Detection of high-level Aminoglycoside resistance in Enterococci  
By Disk diffusion screening method

**Purpose:**
Enterococci are intrinsically resistant to low concentrations of aminoglycosides, due to poor rug uptake by the enterococcal cells. Infections with these isolates can be treated by a combination of an aminoglycoside with a cell wall active agent (ampicillin, penicillin or vancomycin) because a synergistic interaction occurs with this combination of drugs. When enterococci develop high level aminoglycoside resistance, the particular aminoglycoside does not show synergism with the cell wall active agent. Gentamicin resistance is associated with a bifunctional enzyme, 2’-phosphotransferase 6’-acetyltransferase, which possesses phosphotransferase and acetylase activities. This enzyme confers resistance not only to gentamicin, but also to kanamycin, netilmicin, amikacin and tobramycin. Consequently, none of these agents can be used to treat infections caused by enterococci with high level gentamicin resistance. However, if the isolate does not have concomitant high level streptomycin resistance, streptomycin could be used. This is because streptomycin resistance is mediated by a different enzyme, namely streptomycin adenyltransferase. Some isolates have high level resistance to both gentamicin and streptomycin.

**Principle**
High Level Aminoglyside Resistance (HLAR) in Enterococci is generally detected by assessing growth at high concentrations of gentamicin (120µg) and streptomycin (300µg) disks on Mueller Hinton agar

**Procedure:**

*Preparation of inoculum*
Using a sterile loop, 2-3 colonies of enterococcus isolate is inoculated into 3.0ml Mueller-Hinton broth and incubated at 35±2ºC for 4-6h. The turbidity of the growth is adjusted to 0.5 McFarland standards using fresh broth.

*Inoculation and incubation*
- a. A sterile cotton swab is dipped into the organism suspension.
- b. Any excess fluid is expressed by pressing the swab against the side of the tube.
- c. Swab is streaked over the dry surface of a Mueller-Hinton agar plate.
- d. Gentamicin and a streptomycin disk are placed on the inoculated agar surface.
- e. Plates are incubated at 35±2ºC for 18-24 h.

**Observation:**
Examine bacterial lawn culture for adequate growth and measure zone of inhibition as for the standard disk diffusion test.
Interpretation:

**Resistant:** Zone of inhibition = 6mm indicates high level resistance to gentamycin/streptomycin. Strains that show resistance to high level gentamicin will not be synergistically killed by combinations of cell wall active drugs (e.g., ampicillin, penicillin or vancomycin) with any aminoglycoside except streptomycin. Strains that show resistance to high level streptomycin will not be killed by combinations of cell wall active drugs with streptomycin.

**Susceptible:** Zone diameter ≥ 10mm indicates that the isolate is susceptible to high level gentamycin/streptomycin. Strains that show susceptibility to high level aminoglycoside will be synergistically killed by combination of that aminoglycoside with a cell wall active agent (ampicillin, penicillin or vancomycin) that is also susceptible.

**Inconclusive:** Zone diameter of 7-9mm is inconclusive: perform agar dilution or broth microdilution tests to confirm.

### Quality control strains:

<table>
<thead>
<tr>
<th>Control</th>
<th>Bacterial strain</th>
<th>ATCC No.</th>
<th>Zone size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible strain</td>
<td><em>Enterococcus faecalis</em></td>
<td>ATCC 29212</td>
<td>16-23mm for Gentamicin &amp; 14-20mm for streptomycin</td>
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<tr>
<td>Resistant strain</td>
<td><em>Enterococcus faecalis</em></td>
<td>ATCC 51559</td>
<td>≤ 6mm for both Gentamicin &amp; streptomycin</td>
</tr>
</tbody>
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### References:


