April 29, 2019

Hardy Diagnostics
Rianna Malherbe
Performance Studies Coordinator
1430 West McCoy Lane
Santa Maria, California 93455

Re: K190553
Trade/Device Name: HardyCHROM CRE
Regulation Number: 21 CFR 866.1700
Regulation Name: Culture medium for antimicrobial susceptibility tests
Regulatory Class: Class II
Product Code: JSO
Dated: March 4, 2019
Received: March 5, 2019

Dear Rianna Malherbe:

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

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

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

requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/CombinationProducts/GuidanceRegulatoryInformation/ucm597488.htm); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/) and CDRH Learn (http://www.fda.gov/Training/CDRHLearn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (http://www.fda.gov/DICE) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

for

Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

510(k) Summary

I. SUBMITTER

Rianna Malherbe
Performance Studies Coordinator
Hardy Diagnostics
1430 W. McCoy Lane
Santa Maria, CA 93455
Phone: 805-346-2766 x5714
E-mail: MalherbeR@hardydiagnostics.com

II. DEVICE

Name of Device: HardyCHROM™ CRE
Classification Name: Culture medium for anti-microbial susceptibility tests
Regulatory Class: II
Product Code: JSO

III. PREDICATE DEVICE

Bio Rad VRESelect, K122187

IV. DEVICE DESCRIPTION

HardyCHROM™ CRE is a selective and differential chromogenic medium designed to screen for carbapenem non-susceptible *Escherichia coli* and KES (*Klebsiella aerogenes*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Enterobacter cloacae* complex, and *Serratia marcescens*.) from fecal specimens. The selective components in HardyCHROM™ CRE inhibit the growth of yeast, Gram-positive bacteria, and Gram-negative bacteria sensitive to ertapenem. HardyCHROM™ CRE differentiates *Escherichia coli* (pink colonies) from KES (blue colonies).

The CLSI guidelines recommend screening for CREs with an MIC greater than or equal to an established limit to one of several selected agents. Phenotypic confirmation of non-susceptibility to carbapenems is done by demonstrating non-susceptibility to one of the selected agents using MIC or disk diffusion. Further biochemical analysis is required to confirm the production of carbapenemase or any other mechanism of resistance.

V. INDICATIONS FOR USE / 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.

VI. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS WITH THE PREDICATE DEVICE

Attribute	Device	Comparator	Substantially Equivalent?
Name	HardyCHROM™ CRE	Bio Rad VRESelect	
510(k) Details	510(k) number N/A Product Code JSO 21 CFR 866.1700 “66.1700umberCS for anti-microbial susceptibility testsp Class II Panel 83 Microbiology	510(k) number K122187 Product Code JSO 21 CFR 866.1700 “Culture medium for anti-microbial susceptibility tests” Class II Panel 83 Microbiology	Yes

Attribute	Device	Comparator	Substantially Equivalent?
Name	HardyCHROM™ CRE	Bio Rad VRESelect	
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 susceptibility testing and epidemiological typing.	Yes
Methodology	Enzymatic – Chromogenic	Enzymatic – Chromogenic	Yes
Inoculation	Direct	Direct	Yes
Sample Type	Fecal Specimen	Rectal Swab or Fecal Specimen	Yes
Interpretation	Manual, Visual	Manual, Visual	Yes
Subculture	Required for confirmation of identity and antimicrobial susceptibility testing	Required for confirmation of identity and antimicrobial susceptibility testing	Yes
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*	Yes

VII. PERFORMANCE DATA

Performance of HardyCHROM™ CRE was evaluated at three geographically diverse hospitals. Freshly collected stool specimens were used in this study. The recovery of carbapenem non-susceptible *Escherichia coli* and KES (*K. aerogenes, K. oxytoca, K. pneumoniae, E. cloacae* complex, and *S. marcescens*) on HardyCHROM™ CRE was compared to routine culture, defined as selective enrichment in Tryptic Soy Broth (TSB) containing 1 µg/mL meropenem and 3 µg/mL vancomycin, followed by a subculture to MacConkey Agar.
Identity and susceptibility of organisms that grew on both HardyCHROM™ CRE and MacConkey Agar were confirmed using FDA-cleared ID and AST systems. Quality control was performed in parallel every day of testing.

A total of 1,628 samples were tested against routine culture. A total of 144 specimens did not meet enrollment criteria, and were therefore excluded from the analysis. Of the remaining 1,484 valid samples tested, a total of 8 carbapenem non-susceptible *Escherichia coli* and 46 carbapenem non- susceptible KES (*K. aerogenes, K. oxytoca, K. pneumoniae, E. cloacae complex,* and *S. marcescens*) isolates were recovered by expected morphology on HardyCHROM™ CRE with concordant results obtained by the reference method and confirmed by ID and AST.

Product performance is summarized below:

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.

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.

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*

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. Results of this data analysis are summarized below.

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)

To supplement testing of prospectively collected clinical specimens, a total of 203 contrived specimens were also evaluated at Hardy Diagnostics. CRE-negative patient specimens were inoculated with known carbapenem non-susceptible organisms at 2x LoD and tested on HardyCHROM™ CRE. Results are summarized below.

***E. coli* Pink Morphology on HardyCHROM™ CRE at 18 or 24 hours vs Expected**

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

[1]The Positive Percent Agreement and Negative Percent Agreement were the same for both 18 and 24 hours.

KES Blue Morphology on HardyCHROM™ CRE at 18 and 24 hours vs Expected

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

[1]KES = *K. aerogenes, K. oxytoca, K. pneumoniae, E. cloacae* complex, and *S. marcescens*

[2]The Positive Percent Agreement and Negative Percent Agreement were the same for both 18 and 24 hours.

[3]There were 10 False Negative samples for both Raw and Cary Blair samples at 18 and 24 hours. 2/10 (20%) of the organisms were *Klebsiella variicola*. While these organisms are carbapenem non-susceptible, it is not a claimed species. Upon retest, 4/10 (40%) of the organisms 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%) of the organisms were retested and the reason for failure is unknown.

RECOVERY RATE

To determine the recovery (Limit of Detection (LoD)) of HardyCHROM™ CRE, the media was challenged with 10 strains of target carbapenem non-susceptible organisms. Two well-characterized strains each of *E. coli, K. oxytoca, K. pneumoniae, E. cloacae complex* and *S. marcescens* were evaluated for recovery on HardyCHROM™ CRE. Note: The LoD of *K. (Enterobacter) aerogenes* was not evaluated. Each organism was tested at 10-fold decreasing concentrations and evaluated for colony growth and color development. The lowest concentration at which a positive color reaction was seen, indicated by a pink (*E. coli*) or blue (KES) colony color, was determined to be the LoD. The determined LoD was confirmed by testing HardyCHROM™ CRE with five replicate dilutions of the determined LoD concentrations. HardyCHROM™ CRE was able to recover all strains tested at a LoD of $1.5x10^2$ CFU/mL in stool specimen with 100 µL inoculum (spread-plate).

ANALYTICAL REACTIVITY

HardyCHROM™ CRE was evaluated for the recovery of seventy-seven well-characterized carbapenem non-susceptible strains at the limit of detection concentration of $1.5x10^2$ CFU/mL. The strains were tested using a clean suspension in the absence of stool matrix. HardyCHROM™ CRE was able to recover 72 of 77 (93.5%) of the carbapenem non-susceptible strains at $1.5x10^2$ CFU/mL (100 µL inoculum) after 24 hours of incubation. Some strains were either more susceptible to the selective agent or may have struggled to maintain resistance once the organism was serially diluted and inoculated to the media. After 24 hours of incubation, three strains were recovered at $1.5x10^3$ CFU/mL and two strains were recovered at $1.5x10^4$ CFU/mL. All target strains tested were recovered with the expected color development.

Summary of Analytical Sensitivity testing

Species	n	Mechanism	ETP MIC	IMP MIC	MEM MIC	LoD at 24 hours
E. coli	17	OXA-48, NDM (1,5,6) KPC (3, KPC-type)	1->32	4->32	2->32	16 at 10^2 CFU/mL 1 at 10^3 CFU/mL
E. cloacae complex	15	OXA-48, MIR-8, KPC -4, ACT (6, ACT type), NDM-1, VIM (6, 23, 31), IMP(1, 8)	1->32	1->32	0.5->32	13 at 10^2 CFU/mL 1 at 10^3 CFU/mL 1 at 10^4 CFU/mL
K. oxytoca and *K. pneumoniae*	39	OXA (48, 163, 181, 232), KPC (2, 3, 12, KPC-type), NDM-1, IMP (1, 4, 8), VIM-27	0.5->32	2->32	0.5->32	37 at 10^2 CFU/mL 1 at 10^3 CFU/mL 1 at 10^4 CFU/mL
S. marcescens	5	IMP-8, NDM-1, VIM-4, SME (2, SME-type)	0.5->32	8->32	1->32	5 at 10^2 CFU/mL

[1]*K. (Enterobacter) aerogenes* was not evaluated in this study.

ANALYTICAL SPECIFICTY

To determine the potential for cross-reactivity on HardyCHROM™ CRE, internal testing was conducted with carbapenem-susceptible target species as well as non-target species commonly found in stool samples. A total of 110 strains were tested on HardyCHROM™ CRE by streaking 10µL of a $1.5x10^8$ CFU/mL suspension of each organism onto HardyCHROM™ CRE. After 24 hours of incubation, all HardyCHROM™ CRE plates were evaluated for growth and color reaction. The
majority of organisms tested either did not produce a target organism morphology or were inhibited on HardyCHROM™ CRE at 24 hours. One organism (*Aspergillus brasiliensis*) developed blue pigmentation at 24 hours. Three organisms (*Pediococcus, E. faecalis (vanB), E. faecium (vanA)*) developed blue pigmentation at 48 hours of incubation. Organisms that developed a blue color either took 48 hours of incubation (*Pediococcus, Enterococcus* with vancomycin resistance) to develop or had distinct morphology (*Aspergillus*) that allowed differentiation from the target species. None of the non-target organisms tested developed a pink color.

List of non-target organisms tested in Analytical Specificity

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

MICROBIAL INTERFERENCE

HardyCHROM™ CRE was challenged to determine if target organisms at low concentration could be recovered in the presence of non-target organisms at a high concentration. All organisms that were recovered on HardyCHROM™ CRE in the Analytical Specificity study were used in this Microbial Interference study. Non-target organisms at a high concentration ($1.5x10^8$ CFU/mL) were mixed with each target organism at a concentration of $7.5x10^3$ CFU/mL and inoculated to HardyCHROM™ CRE using a 10µL loop. If the target organism was not recovered, the concentration of the non-target organism was lowered 10-fold until the target organism was recovered.

HardyCHROM™ CRE was able to recover all target organisms from the mixed suspension when in the presence of high concentrations of all non-target organisms used in this study. For most of the target organisms, colony color was as expected. The following non-target organisms had an effect on colony size of all target strains tested except *Serratia marcescens*: *Acinetobacter baumanii, Aeromonas hydrophila, Pseudomonas fluorescens,* and *Stenotrophomonas maltophilia*. *Pseudomonas aeruginosa* had an effect on colony size of all target strains tested except *Serratia marcescens* and *Enterobacter asburiae*. One of the target strains, *E. cloacae,* was recovered as purple colonies when mixed with *Stenotrophomonas maltophilia*. Pigmentation of other target organisms tested was not affected by any of the other non-target organisms tested.

INTERFERENCE

Commonly used or encountered endogenous and exogenous substances that may be present in stool specimen were evaluated for potential interference of growth or chromogenic reaction on HardyCHROM™ CRE. The substances tested are listed in the table below. No interference was observed with any substance at the highest clinically relevant concentration in the CRE-negative specimen matrix.

Interfering Substances

Category	Substance	Concentration in Sample Matrix [1]
Antifungal	Nystop (Nystatin)	5% w/v
Antifungal	Lotrimin (Clotrimazole)	5% v/v
Antifungal	Lotrimin Ultra (Butenafine Hydrochloride)	5% v/v
Antifungal	Lamisil (Terbinafine Hydrochloride 1%)	5% v/v
Antiseptic	Bactine (Benzalkonium Chloride)	1% v/v
Antiseptic	Ethanol	1% v/v
Biologic	Whole blood	5% v/v
Contraceptive	Nonoxynol-9	5% w/v
GI Medication	Pepto-Bismol (Bismuth Subsalicylate)	5% v/v
GI Medication	Prilosec OTC (Omeprazole)	5% v/v
GI Medication	Alka-Seltzer (Sodium carbonate/potassium carbonate)	5% v/v
GI Medication	Mylanta ($Al(OH)_3$)	5% v/v
GI Medication	Tums ($CaCO_3$)	5% v/v
GI Medication	Rolaids ($Mg(OH)_2$)	5% w/v
GI Medication	Milk of Magnesia ($Mg(OH)_2$)	5% v/v
GI Medication	Dulcolax (Sodium picosulfate solution)	5% w/v
GI Medication	Immodium AD (Loperamide)	5% v/v
Lubricant	Mineral oil	10% v/v
Lubricant	Petroleum jelly	10% v/v
Lubricant	Fleet (Glycerin)	10% v/v
Lubricant	KY Jelly	10% v/v
Other	C&S Transport Medium	75% v/v
Other	Physiological Saline	10% v/v
Other	Tween80 (Polysorbate80)	10% v/v
Topical Medication	Preparation H (Hemorrhoid Cream)	10% v/v
Topical Medication	Cortizone 10 (Hydrocortizone)	10% v/v

[1]Specific amounts of substance added to stool specimen matrix calculated using $C_1V_1=C_2V_2$ with the assumption that 1g=1mL.

INCUBATION STUDY

In order to determine a recommended incubation time range, the performance of HardyCHROM™ CRE was evaluated using seven carbapenem non-susceptible target organisms at various incubation times. Each target organism was inoculated to HardyCHROM™ CRE at the limit of detection and incubated at 35°C. Plates were evaluated for growth and chromogenic performance every 2 hours from 18-26 hours and 42-50 hours of incubation. All organisms were recovered from HardyCHROM™ CRE with expected color development as early as 18 hours.

STOOL SPECIMEN STABILITY

Stool specimen with and without Cary Blair Transport Media was evaluated to determine the acceptable storage conditions required to recover carbapenem non-susceptible target organisms. Stool specimens were spiked with carbapenem non-susceptible target organisms near LoD and kept at both room temperature and refrigerated conditions. Specimens were inoculated to HardyCHROM™ CRE at 0, 1, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours after inoculating the stool with target organism.

HardyCHROM™ CRE was able to recover 6/6 (100%) of the carbapenem non-susceptible target strains from raw stool specimen and stool specimen in Cary Blair Transport Media when stored at room temperature for up to 24 hours. HardyCHROM™ CRE was able to recover 6/6 (100%) of the carbapenem non-susceptible target strains from raw stool specimen and stool specimen in Cary Blair Transport Media when stored at 2-8°C for up to 7 days.

REPRODUCIBILITY

Prior to initiating the prospective clinical study, a panel of 10 blinded isolates provided by Hardy Diagnostics was tested at three distinct study sites in duplicate on five work days to demonstrate reproducibility and to document proficiency in the performance of the test. Each panel included five CRE-positive [*Enterobacter cloacae* complex (one isolate), *Escherichia coli* (one isolate) and *Klebsiella pneumoniae* (three isolates) at 10^3 CFU/mL] , and 5 CRE negative [*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]. Agreement of >95% with known test results was required before proceeding with the study. The testing was done with at least one operator and two readers, blinded to each other's results, per site. All CRE-positive isolates tested (100%) were detected by HardyCHROM™ CRE on all days of the reproducibility study.

CONCLUSIONS

The non-clinical data support the safety of the device and the device verification and validation demonstrate that the HardyCHROM™ CRE device should perform as intended in the specified use conditions. The analytical and clinical data demonstrate that the HardyCHROM™ CRE device is substantially equivalent to the predicate device.