

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

A. 510(k) Number:

K091025

B. Purpose for Submission:

To obtain Premarket Notification clearance of chromID™ VRE Agar for the qualitative detection of *Enterococcus faecium* and *E.faecalis* showing acquired vancomycin resistance (VRE) in stool specimens.

C. Measurand:

Vancomycin resistant *E. faecium* and *E. faecalis*

D. Type of Test:

Detection of vancomycin resistant *Enterococcus faecium* and *Enterococcus faecalis* using a selective and differential chromogenic medium.

E. Applicant:

bioMérieux, Inc.

F. Proprietary and Established Names:

chromID™ VRE Agar

G. Regulatory Information:

1. Regulation section:

21 CFR 866.1700

2. Classification:

Class II

3. Product code:

JSO Culture media, Antimicrobial susceptibility test, excluding Mueller Hinton Agar

4. Panel:

Microbiology

H. Intended Use:

1. Intended use:

chromID™ VRE agar is a selective and differential chromogenic medium containing 8 µg/mL of vancomycin, for the qualitative detection of *E. faecium* and *E. faecalis* showing acquired vancomycin resistance (VRE) in stool specimens. chromID™ VRE agar can be used as an aid to identify, prevent and control VRE colonization in healthcare settings. chromID™ VRE is not intended to diagnose VRE infection nor to guide or monitor treatment for infections. Subculture to non-selective media (e.g. trypticase soy agar with 5% sheep blood) is needed for further identification, susceptibility testing and epidemiological typing.

2. Indications for use:

chromID™ VRE agar is a selective and differential chromogenic medium containing 8 µg/mL of vancomycin, for the qualitative detection of *E. faecium* and *E. faecalis* showing acquired vancomycin resistance (VRE) in stool specimens. chromID™ VRE agar can be used as an aid to identify, prevent and control VRE colonization in healthcare settings. chromID™ VRE is not intended to diagnose VRE infection nor to guide or monitor treatment for infections. Subculture to non-selective media (e.g. trypticase soy agar with 5% sheep blood) is needed for further identification, susceptibility testing and epidemiological typing.

3. Special conditions for use statement:

Prescription use

4. Special instrument requirements:

Not applicable

I. Device Description:

chromID™ VRE agar is translucent and a light tan color. After the plates are inoculated and incubated, VRE colonies will have either a violet color for *E. faecium* or a blue-to-green color for *E. faecalis*. Non-VRE colonies will be colorless or have colors other than violet or blue-to-green.

J. Substantial Equivalence Information:

1. Predicate device name:

Remel Bile Esculin Azide Agar w/ 6µg/mL Vancomycin

2. Predicate K number:

K972359

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	chromID™ VRE agar is a selective and differential chromogenic medium containing 8µg/mL of vancomycin, for the qualitative detection of <i>E. faecium</i> and <i>E. faecalis</i> showing acquired vancomycin resistance (VRE) in stool specimens.	Remel Bile Esculin Azide Agar w/ 6µg/mL vancomycin is a solid medium recommended for use in qualitative procedures as a screening method for primary isolation and presumptive identification of vancomycin resistant enterococci (VRE) from surveillance cultures.
Reading	Manual	Manual
Inoculum	Direct Specimen	Direct Specimen
Specimen	Stool samples	Urine, stool

Differences		
Item	Device	Predicate
Detection Method	Two chromogenic substrates provide for the direct detection of <i>E. faecium</i> and <i>E. faecalis</i> through characteristic colony color <ul style="list-style-type: none"> • <i>E. faecium</i>: violet color for β-galactosidase-producing strains • <i>E. faecalis</i>: blue-to-green color for α-glucosidase-producing strains 	Organisms positive for esculin hydrolysis hydrolyze the glycoside esculin to esculetin and dextrose. The esculetin reacts with ferric ammonium citrate to form a dark brown or black complex in the medium.
Incubation	35 - 37°C in aerobic	33 - 37° C in aerobic conditions or

Differences		
Item		Predicate
	conditions, in the dark	in 5 – 10% CO ₂

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

Not applicable

L. Test Principle:

chromID™ VRE agar (patents pending) consists of a rich nutritive base including a variety of peptones. It also contains chromogenic substrates and a mixture of antibiotics including vancomycin (8 µg/mL) which enable

- the specific and selective growth of VRE,
- the direct detection of *E. faecium* and *E. faecalis* through the characteristic color of colonies:
 - *E. faecium*: violet color for β-galactosidase producing strains
 - *E. faecalis*: blue-to-green color for α-glucosidase producing strains

The selective mixture inhibits enterococcal strains that do not express acquired vancomycin resistance; enterococcal strains that express intrinsic vancomycin resistance (i.e. *vanC* phenotype: *E. gallinarum* and *E. casseliflavus*); and most Gram-negative and Gram-positive bacteria .

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Reproducibility was demonstrated at three sites using a set of ten organisms which were tested in triplicate each day for three days at three different sites. The study included vancomycin susceptible and vancomycin resistant *E. faecalis* and *E. faecium* (both with *vanA* and *vanB*). The quality control reference strains were also included in the study.

Preliminary results at 24 hours demonstrated 80% reproducibility. After incubation for the full 48 hours, overall reproducibility was 100%.

b. *Linearity/assay reportable range:*

Not applicable

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

The recommended quality control (QC) organisms, *E. faecium* (*vanA*) ATCC 700221 and *E. faecalis* (*vanB*) ATCC 51299 as positive controls, and *E. faecalis* ATCC 29212 as negative control were used. Quality control data was compiled across all three sites and overall QC results were acceptable.

QC Data Summary at 48 hours

Organism	Expected Result on chromID™ VRE	chromID™ VRE Positive	chromID™ VRE Negative
<i>E. faecalis</i> ATCC 29212	No Growth (Negative)	1	96
<i>E. faecalis</i> ATCC 51299	Growth of Blue-to-Green colored Colonies (Positive)	97	0
<i>E. faecium</i> ATCC 700221	Growth of Violet colored Colonies (Positive)	97	0

In one instance, the negative control, *E. faecalis* ATCC 29212 exhibited breakthrough growth on the chromID™ VRE agar medium. A note was added to the table in the Quality Control section of the package insert recommending the use of a light organism inoculum in order to mitigate erroneous quality control results.

d. *Detection limit:*

Not applicable

e. *Analytical specificity:*

Cross-reactivity Study

A Cross-reactivity study was performed using a total of 99 strains representing various organism groups.

One strain of *Enterococcus raffinosus* produced colorless colonies after 24 hours of incubation which subsequently developed violet pigmentation after 48 hours of incubation.

One strain of *Citrobacter freundii* produced violet *Klebsiella pneumoniae* pigmented colonies after 24 hours incubation. Two strains of produced violet colored colonies after 24 and 48 hours of incubation.

Two strains of *P. aeruginosa*, five strains of *P. fluorescens*, and one strain each of *S. maltophilia* and *A. baumannii* grew producing colorless and/or atypically pigmented colonies.

One strain each of *C. albicans*, *C. tropicalis*, *C. glabrata*, *Geotrichum candidum*, *Saccromyces cerevisiae*, *Aspergillus niger*, and *Penicillium abeanum*, all grew producing colorless or blue colonies on the chromID™ VRE medium.

The study results are reflected in the Limitation and Cross Reactivity sections of the device package insert.

Interference Study

Commonly used medicinal substances namely, Preparation H Ointment, Boudreaux's Butt Paste, Glycerin, Dulcolax, Vaseline and Tucks Pads, as well as human blood and physiological saline were found to decrease growth of VRE isolates on the chromID™ VRE medium in comparison to the control. However, the chromogenic reaction remained unaffected.

Testing of both Preparation H Cream and Miconazole 7 resulted inhibition or delayed growth of VRE isolates on the chromID™ VRE medium at 24 and 48 hours incubation. However, the chromogenic reaction remained unaffected.

Cary Blair transport medium was tested and found to have no detrimental effect on the growth, recovery or chromogenic reaction of VRE isolates on the chromID™ VRE medium.

Collection Swab Study

The influence of collection swabs on the recovery of VRE was also evaluated. Rayon and nylon flocked swabs were tested dry and coupled with Amies transport media. After 1 and 4 hours of contact there was found to be no significant difference in the sensitivity of detection of VRE between dry swabs and those in Amies transport medium. After 18 hours of contact at room temperature, the nylon flocked swab with Amies transport media resulted in the best recovery for VRE, followed by nylon flocked dry swabs and rayon swab with Amies transport media. After 18 hours at 2-8°C, the nylon flocked swabs with and without Amies transport medium resulted in the best recovery of VRE, followed by the rayon swabs with and without Amies transport medium.

f. Analytical Sensitivity

Recovery Study

A recovery study was performed to determine the lowest number of colony forming units (CFU) of vancomycin resistant enterococci (VRE) that will grow on the chromID™ VRE medium. One strain of *E. faecalis* (*vanA*) bioMerieux stock no. 05.02.228; and one strain of *E. faecium* (*vanA*) ATCC 700221; were evaluated. Ten-fold serial dilutions were prepared in saline and then plated in duplicate onto chromID™ VRE medium. After 48 hours incubation, the number of CFU was counted on each plate. The number of CFU obtained on the two plates from each dilution was averaged. The percent recovery on chromID™ VRE was determined to be equivalent to 100 CFU/mL of sample.

g. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

The performance of the chromID™ VRE agar was evaluated at four geographically diverse laboratory sites. A total of 1299 stool specimens were evaluated. Stool specimens were inoculated on chromID™ VRE agar medium and the Bile Esculin Azide Agar with 6µg/ml of Vancomycin. (BEAV). Both plates were observed for growth at 24 and 48 hours. Colonies with violet or blue-to-green pigment (chromID™ VRE) or brown to black pigment diffusing into the medium (BEAV), were identified by a combination of conventional reference methods to include Gram stain, catalase, VITEK 2 GP, and 16S-500 sequencing. Vancomycin resistance was confirmed by agar dilution.

Percent agreement of the chromID™ VRE compared to conventional reference methods is presented in Table 1.

Table 1. chromID™ VRE vs Conventional Methods	Positive % Agreement	Negative % Agreement
chromID™ VRE agar @24h incubation	97.1% (334/344) (95% CI = 94.7%, 98.6%)	99.7% (952/955) (95% CI = 99.1%, 99.9%)
chromID™ VRE agar @48h incubation	96.9% (344/355) (95% CI = 94.5%, 98.4%)	99.7% (941/944) (95% CI = 99.1%, 99.3%)

Performance data for the chromID™ VRE agar compared to the VITEK® 2 GP Identification, for both isolates of *E.faecalis* and *E.faecium* is presented in Tables 2 and 3.

In Table 2, of the nine isolates that were not identified as *E.faecium* by VITEK®2, one isolate was negative on the chromID™ VRE agar at 24 hours but was false positive after 48 hours. The other eight isolates were false positive on chromID™ VRE at 24 and 48 hours.

Table 2. chromID™ VRE vs VITEK® 2 Identification <i>E. faecium</i>	Positive % Agreement	Negative % Agreement
chromID™ VRE agar @ 24h incubation	94.3% (316/335) (95% CI = 91.3%, 96.6%)	11.1% (1/9)* (95% CI = 0.3%, 42.3%)
chromID™ VRE agar @ 48h incubation	96.7% (324/335) (95% CI = 94.2%, 98.4%)	0.0% (0/9)* (95% CI = 0.0%, 33.6%)

In Table 3, all *E.faecalis* isolates were identified by VITEK® 2 however four isolates did not produce the characteristic blue-to-green color at 24 hours. At 48 hours incubation, three of the four isolates developed the characteristic blue-to-green color.

Table 3. chromID™ VRE vs VITEK® 2 Identification <i>E. faecalis</i>	Positive % Agreement	Negative % Agreement
chromID™ VRE agar @ 24h incubation	87.1% (27/31) (95% CI = 70.2%, 96.4%)	0/0*
chromID™ VRE agar @ 48h incubation	96.8% (30/31) (95% CI = 83.3%, 99.9%)	0/0*

Performance data for the chromID™ VRE agar compared to the vancomycin MIC, for both isolates of *E.faecalis* and *E.faecium* is presented in Tables 4 and 5.

In Table 4, three of four isolate were false positive as they produced violet colonies on chromID™ VRE but were confirmed to be vancomycin susceptible *E. faecium* by MIC. The fourth isolate did not produce violet pigmented colonies.

Table 4. chromID™ VRE vs Vancomycin MIC <i>E. faecium</i>	Positive % Agreement	Negative % Agreement
chromID™ VRE agar @ 24h incubation	94.4% (321/340) (95% CI = 91.4%, 96.6%)	25% (1/4)* (95% CI = 0.6%, 80.6%)
chromID™ VRE agar @ 48h incubation	97.1% (330/340) (95% CI = 94.7%, 98.6%)	25% (1/4)* (95% CI = 0.6%, 80.6%)

In Table 5, one sample produced blue-to-green colonies on chromID™ VRE and was vancomycin susceptible by MIC.

Table 5. chromID™ VRE vs Vancomycin <i>E. faecalis</i>	Positive % Agreement	Negative % Agreement
chromID™ VRE agar @ 24h incubation	86.7% (26/30) (95% CI = 69.3%, 96.3%)	0.0% (0/1)* (95% CI = 0.0%, 97.5%)
chromID™ VRE agar @ 48h incubation	96.7% (29/30) (95% CI = 82.8%, 99.9%)	0.0% (0/1)* (95% CI = 0.0%, 97.5%)

A challenge set of 75 well characterized isolates were tested at one external site. The challenge isolates included vancomycin resistant and vancomycin susceptible *E. faecalis* and *E. faecium*, *E. gallinarum* and *E. casseliflavus*, and other gram positive microorganisms commonly isolated from the stools. Results of the challenge study were compared to the expected result as determined by the organism identification based on API® results or known reference strain and vancomycin resistance as determined by the vancomycin agar dilution method.

One challenge isolate *E. faecalis* 13029 *vanB* produced blue-purple colonies after 48 hours incubation. This was due to a rare situation of activity of both enzymes (α -glucosidase and β -galactosidase) occurring in one strain. Both enzymes reacted with the chromogen in the chromID™ VRE agar medium and this combination of activity produced the blue-purple color variation.

A second study was performed to evaluate the detection of enterococcal strains with low level vancomycin resistance. Forty-nine enterococcal strains characterized by intermediate or low levels of resistance to vancomycin were evaluated. The strains tested were from several international collections such as ATCC, LMG (*Gent-Laboratorium voor Microbiologie, Belgium*), NCTC (*National Collection of Type Culture, United Kingdom*) and the bioMérieux stock collection. *E. faecium* and *E. faecalis* isolates with a low level of vancomycin resistance were detected if their vancomycin MIC was greater than or equal to 4 $\mu\text{g/mL}$. For a majority of the isolates, the characteristic colony coloration was observed after 48 hours incubation. All the isolates of *E. casseliflavus* in this study were inhibited on the chromID™ VRE medium. Most isolates of *E. gallinarum* in this study were inhibited on the chromID™ VRE medium with the exception of two isolates, the first with the acquired *vanA* gene resulted in growth of violet colonies, a second with an MIC of greater than 64 $\mu\text{g/mL}$ resulted in growth of colorless colonies.

b. *Matrix comparison:*

Not Applicable

3. Clinical studies:

a. *Clinical Sensitivity:*

Not Applicable

b. *Clinical specificity:*

Not Applicable

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:

Vancomycin resistant *E. faecalis* presents as blue-to green color colonies.

Vancomycin resistant *E. faecium* presents as violet color colonies.

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.