

Title: EVALUATION OF A SELECTIVE CHROMOGENIC MEDIUM FOR THE DETECTION OF VANCOMYCIN RESISTANT ENTEROCOCCI PREVIOUSLY CHARACTERIZED FOR PHENOTYPIC AND MOLECULAR METHODS

Authors: Soares, R.O.¹, Tolfo, N.¹, Paim, T.G.S.¹, Sambrano, G.², Caierão, J.¹, d'Azevedo, P.A.¹

Affiliations: ¹ UFCSPA - Universidade Federal de Ciências da Saúde de Porto Alegre (Rua Sarmiento Leite, 245 – Rio Grande do Sul, Porto Alegre, Brasil), ² NUI - National University of Ireland Galway (University Road, Galway, Ireland)

Vancomycin Resistant Enterococci (VRE) have emerged in Healthcare Associated Infections and they are considered a public health problem. The rapid identification can assist in choosing the appropriate treatment and in preventing the spread of this pathogen. The aim of this study was to evaluate the performance of a selective chromogenic medium for the detection and differentiation of *Enterococcus faecium* and *Enterococcus faecalis* showing acquired vancomycin resistance. In this study, 53 VSE clinical isolates, 134 VRE clinical isolates, 4 isolates intrinsically resistant to vancomycin (*E. casseliflavus* and *E. gallinarum*) and 9 ATCC strains were evaluated. All the isolates were previously identified by phenotypic and molecular methods and all isolates belonged to the strains bank of the Gram-positive Cocci Laboratory - UFCSPA and were preserved in Skim Milk (Difco™) at -20°C. The minimum inhibitory concentration to vancomycin was performed by broth microdilution method according to CLSI (2015) and confirmed by E-test®. The ChromID™ VRE assays were performed in two steps: first the isolates preserved in Skim Milk were grown in Bile Esculin agar and in Trypticase Soy agar; after growth, a pure colony was grown in ChromID™ VRE agar and all the plates were incubated at 35°-37°C. After 24 hours, the observation of existing growth on the plates was performed. Of the 47 *E. faecalis* VRE tested, 41 showed the blue-green coloration and all the 88 *E. faecium* VRE tested showed the violet staining, according to the manufacturer's instructions. Four *E. faecalis* VRE showed grayish staining, that can cause to confusion on observation, and one isolate did not grow. Among the 55 VSE isolates, 20 did not show growth and 35 isolates showed some growth at the edges of the plates that may suggest a false-positive result. The VSE-ATCC isolates, as well as, *E. casseliflavus* and *E. gallinarum* did not exhibit any growth in the chromogenic medium. As noted, the chromID™ VRE agar showed 97.87% and 100% of sensivity to *E. faecalis* VRE and *E. faecium* VRE detections, respectively. The slow growth observed in the agar by susceptible isolates can be related to the poor stability of the glycopeptide molecule in the medium. It is suggested that tests for determining the optimum time of use and medium conservation be performed to the maintenance of the initial concentration of vancomycin indicated by the manufacturer, thereby avoiding the growth of *Enterococcus* susceptible to this antibiotic.

Keywords: ChromID™ VRE, chromogenic medium, *Enterococcus faecalis*, *Enterococcus faecium*, VRE