HardyCHROM™ CRE

Cat. no. G323	HardyCHROM™ CRE, 15x100mm Plate, 18ml	10 plates/bag

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.

SUMMARY AND PRINCIPLES

The selective components in HardyCHROM™ CRE Agar are agents that inhibit the growth of yeast, gram-positive bacteria and gram-negative carbapenem-sensitive bacteria (e.g. a carbapenem). 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.[1] The colonies of those bacteria that release products when they use the chromogens as a substrate source appear colored.

FORMULA

Ingredients per liter of deionized water:*

Peptones	20.0gm
Sodium Chloride	5.0gm
Chromogenic Mixture	5.0gm
Bile Salts	1.5gm
Selective Agents	1.0gm
Agar	13.5gm

* Adjusted and/or supplemented as required to meet performance criteria.

Final pH 6.5 +/- 0.1 at 22.5°C +/- 2.5°C

STORAGE AND SHELF LIFE

This product is temperature sensitive. Upon receipt store at 2-8°C; protect from light, excessive heat, moisture, and freezing. Media should not be used if there are any signs of deterioration, contamination, or if the expiration date has passed.

Product is extremely light sensitive: protect against damage from excessive illumination and store away from any direct light source.

Do not use media after the expiration date. Sensitivity is not optimal after expiration date or if the product has been stored inadequately.

The expiration dating on the product label applies to the product in its intact packaging when stored as directed.

Refer to the document "Storage" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as 1µL inoculating loops, specimen transport materials, other culture media, swabs, incubators, etc. as well as serological and biochemical reagents, are not provided.

Please refer to the Quality Control section below for Quality Control organisms required.

PRECAUTIONS

For *in vitro* diagnostic use.

Federal Law restricts this device to sale by or on the order of a licensed practitioner.

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious and handled observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only and is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." The "Guideline for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdcgov/ncidod/dhqp/gl_isolation.html.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

Refer to the SDS Search instructions on the Hardy Diagnostics' website for more information.

PROCEDURE

Clinical Procedure

Specimen Transport and Storage:

Stool specimen can be stored at room temperature for 24h or at 2-8°C for up to 168h (7 days). Other storage conditions were not evaluated during the clinical study. Please refer to the Stool Specimen Stability Study below for a description of the analytical stool (raw and stool preserved in Cary Blair) specimen stability study performed in spiked samples.

Method of Use:

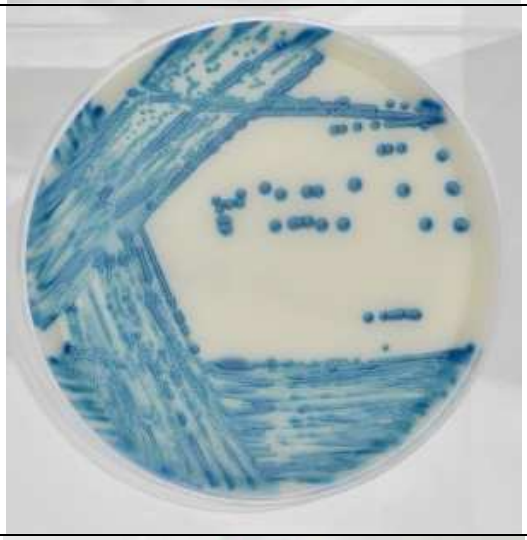
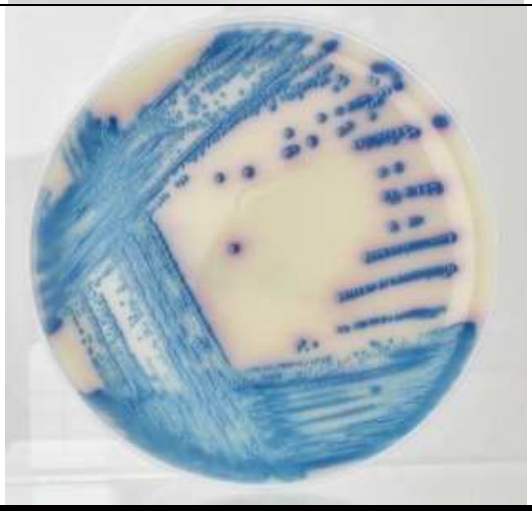
1. Allow the plates to equilibrate to room temperature. The agar surface should be dry prior to inoculating.
2. Inoculate the stool specimen onto the medium as soon as possible after it is received in the laboratory.
3. Using a 1µl loop, apply the stool to a small area of the agar surface and streak for isolation.
4. Incubate plates aerobically at 35 to 37°C for 18-24 hours. **Do not incubate plates in CO_2**.
5. Observe plates for characteristic colonial morphology and color.
6. HardyCHROM™ CRE can detect carbapenem non-susceptible strains of *E. coli* and KES (*K. aerogenes, K. oxytoca, K. pneumoniae, E. cloacae* complex, and *S. marcescens*) within 18-24 hours. HardyCHROM™ CRE should not be read after 24 hours because specificity may be reduced if incubation is extended beyond 24 hours.

INTERPRETATION OF RESULTS

Organism	Description
Pink to magenta colonies	Presumptive positive for carbapenem non-susceptible *Escherichia coli*
Blue or Blue with a pink halo	Presumptive positive for carbapenem non-susceptible KES (*Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Enterobacter cloacae* complex, and *Serratia marcescens*)
Colonies that are not pink to magenta, blue, or blue with pink halos	Negative – No carbapenem non-susceptible *Escherchia coli* or KES detected.

Note: All pink or blue colonies should be sub-cultured to non-selective medium for bacterial identification and susceptibility testing. Pink or blue colonies should also undergo additional testing according to standardized methods to confirm the CRE phenotype.

VARIATION IN COLOR DEVELOPMENT

Color	Interpretation	Variation	Image
Pink	**True positive** *Escherichia coli*	**Pink**	
Blue	**True positive** KES (*Klebsiella aerogenes*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Enterobacter cloacae* complex, and *Serratia marcescens*)	**Blue to Light Blue**	
		Blue with Pink Halo	

Note: Color-blind individuals may encounter difficulty in distinguishing color differences on HardyCHROM™ CRE.

LIMITATIONS

1. **Do not incubate plates in a CO_2 atmosphere.**
2. Prolonged exposure of the medium to light may result in reduced recovery or may affect the intensity of the chromogenic reaction. Minimize exposure of HardyCHROM™ CRE to light both before and during incubation.
3. 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.
4. Color-blind individuals may encounter difficulty in distinguishing color differences on HardyCHROM™ CRE.
5. 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.
6. Use of transport media with swabs has not been evaluated on HardyCHROM™ CRE.
7. The performance of HardyCHROM™ CRE has not been evaluated with rectal or perianal swabs.
8. Pediatric samples were not extensively analyzed during the clinical investigation; therefore, the performance of this medium for evaluating pediatric samples is unknown.
9. Colonies that are colorless, off-white, yellow or green, or have fuzzy/mold appearance should not be considered carbapenem non-susceptible *Escherichia coli* or KES (*Klebsiella aerogenes*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Enterobacter cloacae* complex, and *Serratia marcescens*).
10. 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.
11. 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 false-negatives.
12. 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.
13. 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.
14. Analytical testing of HardyCHROM™ CRE has shown that *Aspergillus brasiliensis* may appear as blue and fuzzy at 22 to 24 hours.
15. The colony size of all target carbapenem non-susceptible organisms (except *Serratia marcescens*) may appear smaller in the presence of high concentration 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.
16. In the analytical mixed infection study, 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.

17. Do not use Kovacs Indole Reagent on dark rose or pink colonies as the colony color may interfere with the red color of a positive indole reaction. Use only dimethylaminocinnamaldehyde (DMACA - Indole Spot Reagent, Cat. no. Z65) for indole testing.
18. Some *Enterobacter cloacae* complex isolates may appear as pink colonies on HardyCHROM™ CRE and be falsely considered as *E. coli*.
19. 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 incubation, plates should be incubated at 35 to 37°C for up to 24 hours.
20. 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.

Refer to the document "Limitations of Procedures and Warranty" for more information.

PERFORMANCE CHARACTERISTICS

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.

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 or 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%) 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) color, was determined to be the LoD. The determined LoD was confirmed by testing HardyCHROM™ CRE with five replicate dilutions of the determined LoD concentration. HardyCHROM™ CRE was able to recover all strains tested at a LoD of $1.5x10^2$ CFU/mL in CRE-free stool specimen with 100 µL inoculum.

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[1]	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
Enterobacter 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 SPECIFICITY

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 5x LoD ($7.5x10^3$ CFU/mL) and inoculated to HardyCHROM™ CRE using a 10µL. 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.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Klebsiella pneumoniae ATCC® BAA-1705**	A	18-24hr	35ºC	Aerobic	Growth; dark blue colonies
Escherichia coli ATCC® BAA-2469	A	18-24hr	35ºC	Aerobic	Growth; medium sized rose to magenta colonies with darker pink centers
Klebsiella pneumoniae ATCC® 700603	B	18-24hr	35ºC	Aerobic	Inhibited

Refer to the document "Inoculation Procedures for Media QC" on the Hardy Diagnostics Technical Document website for more information.

***Inoculation Method**

METHOD A

Suspend three to five isolated colonies in a small volume of Tryptic Soy Broth (TSB) and incubate for 4 to 5 hours. Adjust the turbidity to match that of a 0.5 McFarland standard. Dilute the cell suspension to 1:100 in TSB or normal saline. Inoculate the test plate with a 10uL calibrated loop of the diluted suspension. This will provide approximately 10^3 to 10^4 CFU per plate. Plates are streaked in four quadrants for isolation.

METHOD B

Use the same cell suspension (equivalent to a 0.5 McFarland standard) described in "Method A" and dilute to 1:10 in Tryptic Soy Broth (TSB). Inoculate the plate as described in "Method A" with a 10uL calibrated loop.

This should result in 10^4 to 10^5 CFU per plate. A non-inhibitory plate (e.g. TSA) is inoculated at the same time to serve as a positive control.

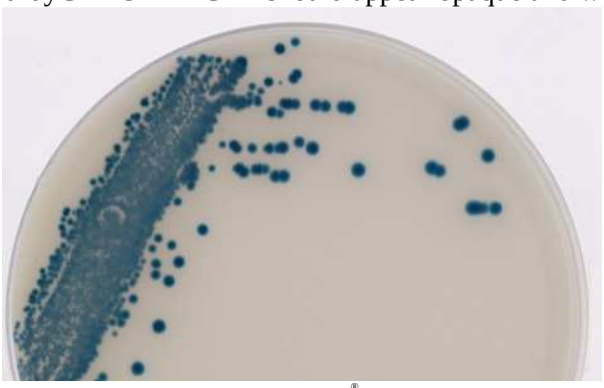
******According to CLSI document M100, *Klebsiella pneumoniae* ATCC® BAA-1705 may undergo a spontaneous loss of the plasmid encoding the carbapenemase, leading to false-negative QC results.(11) To avoid false-negative QC results, K. pneumoniae ATCC® BAA-1705 should be subbed to or maintained in a carbapenem-containing medium prior to inoculating onto HardyCHROM™ CRE Agar. Users can meet this maintenance requirement by following either of these options:

a. Maintain *K. pneumoniae* ATCC® BAA-1705 by subbing week-to-week on non-selective media, such as Tryptic Soy Agar (Cat. no. G60) or Blood Agar (Cat. no. A10). When preparing to QC HardyCHROM™ CRE Agar, directly sub K. pneumoniae ATCC® BAA-1705 to a HardyCHROM™ CRE Agar plate and incubate overnight. Use this 18-24 hour growth to prepare suspension for QC testing.
b. Directly inoculate *K. pneumoniae* ATCC® BAA-1705 into 5ml of Tryptic Soy Broth (Cat. no. K89 or R30), add a 10µg ertapenem (Cat. no. 232174) or meropenem (Cat. no. 231703) disc, and incubate overnight. When preparing to QC HardyCHROM™ CRE Agar, use this turbid growth to prepare suspension for QC testing. After overnight incubation of the suspension, the tube may be stored at 2-8°C (tightly capped) for up to one month and used to prepare future suspensions for QC testing.

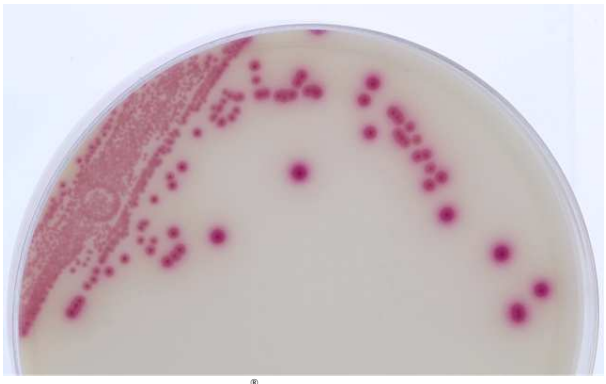
Check for signs of contamination and deterioration. Users of commercially prepared media may be required to perform quality control testing with at least one known organism to demonstrate growth or a positive reaction, and at least one organism to demonstrate inhibition or a negative reaction (where applicable).

PHYSICAL APPEARANCE

HardyCHROM™ CRE should appear opaque and white in color.



Klebsiella pneumoniae (ATCC® BAA-1705) colonies growing on HardyCHROM™ CRE (Cat. no. G323) incubated aerobically for 24 hours at 35 deg. C.



Escherichia coli (ATCC® BAA-2469) colonies growing on HardyCHROM™ CRE (Cat. no. G323) incubated aerobically for 24 hours at 35 deg. C.

Uninoculated plate of HardyCHROM™ CRE (Cat. no. G323)

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HDQA 2207D [C]

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