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

A. 510(k) Number:

K160512

B. Purpose for Submission:

To obtain a substantial equivalence determination for the HardyCHROM ESBL agar

C. Measurand:

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

Extended Spectrum β-Lactamase (ESBL) producing *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli*

D. Type of Test:

Selective and differential culture medium

E. Applicant:

Hardy Diagnostics

F. Proprietary and Established Names:

HardyCHROM ESBL

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.1700: Culture medium for antimicrobial susceptibility tests.

2. Classification:

   Class II

3. Product code:

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

4. Panel:

   83: Microbiology

H. Intended Use:

1. Intended use(s):

   HardyCHROM 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.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only.

   Do not incubate plates in a CO_2 atmosphere.

   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.

   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.

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*.

A lack of growth or the absence of pink or blue colonies on HardyCHROM ESBL does not preclude the presence of ESBL-producing organisms.

It is important to subculture isolated colonies to non-selective medium to confirm the identity, susceptibility and ESBL-phenotype of pink or blue colonies from HardyCHROM ESBL when screening for ESBL-producing microorganisms.

4. Special instrument requirements:

   None

I. Device Description:

HardyCHROM ESBL is a selective and differential chromogenic medium that contains extended-spectrum β-lactam antimicrobial agents. The medium is intended for use in screening of stool specimens for:

1) Detection of 3rd generation cephalosporin non-susceptible *Enterobacteriaceae*;

2) Detection and differentiation of strains of *E. coli* and *K. pneumoniae*/*K. oxytoca* that produce an Extended-Spectrum β-Lactamase (ESBL).

All isolates must be subcultured for confirmatory identification, antimicrobial susceptibility testing and, if required, epidemiological typing.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Bio-Rad VRESelect

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

   K122187

3. Comparison with predicate:

Similarities		
Item	**Device** **HardyCHROM ESBL** **K160512**	**Predicate** **Bio-Rad VRESelect** **K122187**
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.	VRESelect is a selective and differential chromogenic medium, containing 8 µg/mL of vancomycin, for the qualitative detection of gastrointestinal colonization of vancomycin-resistant *Enterococcus faecium* (VREfm) and vancomycin-resistant *Enterococcus faecalis* (VREfs) and to aid in the prevention and control of vancomycin-resistant *Enterococcus* (VRE) in healthcare settings. The test is performed on rectal swabs, or fecal specimens from patients to be screened for VRE colonization. VRESelect is not intended to diagnose VRE infection nor to guide or monitor treatment of infection. Results can be interpreted after 24 to 28 hours incubation. Subculture to non-selective media (*e.g.*, Trypticase Soy Agar with 5% sheep blood) is needed for further identification, susceptibility testing and epidemiological typing.
Technology	Bacterial growth with enzymatic metabolism of chromogenic substrate	Same

Similarities		
Item	**Device HardyCHROM ESBL K160512**	**Predicate Bio-Rad VRESelect K122187**
Sample	Stool	Rectal swab or stool
Inoculation	Direct	Same
Interpretation	Manual, visual	Same
Subculture	Required for confirmation of identity and antimicrobial susceptibility testing	Same

Differences		
Item	**Device HardyCHROM ESBL K160512**	**Predicate Bio-Rad VRESelect K122718**
Organisms Detected	*Cephalosporin non-susceptibility:* 3rd generation cephalosporin non-susceptible *Enterobacteriaceae* *ESBL:* ESBL-producing strains of *E. coli*, *K. pneumoniae* and *K. oxytoca*	Vancomycin resistant *E. faecalis* and *E. faecium*
Reference Method	Growth in Trypticase Soy enrichment broth with ceftazidime or cefotaxime, followed by subculture to MacConkey agar with biochemical identification and antimicrobial susceptibility testing of isolated colonies	Growth on Bile Esculin Azide Agar with 6µg/mL Vancomycin followed by biochemical identification and vancomycin MIC

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

CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 25th ed. CLSI supplement M100S. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.

L. Test Principle:

HardyCHROM ESBL is a selective and differential medium for the detection of bacteria belonging to the *Enterobacteriaceae* family that are non-susceptible to 3rd generation cephalosporins, including those that produce Extended Spectrum β-Lactamases.

The selective components in HardyCHROM ESBL culture medium are designed to inhibit the growth of yeasts and gram-positive bacteria, as well as gram-negative bacteria that are sensitive to 3rd generation cephalosporins. Organisms that grow on HardyCHROM ESBL are differentiated based on the metabolism of chromogenic substrates. ESBL-producing strains of *K. pneumoniae* and *K. oxytoca* exhibit blue colonies, whereas those of *E. coli* appear pink.

Colonies of *Enterobacteriaceae* that are non-susceptible to 3rd generation cephalosporins may appear pink, blue or yellow/gold.

Testing is performed by direct inoculation of the stool onto the culture medium. Cultures are read after incubation of the plates for 18-24 hours at 35-37°C in an aerobic atmosphere. All presumptive cephalosporin non-susceptible and ESBL-producing isolates must be sub-cultured for identification and antimicrobial susceptibility testing according to established methods.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

   The reproducibility of results obtained with HardyCHROM ESBL was evaluated in a study conducted at 3 sites using blinded panels comprised of duplicate suspensions of 10 strains of bacteria in transport medium. The panel members included 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 (1 each of *E. coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Shigella flexneri* and *Shigella sonnei* at 10^6 CFU/mL). The bacterial suspensions were prepared and shipped overnight to the test sites on ice prior to each day of testing. At the test sites, 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 2 independent operators. Cultures were inoculated on each of 5 days at each site (2 replicates x 5 days x 2 operators x 3 sites = 60 results per strain). The results of the study are summarized in **Tables 1** and **2** according to the respective criteria for detection of 3rd generation cephalosporin non-susceptible *Enterobacteriaceae* and ESBL-producing *E. coli*, *K. pneumoniae*/*K. oxytoca*.

Table 1. Reproducibility Study for detection of 3rd generation cephalosporin non-susceptible *Enterobacteriaceae*, stratified by day

	Positive Agreement			Negative Agreement		
	N	**HardyCHROM Positive**	**%**	***N***	**HardyCHROM Negative**	**%**
Day 1	60	59	98.3	60	59	98.3
Day 2	60	60	100	60	60	100
Day 3	52 [1]	50	96.2	60	60	100
Day 4	40 [2]	40	100	40 [2]	40	100
Day 5	60	58	96.7	60	60	100
Overall	**272**	**267**	**98.2**	**280**	**279**	**99.6**

Expected colony colors: Pink, Blue or Yellow/Gold
[1] 8 samples at Site 1 (4 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
Note: No 3rd generation cephalosporin non-susceptible organisms expected to produce yellow/gold colonies were included in the study

Table 2. Summary of the results of the Reproducibility Study for detection of ESBL-producing *E. coli* and *K. pneumoniae/K. oxytoca*, stratified by day

	Positive Agreement			Negative Agreement		
	N	**HardyCHROM Positive**	**%**	***N***	**HardyCHROM Negative**	**%**
Day 1	48	48	100	72	60	83.3
Day 2	48	48	100	72	60	83.3
Day 3	40 [1]	38	95.0	72	60	83.3
Day 4	32 [2]	32	100	48 [2]	40	83.3
Day 5	48	46	95.8	72	60	83.3
Overall	**216**	**212**	**98.1**	**336**	**280**	**83.3**

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%).

b. Linearity/assay reportable range:

Not applicable.

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

External Controls
External control strains of confirmed ESBL phenotype were tested on day of the Clinical Study as described in **Section M(3)**, below. Instructions for appropriate testing of External Controls are included in the Package Insert.

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

Species	Phenotype	ATCC No.	Expected Result
Escherichia coli	ESBL Negative	25922	No growth
Escherichia coli	ESBL Positive	51983	Pink Colonies
Klebsiella oxytoca	ESBL Positive	51983	Blue Colonies
Klebsiella pneumoniae	ESBL Positive	700603	Blue Colonies
Proteus mirabilis	3rd generation cephalosporin non-susceptible	BAA-856	Yellow/Gold Colonies

ATCC: American Type Culture Collection

Specimen Stability

A study was conducted to determine the acceptable duration of storage of stool specimens prior to inoculation on HardyCHROM ESBL. Testing was performed with "raw", unpreserved stool and stool in Culture & Sensitivity (C&S) Medium Transport. One strain each of ESBL-producing and 3rd generation cephalosporin non-susceptible *E. coli*, *K. pneumoniae* and *K. oxytoca* was included in the study. Each species was used to seed 3 ESBL-negative stool specimens (raw or in transport medium) which were then then stored at 2-8°C or ambient temperature (23-24°C). At specified intervals, samples of each stool specimen were inoculated onto HardyCHROM ESBL. Acceptable results were defined as recovery of the target organism with <1 $\log_{10}$ decrease in colony counts compared to baseline. The acceptance criteria were met for all 3 species in raw, unpreserved stool after storage for up to 4 hours at ambient temperature or up to 168 hours (7 days) at 2-8°C. For stool specimens diluted in C&S Medium, the acceptance criteria were met after storage for up to 24 hours at ambient temperature and up to 7 days at 2-8°C. Appropriate storage conditions for stool specimens prior to plating on HardyCHROM ESBL are included in the Package Insert.

d. Detection limit:

Limit of Detection

The analytical sensitivity of HardyCHROM ESBL was determined for two strains of each of *E. coli*, *K. pneumoniae*, *K. oxytoca* and *E. cloacae* (**Table 4**). Suspensions of each organism were diluted in stool matrix and plated on HardyCHROM ESBL. Parallel dilutions were also prepared in Tryptic Soy Broth/saline and plated on blood agar. All culture plates were incubated aerobically at 35°C and read after 24 hours. The LOD was estimated based on the highest dilution of the parental bacterial suspension at which growth was visible on the HardyCHROM medium and assuming a standard inoculum of 10µL stool per plate. The estimated LOD was verified by testing 5 additional replicates of each bacterial strain at the LOD target level. For each strain the LOD was estimated to be 10^3 CFU/mL stool (10 CFU/plate).

Table 4. Bacterial strains used to determine the analytical LOD of HardyCHROM ESBL

Species	Strain [1]	β-lactamase Genotype	Antimicrobial Susceptibility			EBSL Phenotype [2]	3rd Generation Cephalosporin Susceptibility [3]
			CT	TZ	CPD		
E. cloacae	856282	SHV-12(2be); TEM1(2b)	R	R	R	Not applicable	Non-Susceptible
E. cloacae	871550	CTX-M-9	R	R	R	Not applicable	Non-Susceptible
E. coli	900691	CTX-M-8	R	S	R	ESBL+	Non-Susceptible
E. coli	937062	TEM-19(2be)	S	S	R	Non-ESBL	Non-Susceptible
K. oxytoca	846222	TEM-52(2be)	R	R	R	ESBL+	Non-Susceptible
K. oxytoca	870581	CTX-M-30; CTX-M-75	R	S	R	ESBL+	Non-Susceptible
K. pneumoniae	866353	CTX-M-12	R	S	R	ESBL+	Non-Susceptible
K. pneumoniae	871347	SHV-2(2be)	R	R	R	ESBL+	Non-Susceptible

CT: Cefotaxime (S: ≤1; I = 2; R ≥4µg/mL); TZ: Ceftazidime (S: ≤4; I = 8; R: ≥16µg/mL); CPD: Cefpodoxime (S: ≥21; I = 18-20; R ≤17mm)
S: Susceptible; I: Intermediate: R: Resistant
[1] All strains were obtained from IHMA (International Health Management Associates)
[2] As determined by cefotaxime-cefotaxime/clavulanic acid (CT/CTL) and ceftazidime-ceftazidime/clavulanic acid (TZ/TZL)
[3] Defined as I or R for any one of CT, TZ or CPD

Analytical Reactivity/Inclusivity

The ability of HardyCHROM ESBL to recover potential ESBL-producing and/or 3rd generation cephalosporin non-susceptible strains of *E. coli*, *K. oxytoca* and *K. pneumoniae* was determined by testing a representative panel of strains with known cefotaxime, ceftazidime and cefpodoxime MICs, known genotypic resistance markers and confirmed ESBL phenotypes. The study included a total of 54 ESBL producing strains as shown in **Table 5**, all (100%) of which produced the expected colony color on HardyCHROM ESBL when inoculated at a concentration of 10 CFU/plate. Fifty-three of 54 strains (98.1%) were also non-susceptible to at least one 3rd generation cephalosporin and produced colony colors on HardyCHROM ESBL that were consistent with this phenotype.

Table 5. Summary of the genotypic characteristics of the strains of *E. coli*, *K. pneumoniae* and *K. oxytoca* evaluated in the Analytical Reactivity Study

Species	N	β-lactamase Genotypes Represented
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 (i.e., pink or blue colonies). The exception was 1 strain of *K. pneumoniae* that was ESBL positive and carried the SHV-11 and TEM-11 β-lactamase genes but which was susceptible to ceftazidime, cefotaxime and cefpodoxime.

In addition to the strains of ESBL-producing organisms in **Table 5**, testing was also conducted 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. Of

these, 15 strains (71.4%) exhibited pink, blue or yellow-gold colonies on HardyCHROM ESBL after incubation for 24 hours. Colonies of the remaining 6 strains (*H. alvei* (1), *M. morganella* (2), *P. stuartii* (2) and *S. sonnei* (1)) were recovered but did not exhibit one of the expected colony colors. The potential to obtain false negative results with HardyCHROM ESBL due to a lack of growth or failure to develop the expected colony color is noted in the Intended Use and is mitigated by including a Limitation in the device labeling (see **Section H(3)**, above).

e. Analytical specificity:

The analytical specificity of HardyCHROM ESBL was evaluated by testing a panel of organisms that are phylogenetically related to ESBL-producing species or that are likely to be found in stool specimens. Testing was performed by streaking 10µL of a 10^8 CFU/mL suspension of each organism on HardyCHROM ESBL and incubating at 35°C for 24 hours. The organisms tested are listed in **Tables 6** and **7** for *Enterobacteriaceae* (n = 52) and non-*Enterobacteriaceae* (n = 47), respectively.

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 colonies that were not pink, blue or yellow/gold. Three carbapenem resistant and 3rd generation cephalosporin non-susceptible strains (*E. coli* (1) and *K. pneumoniae* (2)) produced pink and blue colonies, as expected. Four strains (1 each of *C. braakii*, *C. freundii*, *E. cloacae* and *Y. kristensenii*) that were non-susceptible to cefpodoxime were not recovered on HardyCHROM ESBL. These results are mitigated by including appropriate Limitations in the device labeling (see **Section H(3)**, above).

Table 6. Summary of results from testing strains of *Enterobacteriaceae* in the HardyCHROM ESBL Analytical Specificity Study

Genus or Species	Number Tested	Number Non-susceptible			Growth on HardyCHROM ESBL [1]
		Cefotaxime	Ceftazidime	Cefpodoxime	
Citrobacter	6	0	0	2 [2]	0
Cronobacter	3	0	0	0	0
Enterobacter	4	1	0	1	0
Escherichia coli	4	1	1	1	1 [3]
Hafnia	3	0	0	0	0
Klebsiella oxytoca	3	0	0	0	0
Klebsiella pneumoniae	5	2	2	2	2 [4]
Morganella	2	0	0	0	0
Plesiomonas	1	0	0	0	0
Proteus	3	0	0	0	0
Providencia	3	0	0	0	0
Raoultella	3	0	0	0	0
Salmonella	3	0	0	0	0
Serratia	3	0	0	0	0
Shigella	3	0	0	0	0
Yersinia	3	0	0	1 [5]	0

NA: Not available

[1] Number with Pink or Blue colonies after 24 hours

[2] 1 *C. braakii* and 1 *C. freundii*; both with intermediate susceptibility to cefpodoxime

[3] Carbapenem resistant and resistant to cefotaxime, ceftazidime and cefpodoxime; ESBL non-determinable

[4] Both strains carbapenem resistant and resistant to cefotaxime, ceftazidime and cefpodoxime; 1 ESBL non-producer and 1 ESBL non-determinable

[5] *Y. kristensenii*

Table 7. Summary of non-*Enterobacteriaceae* species tested in the HardyCHROM ESBL Analytical Specificity Study

Species Name [1]	
Acinetobacter baumannii [2]	*Lactobacillus gasseri*
Aeromonas hydrophila	*Lactobacillus lactis*
Aspergillus sp.	*Listeria grayi*
Bacillus cereus	*Listeria monocytogenes*
Bacillus subtilis	*Micrococcus luteus*
Campylobacter coli	*Pediococcus acidilactici*
Campylobacter jejuni ssp. *jejuni*	*Penicillium aurantiogriseum*
Candida albicans	*Penicillium chrysogenum*
Candida glabrata	*Penicillium rubens* [2]
Candida tropicalis	*Pseudomonas aeruginosa*
Corynebacterium jeikeium	*Pseudomonas fluorescens* [2]
Enterococcus cassiflavus	*Saccharomyces cerevisiae*
Enterococcus durans	*Staphylococcus aureus* (3)
Enterococcus faecalis (3)	*Staphylococcus epidermidis*
Enterococcus faecium (2)	*Stenotrophomonas maltophilia*
Enterococcus hirae	*Streptococcus agalactiae* (2)
Enterococcus raffinosus	*Streptococcus gallolyticus*
Enterococcus saccharolyticus	*Streptococcus mitis*
Geotrichum candidum [2]	*Streptococcus pyogenes*
Geotrichum guilliermondii [2]	*Vibrio cholerae*
Lactobacillus acidophilus	

[1] The number of strains tested is shown in parenthesis if >1
[2] Growth observed on HardyCHROM ESBL but colonies not pink, blue or yellow/gold

f. Assay cut-off:

Not applicable.

g. Assay Interference:

Interfering Substances
A study was conducted to determine the ability to recover target organisms in the presence of potentially interfering substances. In order to represent a "worst case" scenario, the study was performed with representative strains of ESBL-producing and/or 3rd generation cephalosporin non-susceptible *E. coli*, *K. pneumoniae*, *K. oxytoca*, *E. cloacae* and *P. mirabilis* seeded into "raw", unpreserved stool at levels close to the LOD of HardyCHROM ESBL. The absence of interference was determined by the recovery of each species with the expected colony color and <1 $\log_{10}$ reduction in colony count compared with a control condition without the interfering substance. A list of the potentially interfering substances tested and their concentrations is provided in **Table 8**. None of the substances was found to interfere

with recovery of the target organisms at the concentrations tested.

Table 8. Substances tested for potential interference with growth on HardyCHROM ESBL

Category	Substance	Concentration (%) [1]
Antifungal	Nystop (Nystatin)	5%
	Lotrimin (Clotrimazole)	5%
	Lotrimin Ultra (Butenafine Hydrochloride)	5%
	Lamisil (Terbinafine Hydrochloride 1%)	5%
Antiseptic	Bactine (Benzalkonium Chloride)	1%
	Ethanol	1%
Biologic	Whole blood	5%
Contraceptive	Nonoxynol-9	5%
GI Medication	Pepto-Bismol (Bismuth Subsalicylate)	5%
	Prilosec OTC (Omeprazole)	5%
	Alka-Seltzer (Sodium carbonate/potassium carbonate)	5%
	Mylanta ($Al(OH)_3$)	5%
	Tums ($CaCO_3$)	5%
	Rolaids ($Mg(OH)_2$)	5%
	Milk of Magnesia ($Mg(OH)_2$)	5%
	Dulcolax (Sodium picosulfate solution)	5%
	Immodium AD (Loperamide)	5%
Lubricant	Mineral oil	10%
	Petroleum jelly	10%
	Fleet (Glycerin)	10%
	KY Jelly	10%
Other	C&S Medium Transport	75%
	Physiological Saline	10%
	Tween 80 (Polysorbate 80)	10%
Topical Medication	Preparation H (Hemorrhoid Cream)	10%
	Cortizone 10 (Hydrocortizone)	10%

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

Interfering Microorganisms

A study was performed to demonstrate the ability to discriminate ESBL-producing target organisms from non-target species that are capable of growing on HardyCHROM ESBL. Plates of HardyCHROM ESBL were inoculated with one strain each of *E. coli*, *K. pneumoniae* and *K. oxytoca* (~10^2 CFU/plate) in the presence and absence of high concentrations (>10^6 CFU/plate) of various "co-infecting" species that grew on HardyCHROM ESBL in the Analytical Specificity Study (**Table 9**).

Table 9. Species of potentially interfering microorganisms included in the Interference Study

Species Name	
Candida guilliermondii	*Pediococcus acidilactici*
Geotrichum candidum	*Providencia stuartii*
Aspergillus sp.	*Acinetobacter baumannii*
Penicillium rubens	*Pseudomonas fluorescens*
Penicillium chrysogenum	*Stenotrophomonas maltophilia*

All species listed had growth on HardyCHROM ESBL in the Analytical Specificity Study, although none produced pink, blue or yellow-gold colonies

In the presence of high levels of *Acinetobacter baumannii*, the number of colonies *E. coli* observed was substantially diminished compared to that obtained in the absence of the interfering species. In addition, in the presence of *A. baumannii* and *Pseudomonas fluorescens*, colonies of *K. pneumoniae* appeared smaller than those grown under control conditions. No other anomalies were observed in the presence of any of the potentially interfering microorganisms. The potential for an adverse effect on the performance of HardyCHROM from the presence of high concentrations of *A. baumannii* and *P. fluorescens* is mitigated by including a Limitation in the device labeling.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable.

b. Matrix comparison:

Comparison of Fresh vs Frozen Stool
To enable use of frozen stool specimens for analytical testing, a study was performed to compare the performance of HardyCHROM ESBL with fresh and frozen stool matrix. Stool samples (raw or diluted 1:5 in Culture & Sensitivity Medium Transport, Cary Blair Formula) were stored overnight at 2-8°C or frozen at -20°C, equilibrated to ambient temperature and then inoculated with representative strains of ESBL-producing and/or 3rd generation cephalosporin non-susceptible *Enterobacteriaceae*. A list of the organisms tested is provided in **Table 10**. An aliquot of each sample was plated on HardyCHROM ESBL culture medium. The number and color of colonies obtained were recorded after incubation of the culture plates for 18 and 24 hours. All the organisms tested were successfully recovered with the expected colony color from ≥9/10 fresh or frozen stool samples. No important differences in colony counts between cultures from fresh and frozen stool samples were observed. These results support the use of frozen stool samples (raw or diluted in C&S Medium Transport, Cary Blair Formula) for analytical testing.

Table 10. Organisms tested in the comparison of fresh and frozen stool matrix

Species	IHMA Strain [1]	ESBL	3rd Generation Cephalosporin
E. coli	946852	Positive	Non-susceptible
K. oxytoca	846222	Positive	Non-susceptible
K. pneumoniae	871347	Positive	Non-susceptible
E. cloacae	856282	Not applicable	Non-susceptible
P. mirabilis	862552	Not applicable	Non-susceptible

[1] International Health Management Associates strain designation

3. Clinical studies:

a. Clinical Sensitivity:

The performance of HardyCHROM ESBL was evaluated in a prospective Clinical Study conducted at three geographically diverse sites in the US. Recovery of ESBL-producing *E. coli* and *K. pneumoniae*/*K. oxytoca* from stool samples on HardyCHROM ESBL was compared to the results of a reference method comprised of enrichment culture of stool in Trypticase Soy Broth containing ceftazidime (1µg/mL) or cefotaxime (1µg/mL), followed by subculture to MacConkey agar. The same study was also used to evaluate the performance of HardyCHROM ESBL for recovery of cephalosporin non-susceptible *Enterobacteriaceae*. Organisms that grew on either MacConkey agar or HardyCHROM ESBL were identified by standard methods. Cephalosporin susceptibility and confirmed ESBL phenotype were determined using an FDA cleared MIC test and/or by disk diffusion according to CLSI M100.

A total of 1,687 stool samples were enrolled in the study. Of these, 128 were excluded from the analysis of performance due to protocol deviations. Isolates from a total of 1,559 samples were included in the analysis of performance.

To supplement testing of prospectively collected clinical specimens, a total of 50 contrived specimens were also evaluated. These were prepared by spiking organisms of known ESBL and cephalosporin susceptibility phenotype directly into stool matrix at a concentration of 3 x 10^3 CFU/mL. The results obtained on HardyCHROM ESBL were compared to those obtained by the same reference methods used for the prospective clinical specimens.

Detection of 3rd Generation Cephalosporin Non-Susceptible Enterobacteriaceae

Prospectively Collected Clinical Specimens
The performance of HardyCHROM ESBL for recovery of 3rd generation cephalosporin non-susceptible *Enterobacteriaceae* from prospectively collected clinical specimens is shown in **Table 11.** The same sensitivity and specificity were observed when plates were read after 18 and 24 hours. **Table 12** shows the agreement

between the colony color observed on the HardyCHROM ESBL plates and the identity and 3rd generation cephalosporin susceptibility of the isolates recovered.

Table 11. Performance of HardyCHROM ESBL with prospectively collected clinical specimens in comparison to the reference method, stratified by clinical site

3rd Generation Cephalosporin Non-susceptible *Enterobacteriaceae*				
Site 1		**Reference Method**		
		Positive	**Negative**	**Total**
HardyCHROM ESBL	**Positive** [1]	85	15	**100**
	Negative [2]	9	526	**535**
	Total	**94**	**541**	**635**
	Sensitivity	85/94 = 90.4% (82.8-94.9%) [3]		
	Specificity	526/541 = 97.2% (95.5-98.3%)		
Site 2		**Reference Method**		
		Positive	**Negative**	**Total**
HardyCHROM ESBL	**Positive**	71	25	**96**
	Negative	6	678	**684**
	Total	**77**	**703**	**780**
	Sensitivity	71/77 = 92.2% (84.0-96.4%)		
	Specificity	678/703 = 96.4% (94.8-97.6%)		
Site 3		**Reference Method**		
		Positive	**Negative**	**Total**
HardyCHROM ESBL	**Positive**	217	53	**270**
	Negative	22	638	**660**
	Total	**239**	**691**	**930**
	Sensitivity	217/239 = 90.8% (86.5-93.8%)		
	Specificity	638/691 = 92.3% (90.1-94.1%)		
Overall		**Reference Method**		
		Positive	**Negative**	**Total**
HardyCHROM ESBL	**Positive**	373	93	**466**
	Negative	37	1842	**1879**
	Total	**410**	**1935**	**2345**
	Sensitivity	373/410 = 91.0% (87.8-93.4%)		
	Specificity	1842/1935 = 95.2% (94.1-96.1%)		
	PPV	373/466 = 80.0% (76.2%-83.4%)		
	NPV	1842/1879 = 98.0% (97.3-98.6%)		

PPV: Positive Predictive value; NPV: Negative Predictive Value

Note: The same sensitivity and specificity were observed when plates were read after 18 and 24 hours

[1] Pink, Blue or Yellow/Green colonies

[2] Colonies other than Pink, Blue or Yellow/Green, or No Growth

[3] 95% Score Confidence Interval

Table 12. Agreement between colony color observed on HardyCHROM ESBL with prospectively collected clinical specimens and the identity and 3rd generation cephalosporin susceptibility of the isolates recovered from the HardyCHROM ESBL culture medium

		3rd Generation Cephalosporin Non-susceptible *Enterobacteriaceae*		
		Positive	**Negative**	**Total**
HardyCHROM ESBL	**Positive** [1]	448	18	**466**
	Negative [2]	21	1858	**1879**
	Total	**469**	**1876**	**2345**
	PPA	448/469 = 95.5% (93.3-97.1%)		
	NPA	1858/1876 = 99.0% (98.5-99.4%)		
	PPV	448/466 = 96.1% (94.0-97.5%)		
	NPV	1858/1879 = 98.9% (98.3-99.3%)		

PPA: Positive Percent Agreement; NPA: Negative Percent Agreement; PPV: Positive Predictive Value; NPV: Negative Predictive Value

[1] Pink, Blue or Yellow/Green colonies

[2] Colonies other than Pink, Blue or Yellow/Green or No Growth

Contrived Specimens

Table 13 shows the agreement between colony color observed on HardyCHROM ESBL and the identity and 3rd generation cephalosporin susceptibility of the isolates recovered from contrived specimens as determined by the reference method. There were no differences in positive or negative agreement when plates were read either 18 or 24 hours after inoculation.

Table 13. Agreement between the colony color observed on HardyCHROM ESBL with contrived specimens and organism identity and cephalosporin susceptibility as determined by the reference method

		3rd Generation Cephalosporin Non-susceptible *Enterobacteriaceae*		
		Positive	**Negative**	**Total**
HardyCHROM ESBL	**Positive** [1]	47	3	**50**
	Negative [2]	1	2	**3**
	Total	**48**	**5**	**53**
	PPA	47/48 = 97.9% (89.1-99.6%)		
	NPA	3/5 = 60.0% (23.1-88.2%)		

PPA: Positive Percent Agreement; NPA: Negative Percent Agreement

Note: The same positive and negative agreement were observed when plates were read after 18 and 24 hours

[1] Pink, Blue or Yellow/Green colonies

[2] Colonies other than Pink, Blue or Yellow/Green or No Growth

Detection of ESBL Producing E. coli and K. pneumoniae/K. oxytoca

Prospectively Collected Clinical Specimens

The performance of HardyCHROM ESBL for recovery of ESBL-producing strains of *E. coli* and *K. pneumoniae/K. oxytoca* from prospectively collected clinical samples is

shown in **Table 14**. The same sensitivity and specificity were observed when plates were read after 18 and 24 hours. **Table 15** summarizes HardyCHROM ESBL performance according to the species of ESBL-producing organisms recovered by the reference method. **Table 16** shows a comparison of the morphology observed on HardyCHROM ESBL and the identity and ESBL phenotype of the isolates recovered from the HardyCHROM ESBL plates as determined by conventional methods.

Table 14. Performance of HardyCHROM ESBL with prospectively collected clinical specimens in comparison to the reference method, stratified by clinical site

ESBL Producing *E. coli* and *K. pneumoniae/K. oxytoca*				
Site 1		**Reference Method**		
		Positive	**Negative**	**Total**
HardyCHROM ESBL	**Positive** [1]	26	67	**93**
	Negative [2]	1	456	**457**
	Total	**27**	**523**	**550**
	Sensitivity	26/27 = 96.3% (81.7-99.3%) [3]		
	Specificity	456/523 = 87.2% (%)		
Site 2		**Reference Method**		
		Positive	**Negative**	**Total**
HardyCHROM ESBL	**Positive**	25	65	**90**
	Negative	0	564	**564**
	Total	**25**	**629**	**654**
	Sensitivity	25/25 = 100% (86.7-100%)		
	Specificity	564/629 = 89.7% (87.0-91.9%)		
Site 3		**Reference Method**		
		Positive	**Negative**	**Total**
HardyCHROM ESBL	**Positive**	115	148	**263**
	Negative	4	471	**475**
	Total	**119**	**619**	**738**
	Sensitivity	115/119 = 96.6% (91.7-98.7%)		
	Specificity	471/619 = 76.1% (72.6-79.3%)		
Overall		**Reference Method**		
		Positive	**Negative**	**Total**
HardyCHROM ESBL	**Positive**	166	280	**446**
	Negative	5	1894	**1899**
	Total	**171**	**2174**	**2345**
	Sensitivity	166/171 = 97.1% (93.3-98.7%)		
	Specificity	1894/2174 = 87.1% (85.6-88.5%)		
	PPV	166/446 = 37.2% (32.9-41.8%)		
	NPV	1894/1899 = 99.7% (99.4-99.9%)		

PPV: Positive Predictive Value; NPV: Negative Predictive Value

Note: The same sensitivity and specificity were observed when plates were read at 18 and 24 hours

[1] Pink, or Blue colonies

[2] Colonies other than Pink or Blue, or No Growth

[3] 95% Score Confidence Interval

Table 15. Summary of HardyCHROM ESBL performance with prospectively collected clinical specimens in comparison to the reference method, stratified by ESBL-producing species

ESBL-producing Species	Sensitivity	PPV	Specificity	NPV
E. coli	110/115 95.7% (90.2-98.1%) [1]	110/224 49.1% (42.6-55.6%)	2116/2230 94.9% (93.9-95.7%)	2116/2121 99.8% (99.4-99.9%)
K. pneumoniae/K. oxytoca	56/56 100% (93.6-199%)	56/222 25.2% (20.0-31.3%)	2123/2289 92.7% (91.6-93.7%)	2123/2123 100% (99.8-100%)
E. coli and *K. pneumoniae/K. oxytoca* Combined	166/171 97.1% (93.3-98.7%)	166/446 37.2% (32.9-41.8%)	1894/2174 87.1% (85.6-88.5%)	1894/1899 99.7% (99.4-99.9%)

PPV: Positive Predictive Value; NPV: Negative Predictive Value
[1] 95% Score Confidence Interval

Table 16. Agreement between the colony color observed on HardyCHROM ESBL with prospectively collected clinical specimens and the identity and ESBL phenotype of the isolates recovered from the HardyCHROM ESBL culture medium

HardyCHROM ESBL		Incubation	Agreement (% with 95% CI) [1]	
Colony Color	Species		Positive	Negative
Pink	*E. coli*	18 hours	123/126 97.6% (93.2-99.2%)	2118/2219 95.4% (94.5-99.2%)
		24 hours	123/126 97.6% (93.2-99.2%)	2125/2219 95.8% (94.8-96.5%)
Blue	*K. pneumoniae/K. oxytoca*	18 hours	61/62 98.4% (91.4-99.7%)	2122/2283 92.9% (91.8-93.9%)
		24 hours	61/62 98.4% (91.4-99.7%)	2115/2293 92.6% (91.5-93.6%)

[1] 95% Score Confidence Interval

Contrived Specimens

Table 17 shows the agreement between colony color observed on HardyCHROM ESBL and the identity and ESBL phenotype of the isolates recovered from contrived specimens as determined by the reference method. There were no differences in positive or negative agreement when plates were read either 18 or 24 hours after inoculation.

Table 17. Agreement between the colony color observed on HardyCHROM ESBL with contrived specimens and organism identity and ESBL phenotype as determined by the reference method

HardyCHROM ESBL		Agreement (% with 95% CI) [1, 2]	
Colony Color	**Species**	**Positive**	**Negative**
Pink	*E. coli*	19/19 100% (83.2-100%)	31/34 91.2% (77.0-97.0%)
Blue	*K. pneumoniae/K. oxytoca*	26/26 100% (87.1-100%)	25/27 92.6% (76.6-97.9%)

[1] 95% Score Confidence Interval
[2] The same positive and negative agreement were observed when plates were read after 18 and 24 hours

b. Clinical specificity:

Refer to **Section M3(a)**, above.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

In the prospective Clinical Study described in **Section M(3)**, above, the prevalence of 3rd generation cephalosporin non-susceptible *Enterobacteriaceae* that was observed on HardyCHROM ESBL was 23.9%. The prevalence of ESBL-producing *E. coli* and *K. pneumoniae*/*K. oxytoca* as determined using HardyCHROM ESBL was 10.6%.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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