IFU

Instructions for Use

HARDY DIAGNOSTICS
A Culture of Service™

HardyCHROM™ ESBL

Cat. no. G321	HardyCHROM™ ESBL, 15x100mm Plate, 18ml	10 plates/bag

INTENDED USE

HardyCHROM™ ESBL is a selective and differential chromogenic medium which is intended for the qualitative and presumptive detection from stool specimens of: 1) *Enterobacteriaceae* that are potentially non-susceptible to ceftazidime and cefpodoxime; and 2) Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli, Klebsiella pneumoniae* and *Klebsiella oxytoca*.

The test is performed on stool specimens from patients at risk of harboring *Enterobacteriaceae* that are non-susceptible to 3rd generation cephalosporins or ESBL-producing *E. coli*, *K. pneumoniae* and *K. oxytoca*, and is intended as an aid in the detection, identification of colonization and control of these bacteria in a healthcare setting. HardyCHROM™ ESBL is not intended to diagnose infection or to guide or monitor treatment for infections. 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, blue or yellow/gold colonies on HardyCHROM™ ESBL does not preclude the presence of *Enterobacteriaceae* that are non-susceptible to 3rd generation cephalosporins or ESBL producing organisms.

SUMMARY AND PRINCIPLES

HardyCHROM™ ESBL is a selective and differential chromogenic medium containing an extended-spectrum beta-lactam that is intended for the presumptive detection and isolation of *Enterobacteriaceae* non-susceptible to 3rd generation cephalosporins. Chromogenic substrates in the medium allow for differentiation of *Enterobacteriaceae* non-susceptible to 3rd generation cephalosporins as bacteria that can grow and utilize the chromogens and produce a colored colony. *Enterobacteriaceae* not susceptible to 3rd generation cephalosporins will produce colonies of varying size that are pink, blue, blue with pink halos, and yellow/gold.

HardyCHROM™ ESBL can also be used as a screening medium for *K. pneumoniae, K. oxytoca,* and *E. coli* that produce an extended-spectrum beta-lactamase (ESBL). ESBL-producing *K. pneumoniae* and *K. oxytoca*. produces large, dark blue colonies. ESBL-producing *Escherichia coli* produce colonies that are rose to magenta in color, with darker pink centers.

The selective components in HardyCHROM™ ESBL are designed to inhibit the growth of yeast, gram-positive bacteria, and gram-negative extended-spectrum beta-lactam-sensitive bacteria (i.e. strains susceptible to a cephalosporin).

FORMULA

Ingredients per liter of deionized water:*

Peptones	20.0gm
Sodium Chloride	5.0gm
Chromogenic Mixture	0.2gm
Inhibitory and Selective Agents	3.1gm
Agar	15.0gm

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

Final pH 7.0 +/- 0.2 at 25°C

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

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

Refer to the document "Storage" on the Hardy Diagnostics Technical Document website for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as 10µL inoculating loops, specimen transport materials, other culture media, swabs, incubators, etc., as well as serological and biochemical reagents, are not provided.

Stool specimens unpreserved and preserved in C&S Medium Transport, Cary Blair Formula by Medical Chemical (Cat. no. 28050510, 280505 or 280505WB) have been validated as appropriate specimens for use on HardyCHROM™ ESBL.

ESBL-positive Quality Control Organisms for target species: ESBL+ *Klebsiella pneumoniae* (ex: ATCC® 700603), ESBL+ *Klebsiella oxytoca* (ex: ATCC® 51983), ESBL+ *Escherichia coli* (ATCC® BAA-196), 3rd generation cephalosporin non-susceptible *Proteus mirabilis* (ex: ATCC® BAA-856**),** and negative Quality Control organism (ex: *Escherichia coli* ATCC® 25922).

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 the media 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" on the Hardy Diagnostics Technical Document website for more information.

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

PROCEDURE

Clinical Procedure

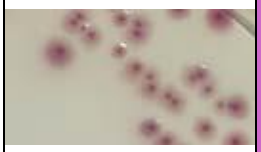
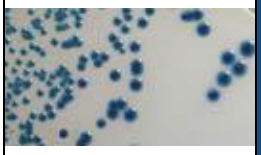
Specimen Transport and Storage:

The medium has been evaluated for use with stool specimens, raw and preserved in C&S Medium Transport, Cary Blair formula (Cat. no. 28050510, 280505 or 280505WB). Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated into C&S Medium Transport, Cary Blair Formula and refrigerated until inoculation. An internal investigation found that all target organisms tested (*E. coli, K. oxytoca, K. pneumoniae, E. cloacae, P. mirabilis*) were stable and recoverable in raw, unpreserved stool and stool preserved in C&S Medium Transport, Cary Blair formula for up to seven days when stored between 2-8°C. However, when stored at room temperature (23-24°C), organisms are only recoverable from raw stool for up to four hours and from stool preserved in C&S Medium Transport, Cary Blair formula for up to 24 hours. Consult listed references for more information on specimen collection.[2-5]

Method of Use:

1. Allow the plates to equilibrate to room temperature. The agar surface should be dry prior to inoculating.
2. Inoculate the specimen onto the medium as soon as possible after it is received in the laboratory.
3. Using a 10µl 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™ ESBL can detect ESBL-producing and cephalosporin non-susceptible strains of *Enterobacteriaceae* within 18-24 hours. HardyCHROM™ ESBL should not be read after 24 hours because specificity may be reduced if incubation is extended beyond 24 hours.

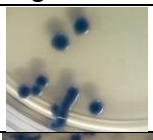
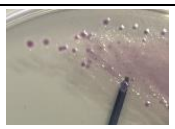
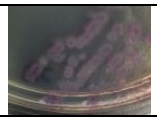
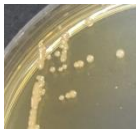
INTERPRETATION OF RESULTS

24 hour Incubation	Interpretation /Recommended Action	Photo	Color
Pink to magenta colonies	Presumptive positive for 3rd generation cephalosporin non-susceptible *Enterobacteriaceae*. Subculture required for identification and antimicrobial susceptibility testing. Presumptive positive for ESBL-producing *E. coli*. Subculture required for identification and confirmation of ESBL phenotype.		
Blue to purple colonies with or without pink halo	Presumptive positive for 3rd generation cephalosporin non-susceptible *Enterobacteriaceae*. Subculture required for identification and antimicrobial susceptibility testing. Presumptive positive for ESBL-producing *K. pneumoniae* or *K. oxytoca*. Subculture required for identification and confirmation of ESBL phenotype.		
Yellow/Gold colonies with golden-orange halo in the surrounding medium	Presumptive positive for 3rd generation cephalosporin non-susceptible *Enterobacteriaceae*. Subculture required for identification and antimicrobial susceptibility testing.		
Colonies that are not pink to magenta, blue, blue with pink halos, or yellow/gold*	Negative – No ESBL-producing or 3rd generation cephalosporin non-susceptible *Enterobacteriaceae* detected.		

* Colonies that are colorless, white, off-white, yellow or green should not be considered as ESBL-producing or 3rd generation cephalosporin non-susceptible *Enterobacteriaceae*.

Note: All pink, blue or yellow/gold 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 ESBL phenotype.

Variations of color development

Color	Variants	Image	Color	Variants	Image
Blue	**Blue** (i.e., *Klebsiella pneumoniae or Klebsiella oxytoca)*		Pink	**Pink** (i.e., *Escherichia coli)*	
	Blue with Pink Halo (i.e., *Klebsiella oxytoca)*			**White with Pink Center** (i.e., ***Enterobacteriaceae*** *Hafnia alvei*)	
	Blue and Pink or Pink with Blue Center (i.e., ***Enterobacteriaceae*** *Citrobacter freundii and Enterobacter cloacae*)			**White with Purple Center** (i.e., ***Enterobacteriaceae*** *Hafnia alvei*)	
	Light Blue (i.e., ***Enterobacteriaceae*** *Citrobacter amalonaticus*)			**Pink and White** (i.e., ***Enterobacteriaceae*** *Hafnia alvei*)	
	Dark Blue (i.e., ***Enterobacteriaceae*** *Enterobacter aerogenes*)		Yellow/Gold	**Yellow/ Gold Media** (i.e., *Proteus mirabilis*)	
	White with Blue Center (i.e., ***Enterobacteriaceae*** *Citrobacter amalonaticus*)				

LIMITATIONS

General

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™ ESBL to light both before and during incubation.
3. Cultures on HardyCHROM™ ESBL 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™ ESBL.
5. The clinical performance of HardyCHROM™ ESBL was established with fresh stool samples. Compatibility with stools in C&S Medium Transport (Cary Blair Formula) was established analytically. Clinical performance with stools preserved in C&S Medium Transport (Cary Blair Formula) has not been established.
6. Use of transport media with swabs has not been evaluated on HardyCHROM™ ESBL.
7. The performance of HardyCHROM™ ESBL 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. A small percentage of *E. coli* lacking the enzyme beta-D-glucuronidase may appear as white to off-white colonies on HardyCHROM™ ESBL and would be considered false-negatives. Clinical studies using HardyCHROM™ ESBL identified two of these organisms out of 549 *E. coli* recovered.
10. A small percentage of *Candida tropicalis* may appear as pink colonies on HardyCHROM™ ESBL and would be considered false-positives. Only one of these organisms was recovered out of 1,559 specimens tested during clinical studies.

Detection of 3rd-generation cephalosporin non-susceptible *Enterobacteriaceae*

1. A lack of growth or the absence of pink, blue or yellow/gold colonies on HardyCHROM™ ESBL does not preclude the presence of *Enterobacteriaceae* that are non-susceptible to 3rd generation cephalosporins. 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™ ESBL medium but which are non-susceptible to other 3rd generation cephalosporins. In analytical testing, 3rd generation cephalosporin non-susceptible strains of *Hafnia alvei*, *Morganella morganii*, *Providencia stuartii* and *Shigella sonnei* were recovered but did not produce pink, blue or yellow/gold colonies after incubation for 24h on HardyCHROM™ ESBL.
2. *Acinetobacter baumannii* may grow as white/colorless colonies on HardyCHROM™ ESBL and when present at high concentration may suppress growth of 3rd generation cephalosporin non-susceptible *E. coli*.
3. In the presence of *Acinetobacter baumannii* or *Pseudomonas fluorescens*, colonies of 3rd generation cephalosporin non-susceptible *K. pneumoniae* may appear dark blue and small, even after incubation for 24 hours.
4. In the event of a mixed infection, the accuracy of this device for detecting 3rd-generation cephalosporin non-susceptible *Enterobacteriaceae* in the presence of other bacteria at a concentration higher than 1×10^8 CFU/mL has not been determined and is unknown.

5. Some 3rd generation cephalosporin non-susceptible strains of *Hafnia* may produce white colonies with pink centers to fully pink colonies causing misinterpretation as *E. coli*. It is important to subculture isolated colonies to non-selective medium to confirm the identity and susceptibility of pink or blue colonies from HardyCHROM™ ESBL when screening for 3rd-generation cephalosporin non-susceptible *Enterobacteriaceae*.
6. The performance of HardyCHROM™ ESBL has not been evaluated with *Cronobacter*, *Salmonella*, and *Yersinia*.

Detection of ESBL-producing organisms

1. A lack of growth or the absence of pink or blue colonies on HardyCHROM™ ESBL does not preclude the presence of ESBL-producing organisms. 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™ ESBL medium but which are non-susceptible to other 3rd generation cephalosporins.
2. ESBL-producing organisms with ceftazidime MIC's below 2µg/mL or disk zone diameter > 22mm may not be recovered on HardyCHROM™ ESBL.
3. *Acinetobacter baumannii* may grow as white/colorless colonies on HardyCHROM™ ESBL and when present at high concentration may suppress growth of ESBL-producing *E. coli*.
4. In the presence of *Acinetobacter baumannii* or *Pseudomonas fluorescens*, colonies of ESBL producing *K. pneumoniae* may appear dark blue and small, even after incubation for 24 hours.
5. In the event of a mixed infection, the accuracy of this device for detecting ESBL-producing *E. coli*, *K. pneumoniae*, and *K. oxytoca* in the presence of other bacteria at a concentration higher than 1×10^8 CFU/mL has not been determined and is unknown.
6. Some 3rd generation cephalosporin non-susceptible strains of *Hafnia* may produce white colonies with pink centers to fully pink colonies causing misinterpretation as *E. coli*. It is important to subculture isolated colonies to confirm the identity and ESBL-phenotype of pink or blue colonies from HardyCHROM™ ESBL when screening for ESBL-producing microorganisms.
7. While HardyCHROM™ ESBL is a screening medium for ESBL-producing microorganisms, *Enterobacter* spp., *Hafnia* spp.,*Citrobacter* spp., and other *Enterobacteriaceae* that do not have CLSI interpretive screening guidelines for ESBL production may appear as false positive on HardyCHROM™ ESBL (growth with pink, variations of blue, or yellow/gold colony coloration). It is important to subculture isolated colonies to non-selective medium to confirm identification, susceptibility and ESBL phenotype of pink or blue colonies from HardyCHROM™ ESBL when screening for ESBL-producing microorganisms.
8. *Enterobacteriaceae* with de-repressed AmpC enzyme production or those that produce a carbapenemase are expected to grow on HardyCHROM™ ESBL. It is important to subculture isolated colonies to confirm identification and ESBL-resistance of pink or blue colonies from HardyCHROM™ ESBL when using the product to screen for ESBL-producing microorganisms (*E. coli*, *K. oxytoca*, and *K. pneumoniae)*.
9. Some ESBL-producing strains that produce enzymes such as CTX-M-116 and CTX-M-130 are more susceptible to the selective agent in the medium and were recovered on HardyCHROM™ ESBL at a concentration of 10^3 CFU/mL (100 CFU/plate). All other ESBL genotypes evaluated were recovered at a concentration of 10^2 CFU/mL (10 CFU/plate).

Refer to the document "Limitations of Procedures and Warranty" on the Hardy Diagnostics Technical Document website for more information.

EXPECTED VALUES

In the prospective clinical evaluation described below, the overall prevalence of 3rd generation cephalosporin non-susceptible *Enterobacteriaceae* organisms was 23.9%. The overall prevalence of ESBL-producing *E. coli* and *K. pneumoniae*/*oxytoca* was 10.6% among the specimens tested.

PERFORMANCE CHARACTERISTICS

Performance of HardyCHROM™ ESBL was evaluated at three geographically diverse hospitals with fresh stool specimens. The recovery of ESBL-producing *K. pneumoniae, K. oxytoca, and E. coli,* on HardyCHROM™ ESBL was compared to routine culture, defined as the selective enrichment of microorganisms in Tryptic Soy Broth (TSB) containing either 1µg/mL ceftazidime or 1µg/mL cefotaxime, followed by subculture to MacConkey Agar. Organisms that grew on MacConkey Agar were identified using an FDA-cleared identification system. Quality control was performed in parallel every day of testing. Results from days of QC failure were excluded from the analysis.

Confirmation of ESBL-resistance and 3rd generation cephalosporin non-susceptibility was performed using traditional Kirby-Bauer Antimicrobial Susceptibility Test following the device manufacturer's instructions. (6) Identification of organisms that grew on HardyCHROM™ ESBL was confirmed using an FDA-cleared identification system.

A total of 1,687 samples were tested against routine culture, 128 specimens did not meet enrollment criteria, and were therefore excluded from the analysis. Of the remaining 1,559 valid samples tested, a total of 166 isolates were recovered on HardyCHROM™ ESBL as ESBL-producing with concordant results obtained on traditional culture and confirmed by antimicrobial susceptibility testing and identification. A total of 373 isolates were recovered on HardyCHROM™ ESBL as non-susceptible to 3rd generation cephalosporins with concordant results obtained on traditional culture and confirmed by antimicrobial susceptibility testing and identification.

Product performance with prospectively collected clinical samples is summarized below:

HardyCHROM™ ESBL for use in confirming the presence of 3rd generation cephalosporin non-susceptible *Enterobacteriaceae* vs. confirmation of 3rd generation cephalosporin non-susceptible *Enterobacteriaceae* from traditional culture

Morphology vs. Confirmed Non-Susceptible 18 hr[1]

Site	TP	FP	FN	TN	% Sensitivity	95% CI		% Specificity	95% CI	
1	85	15	9	526	**90.4**	**82.8**	**94.9**	**97.2**	**95.5**	**98.3**
2	71	25	6	678	**92.2**	**84.0**	**96.4**	**96.4**	**94.8**	**97.6**
3	217	53	22	638	**90.8**	**86.5**	**93.8**	**92.3**	**90.1**	**94.1**
Overall	**373**	**93**	**37**	**1842**	**91.0**	**87.8**	**93.4**	**95.2**	**94.1**	**96.1**

[1]The same performance was observed at 18 and 24 hours

HardyCHROM™ ESBL used to screen for ESBL-producing *K. oxytoca, K. pneumoniae,* and *E. coli* vs. confirmation of ESBL-resistance from traditional culture

Morphology vs. Confirmed ESBL 18 hr[1]

Site	TP	FP	FN	TN	% Sensitivity	95% CI		% Specificity	95% CI	
1	26	67	1	456	**96.3**	81.7	99.3	**87.2**	84.1	89.8
2	25	65	0	564	**100.0**	86.7	100.0	**89.7**	87.0	91.8
3	115	148	4	471	**96.6**	91.7	98.7	**76.1**	72.6	79.3
Overall	**166**	**280**	**5**	**1894**	**97.1**	**93.3**	**98.7**	**87.1**	**85.6**	**88.5**

[1]The same performance was observed at 18 and 24 hours

To supplement testing of prospectively collected clinical specimens, a total of 50 contrived specimens were also evaluated. Patient specimens were inoculated with known resistant (ESBL producing or non-susceptible to third generation cephalosporin) organisms at LoD and tested against routine culture. Results are summarized below.

Contrived Specimen Morphology vs. Ref. NS 18 hr[1]

Performance Metric	Overall (n=53)
PPA	47/48 97.9% (89.1% - 99.6%)
NPA	3/5 60.0% (23.1% - 88.2%)

[1] The same performance was observed at 18 hr and 24 hr

Contrived Specimen Morphology vs. ESBL Ref 18 hr[1]

Performance Metric	"EC" Pink	"KP/KO" Blue
PPA	19/19 100% (83.1% - 100%)	26/26 100% (87.1% - 100%)
NPA	31/34 91.2% (77.0% - 97.0%)	25/27 92.6% (76.6% - 97.9%)

[1] The same performance was observed at 18 hr and 24 hr

RECOVERY RATE

HardyCHROM™ ESBL was evaluated to determine the recovery (limit of detection (LoD)) of 3rd generation cephalosporin non-susceptible *Enterobacteriaceae* in specimen matrix. One isolate of five representative genera of *Enterobacteriaceae* with ceftazidime MIC's varying between 1µg/mL and >32µg/mL and cefotaxime MICs varying between 1µg/mL and >16µg/mL were evaluated for recovery on HardyCHROM™ ESBL. Sheep Blood Agar (BAP) plates were used to determine the concentration of organisms present in each dilution. At 10^3 CFU/mL, there was no discernable difference in recovery. Variable recovery was seen at lower concentrations.

In addition, HardyCHROM™ ESBL was also evaluated to determine the recovery of ESBL-producing *E. coli*, *K. oxytoca* and *K. pneumoniae* in stool specimen matrix. Two strains of each species with varying ceftazidime MIC values and different β-lactamase genotypes were evaluated for recovery on HardyCHROM™ ESBL. Sheep Blood Agar (BAP) plates were used to determine the concentration of organisms present in each dilution. At 10^3 CFU/mL of stool (10 CFU/plate when using a 10µL inoculating loop), there was no discernable difference in recovery. Variable recovery was seen at lower concentrations.

ANALYTICAL REACTIVITY

Internal testing was performed using 54 characterized ESBL-producing strains of *E. coli*, *K. pneumoniae* and *K. oxytoca* with confirmed ESBL phenotype that were associated with various β-lactamase genotypes. The strains were tested using a clean suspension in the absence of stool matrix. HardyCHROM™ ESBL was able to recover 54 of 54 (100%) of the ESBL-producing strains after 24 hours of incubation. Some strains containing enzymes such as CTX-M-116 and CTX-M-130 are more susceptible to the selective agent in the medium and were recovered on HardyCHROM™ ESBL at an inoculum of 100 CFU/plate; all other strains were recovered from an inoculum of 10 CFU/plate.

Summary of Analytical Sensitivity Testing

Species	n	β-lactamase Genotype
E. coli	24	**TEM** (6, 12, 19, 21, 29, 210, 214, 215), **CTX-M** (1, 2, 3, 8, 14, 15, 24, 27, 28, 40, 55, 75, 79, 116, 125, 130), TEM-OSBL
K. pneumoniae	26	**SHV** (2, 11, 14, 18, 31, 55, 83, 89, 90, 108, 120, 133, 173, 178, 179, 180, 182), **TEM** (4, 11, 129), **CTX-M** (12, 14, 15, 22, 38, 40, 64, 74, 124), **VEB-1, SHV-OSBL, TEM-OSBL**
K. oxytoca	4	**TEM**(52), **CTX-M** (22, 30, 75), **SHV** (7, 12), DHA-1

Note: 53/54 strains (98.1%) were also non-susceptible to at least one 3rd generation cephalosporin and produced results on HardyCHROM™ ESBL that were consistent with this phenotype

In addition, testing was performed with 21 representative strains of *Citrobacter*, *Enterobacter*, *Escherichia*, *Hafnia*, *Klebsiella*, *Morganella*, *Proteus*, *Providencia*, *Raoultella*, *Serratia*, and *Shigella* that were non-susceptible to at least one 3rd generation cephalosporin. Organisms showed a range of MICs to each antibiotic: CAZ (2 µg/mL to >32 µg/mL), CTX (0.38 µg/mL to >16 µg/mL), and CPD disk zone sizes (6-27 mm). The strains were tested using a clean suspension in the absence of stool matrix containing an inoculum of approximately 10 CFU/plate. HardyCHROM™ ESBL was able to recover 21 of 21 (100%) of the 3rd generation cephalosporin non-susceptible strains after 24 hours of incubation, although, only 15 of 21 (71.4%) showed Pink, Blue, or Yellow/Gold color development. Non-susceptible isolates of *Cronobacter*, *Salmonella*, and *Yersinia* were not evaluated.

ANALYTICAL SPECIFICITY

To determine the potential for cross-reactivity on HardyCHROM™ ESBL, internal testing was conducted with ESBL non-producing and 3rd generation cephalosporin susceptible species as well as non-*Enterobacteriaceae*. A total of 99 strains were tested by plating 10µL of a 10^8 CFU/mL suspension of each organism on HardyCHROM™ ESBL. Included in the study were 52 strains of *Enterobacteriaceae* and 47 non-*Enterobacteriaceae*. The majority of non-*Enterobacteriaceae* species did not grow on HardyCHROM™ ESBL medium and none exhibited pink, blue or yellow/gold colonies. Of the *Enterobacteriaceae*, 45/45 (100%) of those that were 3rd generation cephalosporin non-susceptible either did not grow on HardyCHROM™ ESBL or produced colony colors other than pink, blue or yellow/gold. Four strains (one each of *C. braakii*, *C. freundii*, *E. cloacae* and *Y. kristensenii*) that were non-susceptible to cefpodoxime were not recovered on HardyCHROM™ ESBL. Three carbapenem resistant and 3rd generation cephalosporin non-susceptible strains (*E. coli* (1) and *K. pneumoniae* (2) produced pink or blue colonies, as expected (one of these strains was ESBL negative; two were ESBL non-determinable).

Because non-ESBL producing organisms that are carbapenem resistant or which exhibit other beta-lactamase resistance mechanisms such as AmpC may produce pink, blue or yellow/gold colonies on HardyCHROM™ ESBL, it is important to subculture such colonies for identification and antimicrobial susceptibility testing to confirm the ESBL-producing phenotype.

MICROBIAL INTERFERENCE

A study was conducted using known concentrations of ESBL-producing *K. pneumoniae*, *K. oxytoca*, and *E. coli* with ten non-target organisms that grow, but which exhibit non-target colors on HardyCHROM™ ESBL. Suspensions of non-ESBL organisms were prepared at a concentration of at least 10^8 CFU/mL and mixed with suspensions of ESBL strains at the detection limit concentration (10^3 CFU/mL). HardyCHROM™ ESBL recovered all target organisms used in the presence of high concentrations of non-target organisms, although the number of colonies of *E. coli* recovered was reduced in the presence of high concentrations of *A. baumannii* and colonies of *K. pneumoniae* appeared unusually small in the presence of *Pseudomonas fluorescens.*

Non-target organisms used in microbial interference study
Geotrichum guilliermondii
Geotrichum candidum
Aspergillus brasiliensis
Penicillium rubens
Penicillium chrysogenum
Pediococcus acidilactici
Provicencia stuartii
Acinetobacter baumannii
Pseudomonas fluorescens
Stenotrophomonas maltophilia

INTERFERENCE STUDY

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

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

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

REPRODUCIBILITY

Prior to the start of the prospective clinical study, each site tested blinded panels of bacterial suspensions in transport medium on five separate days to evaluate the reproducibility of HardyCHROM™ ESBL. Each panel included duplicate suspensions of five 3rd generation cephalosporin non-susceptible strains of *Enterobacteriaceae* (*E. coli* (2), *Enterobacter cloacae* (1), *Klebsiella pneumoniae* (1), and *Klebsiella oxytoca* (1) at 10^3 CFU/mL) and five susceptible strains of bacteria (one each of *E. coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Shigella flexneri* and *Shigella sonnei* at 10^6 CFU/mL). Each day of testing, a 10µL loop of each bacterial suspension was used to inoculate a plate of HardyCHROM™ ESBL culture medium, which was then incubated for 24 hours at 35°C prior to being read by two independent operators (10 strains x 2 replicates x 5 days x 2 operators x 3 sites = 600 results). With regards to detection of 3rd generation cephalosporin non-susceptible *Enterobacteriaceae*, the overall PPA for all three sites was 98.2% (95.7% – 99.2%) and the overall NPA was 99.6% (98.0% - 99.9%).With regards to ESBL-producing *E. coli*, *K. oxytoca*, and *K. pneumoniae*, the overall PPA for all three sites was 98.1% (95.3% - 99.3%) and the overall NPA was 83.3% (79.0% - 86.9%).

Results of the Reproducibility Study at 24 hours for detection of **3rd generation cephalosporin non-susceptible** producing organisms.

	Positive Percent Agreement					Negative Percent Agreement				
	N	HC Positive	PPA %	low 95%	high 95%	*N*	HC Negative	NPA %	low 95%	high 95%
Day 1	60	59	98.3	91.1	99.7	60	59	98.3	91.1	99.7
Day 2	60	60	100.0	94.0	100.0	60	60	100.0	94.0	100.0
Day 3	52 [1]	50	96.2	87.0	98.9	60	60	100.0	94.0	100.0
Day 4	40 [2]	40	100.0	91.2	100.0	40 [2]	40	100.0	91.2	100.0
Day 5	60	58	96.7	88.6	99.1	60	60	100.0	94.0	100.0
Overall	272	267	98.2	95.7	99.2	280	279	99.6	98.0	99.9

Expected colony colors: Pink, Blue or Yellow/Gold

[1] Eight samples at Site 1 (four from each operator) were excluded due to an error in preparation

[2] 20 positive and 20 negative samples excluded from analysis due to control failure at Site 3

Results of the Reproducibility Study at 24 hours for detection of **ESBL-producing organisms**

	Positive Percent Agreement					Negative Percent Agreement				
	N	HC Positive	PPA %	low 95%	high 95%	*N*	HC Negative	NPA %	low 95%	high 95%
Day 1	48	48	100.0	92.6	100.0	72	60	83.3	73.1	90.2
Day 2	48	48	100.0	92.6	100.0	72	60	83.3	73.1	90.2
Day 3	40 [1]	38	95.0	83.5	98.6	72	60	83.3	73.1	90.2
Day 4	32 [2]	32	100.0	89.3	100.0	48 [2]	40	83.3	70.4	91.3
Day 5	48	46	95.8	86.0	98.9	72	60	83.3	73.1	90.2
Overall	216	212	98.1	95.3	99.3	336	280	83.3	79.0	86.9

Expected colony colors: *E. coli*: Pink; *K. pneumoniae/K. oxytoca*: Blue

[1] 8 samples at Site 1 (4 from each operator) were excluded due to an error in preparation

[2] 16 positive and 24 negative samples excluded from analysis due to control failure at Site 3

Note: Samples that contained *E. cloacae* and which exhibited blue colonies on HardyCHROM™ ESBL were considered "false positive" for ESBL-producing *E. coli* and *K. pneumoniae/K. oxytoca*. If these samples are omitted from the analysis, negative agreement was 279/280 (99.6%).

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® 700603*	A	24hr	35ºC	Aerobic	Growth; dark blue colonies
Klebsiella oxytoca Clinical strain	A	24hr	35ºC	Aerobic	Growth; dark blue colonies
Escherichia coli Clinical strain	A	24hr	35ºC	Aerobic	Growth; rose to magenta colonies
Proteus mirabilis Clinical strain	A	24hr	35ºC	Aerobic	Growth; yellow colonies with a gold halo
Candida albicans *ATCC® 10231*	B	24hr	35ºC	Aerobic	Partial to complete inhibition
Escherichia coli ATCC® 25922	B	24hr	35ºC	Aerobic	Inhibited

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

****METHOD A**

Suspend three to five isolated colonies in a small volume of Tryptic Soy Broth (TSB) and incubate for four to five 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.

USER QUALITY CONTROL

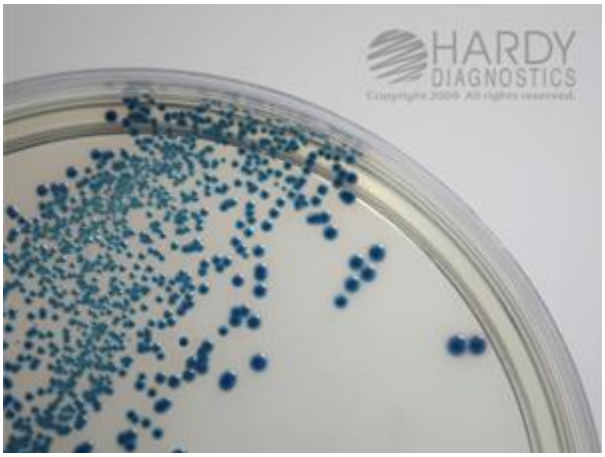
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificates of analysis (CofA) available from Hardy Diagnostics Certificates of Analysis website. In addition, refer to the following documents on the Hardy Diagnostics Technical Document website for more information on QC: "Introduction to Quality Control" and "Finished Product Quality Control Procedures," or see reference(s) for more specific information.

Example of strains appropriate for Quality Control testing

Organism	Strain	Expected Colony Color/Result
ESBL+ *Klebsiella pneumoniae*	ATCC® 700603	Blue
ESBL+ *Klebsiella oxytoca*	ATCC® 51983	Blue
ESBL+ *Escherichia coli*	ATCC® BAA-196	Pink
3rd generation cephalosporin non-susceptible *Proteus mirabilis*	ATCC® BAA-856	Yellow/gold
Escherichia coli (negative control)	ATCC® 25922	No Growth

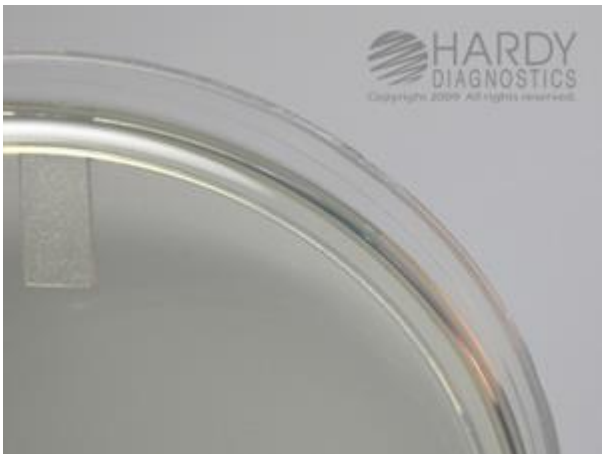
PHYSICAL APPEARANCE

HardyCHROM™ ESBL should appear translucent, and light amber in color.



Klebsiella pneumoniae (ATCC® 700603) colonies growing on HardyCHROM™ ESBL (Cat. no. G321). Incubated aerobically for 24 hours at 35 deg. C.

Escherichia coli (clinical strain) colonies growing on HardyCHROM™ ESBL (Cat. no. G321). Incubated aerobically for 24 hours at 35 deg. C.



Uninoculated plate of HardyCHROM™ ESBL (Cat. no. G321).

ATCC® is a registered trademark of the American Type Culture Collection.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Versalovic, J., et al. *Manual of Clinical Microbiology*. American Society for Microbiology, Washington, D.C.

3. Tille, P.M., et al. *Bailey and Scott's Diagnostic Microbiology,* C.V. Mosby Company, St. Louis, MO.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook,* Vol. I, II & III. American Society for Microbiology, Washington, D.C.

5. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*. J.B. Lippincott Company, Philadelphia, PA.

6. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. M100. CLSI, Wayne, PA.

7. Siegel, J.D. et al., and the Healthcare Infection Control Practices Advisory Committee, *2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings*, available from the Centers for Disease Control and Prevention at http://www.cdc.gov/hicpac/pdf/isolation/Isolation2007.pdf .

8. CLSI. *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline*. CLSI document M29. Wayne, PA: Clinical and Laboratory Standards Institute.

9. CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard*. CLSI document M2. Wayne, PA: Clinical and Laboratory Standards Institute.

10. CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard.* CLSI document M7. Wayne, PA: Clinical and Laboratory Standards Institute.

111516rlm

HARDY DIAGNOSTICS

1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: www.HardyDiagnostics.com
Email: TechService@HardyDiagnostics.com

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · Florida · New York · North Carolina

The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.