


Journal of Antimicrobial Chemotherapy
doi:10.1093/jac/dkl543
Advance Access publication 6 February 2007

Direct detection of vanB2 using the Roche LightCycler vanA/B detection assay to indicate vancomycin-resistant enterococcal carriage – sensitive but not specific

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Keywords: VRE, PCR, anaerobes, diagnostics

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Sir, Vancomycin-resistant enterococci (VRE) are an infection control issue in many hospitals, such that rapid, accurate detection has important clinical consequences. There are five genotypes responsible for acquired vancomycin resistance (vanA, vanB, vanD, vanE, vanG), with vanB the most prevalent in Australia.1,2 Detection of VRE is generally by culture-based methods, with confirmation of species and van genotype based on molecular methods.2

The Roche LightCycler VRE Detection Kit (Roche Diagnostics, Mannheim, Germany) has been developed to rapidly assess for VRE colonization.3 This kit provides the primers and the hybridization probes for the amplification and sequence-specific detection of vanA, vanB1 and vanB2/3, along with an internal control for detection of sample inhibition. It has a processing time of approximately 1.5 h. Using this kit with DNA directly extracted from clinical specimens, detection of VRE can be reduced to less than 24 h. However, when used in this manner, it is important to note that the system detects van genes rather than VRE per se. Thus, the presence of vanB in non-enterococcal species may influence the sensitivity and specificity of this approach for detection of VRE.4–7

We assessed the usefulness and accuracy of the LightCycler VRE detection system in the rapid identification of vanB VRE colonization in 59 faecal samples previously obtained from hospital inpatients (53 haemodialysis, 6 non-haemodialysis patients) and stored at −80°C. Twelve of these faecal specimens (from 6 haemodialysis and 6 non-haemodialysis patients) were shown previously to contain vanB2 Enterococcus faecium, while 42 specimens were repeatedly shown to be culture-negative for VRE.4 As we have previously reported, four specimens were shown to be vanB2 anaerobe culture-positive, but were VRE culture-negative (one specimen contained Clostridium bolteae and Ruminococcus sp., two specimens contained Clostridium hathewayi and one specimen contained an un-specified Clostridium), while a further specimen contained a vanB2 anaerobe (C. hathewayi) and vanB2 E. faecium.4 Faecal specimens were defrosted and DNA was extracted directly from 200–220 mg of faeces using the QIAamp® DNA Stool Mini Kit (Qiagen GmbH, Germany) according to the manufacturer’s recommendations. In addition, 17 h enrichment cultures in antibiotic-free Enterococcus broth (BBL, Cockeysville, MD, USA) of these faecal samples were prepared and DNA extracted as described previously.4 Control isolates were Enterococcus faecalis ATCC 29212 (van A/B negative), E. faecalis ATCC 51299 (vanB positive) and a clinical vanA E. faecium isolate obtained from our institution. All DNA was stored at −20°C then tested for the presence of vanA or vanB using the LightCycler VRE Detection Kit (Roche Diagnostics, Catalogue no. 3 334 996) and the LightCycler instrument (Roche Diagnostics) with software version 3.5, according to the manufacturer’s instructions. The limit of detection was determined by dilution of the plasmid DNA positive control (1000 copies per 5 μL) provided in the kit. Dilutions were performed in triplicate with the resulting standard curve imported into the data analysis mode. The sensitivity and specificity for detection of VRE was calculated. Statistical differences in the rates of accurate VRE identification and PCR inhibition were compared using χ²/Fisher’s exact test.

All 12 vanB2 E. faecium culture-positive samples were positive for vanB by LightCycler on both the direct faecal specimen and the 17 h enrichment culture (100% sensitivity; Table 1). However, specificity for detecting VRE with LightCycler was 36% and 30% using direct DNA extraction and 17 h enrichment broth culture, respectively. Of the four specimens known to contain vanB anaerobes, three of these were positive by LightCycler by both methods (Table 1). The fourth specimen was most likely below the limit of detection as this was only positive following 36 h enrichment broth culture (data not shown). In addition, the specimen that contained both a vanB2 anaerobe (C. hathewayi) and vanB2 E. faecium was also positive for vanB by LightCycler. Overall, the LightCycler demonstrated a lower detection limit of 40 genome equivalents per reaction (data not shown). The rate of assay inhibition using the LightCycler procedure was 0% for 17 enrichment broth cultures and 3% for direct detection, similar to that observed previously for the Roche LightCycler VRE kit.3

The Roche LightCycler VRE detection assay demonstrates high sensitivity (100%), but limited specificity (30–36%) for the detection of vanB VRE in surveillance specimens of faeces. This
could potentially result in an over-estimation of the rate of faecal VRE colonization if the patient population assessed had high rates of faecal carriage of non-enterococcal species that contain vanB, as previously reported.6 Although very low density VRE faecal colonization (below the detection limit of routine detailed culture methods) could explain these results, this seems less likely in our study faecal collection given our repeated detailed analysis. Based on our data, use of the LightCycler VRE detection assay directly on clinical specimens provides rapid results, but can lead to a substantive number of specimens that are LightCycler-positive, but VRE culture-negative, that will require confirmatory culture follow-up (with its associated cost). In a number of cases, infection control interventions based on initial positive LightCycler results are likely to subsequently prove unnecessary if VRE is not isolated. However, as our understanding of vanB epidemiology and VRE emergence improves, one might speculate that carriage of vanB, regardless of the host species, may become the priority rather than simply VRE.

Acknowledgements

Presented in part at the Second International ASM-FEMS Conference on Enterococci, Helsingor, Denmark, August 2005. We gratefully acknowledge the support and assistance of Dr B. Mayall, Dr P. Ward and the laboratory staff of the Department of Microbiology, Austin Hospital, Victoria, Australia. No specific funding was provided, but the LightCycler and some detection kits were provided gratis by Roche.

Transparency declarations

None to declare.

References


Correspondence

Table 1. Accuracy of the LightCycler VRE detection assay for detecting VRE colonization in faeces (n = 58a)

<table>
<thead>
<tr>
<th>Protocol</th>
<th>No. of specimens PCR-positive</th>
<th>VRE-negativeb</th>
<th>Accuracy in detecting VRE in non-inhibited specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct assessment of faeces</td>
<td>VRE-positive</td>
<td>anaerobe culture-negativec</td>
<td>anaerobe culture-positivec</td>
</tr>
<tr>
<td>n = 12</td>
<td>n = 42</td>
<td>n = 4</td>
<td></td>
</tr>
<tr>
<td>12/12d</td>
<td>25/40d,e</td>
<td>3/4d</td>
<td>100%</td>
</tr>
<tr>
<td>17 h enrichment broth cultures of faeces</td>
<td>12/12e</td>
<td>29/42e</td>
<td>3/4e</td>
</tr>
</tbody>
</table>

aIn addition to these 58 specimens there was one further faecal specimen that contained both vanB VRE and a vanB anaerobe, which has been excluded from this analysis (see text).
bNo VRE detectable using routine culture techniques (see text).
cAll VRE-negative specimens positive for vanB by PCR were screened for the presence or absence of vanB-containing anaerobes.4
dStatistically significant difference: 12/12 versus 28/44; P = 0.012; Fisher’s exact test.
eStatistically significant difference: 12/12 versus 32/46; P = 0.028; Fisher’s exact test.
fTwo specimens were inhibited in PCR and excluded from analysis.

Evaluation of the Etest method for fosfomycin susceptibility of ESBL-producing Klebsiella pneumoniae

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doi:10.1093/jac/dkl545
Advance Access publication 9 February 2007