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).

**1. INTENDED USE**

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 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.

**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**

VRESelect™ 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 VRESelect™ 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 vancomycin-resistant *Enterococcus faecium* (VREfm). Blue colonies should be confirmed by a catalase test and susceptibility (see limitation 9).

**4. REAGENTS**

VRESelect™ (catalog # 63751) contains 20 plates per package.

**Approximate media formulation (g/L):**
- Peptone: 61.0g
- Contrast 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 Material Safety Data Sheet (MSDS) is available upon request or on [www.bio-rad.com](http://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. 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 VRESelect™.
Inoculation until processed. If rectal swabs are not processed immediately upon receipt, refrigerate swabs aseptic technique when using the media. If rectal swabs are processed, follow the results by susceptibility testing.† In rare cases, colony color development may not occur before 28 hours.

Materials Provided
- Bio-Rad VRESelect™ agar plates
- Ancillary culture media
- QC organisms – Refer to section 12 for recommended strains
- Other laboratory equipment as required

10. TEST PROCEDURE
The VRESelect™ 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 rectal swabs are not processed immediately upon receipt, refrigerate swabs 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.

A. Direct Inoculation:
Inoculate the sample onto the plate and 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.

Incubation
Incubate the inoculated VRESelect™ 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 susceptibility testing.

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.

Materials Required but not Provided
- 3- Tuck’s Medicated Cooling Pads®
- Miconazole cream

Recommended Quality Control (QC) Strains:
- Enterococcus faecium ATCC 700221
- Enterococcus faecalis ATCC 51299
- Enterococcus casseliflavus ATCC 29212

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

<table>
<thead>
<tr>
<th>QC Strains</th>
<th>Expected Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus faecium ATCC 700221</td>
<td>Small pink colonies after 24 to 28 hours</td>
</tr>
<tr>
<td>Enterococcus faecalis ATCC 51299</td>
<td>Small blue colonies after 24 to 28 hours</td>
</tr>
<tr>
<td>Enterococcus faecalis ATCC 29212</td>
<td>No growth after 24 to 28 hours</td>
</tr>
</tbody>
</table>

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 VRESelect™ agar medium at the point of inoculation. Interpretation of colony color must be done on isolated colonies.
3- In rare instances VRE strains such as ATCC 51299, which display low-level vancomycin resistance (MIC < 32 mg/mL), 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.
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 (blue or pink with tightly clustered colonies).
7- In rare instances VRE strains such as ATCC 51299, which display low-level vancomycin resistance (MIC < 32 mg/mL), 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- 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 confirmed.
10. Prolonged exposure to light may result in reduced coloration of the QC organisms or patient isolates. Minimize exposure of VRESelect™ plates to light both before and during incubation.

11. The performance of VRESelect™ 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 VRESelect™ medium a blinded panel of 6 ATCC reference strains (2 VREs, 3 VREFm, and 1 vancomycin-susceptible Enterococcus) was tested at three sites. At each site three technicians tested the panel on three lots of VRESelect™ each day for three days. The strains produced the expected results with VRESelect™ 100% of the time at 24 and 28 hours.

Interfering Substances
The following substances were evaluated for potential interference with the performance of the VRESelect™ 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 Pepsid 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 VRESelect™ 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 VRESelect™ plates that had been inoculated with VREFm (ATCC 700221). Regarding Miconazole cream, an inhibitory effect on the growth of Enterococcus colonies on the VRESelect™ 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 VRESelect™. One hundred nineteen (119) microorganisms representing Gram-negative rods, Gram-positive cocci, and yeast were evaluated on the VRESelect™. 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 VRESelect™ is 10³ CFU/mL.

To determine the percent recovery for the VRESelect™ media a panel of eighteen vancomycin-resistant enterococci – 9 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 VRESelect™ 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 VRESelect™ is 10³ CFU/mL.

Clinical Accuracy
Seven hundred and fifty seven (757) swab specimens were tested on VRESelect™ 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. VRESelect™ results

<table>
<thead>
<tr>
<th>VRESelect™</th>
<th>% Positive Agreement</th>
<th>% Negative Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hrs</td>
<td>98% (118/120, [C.I. 0.94, 1.00])</td>
<td>97% (615/637, [C.I. 0.95, 0.98])</td>
</tr>
<tr>
<td>28 hrs</td>
<td>99% (119/120, [C.I. 0.95, 1.00])</td>
<td>96% (610/637, [C.I. 0.94, 0.97])</td>
</tr>
</tbody>
</table>

* Ten of the 27 specimens that were BEAV plus confirmation negative and grew pink and/or blue colonies on VRESelect™ media, after subculture from VRESelect™ to blood agar plates (BAPs), were confirmed to be vancomycin-resistant E. faecium and/or E. faecalis by Vitek® 2 biochemical identification and vancomycin MIC E-Test. They showed the following results.

Table 2 Biochemical identification (Vitek® 2) vs. VRESelect™ results

<table>
<thead>
<tr>
<th>Vitek 2 Biochemical Identification</th>
<th>VREfm</th>
<th>VRESelect™</th>
</tr>
</thead>
<tbody>
<tr>
<td>@ 24 hours</td>
<td>97% (94/97, [C.I. 0.91, 0.99])</td>
<td>97% (639/660, [C.I. 0.95, 0.98])</td>
</tr>
<tr>
<td>@ 28 hours</td>
<td>98% (95/97, [C.I. 0.92, 0.99])</td>
<td>97% (639/660, [C.I. 0.95, 0.98])</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>VRESelect™</th>
<th>% Positive Agreement</th>
<th>% Negative Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hrs</td>
<td>97% (30/38, [C.I. 0.63, 0.89])</td>
<td>97% (696/719, [C.I. 0.95, 0.98])</td>
</tr>
<tr>
<td>28 hrs</td>
<td>82% (31/38, [C.I. 0.66, 0.91])</td>
<td>97% (701/719, [C.I. 0.96, 0.98])</td>
</tr>
</tbody>
</table>
Twenty-one (21) specimens not identified as *E. faecium* on the reference arm of the study grew pink colonies on VRESelect™ media. Twenty (20) of those specimens, after subculture from VRESelect™ to BAPs, were confirmed as vancomycin-resistant *E. faecium* or *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 VRESelect™ media, six were shown to be vancomycin susceptible by the reference arm of the study. One specimen grew blue colonies on VRESelect™ after 28 hours and one 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 VRESelect™ media. Thirteen (13) of those specimens, after subculture from VRESelect™ 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).

VRESelect™ (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. VRESelect™ results

<table>
<thead>
<tr>
<th></th>
<th>VREfm</th>
<th>VRESelect™</th>
<th>VRESelect™</th>
<th>VRESelect™</th>
<th>VRESelect™</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>@ 24 hours</td>
<td>@ 24 hours</td>
<td>@ 28 hours</td>
<td>@ 28 hours</td>
<td>@ 28 hours</td>
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<tr>
<td>% Positive</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>% Negative</td>
<td></td>
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<tr>
<td>Agreement</td>
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<tr>
<td>Agreement</td>
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<td></td>
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</tr>
<tr>
<td>% Positive</td>
<td>99%</td>
<td>99%</td>
<td>100%</td>
<td>96%</td>
<td>96%</td>
</tr>
<tr>
<td>Agreement</td>
<td>(93/94, [C.I. 0.94, 0.99])</td>
<td>(93/94, [C.I. 0.94, 0.99])</td>
<td>(94/94, [C.I. 0.95, 1.00])</td>
<td>(27/28, [C.I. 0.81, 0.99])</td>
<td>(27/28, [C.I. 0.81, 0.99])</td>
</tr>
<tr>
<td>Agreement</td>
<td>98%</td>
<td>98%</td>
<td>98%</td>
<td>97%</td>
<td>97%</td>
</tr>
<tr>
<td></td>
<td>(626/637, [C.I. 0.97, 0.99])</td>
<td>(626/637, [C.I. 0.97, 0.99])</td>
<td>(626/637, [C.I. 0.97, 0.99])</td>
<td>(617/637, [C.I. 0.95, 0.98])</td>
<td>(617/637, [C.I. 0.95, 0.98])</td>
</tr>
</tbody>
</table>

**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 VRESelect™ were considered in positive agreement.

Eleven (11) specimens not identified as vancomycin-resistant on the reference arm of the study grew pink colonies on VRESelect™; the colonies which grew from 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 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 VRESelect™. 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).

**16. ORDERING INFORMATION**

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

**17. REFERENCES**


**Symbol**

Stored plates must be protected from light.


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Bio-Rad Laboratories
6565 185th Avenue NE
Redmond, WA 98052 U.S.A.

Manufactured in France by:
Bio-Rad
3, boulevard Raymond Poincaré
92430 Marnes-la-Coquette France
Tel.: +33 (0) 1 47 95 60 00
Fax: +33 (0) 1 47 41 91 33
www.bio-rad.com

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