

# remel

## Spectra™ VRE

### INTENDED USE

Remel Spectra™ VRE is a selective and differential chromogenic medium, containing 6 µg/ml of vancomycin, intended for use in the qualitative detection of gastrointestinal colonization with vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* (VRE) to aid in the prevention and control of VRE in healthcare settings. The test is performed with rectal swabs and fecal specimens from patients to screen for VRE colonization. Spectra™ VRE is not intended to diagnose VRE infection or to guide or monitor treatment for infections. Subculture to non-selective media (e.g., Tryptic Soy Agar with 5% sheep blood) is needed for further identification, susceptibility testing, and epidemiological typing.

### SUMMARY AND EXPLANATION

Information collected by the CDC during 2006 and 2007 showed that enterococci caused about 1 of every 8 infections in hospitals, with roughly 30% of isolates resistant to vancomycin.<sup>1</sup> Vancomycin-resistant enterococci (VRE) are the third leading cause of hospital-acquired infections resulting in higher morbidity, mortality, and increased hospital costs.<sup>2</sup> Patients colonized with VRE are at increased risk of developing a VRE infection.<sup>3,4</sup>

The *vanA* gene confers high-level vancomycin resistance in enterococci. The transfer of the genetic element containing *vanA* from *Enterococcus faecalis* to *Staphylococcus aureus* resulted in the first vancomycin-resistant *S. aureus* (VRSA) clinical infection in 2002.<sup>5</sup> Patients at risk for VRSA are co-infected or co-colonized with VRE and methicillin-resistant *Staphylococcus aureus* (MRSA). A study by Warren et al. revealed a 9.5% incidence rate of co-colonization or co-infection with VRE and MRSA.<sup>6</sup> To date, eleven cases of VRSA have been reported in the U.S. and it remains a serious public health concern due to limited treatment options.<sup>7</sup>

VRE can be transmitted directly by patient-to-patient contact or indirectly by transient carriage on the hands of healthcare workers or by contaminated environmental surfaces and patient-care equipment. The Healthcare Infection Control Practices Advisory Committee (HICPAC) issued recommendations for the management of Multidrug-Resistant Organisms (MDROs), including VRE, in healthcare settings in 2006.<sup>8</sup> Implementation of active surveillance cultures (ASC) to identify colonized patients and Contact Precautions are strongly recommended as control measures to reduce MDRO transmission.

### PRINCIPLE

Remel Spectra™ VRE is an opaque medium allowing differentiation of vancomycin-resistant *E. faecium* from vancomycin-resistant *E. faecalis* by incorporation of two chromogens that are targeted by phosphatase and α-galactosidase. The action of these enzymes on the chromogens results in a build-up of color within the colony. The presence of phosphatase enzymes in both *E. faecium* and *E. faecalis* results in a light blue or blue colony. However, *E. faecium* also produces α-galactosidase, resulting in a mix of blue and pink chromophores within the bacterium producing navy blue or pink-purple colonies, which are distinguished from the light blue or blue *E. faecalis* colonies. Additional antibiotics, in combination with vancomycin, are present to suppress the growth of competing flora including *E. gallinarum* and *E. casseliflavus*, both of which are intrinsically resistant to vancomycin, possessing the chromosomally encoded VanC resistance mechanism.

### REAGENTS (CLASSICAL FORMULA)\*

Peptone Mix .....	25.0 g
Salt Mix .....	25.0 g
Titanium Dioxide.....	1.0 g
Chromogenic Mix .....	0.45 g
Selective Agents.....	5.0 ml
Agar.....	12.5 g
Demineralized Water.....	1000.0 ml
pH 6.5 ± 0.2 @ 25°C	

\*Adjusted as required to meet performance standards.

### PRECAUTIONS

- For *in vitro* diagnostic use only.
- For professional use only.
- Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use.
- Directions should be read and followed carefully. Further interpretation of test results should be considered based on patient history, the source of the specimen, colonial and microscopic morphology, and the result of any other test performed.
- Use of this medium may be difficult for those who have problems recognizing colors.

### STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use. Do not incubate prior to use.

### PRODUCT DETERIORATION

This product should not be used if (1) there is evidence of dehydration, (2) the product is contaminated, (3) the color has changed from off-white, (4) the expiration date has passed, or (5) there are other signs of deterioration.

### SPECIMEN COLLECTION, STORAGE, TRANSPORT

Specimens should be collected and handled following recommended guidelines.<sup>9</sup>

### MATERIALS REQUIRED BUT NOT SUPPLIED

- (1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms.

### PROCEDURE

Allow plates to equilibrate to room temperature prior to inoculation.

1. Inoculate the specimen and streak for isolation as soon as possible after it is received in the laboratory.
2. Incubate plates in ambient air for 24 hours at 33-37°C.
3. Observe colony characteristics, morphology, and color reactions.

### INTERPRETATION OF THE TEST

At 24 hours incubation, vancomycin-resistant *E. faecium* will appear as navy blue or pink-purple colonies against a white background. Vancomycin-resistant *E. faecalis* colonies will appear as light blue to blue colonies at 24 hours. Colonies of VRE are raised, smooth, and small to medium in size. If after 24 hours incubation no colonies matching the description for vancomycin-resistant *E. faecium* or vancomycin-resistant *E. faecalis* are observed, the specimen is considered negative and plates should be discarded.

24 hours incubation	Interpretation
Light blue to blue colonies	Positive – Vancomycin-resistant <i>E. faecalis</i> colonization
Navy blue or pink-purple colonies	Positive – Vancomycin-resistant <i>E. faecium</i> colonization
No colored colonies	Negative – No VRE colonization

**Note:** Color reactions are key to all chromogenic media. Interpretation charts or comparison to a positive control can assist new users (call Technical Service).

#### QUALITY CONTROL

All lot numbers of Spectra™ VRE have been tested using the following quality control organisms and have been found to be acceptable. Quality control requirements must be performed in accordance with applicable local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
<i>Enterococcus faecalis</i> ATCC® 51299	Ambient, 24 h @ 33-37°C	Light blue colonies
<i>Enterococcus faecium</i> ATCC® 51559	Ambient, 24 h @ 33-37°C	Pink-purple colonies
<i>Enterococcus faecalis</i> ATCC® 29212	Ambient, 24 h @ 33-37°C	No growth

#### PROCEDURE FOR TESTING QUALITY CONTROL ORGANISMS<sup>a</sup>

1. Use a pure, 18-24 hour culture of each quality control organism.
2. Prepare a suspension of each organism in sterile, nonbacteriostatic saline (0.85% w/v NaCl) equal in density to a 0.5 McFarland standard (1x10<sup>7</sup> to 1x10<sup>8</sup> cfu/ml).
3. Dilute the suspension 1:10 in sterile broth or nonbacteriostatic saline.
4. Inoculate Spectra™ VRE with 10 µl (0.01 ml) of the diluted suspension using a calibrated loop or pipette and streak for isolation.
5. Incubate and read results at 24 hours.

<sup>a</sup>Refer to Clinical and Laboratory Standards Institute (CLSI) M22.<sup>12</sup>

#### LIMITATIONS

1. VRE with atypical enzyme patterns (phosphatase and α-galactosidase) may yield uncharacteristic results (false-positive or false-negative).
2. Fecal samples may cause some localized pink-purple media discoloration in the primary area of inoculation. This should not be confused with a chromogenic reaction with the color expressed in the colony.
3. The growth requirements of certain VRE may lead to their partial or complete inhibition in culture.<sup>10,11</sup>
4. Rarely, strains of *E. faecalis* or *E. faecium* with intermediate resistance to vancomycin may yield positive results and require subculture for further confirmation.
5. Surveillance testing determines the colonization status at a given time and can vary depending on patient treatment, patient status (actively shedding), or exposure to high-risk environments. Monitoring of colonization status should be performed in accordance with hospital policies and procedures.
6. In studies conducted, two vancomycin-resistant *E. faecium* strains with vancomycin MICs > 256 µg/ml were falsely negative at 24 hours on Spectra™ VRE and falsely negative at 48 hours by the comparator method.
7. Three *Lactobacillus* strains were encountered in this study that demonstrated false positives by the comparator method at 48 hours. One of these strains produced pink colonies on Spectra™ VRE at 24 hours and was considered falsely positive.

8. Forty perianal swabs (twelve positive and twenty-eight negative) were tested which did not yield a statistically sound 95% lower bound confidence interval. The results are not included in the data.
9. Some strains of KPC-producing *Klebsiella pneumoniae* may develop large blue colonies on Spectra™ VRE at 24 hours.
10. Blood, Pepto-Bismol®, glycerin, vancomycin, miconazole, and Preparation H® may reduce the recovery of VRE.
11. A negative result does not rule out the possibility of VRE colonization.

#### EXPECTED VALUES

Information collected by the Centers for Disease Control and Prevention during 2006 and 2007 showed that enterococci caused about 1 of every 8 infections in hospitals, with roughly 30% of isolates resistant to vancomycin (i.e., VRE). VRE are the third leading cause of hospital-acquired infection. Hospital-acquired enterococcal infections typically occur in very ill, debilitated patients who have been exposed to broad-spectrum antibiotics. They are also the third most common cause of hospital-acquired bloodstream infections in the U.S. The overall prevalence rate of VRE colonization in this study was 35%.

#### PERFORMANCE CHARACTERISTICS

##### Clinical Accuracy:

The performance of Spectra™ VRE was evaluated at three geographically diverse regions of the United States. A total of six hundred twenty-three (623) prospective rectal swab and fecal surveillance specimens (yielding 629 data points) were evaluated. Results from Spectra™ VRE at 24 hours incubation were compared to results obtained from traditional culture on Bile Esculin Azide Agar with 6 µg/ml Vancomycin (BEAV) after 48 hours incubation. Two hundred twenty VRE with minimal inhibitory concentration MICs to vancomycin of >256 µg/ml were recovered from six hundred twenty three specimens (191 vancomycin-resistant *E. faecium* and 29 vancomycin-resistant *E. faecalis*). The overall recovery of VRE on Spectra™ VRE at 24 hours was 99.1% (218/220) compared to recovery of 95.5% (210/220) on BEAV at 48 hours.

Suspect isolates of VRE were evaluated using the Vitek® 2 system and biochemical tests, and an antibiotic gradient method for determination of vancomycin MIC. For detection of VRE by colored colonies isolated on Spectra™ VRE at 24 hours compared to identification and susceptibility testing as described, the overall agreement was 99.5% (626/629).

#### Spectra™ VRE vs. Conventional Methods

Table 1:

	Positive % Agreement	Negative % Agreement
Spectra™ VRE vs. conventional methods	99.1% (218/220) (95% CI = 96.8–99.9%)	99.8% (408/409) (95% CI = 98.6–100%)

Note: CI = Confidence Interval

Forty perianal swabs (twelve positive and twenty-eight negative) were tested which did not yield a statistically sound 95% lower bound confidence interval. The results are not included in the data.

Table 2:

	Positive % Agreement	Negative % Agreement
VR- <i>E. faecium</i>	99.0% (189/191) <sup>a</sup> (95% CI = 96.3–99.9%)	99.8% (437/438) <sup>b</sup> (95% CI = 98.7–100%)
VR- <i>E. faecalis</i>	100% (29/29) (95% CI = 88.1–100%)	100% (600/600) (95% CI = 99.4–100%)

Note: CI = Confidence Interval

<sup>a</sup> One isolate showed expected results at 28 hours and one isolate showed expected results at 48 hours.

<sup>b</sup> One isolate developed pink colonies and was identified as *Lactobacillus* sp.

**Table 3: Spectra™ VRE vs. Conventional Methods (fecal specimens)**

	Positive % Agreement	Negative % Agreement	Total
<i>VR-E. faecium</i>	98.3% (113/115) <sup>a</sup> (95% CI = 93.9–99.8%)	99.7% (288/289) <sup>b</sup> (95% CI = 98.1–100%)	99.3% (401/404) (95% CI = 97.8–99.8%)
<i>VR-E. faecalis</i>	100% (3/3) (95% CI = 29.2–100%)	100% (401/401) (95% CI = 99.1–100%)	100% (404/404) (95% CI = 99.1–100)
<b>Total</b>	98.3% (116/118) (95% CI = 94.0–99.8%)	99.7% (285/286) (95% CI = 98.1–100%)	99.3% (401/404) (95% CI = 97.8–99.8%)

<sup>a</sup> One isolate showed expected results at 28 hours and one isolate showed expected results at 48 hours.

<sup>b</sup> One isolate developed pink colonies on Spectra™ VRE and was identified as *Lactobacillus* sp.

**Table 4: Spectra™ VRE vs. Identification (Vitek® 2 and biochemicals) (fecal specimens)**

	Positive % Agreement	Negative % Agreement	Total
<i>VR-E. faecium</i>	98.3% (113/115) <sup>a</sup> (95% CI = 93.9–99.8%)	99.7% (288/289) <sup>b</sup> (95% CI = 98.1–100%)	99.3% (401/404) (95% CI = 97.8–99.8%)
<i>VR-E. faecalis</i>	100% (3/3) (95% CI = 29.2–100%)	100% (401/401) (95% CI = 99.1–100%)	100% (404/404) (95% CI = 99.1–100)
<b>Total</b>	98.3% (116/118) (95% CI = 94.0–99.8%)	99.7% (285/286) (95% CI = 98.1–100%)	99.3% (401/404) (95% CI = 97.8–99.8%)

<sup>a</sup> One isolate showed expected results at 28 hours and one isolate showed expected results at 48 hours.

<sup>b</sup> One isolate developed pink colonies on Spectra™ VRE and was identified as *Lactobacillus* sp.

**Table 5: Spectra™ VRE vs. Vancomycin MIC (gradient method) (fecal specimens)**

	Positive % Agreement	Negative % Agreement	Total
<i>VR-E. faecium</i>	98.3% (113/115) <sup>a</sup> (95% CI = 93.9–99.8%)	99.7% (288/289) <sup>b</sup> (95% CI = 98.1–100%)	99.3% (401/404) (95% CI = 97.8–99.8%)
<i>VR-E. faecalis</i>	100% (3/3) (95% CI = 29.2–100%)	100% (401/401) (95% CI = 99.1–100%)	100% (404/404) (95% CI = 99.1–100)
<b>Total</b>	98.3% (116/118) (95% CI = 94.0–99.8%)	99.7% (285/286) (95% CI = 98.1–100%)	99.3% (401/404) (95% CI = 97.8–99.8%)

<sup>a</sup> One isolate showed expected results at 28 hours and one isolate showed expected results at 48 hours.

<sup>b</sup> One isolate developed pink colonies on Spectra™ VRE and was identified as *Lactobacillus* sp.

**Table 6: Spectra™ VRE vs. Conventional Methods (rectal swabs)**

	Positive % Agreement	Negative % Agreement	Total
<i>VR-E. faecium</i>	100% (76/76) (95% CI = 95.3–100%)	100% (149/149) (95% CI = 97.6–100%)	100% (225/225) (95% CI = 98.4–100%)
<i>VR-E. faecalis</i>	100% (26/26) (95% CI = 86.8–100%)	100% (199/199) (95% CI = 98.2–100%)	100% (225/225) (95% CI = 98.4–100%)
<b>Total</b>	100% (102/102) (95% CI = 96.4–100%)	100% 123/123) (95% CI = 97.0–100%)	100% (225/225) (95% CI = 98.4–100%)

**Table 7: Spectra™ VRE vs. Identification (Vitek® 2 and biochemicals) (rectal swabs)**

	Positive % Agreement	Negative % Agreement	Total
<i>VR-E. faecium</i>	100% (76/76) (95% CI = 95.3–100%)	100% (149/149) (95% CI = 97.6–100%)	100% (225/225) (95% CI = 98.4–100%)
<i>VR-E. faecalis</i>	100% (26/26) (95% CI = 86.8–100%)	100% (199/199) (95% CI = 98.2–100%)	100% (225/225) (95% CI = 98.4–100%)
<b>Total</b>	100% 102/102) (95% CI = 96.4–100%)	100% (123/123) (95% CI = 97.0–100%)	100% (225/225) (95% CI = 98.4–100%)

**Table 8: Spectra™ VRE vs. Vancomycin MIC (gradient method) (rectal swabs)**

	Positive % Agreement	Negative % Agreement	Total
<i>VR-E. faecium</i>	100% (76/76) (95% CI = 95.3–100%)	100% (149/149) (95% CI = 97.6–100%)	100% (225/225) (95% CI = 98.4–100%)
<i>VR-E. faecalis</i>	100% (26/26) (95% CI = 86.8–100%)	100% (199/199) (95% CI = 98.2–100%)	100% (225/225) (95% CI = 98.4–100%)
<b>Total</b>	100% (102/102) (95% CI = 96.4–100%)	100% (123/123) (95% CI = 97.0–100%)	100% (225/225) (95% CI = 98.4–100%)

**Challenge Testing:**

Spectra™ VRE was evaluated with fifty well-characterized strains of enterococci (vancomycin susceptible and resistant enterococci) from the Centers for Disease Control and Prevention. Three strains of *E. faecium* with vancomycin MICs between 128-1024 µg/ml failed to grow at 24 hours. One strain of *E. faecium* with a vancomycin MIC of 16 µg/ml grew and produced pink-purple colonies.

**Interfering Substances:**

The following substances were evaluated for potential interference of the chromogenic reaction of Spectra™ VRE. These substances were tested in combination with vancomycin-resistant *E. faecalis* and *E. faecium* isolates at a concentration of 50 CFU: blood, mucous, MYLANTA® Maximum Strength, Pepto-Bismol®, Imodium® A-D, Kaopectate®, Fletcher's Castoria®, PEPCID® AC Maximum Strength, Tagamet HB 200®, Prilosec OTC®, vancomycin, metronidazole, barium sulfate, Preparation H®, petroleum jelly, glycerin, bisacodyl, witch hazel, miconazole, nonoxynol-9, KY® Jelly. Hydrocortisone acetate was not evaluated. Blood, Pepto-Bismol®, glycerin, vancomycin, miconazole, and Preparation H® may reduce the recovery of vancomycin resistant *E. faecalis* and *E. faecium* strains.

**Cross Reactivity:**

Two hundred twenty-nine (229) microorganisms representing gram-negative rods, yeast, streptococci, enterococci, staphylococci, and related organisms were evaluated with Spectra™ VRE. Six of eleven KPC-producing *Klebsiella pneumoniae* developed large blue colonies on Spectra™ VRE at 24 hours. No other cross reactivity was observed following 24 hours incubation.

**Reproducibility:**

Reproducibility testing was conducted at four sites on three separate days with twenty blinded strains including vancomycin susceptible and resistant *E. faecium* and *E. faecalis*, as well as quality control reference strains. The strains produced the expected result with Spectra™ VRE 100% of the time at 24 hours.

**Limit of Detection Study:**

A study was conducted to determine the limit of detection of Spectra™ VRE for the recovery of VRE. Fifty-eight strains (40 vancomycin-resistant *E. faecium* and 18 vancomycin-resistant *E. faecalis*) were evaluated. The limit of detection for Spectra™ VRE was determined to be 50 colony forming units per milliliter (cfu/ml).

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**PACKAGING**

**Spectra™ VRE**

- REF R01830, 85 mm Plate ..... 10/Pk
- REF R01832, 85 mm Plate ..... 100/Pk

**Symbol Legend**

<b>REF</b>	Catalog Number
<b>IVD</b>	In Vitro Diagnostic Medical Device
<b>LAB</b>	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
<b>LOT</b>	Batch Code (Lot Number)
	Use By (Expiration Date)

U.S. Patent Pending

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