VRE*Select*™

REF 63751

A selective and differential chromogenic medium for the qualitative detection of gastrointestinal colonization of vancomycin-resistant *Enterococcus faecium* (VREfm) and vancomycin-resistant *Enterococcus faecalis* (VREfs).

IVD CE 506521 – 2012/11

1. INTENDED USE

VRE*Select*™ 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. **VRE***Select*™ 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.

2. SUMMARY AND EXPLANATION

The Centers for Disease Control and Prevention reported that during 2006 and 2007 enterococci caused about 12% of all hospital infections; 30% of the isolates were resistant to vancomycin. Hospital acquired enterococcal infections typically occur in the very ill, debilitated patients that have been exposed to broad spectrum antibiotics. They are also the third most common cause of hospital-acquired infections in the US.
The Healthcare Infection Control Practices Advisory Committee (HICPAC) issued recommendations for the management of multidrug-resistant organisms (MDROs) including VRE, in 2006. Active surveillance cultures to identify colonized patients and control precautions are strongly recommended as control measures to reduce MDRO transmission.

3. PRINCIPLES OF THE PROCEDURE

VRE*Select*™ is a selective medium for the detection of vancomycin-resistant *Enterococcus* (VRE). The selectivity of this medium is based on the presence of an antifungal/antibiotic mixture that inhibits the growth of most yeasts, Gram negative and Gram positive bacteria, with the exception of VRE.
Detection is based on the cleavage of chromogenic substrates by specific enzymes of *Enterococcus faecium* which produces pink colonies and *Enterococcus faecalis* which produces blue colonies.
Enterococcus gallinarum and *Enterococcus casseliflavus* are intrinsically resistant to vancomycin and may grow on the **VRE***Select*™ medium as colorless or white colonies because they do not metabolize the chromogenic substrates Vancomycin-susceptible enterococci are inhibited.
After 24 to 28 hours incubation pink colonies can be reported as VREfm. Blue colonies should be confirmed by a catalase test and susceptibility (see limitation 9).

4. REAGENTS

VRE*Select*™ (catalog # 63751) contains 20 plates per package.

Approximate media formulation (g/L):

○ Peptone:	61.0g
○ Contrasting reagents:	3.0g
○ Chromogenic substrates:	0.3g
○ Antimicrobial and antifungal:	0.1g
○ Salts Mixture:	16.0g
○ Agar:	15.0g

5. WARNINGS AND PRECAUTIONS

•For *in vitro* diagnostic use only.
•For professional use only.
•Observe aseptic technique and established precautions against microbiological hazards throughout all procedures.
•After use, prepared plates, specimen containers and other potentially contaminated materials must be sterilized or disposed of in accordance with defined laboratory procedures and local/regional regulations.
•Pathogenic microorganisms, including hepatitis viruses and human immunodeficiency virus (HIV), may be present in clinical samples. Universal precautions and institutional guidelines should be followed in handling all items contaminated with blood or other body fluids.
•Use of this medium may be difficult for those who have problems recognizing colors.
•Directions should be read and followed carefully. Interpretation of test results should be considered based on patient history, the source of the specimen, colony morphology, and the results of any other tests performed.
•The Safety Data Sheet (SDS) is available upon request at www.bio-rad.com.

6. STORAGE INSTRUCTIONS

Store plates at 2 – 8°C protected from light.
Prolonged exposure to light may result in reduced coloration of the QC organisms or patient isolates. Store plates in original packaging until ready for use. Close plate packaging each time after plates are removed. Plates should not be used after the expiration date indicated on the label and printed on the plate.

7. PRODUCT DETERIORATION

Do not use plates if they show any evidence of contamination, drying, cracking or any other sign of deterioration.

8. SPECIMEN COLLECTION AND HANDLING

This device has been evaluated with rectal swabs or fecal specimens. Use of transport devices approved for collection of such specimens may be used. Follow the transport device manufacturer's recommended procedures. Amies without charcoal, Cary Blair, and LQ Stuart transport devices were evaluated and the performance was found to be acceptable for use with **VRE***Select*™.

BIO-RAD

9. MATERIALS

Materials Provided

- Bio-Rad **VRE***Select* ™ agar plates

Materials Required But Not Provided

- Ancillary culture media
- QC organisms – Refer to Section 12 for recommended strains
- Other laboratory equipment as required
- Sterile saline solution

10. TEST PROCEDURE

The **VRE***Select*™ agar surface should be smooth and moist. Allow the media to warm to room temperature (15-30°C) protected from light before inoculation. Follow aseptic technique when using the media. If specimens are not processed immediately upon receipt, refrigerate until processed.

Inoculation

Once the specimen is inoculated, it is important to streak for isolation in order to obtain well isolated colonies. Use the quadrant method with a loop, starting from the original point of specimen inoculation.

From rectal swabs

A. Direct Inoculation:
   Inoculate by touching the swab directly onto the agar surface. Streak for isolation.

B. Indirect Inoculation:
   1. Place the swab in 0.5 mL sterile saline.
   2. Vortex the swab in the saline for approximately 20 seconds and immediately proceed to plate inoculation.
   3. Using a swab or disposable loop, transfer approximately 10-50 µL of the vortexed suspension onto the agar surface and streak for isolation.

From fecal specimens - Indirect inoculation

1. Place a portion of stool into sterile saline (approximately 0.5g/mL).
2. Vortex for approximately 20 seconds and inoculate immediately.
3. Using a swab or disposable loop, transfer approximately 10-50 µL of the suspension onto the agar surface and streak for isolation.

Incubation

Incubate the inoculated **VRE***Select*™ plate in an inverted position, in ambient air for 24 to 28 hours at 35-37°C, in the dark.

11. RESULTS

After 24 to 28 hours incubation **pink colonies can be reported as vancomycin-resistant** *Enterococcus faecium***. Blue colonies should be confirmed by a direct catalase test and, if negative, by vancomycin susceptibility testing.**

24/28h incubation†	Interpretation	Recommended action
Pink colonies	VREfm	Report VREfm colonization
Blue colonies*	Probable VREfs	Run direct catalase test. **If catalase negative** perform susceptibility testing. If organism is vancomycin resistant, report as VREfs colonization. **If catalase positive**, report as No VREfs isolated.
No colony or colorless colonies	No VREfm or VREfs	Report as No VRE isolated.

† In rare cases, colony color development may not occur before 28 hours.
*Note: Isolation of rare or few blue colonies confirmed to be catalase negative may be vancomycin-susceptible enterococci. In these cases, vancomycin susceptibility should be performed.

12. USER QUALITY CONTROL

Examine plates for signs of deterioration (see Section 7 above). Check performance by inoculating a representative sample of plates with pure cultures of stable control organisms that produce known, desired reactions.

Recommended Quality Control (QC) Strains:

- *Enterococcus faecium* ATCC 700221
- *Enterococcus faecalis* ATCC 51299
- *Enterococcus faecalis* ATCC 29212

Prepare a 0.5 McFarland suspension of each QC strain. Dilute suspension 1:10, transfer 10 µL to the **VRE***Select™* agar surface and streak for isolation.

QC Strains	Expected Results
Enterococcus faecium ATCC 700221	Pink colonies after 24 to 28 hours
Enterococcus faecalis ATCC 51299	Blue colonies after 24 to 28 hours
Enterococcus faecalis ATCC 29212	No growth after 24 to 28 hours

Quality control testing must be performed in accordance with local, state, and federal regulations or accreditation requirements and your laboratory's standard quality control procedures. Refer to pertinent CLSI (NCCLS) guidance documents and CLIA regulations for appropriate quality control procedures.

13. LIMITATIONS OF THE PROCEDURE

1- Surveillance testing determines the colonization status at a given time and could vary depending on patient treatment (e.g. decolonization regime), patient status (e.g., not actively shedding VRE) or exposure to high-risk environments (e.g., contact with VRE carrier, prolonged hospitalization). Monitoring of colonization status should be done according to hospital policies.

2- Some rectal specimens may lead to non-specific coloration of the **VRE***Select*™ agar medium at the point of inoculation. Interpretation of colony color must be done on well isolated colonies.
3- Tuck's Medicated Cooling Pads® and Miconazole cream may delay the colonies coloration or inhibit the growth of VRE on **VRE***Select*™.
4- Blood (30%-50%) may reduce or inhibit the recovery of VRE.
5- Tightly clustered colonies of *Enterococcus gallinarum* and *Enterococcus casseliflavus*, could appear as gray, faint blue or faint pink colonies.
6- *Leuconostoc*, *Pediococcus* and *Lactobacillus* are intrinsically resistant to vancomycin. They are inhibited or appear as colorless pinpoint/colonies (tightly clustered colonies may appear blue or pink).
7- In rare instances VREfs strains such as ATCC 51299, which display low-level vancomycin resistance (MIC<32 mg/L), may not develop the characteristic blue color until a full 28 hours incubation (specifically if sample inoculum is close to defined limit of detection). If white or bluish colonies appear at 24 hours, incubate and read after 28 hours incubation.
8- Some strains of *Staphylococcus haemolyticus, Staphylococcus similans, Staphylococcus lentus* and *Staphylococcus aureus* may grow and produce blue colonies. Catalase testing should be performed on all blue colonies isolated.
9- In rare instances, the growth of vancomycin-susceptible *E. faecalis* may produce blue colonies. In case of doubt, vancomycin susceptibility of the isolates in question should be performed.
10- Prolonged exposure to light may result in reduced coloration of the QC organisms or patient isolates. Minimize exposure of **VRE***Select*™ plates to light both before and during incubation.
11- The performance of **VRE***Select*™ has not been evaluated with vancomycin resistant strains of *Staphylococcus aureus*.
12- A negative result does not rule out the possibility of VRE colonization

14. EXPECTED VALUES

The Centers for Disease Control and Prevention reported that during 2006 and 2007 enterococci caused about 12% of all hospital infections; 30% of the isolates were resistant to vancomycin. Hospital acquired enterococcal infections typically occur in the very ill, debilitated patients that have been exposed to broad spectrum antibiotics. Enterococci are also reported as the third most common cause of hospital-acquired infections in the US.

15. PERFORMANCE CHARACTERISTICS

Reproducibility
In order to confirm the reproducibility of the **VRE***Select*™ medium a blinded panel of 6 ATCC reference strains (2 VREfs, 3 VREfm, and 1 vancomycin-susceptible *Enterococcus*) were tested at three sites. At each site three technicians tested the panel on three lots of **VRE***Select*™ each day for three days. The strains produced the expected results with **VRE***Select*™ 100% of the time at 24 and 28 hours.

Transport media
Three commonly used transport media – Amies without charcoal, Cary Blair. and LQ Stuart, were found to be acceptable for use with **VRE***Select™.*

Interfering Substances
The following substances were evaluated for potential interference with the performance of the **VRE***Select™* medium:

- Dulcolax®, Adult Glycerin Suppositories, Vaseline®, Preparation H®, Original Boudreaux's Butt Paste®, Tuck's Medicated Cooling Pads®, Pepto-Bismol®, Miconazole cream, Nonoxynol-9 (spermicide), KY®Jelly, and Maximum Strength Pepcid AC®.
- Blood and Mucins.

The interfering substances tested caused no significant differences between the number of colonies observed on the control plates and the number of colonies observed on the **VRE***Select™* plates. The only exceptions were Tuck's Medicated Cooling Pads® and Miconazole cream. Regarding Tuck's Medicated Pads®, no pink coloration was observed after 24 hours on the **VRE***Select™* plates that had been inoculated with VREfm (ATCC 700221). Regarding Miconazole cream, an inhibitory effect on the growth of *Enterococcus* colonies on the **VRE***Select™* plates was observed. Blood and mucin (3% to 5%) caused delayed colonial growth of one strain of VREfs (ATCC 51299) tested. The growth of the same strain of VREfs was inhibited at blood and mucin concentrations of 30% to 50%.

Cross Reactivity Testing (Analytical Specificity)
A cross-reactivity study was performed to determine if strains other than vancomycin-resistant enterococci could grow on **VRE***Select*™. One hundred thirty one (131) microorganisms representing Gram-negative rods, Gram-positive cocci, and yeast were evaluated on the **VRE***Select*™. No cross-reactivity was observed with any of the organisms tested. No variation was seen between the 24 and 28 hour incubation time.

Recovery Study
The minimum concentration of VRE reliably detected by **VRE***Select*™ is 10^3 CFU/mL.

To determine the percent recovery for the **VRE***Select*™ media a panel of eighteen vancomycin-resistant enterococci – 8 VREfm and 10 VREfs – were tested at varying dilutions. For each strain to be tested a 0.5 McFarland suspension of the strain was prepared. A series of 10-fold serial dilutions in saline were carried out and inoculated onto three lots of **VRE***Select*™ plates and one lot of Blood Agar plates. The plates were incubated at 35-37°C ambient air and read at 24 and 28 hours. The color and number of colonies were recorded. The Blood Agar plates were used to confirm the inoculum concentration at each dilution. Data confirm that the minimum concentration of VRE reliably detected by **VRE***Select*™ is 10^3 CFU/mL.

Challenge Panel
VRE*Select™* was evaluated with fifty-six (56) well-characterized strains including vancomycin-resistant and vancomycin-susceptible *E. faecalis* and *E. faecium,* as well as microorganisms commonly isolated from stool, all strains showed expected results.

Clinical Accuracy

Rectal Swab Specimens

Seven hundred and fifty seven (757) swab specimens were tested on **VRE***Select*™ media (pink or blue colonies between 24 and 28 hours incubation) and on Bile Esculin Azide Agar (BEAV) (colonies with dark halos between 24 and 48 hours incubation) plus confirmatory testing (Gram stain, catalase, PYR, Vitek 2 identification and vancomycin MIC E-Test). They showed the following results.

Table 1 BEAV + Confirmation vs. **VRE***Select*™ results

	BEAV + Confirmation	
VRE*Select*™	% Positive Agreement	% Negative Agreement
24 hrs	98% (118/120, [C.I. 0.94, 1.00])	97% (615/637, [C.I. 0.95, 0.98])
28 hrs	99% (119/120, [C.I. 0.95, 1.00])	96% (610/637, [C.I. 0.94, 0.97])*

* Ten (10) of the twenty-seven (27) specimens that were BEAV plus confirmation negative and grew pink and/or blue colonies on **VRE***Select*™ media, after subculture from **VRE***Select*™ to blood agar plates (BAPs), were confirmed to be vancomycin-resistant *E. faecium* and/or *E. faecalis* by Vitek® 2 biochemical identification and vancomycin E-Test. Seventeen (17) specimens grew pink and/or blue colonies on **VRE***Select*™ that **were not** confirmed by Vitek® 2 biochemical identification and vancomycin E-Test to be either vancomycin-resistant *E. faecium* and/or *E. faecalis* and represent false positive results.

VRE*Select*™ (pink or blue colonies observed after 24 to 28 hours incubation) compared to samples with isolates identified as VREfm or VREfs using commercially available biochemical identification system demonstrated the following results.

Table 2 Biochemical identification (Vitek® 2) vs. **VRE***Select*™ results

	Vitek 2 Biochemical Identification	
	% Positive Agreement	% Negative Agreement
VREfm		
VRE*Select*™ @ 24 hours	97% (94/97, [C.I. 0.91, 0.99])	97% (639/660, [C.I. 0.95, 0.98])
VRE*Select*™ @ 28 hours	98% (95/97, [C.I. 0.92, 0.99])	97% (639/660, [C.I. 0.95, 0.98])*
VREfs		
VRE*Select*™ @ 24 hours	79% (30/38, [C.I. 0.63, 0.89])**	97% (696/719, [C.I. 0.95, 0.98])†
VRE*Select*™ @ 28 hours	82% (31/38, [C.I. 0.66, 0.91])	97% (701/719, [C.I. 0.96, 0.98])

* Twenty-one (21) specimens not identified as *E. faecium* on the reference arm of the study grew pink colonies on **VRE***Select*™ media. Twenty (20) of those specimens, after subculture from **VRE***Select*™ to BAPs, were confirmed as vancomycin-resistant *E. faecium* or E. *faecium/E. faecalis* by Vitek® 2 biochemical identification and vancomycin E-Test. One specimen was confirmed to be a false positive result.

** Of the eight (8) specimens that were identified as *E. faecalis* by Vitek® 2 biochemical identification and did not grow blue colonies on **VRE***Select*™ media, six (6) were shown to be vancomycin susceptible by the reference arm of the study. One (1) specimen grew blue colonies on **VRE***Select*™ after 28 hours and one (1) specimen was determined to be false negative result.

† Twenty-three (23) specimens not identified as *E. faecalis* on the reference arm of the study grew blue colonies on **VRE***Select*™ media. Thirteen (13) of those specimens, after subculture from **VRE***Select*™ to BAPs, were confirmed as vancomycin-resistant *E. faecalis* or E. *faecalis /E. faecium* by Vitek 2 biochemical identification and vancomycin E-Test. Ten (10) specimens were confirmed to be false positive (including 6 staphylococci catalase positive organisms isolated).

VRE*Select*™ (pink or blue colonies observed after 24 to 28 hours incubation) compared to vancomycin minimal inhibitory concentration (MIC) testing by E-test, demonstrated the following results.

Table 3 Vancomycin MIC vs. **VRE***Select*™ results

	Vancomycin Resistance (E-Test)	
	% Positive Agreement	% Negative Agreement
VREfm		
VRE*Select*™ @ 24 hours	99% (93/94, [C.I. 0.94, 0.99])	98% (626/637, [C.I. 0.97, 0.99])
VRE*Select*™ @ 28 hours	100% (94/94, [C.I. 0.95, 1.00])	98% (626/637, [C.I. 0.97, 0.99])*
VREfs		
VRE*Select*™ @ 24 hours	96% (27/28, [C.I. 0.81, 0.99])	98% (622/637, [C.I. 0.96, 0.99])
VRE*Select*™ @ 28 hours	96% (27/28, [C.I. 0.81, 0.99])	97% (617/637, [C.I. 0.95, 0.98])**

Note: Specimens that were identified in the reference arm of the study as vancomycin-resistant and identified as *E. faecium* or *E. faecalis* by Vitek® 2 and grew pink or blue colonies on **VRE***Select*™ were considered in positive agreement.

* Eleven (11) specimens not identified as vancomycin-resistant on the reference arm of the study grew pink colonies on **VRE***Select*™, the colonies which grew from ten (10) of those specimens, after subculture to a BAP, were confirmed to be vancomycin-resistant *E. faecium* by Vitek® 2 biochemical identification and vancomycin E-Test. One (1) specimen was confirmed to be false positive.

** Twenty (20) specimens not identified as vancomycin-resistant on the reference arm of the study grew blue colonies on **VRE***Select*™. When colonies from those specimens were subcultured to BAPs five (5) were identified as vancomycin-resistant *E. faecalis* / *E. faecium* and fifteen (15) were not confirmed to be vancomycin-resistant *E. faecalis* / *E. faecium*, or were vancomycin-susceptible (including 8 staphylococci catalase positive).

Fecal Samples

The performance of the **VRE***Select*™ for use with fecal samples was evaluated at three (3) geographically diverse locations within the United States. A total of nine hundred forty-six (946) fecal samples were evaluated. The following results were obtained.

Specimens that were positive on **VRE***Select*™ (i.e. test specimens grew pink or blue colonies between 24 and 28 hours incubation) compared to specimens that were confirmed BEAV positive (i.e. grew colonies with dark halos confirmed by Gram-stain, Catalase test, PYR test, biochemical identification, and E-test).

Table 4 BEAV plus Confirmation vs. **VRE***Select*™ results

	BEAV plus Confirmation	
VRE*Select*™	% Positive Agreement	% Negative Agreement
24 hours	96% (182/189, [CI. 0.92, 0.98])	96% (727/757, [CI. 0.94, 0.97])
28 hours	98% (186/189, [CI. 0.95, 0.99])	95% (721/757, [CI. 0.93, 0.96])*

* Thirty-three (33) of the Thirty-six (36) specimens that were BEAV plus Confirmation negative and that grew pink and/or blue colonies on **VRE***Select*™ media, after subculture to blood agar plates (BAPs), were confirmed to be vancomycin resistant *E. faecium* and/or *E. faecalis* by biochemical identification and vancomycin E-Test. Three (3) specimens that were BEAV plus Confirmation negative and that grew pink and/or blue colonies on **VRE***Select*™ media, after subculture to blood agar plates (BAPs), were not confirmed biochemical identification and vancomycin E-Test to be *E. faecium* and/or *E. faecalis* and represent false positive results.

VRE*Select*™ (pink or blue colonies observed after 24 to 28 hours incubation) compared to samples with isolates identified as VREfm or VREfs using commercially available biochemical identification system demonstrated the following results.

Table 5 Biochemical Identification (Vitek) vs. **VRE*Select*™** results

	Vitek 2 Biochemical Identification	
	% Positive Agreement	% Negative Agreement
VREfm		
VRE*Select*™ @ 24 hours	94% (171/181, [CI. 0.90, 0.97])	97% (740/765, [CI. 0.95, 0.98])
VRE*Select*™ @ 28 hours	97% (175/181, [CI. 0.93, 0.99])	96% (734/765, [CI. 0.94, 0.97])
VREfs		
VRE*Select*™ @ 24 hours	94% (15/16, [CI. 0.70, 0.99])	98% (910/930, [CI. 0.97, 0.99])
VRE*Select*™ @ 28 hours	94% (15/16, [CI. 0.70, 0.99])	98% (909/930, [CI. 0.97, 0.99])

VRE*Select*™ (pink or blue colonies observed after 24 to 28 hours incubation) compared to vancomycin minimal inhibitory concentration (MIC) testing by E-test, demonstrated the following results.

Table 6 Vancomycin Resistance (E-Test) vs. **VRE*Select*™** results

	Vancomycin Resistance (E-Test)	
	% Positive Agreement	% Negative Agreement
VREfm		
VRE*Select*™ @ 24 hours	96% (171/178, [CI. 0.92, 0.98])	97% (743/768, [CI. 0.95, 0.98])
VRE*Select*™ @ 28 hours	98% (175/178, [CI. 0.95, 0.99])	96% (737/768, [CI. 0.94, 0.97])
VREfs		
VRE*Select*™ @ 24 hours	100% (12/12, [CI. 0.82, 1.00])	98% (911/934, [CI. 0.96, 0.99])
VRE*Select*™ @ 28 hours	100% (12/12, [CI. 0.82, 1.00])	97% (910/934, [CI. 0.96, 0.98])

Note: Specimens that were identified in the reference arm of the study as vancomycin-resistant and identified as *E. faecium* or *E. faecalis* by Vitek 2 and grew pink or blue colonies on **VRE*Select*™** were considered in positive agreement.

16. ORDERING INFORMATION

Product: **VRE*Select*™**
Catalog Number: 63751 (20 plates)
For Customer Orders and Technical Service Call:
1-800-2-BIORAD (1-800-224-6723)

17. REFERENCES

1. Basic Laboratory Procedures Clinical Bacteriology. World Health Organization. Geneva. 1991. 1st edition.
2. Quality Control for Commercially Prepared Microbiological Culture Media. Clinical and Laboratory Standards Institute - Volume 24-Number 19 - M22A3; Approved Standard-Third Edition.

Symbol

Stored plates must be protected from light.

Certificate of analysis available on
http://www.bio-rad.com/certificate.

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