

Evaluation of Chromogenic Agar for Screening Vancomycin-resistant Enterococcus (VRE)

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BACKGROUND

Currently, our institution uses an in-house mEnterococcus agar to differentiate between *Enterococcus faecalis* and *Enterococcus faecium*, but it has a long incubation time of 72 hours. New chromogenic agars, such as Colorex™ VRE media from PML Microbiologicals and chromID™ VRE media from bioMérieux, have recently become commercially available, and have been shown to be superior for VRE identification.

OBJECTIVES

- To verify and validate the role of these agars in screening for VRE in an acute-care pediatric hospital setting
- To evaluate the effectiveness of the commercial chromogenic agars when compared with the in-house mEnterococcus agar

METHODS

The lower limit of detection was calculated by spiking a known quantity (CFU/mL) of VRE into normal stool specimens.

To quantify analytical sensitivity and specificity, a panel of 32 well-defined strains, including *E. faecium*, *E. faecalis*, *Leuconostoc* spp., *Pediococcus* spp., *Candida* spp., and *Enterobacteriaceae*, was used.

Inter-observer variability was evaluated by asking 4 experienced medical lab technologists to independently read each plate and document their findings, generating a Kappa (K) score to measure consistency.

Clinical specimens (n = 127) were plated onto the chromogenic agars to assess their clinical sensitivity and specificity.

Confirmation of VRE was done through the Roche LightCycler VRE Detection Kit, which detects the *vanA* and *vanB* vancomycin resistance genes.

RESULTS

The detection limit for the 3 plates in this study was 10⁻⁴ CFU/mL. Visual discrimination of VRE colonies was easiest on the Colorex™ plate.

Inter-observer variability was noted for 2 of 32 specimens for the Colorex™ and chromID™ VRE agar (K = 0.875).

mEnterococcus agar had variability for 3 of 32 specimens (K = 0.811). Eight of 32 isolates on mEnterococcus agar were too small to identify and were noted to be "pinpoint" after 72 hours of growth; these were found to not be *Enterococcus*.

Table 1: Analytical sensitivity and specificity using n = 32 well-defined strains

Medium	Sensitivity (%)	Specificity (%)
Colorex™	100	100
chromID™	89	100
mEnterococcus	100	79

Chromogenic Agars at 24 hours After Inoculation with Stool Containing 10⁶ CFU/mL of VRE

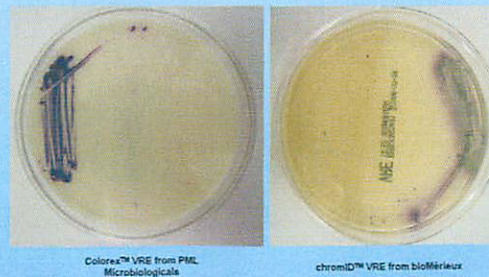


Table 2: Clinical sensitivity of chromogenic agars using 127 patient samples

Medium	Number of True Positives	Sensitivity (%)
Colorex™	6	100
chromID™	5	83

CONCLUSIONS

- The Colorex™ agar was superior to both the chromID™ and in-house mEnterococcus agar
- The Colorex™ agar correctly identified the small colony variant VRE previously observed in Ontario, which was not found on either of the mEnterococcus or chromID™ agars
- The Colorex™ agar exhibited superior sensitivity when clinical specimens were evaluated

REFERENCES

- Landis, JR; Koch, GG. 1977. The measurement of observer agreement for categorical data. *Biometrics*. 33:159-174.
- Ledeboer, N.A., K. Das, M. Eveland, C. Roger-Dalbert, S. Mailler, S. Chatellier, and W.M. Dunne. 2007. Evaluation of a novel chromogenic agar medium for isolation and differentiation of vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* isolates. *J. Clin. Microbiol.* 45:1556-1560.
- Ledeboer, N.A., R.J. Tibbells, and W.M. Dunne. 2007. A new chromogenic agar medium, chromID VRE, to screen for vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis*. *Diagn. Microbiol. Infect. Dis.* 59:477-479.
- Miles, A.A. S. S. Misra, and J. O. Irwin. 1938. The estimation of the bactericidal power of the blood. *J. Hyg.* 38:732-749.
- Poutanen, S.M., B.M. Willey, A. McGeer, M. Lum, L. Louie, S. Callery, S.H. Goh, Y. Yau, F. Jamieson, and S. Krajden. Characteristics of a Small Colony Variant Vancomycin-Resistant *Enterococcus faecium* (scvVRE) Identified in Ontario. *Abstr. Xxth Intersci. Conf. Antimicrob. Agents Chemother.*, abstr. C2-257.
- Sloan, L. M., J. R. Uhl, E. A. Vetter, C. D. Schleck, W. S. Harnsen, J. Manahan, R. L. Thompson, J. E. Rosenblatt, and F. R. Cockerill III. 2004. Comparison of the Roche LightCycler vanA/vanB detection assay and culture for detection of vancomycin-resistant enterococci from perianal swabs. *J. Clin. Microbiol.* 42:2636-2643.

