Evaluation of Three Disk Tests for Identification of Enterococci, Leuconostocs, and Pediococci

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Simple rapid tests for presumptive identification of catalase-negative non-beta-hemolytic cocci (i.e., enterococci, leuconostocs, and pediococci) have not previously been available. Seven hundred thirty-four strains of aerobic and facultatively anaerobic, catalase-negative, non-beta-hemolytic gram-positive cocci were tested for susceptibility to vancomycin (Van') by a screening procedure and production of leucine aminopeptidase (LAPase) and pyrrolidonylarylaminidase (PYRase) in disk tests. Three unique patterns of activity in response to the three disks (30 µg of vancomycin, PYRase, and LAPase) can be used to presumptively identify the vancomycin-resistant (Van') enterococci (Van', PYRase and LAPase positive), leuconostocs (Van' and PYRase negative, and LAPase negative), and pediococci (Van', PYRase negative, and LAPase positive). The results indicate that, together with Gram stain characteristics and the catalase test, the vancomycin, LAPase, and PYRase disk tests can be used to presumptively identify Van' strains of enterococci as well as Leuconostoc and Pediococcus strains from human infections.

Vancomycin-resistant (Van'), facultatively anaerobic and aerobic gram-positive cocci, including Enterococcus, Leuconostoc, and Pediococcus species, are being isolated with increasing frequency from patients (1, 9, 10, 13, 14). The tests used to identify these three Van' genera are complex and sometimes require several days to complete (6, 11, 15). Simple and accurate diagnostic tests are needed to shorten the time required to identify Van' bacteria, because patient management methods may differ according to the infecting organism.

Disk tests such as those using bacitracin and optochin have been used for years to identify group A streptococci and pneumococci, respectively. They are simple to perform and require only isolated colonies on the primary culture plate and an additional blood agar plate for the test. In most cases, the test can be interpreted after overnight incubation. A disk test for determining the presence of pyrrolidonylarylaminidase (PYRase) has been previously described for the identification of group A streptococci as well as enterococci (6, 8, 17). We have previously described a vancomycin susceptibility (Van') screening test to aid in the identification of viridans group streptococci (4). A disk test, the IDENTICULT-AE test (PML Microbiologials, Tualatin, Oreg.), which combines esculin hydrolysis and PYRase production into a single disk to identify the enterococci and group A streptococci, has also been described (3).

This study describes the results of testing of clinical isolates submitted to our laboratory for identification from 1989 to 1993 with three disk tests, consisting of (i) Van' screening, (ii) production of leucine aminopeptidase (LAPase), and (iii) production of PYRase.

MATERIALS AND METHODS

Bacterial cultures were received from city and state health departments in the United States from 1989 to 1993. The strains were isolated from a variety of human sources, including blood and cerebrospinal fluid cultures, wound infections, abscesses, and peritoneal and synovial fluid cultures. Bacterial cultures were identified by previously described procedures (5, 7).

Vancomycin disks and trypticase soy–5% sheep blood agar plates were obtained from BBL Microbiology Systems, Inc., Cockeysville, Md.

PYRase disks, LAPase disks, and the detection reagent (p-dimethylaminocinnamaldehyde) were obtained from Carr Scarborough Microbiological, Inc., Stone Mountain, Ga.

Bacterial strains were cultured on trypticase soy–5% sheep blood agar plates. The inoculum was spread very thickly over one-half of the plate and spread thinly over the remainder of the plate. The vancomycin disk was placed on the section of the plate with the heaviest growth. The plates were incubated overnight in a CO2 incubator at 35 to 37°C. Growth on the thinly spread area of the plate was examined for culture purity after incubation. After incubation, the LAPase and PYRase disks were placed on an area of the plate with little or no growth. One or two large loopfuls of bacteria from the overnight growth were then transferred to the surface of each disk. If the growth was scant, several loopfuls of bacteria were transferred to each disk. The reaction was allowed to proceed at room temperature for 10 min, at which time 1 drop of the detection reagent was added to each disk.

The reaction to vancomycin was recorded as positive if any zone of inhibition was present. Zones of inhibition were not measured. The LAPase and PYRase tests were recorded as positive if a pink or red color developed within 3 min of the addition of the detection reagent. A yellow color or no color change after 3 min indicated a negative reaction.

RESULTS

All of the non-beta-hemolytic streptococci tested (beta-hemolytic strains were not tested) were Van' in the vancomycin screening test (Table 1). The majority of streptococci produced LAPase, but only the nutritional variant streptococci produced PYRase. Two reaction patterns were identified among the enterococcal strains: Van' and Van". All of the Van' strains produced both LAPase and PYRase. Some of the enterococcal strains, notably Enterococcus avium and Enterococcus raffinosus, failed to produce LAPase. If Van' is detected in these species, a new pattern of reactions to the three disks will result when tested: Van', LAPase negative, and PYRase positive. The Lactococcus cultures gave nearly the same results as the Van' enterococci. The Globoctella cultures were all Van' and produced PYRase but did not produce LAPase. The Leuconostoc cultures were all Van' and failed to produce LAPase or PYRase.
Among the gram-positive cocci that did not form chains (Table 1), only the pediococci were Van'. The aerococci and helcococci were all susceptible to vancomycin and produced PYRase but not LAPase. The Aerococcus-like organisms were also Van' but produced LAPase but not PYRase. The alloio-
cocci and gemellae were Van' and produced both LAPase and PYRase. In addition to being Van', the pediococci produced 
LAPase but not PYRase.

The unidentified gram-positive cocci were those that did not fit the genus description given in reference 5. Cultures in this 
category had at least one reaction that was atypical for genus 
identification. For example, one vancomycin-resistant gram-
positive cocci formed chains and produced gas like a Leu-
conostoc strain; however, this organism also produced LAPase, 
which is unlike the leuconostocs. This strain appears to be 
neither identical to the type strain of Leuconostoc paramesen-
teroides, which is also LAPase positive (14). Confirmation of 
the identity of this strain is pending. Other unidentified gram-
positive cocci had two or more characteristics that prevented 
them from being placed into a genus identification.

TABLE 1. Percent positive reactions of gram-positive cocci 
for three diagnostic disk tests

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. tested</th>
<th>% of organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Van'</td>
<td>LAPase positive</td>
</tr>
<tr>
<td>Gram-positive cocci in chains</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus Viridans spp.</td>
<td>282</td>
<td>100  98  99</td>
</tr>
<tr>
<td>S. bovis</td>
<td>10</td>
<td>100  100   100</td>
</tr>
<tr>
<td>NVS</td>
<td>22</td>
<td>100  76    77</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Van'</td>
<td>98</td>
<td>100  90    99</td>
</tr>
<tr>
<td>Van'</td>
<td>62</td>
<td>0      100   100</td>
</tr>
<tr>
<td>Lactococcus spp.</td>
<td>39</td>
<td>100  95    83</td>
</tr>
<tr>
<td>Globicatella spp.</td>
<td>7</td>
<td>100    0    100</td>
</tr>
<tr>
<td>Leuconostoc spp.</td>
<td>53</td>
<td>0      0     0</td>
</tr>
<tr>
<td>Gram-positive cocci in pairs, tetrads, and clusters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerococcus spp.</td>
<td>28</td>
<td>100    0    100</td>
</tr>
<tr>
<td>ALO'</td>
<td>7</td>
<td>100    0    0</td>
</tr>
<tr>
<td>Helcococcus spp.</td>
<td>7</td>
<td>100    0    100</td>
</tr>
<tr>
<td>Alloiococcus spp.</td>
<td>23</td>
<td>100    100  100</td>
</tr>
<tr>
<td>Gemella spp.</td>
<td>34</td>
<td>100    100  94</td>
</tr>
<tr>
<td>Pediococcus spp.</td>
<td>21</td>
<td>0      100   0</td>
</tr>
<tr>
<td>Unidentified</td>
<td>41</td>
<td>90     63    58</td>
</tr>
</tbody>
</table>

a NVS, nutritional variant streptococci. 
b ALO, Aerococcus-like organisms.

Among the gram-positive cocci that did not form chains (Table 1), only the pediococci were Van'. The aerococci and helcococci were all susceptible to vancomycin and produced PYRase but not LAPase. The Aerococcus-like organisms were also Van' but produced LAPase but not PYRase. The alloio-
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**DISCUSSION**

The isolation of additional genera and species of gram-positive cocci has made it necessary for clinical laboratories to 
expand their diagnostic capacities to identify leuconostocs, pe-
diococci, and helcococci (1, 5). In the majority of cases in which 

microbiologic tests are applied to aid in the identification of an 
unknown culture, it is necessary to have some knowledge of the 
culture’s Gram-staining characteristics, cellular arrangement, 
and hemolytic and catalase activity. This is the case for the 
presumptive tests described here.

Leuconostoc and Pediococcus species are intrinsically resis-
tant to vancomycin, while some species of enterococci have 
acquired Van' (16). There is a need for quick, simple, and 
accurate tests to identify Van' gram-positive cocci. Disk tests 
are easy to perform and generally require only overnight 
growth of the bacteria for interpretation. Perhaps the best 
examples of these tests are the bacitracin and optochin tests for 
group A streptococci and pneumococci, respectively. Neither 
requires a standardized inoculum, and in both tests, identifi-
cation of each is determined by inhibition of growth around 
the disk after overnight incubation. Some investigators have 
described a combination of PYRase and esculin hydrolysis for 
rapid identification (within 30 min of obtaining isolated col-
onies) of the enterococci (3). This combination of reactions 
should accurately identify the enterococci but will not identify 
the other genera of gram-positive cocci. Other investigators 
have advocated the use of Gram staining, gas formation from 
glucose broth, hydrolysis of arginine, PYRase production, and 
Van' to identify the Van' strains of enterococci, leuconostocs, 
lactobacilli, and pediococci (11). Although these tests are sim-
ple, they require 2 days or more for a final reading.

We have reported that the 30-µg vancomycin disk test could be used to help identify gram-positive cocci (4, 5). All viridans 
group streptococci and aerococci gave zones of inhibition 
around the vancomycin disk. At this time, among the catalase-
negative, gram-positive cocci and coccobacillary bacteria, only 
Leuconostoc spp., Pediococcus spp., some Enterococcus strains, and some Lactobacillus strains are resistant to vancomycin 
(16). This indicates that, although the vancomycin screening 
test does not selectively identify any given genus or species, it 
may be used in combination with other tests to help identify 
gram-positive cocci.

The PYRase test has been a useful test to aid in the 
identification of enterococci and other gram-positive cocci (4, 8, 
17). Of more than 500 strains of enterococci (160 in this study) 
tested in the past few years, we have identified only 1 strain 
that failed to give a positive PYRase reaction. Many species of 
other genera of gram-positive cocci have PYRase activity, 
including the majority of strains belonging to the Lactococcus, 
Globicatella, Aerococcus, Alloiococcus, and Gemella genera 
(Table 1). However, one of the more useful aspects of the 
PYRase tests is the failure of the viridans group streptococci 
and Streptococcus bovis strains, leuconostocs, and pediococci to 
show any PYRase activity. This “all or none” aspect of the 
PYRase test makes it attractive for differentiating the gram-
positive cocci.

The LAPase test has been a component in the Rapid Strep 
identification system (Biomerieux, Hazelwood, Mo.) and has 
only recently become available as a separate test. This is the 
first report of an evaluation of the LAPase test as an indepen-
dent test. As with PYRase, many gram-positive cocci have 
LAPase activity. The most useful aspect of the LAPase test is 
the failure of all strains of aerococci, globicatellae, helcococci, 
and leuconostocs to have LAPase activity. Only one species of 
the Leuconostoc genus, L. parae fermenteroides, is reported to 
have LAPase activity (14). Not only is this strain atypical in 
that it is not genetically closely related to the other Leuco-
notoc species (2, 12), but it is found only rarely among strains 
identified from human sources. Among 100 strains of leu-
conostocs isolated from human sources, we have confirmed 
only 1 strain that resembles L. parae fermenteroides.
Three unique patterns of activity in response to the three disks (30 μg of vancomycin, PYRase, and LAPase) can be used to presumptively identify the Van" enterococci (Van" and PYRase and LAPase positive), leuconostocs (Van" and PYRase and LAPase negative), and pediococci (Van", PYRase negative, and LAPase positive) (Table 2).

We also suggest that the viridans group streptococci and Streptococcus bovis strains together can be presumptively identified by the combination of reactions Van", LAPase positive, and PYRase negative. Among these strains, there have been no exceptions in any of these three tests (Tables 1 and 2). However, remember that these identifications are assisted by knowledge that the strains are catalase negative, gram-positive cocci.

REFERENCES


