisms on Klebsiella aerogenes colony size and color is unknown. In the presence of Stenotrophomonas maltophilia, colonies of Enterobacter cloacae complex may appear purple and small after incubation for 24 hours".*

- *"In the analytical mixed infection study, the non-target organisms were tested at a concentration of 1.5 x 10^8 CFU/mL. The accuracy of this device for detecting carbapenem non-susceptible Escherichia coli or KES in the presence of the non-target organisms at higher concentrations is unknown."*

2. Comparison studies:

   a. Method comparison with predicate device:

   Not applicable.

   b. Matrix comparison:

   Not applicable.

3. Clinical studies:

   a. Clinical Sensitivity:

   Prospective Study:

The performance of HardyCHROM CRE was evaluated in a prospective Clinical Study conducted at three geographically diverse sites in the US. For the recovery of carbapenem non-susceptible *Escherichia coli,* and *KES (Klebsiella oxytoca, Klebsiella pneumoniae, Klebsiella aerogenes, Enterobacter cloacae complex,* and *Serratia marcescens)* species, fresh stool samples were inoculated onto HardyCHROM CRE agar. The plates were incubated at 35°C and evaluated for colony color at 18 and 24 hours. Results were compared to a culture reference method comprised of a selective enrichment culture of stool in Trypticase Soy Broth (TSB) containing 1µg/mL meropenem and 3µg/mL vancomycin, followed by subculture to MacConkey agar. Plates were first assessed for growth or no growth. Colonies that were observed on either MacConkey agar or HardyCHROM CRE were subcultured and submitted for identification and susceptibility testing using FDA cleared methods. A reference result was considered negative if either no colonies of the targeted species were observed or if the recovered colonies were carbapenem-susceptible organisms or growth of any organism other than *E. coli* (pink) or KES (blue). Identification was performed using an FDA cleared MALDI-TOF test system. Susceptibility and confirmed CRE phenotype were determined using an FDA cleared test (gradient agar diffusion disk diffusion).

A total of 1, 628 specimens were enrolled in the study. Of these, 144 were excluded from the analysis of performance due to protocol deviations. A total of 1,484 stool samples were included in the analysis of performance. As a growth medium, recovery of multiple isolates from each specimen is possible. Two separate analyses were performed, as follows:

The performance of HardyCHROM CRE for recovery of pink colonies (as indicative of carbapenem non-susceptible *Escherichia coli*) from prospectively collected stool specimens at 18h is shown in **Table 8** and at 24h is shown in **Table 9.**

The performance of HardyCHROM CRE for recovery of blue carbapenem non-susceptible KES (*Klebsiella oxytoca, Klebsiella pneumoniae, Klebsiella aerogenes, Enterobacter cloacae complex,* and *Serratia marcescens)* from prospectively collected clinical specimens at 18h is shown in **Table 10** and at 24h is shown in **Table 11.**

Table 8. Performance of HardyCHROM CRE Pink Morphology at 18 hours in comparison to the reference method (stratified by clinical site)

Site 1		**Reference Method: Non-Susceptible *E. coli***		
		Positive	**Negative**	**Total**
HardyCHROM CRE	**Pink (Positive)**	0	5	**5**
	Negative [1]	0	523	**523**
	Total	**0**	**528**	**528**
	Sensitivity	Not applicable		
	Specificity	523/528 = 99.1% (97.8-99.6%)		
Site 2		**Reference Method**		
		Positive	**Negative**	**Total**
HardyCHROM CRE	**Pink (Positive)**	2	2	**4**
	Negative [1]	0	575	**575**
	Total	**2**	**577**	**579**
	Sensitivity	2/2 = 100% (34.2%-100%)		
	Specificity	575/577 = 99.7% (98.7-99.9%)		
Site 3		**Reference Method**		
		Positive	**Negative**	**Total**
HardyCHROM CRE	**Pink (Positive)**	6	4	**10**
	Negative [1]	0	507	**507**
	Total	**6**	**511**	**517**
	Sensitivity	6/6 = 100% (61.0-100%)		
	Specificity	507/511 = 99.2% (98.0-99.7%)		
Overall		**Reference Method**		
		Positive	**Negative**	**Total**
HardyCHROM CRE	**Pink (Positive)**	8	11[2]	**19**
	Negative [1]	0	1605	**1605**
	Total	**8**	**1616**	**1624**
	Sensitivity	8/8 = 100% (67.6-100%)		
	Specificity	1605/1616 = 99.3% (98.8-99.6%)		
	PPV	8/19 = 42.1% (23.1-63.7%)		
	NPV	1605/1605 = 100% (99.7-100%)		

PPV: Positive Predictive Value; NPV: Negative Predictive Value

[1] Negative: No growth, or growth of any color other than pink (Blue, yellow, others)

[2] There were 11 FP isolates observed at 18 hours: 5/11 were confirmed to be carbapenem non-susceptible *E. coli*, but were not recovered from the reference method, 5/11 produced pink colonies but were not the target organism for that color (2 *Enterobacter cloacae complex*, 2 *Citrobacter freundii* and 1 *Citrobacter youngae*), 1/11 *Citrobacter freundii* was confirmed to be susceptible but grew pink

Table 9. Performance of HardyCHROM CRE Pink Morphology at 24 hours in Comparison to the Reference Method, (stratified by clinical site)

Site 1		**Reference Method: Non-Susceptible *E. coli***		
		Positive	**Negative**	**Total**
HardyCHROM CRE	**Pink (Positive)**	0	5	**5**
	Negative [1]	0	530	**530**
	Total	**0**	**535**	**535**
	Sensitivity	Not applicable		
	Specificity	530/535 = 99.1% (97.8-99.6%)		
Site 2		**Reference Method**		
		Positive	**Negative**	**Total**
HardyCHROM CRE	**Pink (Positive)**	2	2	**4**
	Negative [1]	0	582	**582**
	Total	**2**	**584**	**586**
	Sensitivity	2/2 = 100% (34.2%-100%)		
	Specificity	582/584 = 99.7% (98.7-99.9%)		
Site 3		**Reference Method**		
		Positive	**Negative**	**Total**
HardyCHROM CRE	**Pink (Positive)**	6	4	**10**
	Negative [1]	0	507	**507**
	Total	**6**	**511**	**517**
	Sensitivity	6/6 = 100% (61.0-100%)		
	Specificity	507/511 = 99.2% (98.0-99.7%)		
Overall		**Reference Method**		
		Positive	**Negative**	**Total**
HardyCHROM CRE	**Pink (Positive)**	8	11[2]	**19**
	Negative [1]	0	1619	**1605**
	Total	**8**	**1630**	**1638**
	Sensitivity	8/8 = 100% (67.6-100%)		
	Specificity	1619/1630 = 99.3% (98.8-99.6%)		
	PPV	8/19 = 42.1% (23.1-63.7%)		
	NPV	1619/1619 = 100% (99.8-100%)		

PPV: Positive Predictive Value; NPV: Negative Predictive Value

[1] Negative: No growth, or growth of any color other than pink (Blue, yellow, others)

[2] There were 11 FP isolates observed at 24 hours: 5/11 were confirmed to be carbapenem non-susceptible *E. coli*, but were not recovered from the reference method, 5/11 produced pink colonies but were not the target organism for that color (2 *Enterobacter cloacae complex*, 2 *Citrobacter freundii* and 1 *Citrobacter youngae*), 1/11 *Citrobacter freundii* was confirmed to be susceptible but grew pink.

Table 10. Performance of HardyCHROM CRE Blue Morphology at 18 hours in Comparison to the Reference Method, (stratified by clinical site)

Site 1		**Reference Method: Non-Susceptible *KES*[2]**		
		Positive	**Negative**	**Total**
HardyCHROM CRE	**Blue (Positive)**	7	10	**17**
	Negative [1]	1	510	**511**
	Total	**8**	**520**	**528**
	Sensitivity	7/8= 87.5% (52.9%-97.8%)		
	Specificity	510/520 = 98.1% (96.5-99.0%)		
Site 2		**Reference Method**		
		Positive	**Negative**	**Total**
HardyCHROM CRE	**Blue (Positive)**	5	8	**13**
	Negative [1]	1	565	**566**
	Total	**6**	**573**	**579**
	Sensitivity	5/6 = 83.3% (43.7%-97%)		
	Specificity	565/573 = 98.6% (97.3%-99.3%)		
Site 3		**Reference Method**		
		Positive	**Negative**	**Total**
HardyCHROM CRE	**Blue (Positive)**	34	11	**45**
	Negative [1]	0	472	**472**
	Total	**34**	**483**	**517**
	Sensitivity	34/34 = 100% (89.9-100%)		
	Specificity	472/483 = 97.7% (96.0-98.7%)		
Overall		**Reference Method**		
		Positive	**Negative**	**Total**
HardyCHROM CRE	**Blue (Positive)**	46	29[3]	**75**
	Negative [1]	2[4]	1547	**1549**
	Total	**48**	**1576**	**1624**
	Sensitivity	46/48 = 95.8% (86.0-98.9%)		
	Specificity	1547/1576 = 98.2% (97.4-98.7%)		
	PPV	46/75 = 61.3% (50.0-71.5%)		
	NPV	1547/1576 = 99.9% (99.5%-100%)		

PPV: Positive Predictive value; NPV: Negative Predictive Value

[1] Negative: No growth, or growth of any color other than blue

[2] Detection of carbapenem non-susceptible *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Klebsiella aerogenes*, *Enterobacter cloacae complex*, or *Serratia marcescens*.

[3] There were 29 False Positive observed at 18 hours: 23/29 were confirmed as carbapenem non-susceptible KES (11 *Klebsiella pneumoniae*, 12 *Enterobacter cloacae complex*, 2 *Serratia marcescens*, 1 *Klebsiella oxytoca*) that were recovered as blue isolated colonies on HardyCHROM CRE but were not recovered by the reference method; 1/29 was a carbapenem non-susceptible *S. liquefaciens*; 2/29 isolates were non-target Gram positive organisms.

[4] There were 2 False Negative observed at 18 hours: 2/2 carbapenem non-susceptible *Enterobacter cloacae*

Table 11. Performance of HardyCHROM CRE Blue Morphology at 24 hours in Comparison to the Reference Method, (stratified by clinical site)

Site 1		**Reference Method: Non-Susceptible *KES*[2]**		
		Positive	**Negative**	**Total**
HardyCHROM CRE	**Blue (Positive)**	7	12	**19**
	Negative [1]	1	515	**516**
	Total	**8**	**527**	**535**
	Sensitivity	7/8 = 87.5% (52.9-97.8%)		
	Specificity	515/527 = 97.7% (96.1-98.7%)		
Site 2		**Reference Method**		
		Positive	**Negative**	**Total**
HardyCHROM CRE	**Blue (Positive)**	5	10	**15**
	Negative [1]	1	570	**571**
	Total	**6**	**580**	**586**
	Sensitivity	5/6 = 83.3% (43.7-97%)		
	Specificity	570/580 = 98.3% (96.9-99.1%)		
Site 3		**Reference Method**		
		Positive	**Negative**	**Total**
HardyCHROM CRE	**Blue (Positive)**	34	12	**46**
	Negative [1]	0	471	**471**
	Total	**34**	**483**	**517**
	Sensitivity	34/34 = 100% (89.9-100%)		
	Specificity	471/483 = 97.5% (95.7-98.6%)		
Overall		**Reference Method**		
		Positive	**Negative**	**Total**
HardyCHROM CRE	**Blue (Positive)**	46	34[3]	**80**
	Negative [1]	2[4]	1556	**1558**
	Total	**48**	**1590**	**1638**
	Sensitivity	46/48 = 95.8% (86.0-98.9%)		
	Specificity	1556/1590 = 97.9% (97.0-98.5%)		
	PPV	46/80 = 57.5% (46.6-67.7%)		
	NPV	1556/1558 = 99.9% (99.5%-100%)		

PPV: Positive Predictive value; NPV: Negative Predictive Value

[1] Negative: No growth, or growth of any color other than blue

[2] Detection of carbapenem non-susceptible *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Klebsiella aerogenes*, *Enterobacter cloacae complex*, or *Serratia marcescens*.

[3] There were 34 False Positive observed at 24 hours: 23/34 were confirmed as carbapenem non-susceptible KES (11 *Klebsiella pneumoniae*, 12 *Enterobacter cloacae complex*, 2 *Serratia marcescens*, 1 *Klebsiella oxytoca*) that were recovered as blue isolated colonies on HardyCHROM CRE but were not recovered by the reference method; 1/29 was a carbapenem non-susceptible *S. liquefaciens*; 2/29 isolates were non-target Gram positive organisms. 7/34 isolates were non-target Gram positive organisms.

[4] There were 2 False Negative observed at 24 hours: 2/2 carbapenem non-susceptible *Enterobacter cloacae*

The agreement between colony color observed on HardyCHROM CRE with prospectively collected clinical specimens at 24 hours and the identity and carbapenem susceptibility of the isolates recovered from the HardyCHROM CRE culture medium was also analyzed as shown in **Table 12.**

Table 12. Agreement of Carbapenem NS Target Species with color on HardyCHROM CRE

		Carbapenem Non-susceptible Target Species		
		Positive	**Negative**	**Total**
HardyCHROM CRE	**Positive** [1]	84 [3]	15 [4]	**99**
	Negative[2]	0	740	**755**
	Total	**84**	**755**	**854**
Positive Percent Agreement		**100% (91/91); 95.6-100%**		
Negative Percent Agreement		**99.4% (740/755); 98.9-99.7%**		

[1] Pink or Blue colonies
[2] Colonies other than pink or blue
[3] Includes the following species: *K. pneumoniae* (41), *E. cloacae* complex (23), *E. coli* (13), S. *marcescens* (3), *K. aerogenes* (2), *K. oxytoca* (2).
[4] Includes Gram positive cocci (7), *C. freundii* (3), *E. kobei* (3), *C. youngae* (1), *S. liquefaciens* (1)

As a result of this study, the following limitations were included in the labeling:

- "*Some Enterobacter cloacae complex isolates may appear as pink colonies on HardyCHROM CRE and be falsely considered as E. coli."*

- *"Analytical and clinical testing of HardyCHROM CRE has shown that carbapenem non-susceptible Citrobacter spp. may be recovered as pink or blue colonies at 18 and 24 hours."*

- *"The clinical performance of HardyCHROM™ CRE was established with fresh stool samples. Compatibility with stool in C&S Medium Transport (Cary Blair Formula) was evaluated in the contrived study but not in the prospective study."*

Contrived Specimen Study

To supplement testing of prospectively collected clinical specimens, a total of 203 contrived specimens were also evaluated on HardyCHROM CRE: 153 were spiked with isolates with confirmed non- susceptibility to carbapenems, and 50 specimens free of carbapenem non-susceptible organisms. These 153 specimens were prepared by spiking 38 *E. coli* and 115 KES (22 *Enterobacter cloacae* complex, 7 *Klebsiella aerogenes*, 9 *Klebsiella oxytoca*, 65 *Klebsiella pneumoniae*, 2 *Klebsiella variicola* and 10 *Serratia marcescens*) of known susceptibility phenotype directly into CRE negative stool matrix (raw or diluted in C&S Medium Transport, Cary Blair Formula) at a concentration of approximately 3 x 10^4 CFU/mL (1µl inoculum was used to inoculate the HardyCHROM CRE plates). The results obtained on HardyCHROM CRE were compared to the expected results and observed for colony morphology/color at 18 and 24h.

The performance of HardyCHROM CRE for recovery of pink colonies (as indicative of carbapenem non-susceptible *Escherichia coli*) versus expected results of is shown in **Table 13**. Data presented in the table is identical whether the plate readings were made at 18 or at 24 hours.

The performance of HardyCHROM CRE for recovery of blue colonies (as indicative of carbapenem non-susceptible KES) versus expected results is shown in **Table 14**. Data presented in the table is identical whether the plate readings were made at 18 or at 24 hours.

Table 13. HardyCHROM CRE Pink Morphology at 18 or 24 hours vs. Expected Results

Raw Stool		**Expected Result: Non-Susceptible *E. coli***		
		Positive	**Negative**	**Total**
HardyCHROM Color	**Pink**	38	0	**38**
	Negative/Other	0	165	**165**
	Total	**38**	**165**	**203**
Positive Percent Agreement		38/38 = 100% (90.8%-100%)		
Negative Percent Agreement		165/165 = 100% (97.7%-100%)		
Cary Blair		**Expected Result: Non-Susceptible *E. coli***		
		Positive	**Negative**	**Total**
HardyCHROM Color	**Pink**	38	0	**38**
	Negative/Other	0	165	**164**
	Total	**38**	**165**	**202**
PPA[1]		38/38 = 100% (90.8%-100%)		
NPA[1]		165/165 = 100% (97.7%-100%)		

Note: There were no false positives or false negative in either Raw or Cary Blair samples tested.
[1]PPA (Positive Percent Agreement) and NPA (Negative Percent Agreement) were the same at 18 and 24 h

Table 14. HardyCHROM CRE Blue Morphology at 18 or 24 hours vs. Expected Results

Raw Stool		**Expected Result: Non-Susceptible KES[2]**		
		Positive	**Negative**	**Total**
HardyCHROM Color	**Blue**	110	0	**110**
	Negative/Other	5[3]	88	**93**
	Total	**115**	**88**	**203**
PPA		110/115=95.7 %(90.2%-98.1%)		
NPA		88/88=100% (95.8%-100%)		
Cary Blair		**Expected Result: Non-Susceptible KES[2]**		
		Positive	**Negative**	**Total**
HardyCHROM Color	**Blue**	110	0	**110**
	Negative/Other	5[3]	88	**93**
	Total	**115**	**88**	**203**
PPA[1]		110/115=95.7 %(90.1%-98.1%)		
NPA[1]		88/88=100% (95.8%-100%)		

[1]PPA (Positive Percent Agreement) and NPA (Negative Percent Agreement) were the same at 18 and 24 h
[2]KES*: K. aerogenes, K. oxytoca, K. pneumoniae, E. cloacae complex and S. marcescens*
[3] There were 10 false negative results from both raw stool and Cary Blair medium because there was no growth on the HardyCHROM CRE plate for these isolates: 2/10 (20%) of the organisms were *Klebsiella variicola*. While these organisms are carbapenem non-susceptible, it is not a claimed species. 4/10 (40%) of the organisms upon retest were confirmed to have sub populations more susceptible to carbapenems than expected and were not recovered at 2x LoD, as tested in the contrived study. 4/10 (40%) were retested and confirmed carbapenem non-susceptible and the reason for failure were unknow.

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*b. Clinical specificity**:***

Refer to **Section M3(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 M3(a)**, the prevalence of carbapenem non-susceptible isolates based on the target colors (pink for *E.coli* and blue for KES) that were observed on HardyCHROM CRE was 6.0% (99/1638) at 24 hours.

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.