DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

November 15, 2016

HARDY DIAGNOSTICS
ANDRE HSIUNG
DIRECTOR TECHNICAL SERVICES
1430 WEST MCCOU LANE
SANTA MARIA
CA 93455

Re: K160512
Trade/Device Name: HardyCHROM ESBL
Regulation Number: 21 CFR 866.1700
Regulation Name: Culture medium for antimicrobial susceptibility tests
Regulatory Class: II
Product Code: JSO
Dated: October 4, 2016
Received: October 11, 2016

Dear Mr. Hsiung:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

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

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Ribhi Shawar -S

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

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K160512

Device Name
HardyCHROM ESBL

Indications for Use *(Describe)*
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, 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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

Appendix D - 510(k) Summary

I. SUBMITTER

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II. DEVICE

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

III. PREDICATE DEVICE

Bio Rad VRESelect, K122187

IV. DEVICE DESCRIPTION

According to Centers for Disease Control and Prevention (CDC), Extended Spectrum Beta Lactamases (ESBLs) refer to a variety of enzymes which confer resistance to third generation cephalosporins and monobactams. ESBLs are widespread among *Enterobacteriaceae* and have no effect on carbapenems or cephamycins. ESBLs are distinguished from AmpC type beta lactamases by their susceptibility to beta lactamase inhibitors such as clavulanic acid.

HardyCHROM™ ESBL is a selective and differential chromogenic medium containing a broad-spectrum beta-lactam intended for detection and isolation of *Enterobacteriaceae* non-susceptible to 3rd generation cephalosporins. 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).

The selective components in HardyCHROM™ ESBL are designed to inhibit the growth of yeast, Gram-positive bacteria, and Gram-negative bacteria sensitive to broad spectrum beta-lactams (3^{rd} generation cephalosporins). Chromogenic substrates in the medium allow for differentiation of *Enterobacteriaceae* non-susceptible to 3rd generation cephalosporins or that are ESBL-producing, as bacteria that can grow and utilize the chromogens produce a colored colony. ESBL-producing *Klebsiella* spp. produce large, dark blue colonies. ESBL-producing *Escherichia coli* produce colonies that are rose to magenta in color, with darker pink centers. Other *Enterobacteriaceae* not susceptible to 3rd generation cephalosporins will produce colonies of varying size that are pink, blue, blue with pink halos, and yellow/gold.

V. INDICATIONS FOR 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.

NOTE: The Indications for Use statement for HardyCHROM™ ESBL is not identical to the predicate device; however, the differences do not alter the intended use of the device nor do they affect the safety and effectiveness of the device relative to the predicate. Both the subject and predicate devices have the same intended use for screening of stool samples by selective and differential culture for microorganisms that are non-susceptible to antimicrobial agents.

VI. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS WITH THE PREDICATE DEVICE

Attribute	Device	Comparator	Substantially Equivalent?
Name	HardyCHROM™ ESBL agar	Bio Rad VRESelect	
510(k) Details	Product Code JSO 21 CFR 866.1700 "Culture medium for anti-microbial susceptibility tests" Class II Panel 83 Microbiology	Product Code JSO 21 CFR 866.1700 "Culture medium for anti-microbial susceptibility tests" Class II Panel 83 Microbiology	Yes
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 susceptibility testing and epidemiological typing.	Yes
Methodology	Enzymatic - Chromogenic	Enzymatic - Chromogenic	Yes
Inoculation	Direct	Direct	Yes
Sample Type	Fecal Specimen	Rectal Swab or Fecal Specimen	Yes
Interpretation	Manual, Visual	Manual, Visual	Yes

VII. PERFORMANCE DATA

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 ID 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 production and 3rd generation cephalosporin non-susceptibility was performed using traditional Kirby-Bauer AST following the device manufacturer's instructions. ID of organisms that grew on HardyCHROM™ ESBL was confirmed using an FDA-cleared ID 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 AST and ID. 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 AST and ID.

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 genotype 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	Mechanism
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, 21 strains representative 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 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 (1 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 (1 of these strains was ESBL negative; 2 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 10 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 mixed-infection 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.

Summary of Interference Study

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

SPECIMEN STABILITY

The stability of spiked raw stool and in stool preserved in C&S Medium (modified Cary Blair formula) was verified. Stool specimens were inoculated near LoD with ESBL-producing and 3rd generation cephalosporin non-susceptible *Enterobacteriaceae* species (ie. *E. coli*, *K. oxytoca*, *K. pneumoniae*, *E. cloacae*, and *P. mirabilis*) and held at both room temperature (23-24°C) and 2-8°C. Specimens were subcultured to HardyCHROM™ ESBL at 0 hours, 1 hours, 2 hours, 4 hours, 8 hours, 12 hours, and 24 hours, 48 hours, 72 hours, 96 hours, 120 hours, 144 hours, and 168 hours.

Acceptable results were defined as recovery of organism with no appreciable decrease in colony counts (<1 $\log_{10}$ decrease from baseline). Target organisms were stable in raw, unpreserved, stool for up to 4 hours at 23-24°C and 7 days at refrigerated temperature (2-8°C). Target organisms preserved in C&S Medium (modified Cary Blair formula) were stable for 24 hours at 23-24°Cand 7 days at refrigerated temperature.

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 5 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 5 susceptible strains of bacteria (1 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 2 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	40 [2]	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] 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

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	73.5	98.6	72	60	83.3	73.1	90.2
Day 4	32 [2]	32	100.0	89.3	100.0	48	40 [2]	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%).

VIII. CONCLUSIONS

Since the predicate device was cleared based in part on the results of clinical studies, and since the comparison of bench testing to clinical outcomes is still not well understood for this type of device, clinical testing was required to support substantial equivalence. The non-clinical data support the safety of the device and the device verification and validation demonstrate that the HardyCHROM™ ESBL device should perform as intended in the specified use conditions. The analytical and clinical data demonstrate that the HardyCHROM™ ESBL device is substantially equivalent to the predicate device.