Detection of Vancomycin Resistance in *Enterococcus* spp. using E- Test

**Purpose:**

*Enterococcus* spp. generally show two types of resistance to vancomycin. The first type is intrinsic resistance and the other is acquired resistance. Isolates of *Enterococcus gallinarum* and *E. casseliaves/E. flavescens* demonstrate an inherent, low-level resistance to vancomycin, whereas *E. faecium* and *E. faecalis* exhibit acquired resistance. Identification of Vancomycin Resistant Enterococci (VRE) to species level aids in confirming the type of resistance which in turn is critical for infection control purposes.

**Principle:**

The Epsilometer (E) test is an agar diffusion method which utilises a predefined continuous and exponential gradient of antibiotic concentrations immobilized along a rectangular strip. For testing vancomycin susceptibility, a strip with concentration gradient of 0.016 to 256 μg/ml is used. A lawn culture of the test strain of enterococcus spp is spread and grown on Brain heart infusion agar plate and the E test strip is laid on it. Vancomycin diffuses out into the agar producing an exponential gradient. After overnight incubation at 35±2°C an elliptical zone of inhibition is produced and the point at which the ellipse meets the strip gives a reading for the minimum inhibitory concentration (MIC) of vancomycin for the test enterococcus isolate.

**Procedure:**

*Preparation of inoculum*

Randomly selected 2-3 colonies of the *Enterococcus* spp. is inoculated into fresh Brain heart infusion broth and grown overnight at 37±2°C. The turbidity of the overnight broth is adjusted to 0.5 McFarland standards using fresh broth.

*Inoculation and incubation:*

a. 200μl of turbidity adjusted bacterial suspension is pipetted onto a 90-mm Brain heart infusion agar plate and spread out evenly with a swab to obtain a lawn culture.

b. After being dried for approximately 10 minutes, vancomycin E test strip (Himedia Laboratories Pvt. Ltd. Mumbai) is applied on to the surface.

c. The plate is incubated at 35°±2°C for 24 hours, a maximum up to 48 hours.

*Reading of results:*

Results are read at 24 hours and confirmed at 48 hours. The edge of the elliptical zone intersecting the graded strip (Figure-1) is the minimum inhibitory concentration for vancomycin for the test enterococcus isolate. Look for hazes, micro colonies and isolated colonies. Use a magnifying glass, oblique light and/or tilt the plate to look for all growth.

**Interpretation:**

<table>
<thead>
<tr>
<th>CLSI MIC CRITERIA</th>
<th>SUSCEPTIBLE</th>
<th>INTERMEDIATE</th>
<th>RESISTANT</th>
</tr>
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<tbody>
<tr>
<td>(µg/ml)</td>
<td>≤4</td>
<td>8-16</td>
<td>≥32</td>
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</tbody>
</table>
Presence of haze or growth in the zone of inhibition indicates resistance. Those Enterococcus strains which show MIC between 8-16 µg/ml should be tested for motility and pigment production to distinguish species with acquired resistance (VanA and Van B) from those with intrinsic intermediate level resistance to vancomycin (VanC)

**Quality control:**

<table>
<thead>
<tr>
<th>Control</th>
<th>Bacterial strain</th>
<th>ATCC No.</th>
<th>MIC</th>
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</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>Enterococcus faecalis</td>
<td>ATCC 29212</td>
<td>1-4 µg/ml</td>
</tr>
<tr>
<td>Resistant</td>
<td>Enterococcus faecalis</td>
<td>ATCC 51559</td>
<td>≥32 µg/ml</td>
</tr>
</tbody>
</table>

**References:**


**Figure-1**

Vancomycin sensitive *Enterococcus faecalis*  
Vancomycin Resistant *Enterococcus faecalis*