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 asymptptomatically 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).

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-h 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
**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 real-time PCR 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 tested negative; one contained the vanA gene alone, one tested negative, and two 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 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).

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**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>Protocol to detect:</th>
<th>VRE (vanA, vanB, and vanB2/3 genes)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen Extraction</td>
<td>S. aureus</td>
<td>mecA</td>
</tr>
<tr>
<td>Nasal swab</td>
<td>Bacterial colonies</td>
<td>Perianal swab</td>
</tr>
<tr>
<td>Swab broken off into a microcentrifuge tube containing 200 µl of a 1-U/µl achromopeptidase solution</td>
<td>2 or 3 isolated colonies placed into a microcentrifuge tube containing 1% Triton X-100, 0.5% Tween 20, 1 mmol/liter Tris-HCl (pH 8.0), and 10 mmol/liter EDTA</td>
<td>Swab broken off into a microcentrifuge tube containing 100 µl of STAR buffer (Roche)</td>
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<tr>
<td>Processing</td>
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<tr>
<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>
<td>Tube incubated at 100°C for 10 min and then centrifuged for 1 min at &gt;10,000 × g</td>
<td>Tube processed in accordance with protocol for MagNA Pure LC microbiology kit MGRADE, specimens extracted on MagNA Pure LC using the DNA MGRADE protocol</td>
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<tr>
<td>Reaction mix</td>
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<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>5 µl of extracted DNA, 2 µl of LightCycler FastStart DNA master hybridization probe MGRADE mix, 2 µl of LightCycler vanA/vanB primer/hybridization probes, 2 µl of LightCycler vanA/vanB recovery template, 2 µl of MgCl₂, 7 µl of sterile water</td>
</tr>
<tr>
<td>Controls</td>
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<tr>
<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>LightCycler vanA/vanB template set was the positive control; sterile water was the negative control</td>
</tr>
<tr>
<td>Real-time PCR conditions</td>
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<tr>
<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>Melt program</td>
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<tr>
<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>
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$^a$ STAR, stool transport and recovery.
positive, 2 had vanA, 4 had vanB, and 14 had vanB2/3, as determined by the PCR assay.

The costs per test (based on manufacturers’ suggested retail price) plus operator time for processing were $4.00 to $4.50 plus 1 to 2 min for culture, $3.00 plus 2 to 3 min for in-house (S. aureus and mecA) PCR, and $21.00 (S. aureus and mecA) to $42.00 (VRE) plus 2 to 3 min for the commercial ASR tests we describe.

Both the S. aureus and VRE real-time PCR assays yielded results within 3 to 5 h, including extraction and assay run time, compared to culture, which took from 48 to over 72 h. While new chromogenic agar can detect MRSA with overnight incubation, the sensitivity of direct testing is <80%, compared to PCR (15a). The ASR reagents investigated gave reliable results for detection of S. aureus; however, confirmation of MRSA by detection of mecA required growth of S. aureus colonies. We have demonstrated that the use of such a testing strategy to detect S. aureus in presurgical patients can significantly lower their risk of postoperative S. aureus infections (4).

In our VRE testing, we found that while PCR assay of the swab detected vanA, vanB, and vanB2/3 genes, PCR confirmation using the recovered colonies only detected vanA. The 17 specimens with the vanB2/3 signal may represent a false-positive amplification test for VRE. The vanB gene is known to occur in other bacteria (21); however, vanB2/3 containing enterococci can potentially cause outbreaks (7, 9), so this result cannot be ignored. Also, we have previously shown that PCR-positive surveillance swabs for both S. aureus and VRE can indicate persons harboring these pathogens at other sites or prior times (12, 14, 15, 20), so some of the PCR-positive results may represent false-negative cultures. Based on our findings, and those of others (6), the VRE real-time PCR test in a clinical setting appears good for early detection of patients likely infected with VRE; however, it would seem prudent to culture the specimens that signal positive for vanB and vanB2/3 in order to confirm that those patients indeed harbor VRE, as without such confirmation the positive predictive value of the test is only 40%.

Sloan and colleagues (19) found better sensitivity (100%) that we did and a similar specificity when using the Roche LightCycler vanA/vanB ASRs in their laboratory. One of the reasons for higher sensitivity may be that they used a different culture method (19). Also, only PCR-positive samples were evaluated for the presence of VRE colonies.

We have optimized laboratory-developed tests that utilize commercially available ASRs that can detect S. aureus (and that can confirm MRSA after culture) and VRE directly from surveillance swab specimens by using a single set of amplification conditions. These tests can help clinicians make important infection control and surgical prophylaxis decisions. The VRE real-time PCR test using currently available ASR materials reliably detected the VanA phenotype but detected many more vanB and vanB2/3 positives than culture, suggesting that this latter result may need to be confirmed by culture.

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REFERENCES
8. Koh, T. H., L. Y. Hsu, L. L. Chi, and R. V. Lin. 2006. Emergence of


