Optimization of a Laboratory-Developed Test Utilizing Roche Analyte-Specific Reagents for Detection of *Staphylococcus aureus*, Methicillin-Resistant *S. aureus*, and Vancomycin-Resistant *Enterococcus* Species


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Nasal and perianal swab specimens were tested for detection of *Staphylococcus aureus* and vancomycin-resistant *Enterococcus* species (VRE) using a laboratory-developed real-time PCR test and microbiological cultures. The real-time PCR and culture results for *S. aureus* were similar. PCR had adequate sensitivity, but culture was more specific for the detection of VRE.

*Staphylococcus aureus*, especially methicillin-resistant *S. aureus* (MRSA), and vancomycin-resistant *Enterococcus* species (VRE) are important pathogens associated with health care facility outbreaks worldwide (1, 2, 8, 21). It has been reported that identifying asymptomatically colonized individuals and placing them into contact isolation within a short time frame are a good management tool for reducing the spread of these pathogens and for lowering health care-associated infections (5, 11, 16, 18). Furthermore, there is rising interest in identifying all *S. aureus* carriers prior to surgery to decolonize them to reduce postoperative *S. aureus* surgical-site infections (4, 10).

Reliable surveillance requires accurate testing (17), and cultures can take from 2 to 5 days to obtain the final results (3a). PCR offers detection of *S. aureus* and VRE directly from swab specimens within a few hours (4, 12, 14) and can help with the rapid deployment of infection control and prevention measures (16, 18).

The purpose of our study was to develop an optimized test using Roche analyte-specific reagents (ASRs) for detection of *S. aureus* and VRE by real-time PCR with a single set of amplification conditions and to compare the PCR results to the results of conventional culture.

Inpatients at Evanston Northwestern Healthcare during August 2004 made up the patient population. Premoistened, double-headed swabs (culture swab; BBL, Becton Dickinson, Franklin Lakes, NJ) were used to collect paired anterior nasal specimens and paired perianal specimens as part of an infection control activity to determine colonization prevalence. There were 387 nasal specimens (for *S. aureus*) and 309 perianal specimens (for VRE). This investigation was approved by the Institutional Review Board of Evanston Northwestern Healthcare.

For *S. aureus*, one of the paired nasal swabs was plated onto Columbia colistin-nalidixic agar with 5% sheep blood (Remel, Inc., Lenexa, KS) (3a) and incubated at 35°C for 48 h. *S. aureus* was identified by colony morphology and Staphaurex latex agglutination testing (Remel, Inc.). Methicillin resistance was determined on colonies by using PCR as described below. For any swabs that were PCR positive only for *S. aureus* (n = 13), the original cultures were reexamined beyond their initial 48-hour incubation, with five additional swab samples yielding *S. aureus* after reexamination (two yielded MRSA).

VRE was cultured by plating one of the paired perianal swabs on bile esculin azide agar containing 6 μg of vancomycin/ml agar (Remel, Inc.) and by incubation at 35°C for 72 h. Colonies black in color ( bile esculin positive) were confirmed to be *Enterococcus faecium* or *Enterococcus faecalis* by using conventional biochemical testing. Vancomycin resistance was determined via Etest in accordance with the Clinical and Laboratory Standards Institute guidelines (3). For any samples that were PCR positive only, the original swabs were placed into thioglycolate broth (BBL, Becton Dickinson) and incubated for 72 h at 35°C. The broth was then subcultured onto a colistin-nalidixic agar plate; 2 of 22 specimens grew VRE.

Although the swab processing and extraction methods were unique for each assay, we designed identical real-time PCR amplification conditions for all three assays that are presented in Table 1. This was done to facilitate the running of samples for various assay targets at the same time on the LightCycler instrument. The second swab from each surveillance specimen was broken off in a microcentrifuge tube and processed. For *S. aureus*, after the incubation steps, fluid surrounding the swab was aspirated and directly used for real-time PCR analysis (12). For VRE, by using the extraction protocol summarized in Table 1, a final eluate of 100 μl of purified DNA was used for real-time PCR. All ASRs were supplied by Roche Diagnostics.

Each culture that grew an *S. aureus* isolate was tested for the presence of *mecA* from colonies using the Roche LightCycler...
**TABLE 1. Real-time PCR protocols for using the Roche ASRs to detect S. aureus and VRE on the LightCycler instrument**

<table>
<thead>
<tr>
<th>Preparation step and run conditions</th>
<th>S. aureus</th>
<th>meca</th>
<th>VRE (vanA, vanB, and vanB2/3 genes)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specimen Extraction</strong></td>
<td>Nasal swab</td>
<td>Bacterial colonies 2 or 3 isolated colonies placed into a microcentrifuge tube with 1% Triton X-100, 0.5% Tween 20, 1 mmol/liter Tris-HCl (pH 8.0), and 10 mmol/liter EDTA</td>
<td>Perianal swab Swab broken off into a microcentrifuge tube containing 100 µl of STAR buffer (Roche)</td>
</tr>
<tr>
<td>Tube incubated at 100°C for 10 min and then centrifuged for 1 min at &gt;10,000 × g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Processing</strong></td>
<td>Tube vortexed for 3–5 s and then incubated at 37°C for 15 min, followed by 5 min of incubation at 100°C</td>
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<tr>
<td><strong>Reaction mix</strong></td>
<td>2–5 µl of extracted DNA, 2 µl of LightCycler FastStart DNA master hybridization probe MGRADE mix, 2 µl of LightCycler Staphylococcus MGRADE primer/hybridization probes, 1 µl of 1:10 dilution of LightCycler Staphylococcus MGRADE recovery template, 10 µl of sterile water</td>
<td>2 µl of extracted DNA, 2 µl of LightCycler FastStart DNA master hybridization probe MGRADE mix, 2 µl of LightCycler meca primer/hybridization probes, 2 µl of LightCycler meca recovery template, 2.4 µl of MgCl₂, 9.6 µl of sterile water</td>
<td></td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>LightCycler Staphylococcus MGRADE template set was the positive control; sterile water was the negative control</td>
<td>LightCycler meca template DNA was the positive control; sterile water was the negative control</td>
<td></td>
</tr>
<tr>
<td><strong>Real-time PCR conditions</strong></td>
<td>Initial step of 10 min at 95°C, followed by amplification for 45 cycles of 10 s at 95°C, 10 s at 55°C, and 12 s at 72°C, with fluorescence acquisition at the end of each annealing</td>
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<tr>
<td><strong>Melt program</strong></td>
<td>Ramp to 95°C, followed by 20 s at 59°C, 20 s at 45°C at a rate of 0.2°C/s, and a gradual increase to 85°C at a rate of 0.2°C/s with continuous fluorescence acquisition</td>
<td>Ramp to 95°C, followed by 20 s at 59°C, 20 s at 45°C at a rate of 0.2°C/s, and a gradual increase to 85°C at a rate of 0.2°C/s with continuous fluorescence acquisition</td>
<td></td>
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</table>

\(^a\) STAR, stool transport and recovery.

Staphylococcus and LightCycler meca ASRs in an in-house real-time PCR assay (13). For DNA extraction, two or three isolated colonies of S. aureus were touched with a sterile loop, placed into a microcentrifuge tube containing lysis buffer, and processed (Table 1). The PCR results were assessed using culture results as the reference standard. The chi-square statistic was used for determining any significant difference.

The results of our study are shown in Table 2. Eleven specimens were culture positive and PCR negative, of which two were MRSA. Six of these 11 specimens had only one to three S. aureus colonies, indicating very low density colonization, and thus those negative specimens were likely below the detection sensitivity for the PCR assay.

Colonies from each culture that grew S. aureus were tested for the presence of meca by using the Roche LightCycler meca ASR and our in-house real-time PCR assay (13). The meca colony PCR results were identical for both methods. Out of a total of 105 S. aureus specimens, 33 specimens (31%) were meca positive (MRSA).

The laboratory-developed VRE real-time PCR assay detects vanA, vanB, and vanB2/3 genes and differentiates them by using melt curve analysis (Table 2). Melt curve analysis of the 15 culture- and PCR-positive swabs showed that 11 specimens had the vanA gene alone, one contained the vanA and vanB genes, one had the vanB2/3 gene (considered a false-positive test), and two had the vanA and vanB2/3 genes. The PCR results for the cultured colonies showed only the presence of vanA in all 15 specimens. For the four swab specimens that were culture positive and PCR negative for VRE, all four isolates were E. faecium with MICs of 8, 16, 16, and >256 µg/ml. The PCR results for these colonies found that only one harbored the vanA gene while the other three tested negative; a determinant for vancomycin resistance in these is unknown. For the 20 swab specimens that were culture negative and PCR
TABLE 2. Results of real-time PCR testing for \textit{S. aureus}\textsuperscript{a} and VRE\textsuperscript{b} from surveillance swabs with culture used as the reference standard

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of true positives</th>
<th>No. of false positives</th>
<th>No. of false negatives</th>
<th>% Sensitivity (95% CI)</th>
<th>% Specificity (95% CI)</th>
<th>% Positive predictive value (95% CI)</th>
<th>% Negative predictive value (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{S. aureus} (\textit{n} = 106 positive samples)\textsuperscript{c}</td>
<td>95</td>
<td>270</td>
<td>8</td>
<td>11</td>
<td>89.6 (81.8–94.5)</td>
<td>97.1 (94.2–98.7)</td>
<td>92.2 (84.8–96.3)</td>
</tr>
<tr>
<td>VRE (\textit{vanA}, \textit{vanB}, and \textit{vanB2/3} genes; \textit{n} = 19 positive samples)\textsuperscript{d}</td>
<td>14</td>
<td>246</td>
<td>21</td>
<td>5</td>
<td>73.7 (48.6–89.9)</td>
<td>92.1 (88.1–94.9)</td>
<td>40 (24.4–57.8)</td>
</tr>
<tr>
<td>VRE (\textit{vanA} gene only; \textit{n} = 16 positive samples)\textsuperscript{e}</td>
<td>14</td>
<td>268</td>
<td>2</td>
<td>2</td>
<td>87.5 (60.4–97.8)</td>
<td>99.3 (97.1–99.9)</td>
<td></td>
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</tbody>
</table>

\textsuperscript{a} Ten specimens (2.6\%) were inhibited in the real-time PCR test when 5 \textmu L of DNA was used. These were retested using 2 \textmu L of DNA, and seven gave an amplification result, with six being negative and one positive for \textit{S. aureus}, matching the culture results. Three specimens remained inhibited and were excluded from the data analysis, giving a total of 384 analyzed specimens.

\textsuperscript{b} Eighteen specimens were inhibited (5.8\%), and for another 5 specimens, the MagNA Pure LC instrument malfunctioned, with no DNA being extracted, and thus these specimens were not included in the final analysis, giving a total of 286 analyzed specimens.

\textsuperscript{c} Difference between PCR and culture results, the value was not significant.

\textsuperscript{d} The difference between PCR and culture results was significant at a \textit{p} value of 0.005.

\textsuperscript{e} Only PCR-positive samples were evaluated for the presence of VRE colonies.

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REFERENCES

epidemic clones of vancomycin-resistant Enterococcus faecium in Singapore.


