Improved growth of Vancomycin resistant Enterococci on ChromID™ VRE agar by incubation in 5% CO₂

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Introduction
Vancomycin resistant Enterococci (VRE) are increasingly reported in health care institutions world wide. The development of reliable and rapid methods for the identification of vancomycin-resistant enterococci is essential in controlling the spread of this organism. The current culture techniques may take up to 72 hours or more to isolate and identify VRE.

Vancomycin resistant van A and van B phenotypes occur in clinical isolates of Enterococcus faecalis or Enterococcus faecium. Van B phenotype is characterized by inducible resistance to vancomycin. Minimum inhibitory concentration may range from 4 to >1,000 mg/ml.1

ChromID™ VRE (bioMérieux, Marcy l’Etoile, France) was developed for the selective growth of VRE and direct detection of E. faecium and E. faecalis. This selective medium contains chromogenic substrates and vancomycin (8 mg/ml).2

The manufacturer recommends incubation at 37°C in aerobic atmosphere (air) and in an atmosphere of 5% CO₂. This selective agar medium was tested by Delmas, J., Robin, F., Schweitzer, C., Lesens, O., and Bonnet. R., 2007. “Perianal Swabs” Journal of Clinical Microbiology, Vol. 42 : 2636–2643.

Method
32 VRE isolates (van B or vanB2/3 genotypes) were used. Also three isolates of Enterococcus casseliflavus and three isolates of Enterococcus gallinarum with low level intrinsic vancomycin resistance (van C phenotype) were also tested. E. faecalis (ACM 5184) was used as a vancomycin sensitive control.

Bacterial suspensions were made from overnight cultures using 0.9% saline. The turbidity was adjusted to 0.8 cu/ml. This suspension was further diluted 1/100 and 1/10 to give final inocula of 10⁹ cfu (light inoculum) and 10¹⁰ cfu (heavy inoculum). ChromID™ VRE plates and a horse blood agar (HBA) control plates were inoculated with a Streak Replicator using the two suspensions. Duplicate plates were then inoculated in an aerobic atmosphere (air) and in an atmosphere of 5% CO₂ (96.9% and 62.5%) and aerobic atmosphere (air) at 37°C and were examined at 24 and 48 hours. The test plates were compared to HBBA control plates. The growth was recorded and given a score of 1 to 3+ (growth score), using:

3+ = 100% of growth of control
2+ = 50% of growth of control
1+ = 10% of growth of control

Additionally, the effect of the growth of Gram-negative organisms on the ChromID™ VRE agar was tested by inoculating 8 isolates of E. coli and 3 isolates of Pseudomonas species which were selected randomly and incubated in both air and CO₂.

Results
After 24 hours incubation, with the heavy inoculum of 10⁹ cfu/ml, 96.9% (31 of 32) isolates showed visible growth when incubated in CO₂. In comparison, only 43.8% (14 of 32) isolates had visible growth when incubated in air. (x²=11.47, P<0.001).

After 48 hours of incubation, with the heavier inoculum, 100% (32 of 32) isolates grew in 5% CO₂ and 96.9% (31 of 32) isolates grew in air. Similarly, with the lighter inoculum, 62.5% (20 of 32) isolates grew in CO₂ and 46.9% (15 of 32) grew in air (Table 1).

Table 1: The presence of visible colonies of VRE (10⁹ and 10¹⁰ inocula) on chromID™ selective agar in 5% CO₂ and aerobic incubation after 24 and 48 hours incubation

<table>
<thead>
<tr>
<th>Duration of incubation</th>
<th>5% CO₂</th>
<th>Air</th>
<th>10⁹ inoculum</th>
<th>10¹⁰ inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>31/32</td>
<td>14/32</td>
<td>18/32</td>
<td>5/32</td>
</tr>
<tr>
<td>48 h</td>
<td>32/32</td>
<td>31/32</td>
<td>20/32</td>
<td>15/32</td>
</tr>
</tbody>
</table>

With the lighter inoculum of 10⁹ cfu, 56.3% (18 of 32) of isolates grew in CO₂. In comparison, only 15.6% (5 of 32) were positive in air. (x²=11.47, P<0.001).

After 48 hours of incubation, with the heavier inoculum, 100% (32 of 32) isolates grew in 5% CO₂ and 96.9% (31 of 32) isolates grew in air. Similarly, with the lighter inoculum, 62.5% (20 of 32) isolates grew in CO₂ and 46.9% (15 of 32) grew in air (Table 1).

Conclusions
In this study, we observed enhanced growth of VRE both quantitatively and qualitatively on ChromID™ agar when incubated in 5% CO₂ atmosphere. Therefore, it is possible to identify more isolates of vancomycin resistant E. faecalis and E. faecium after 24 hours of incubation when grown in 5% CO₂ compared to an aerobic atmosphere. This would help in earlier identification of Enterococcus species and vancomycin resistance. The colonies can be picked directly from the screening plates for confirmation by the CDS test and/or PCR. Thus potentially more positive results could be issued early (< 48 hours).

However this study was done on identified laboratory isolates, not on direct screening swabs or faecal specimens. Therefore the effect of 5% CO₂ on direct plating of faeces or rectal swabs on the ChromID™ VRE agar medium was not assessed.

Even in an atmosphere of 5% CO₂ if the initial inoculum is low, a minimum of 48 hours incubation is needed for growth to be apparent. All VRE isolates tested were either van B or van B 2/3 genotype, (which has a lower vancomycin MIC compared to van A genotype). Some isolates may be inhibited by the selective ChromID™ VRE agar if the initial inoculum is low. This observation supports the view that parallel inoculation of rectal swabs or faecal specimens in broth is necessary to achieve a satisfactory sensitivity for screening.

Incubating ChromID™ VRE selective agar plates in 5% CO₂ could help in early detection of VRE. Thereby the time taken for the procedure can be reduced by at least 24 hours.

References