JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2004, p. 5739–5744 Vol. 42, No. 12
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.12.5739–5744.2004

Simultaneous Detection of *Staphylococcus aureus* and Coagulase-Negative Staphylococci in Positive Blood Cultures by Real-Time PCR with Two Fluorescence Resonance Energy Transfer Probe Sets

H. Sakai,[1] G. W. Procop,[2]* N. Kobayashi,[1] D. Togawa,[1] D. A. Wilson,[2] L. Borden,[3] V. Krebs,[3] and T. W. Bauer[1]

Department of Anatomic Pathology, Section of Surgical Pathology,[1] Department of Clinical Pathology, Section of Clinical Microbiology,[2] and Department of Orthopaedic Surgery,[3] The Cleveland Clinic Foundation, Cleveland, Ohio

Received 5 March 2004/Returned for modification 4 May 2004/Accepted 5 July 2004

A real-time PCR assay that uses two fluorescence resonance energy transfer probe sets and targets the *tuf* gene of staphylococci is described here. One probe set detects the *Staphylococcus* genus, whereas the other probe set is specific for *Staphylococcus aureus*. One hundred thirty-eight cultured isolates, which contained 41 isolates of staphylococci representing at least nine species, and 100 positive blood cultures that contained gram-positive cocci in clusters were tested. This assay was 100% sensitive and 100% specific for the detection of the *Staphylococcus* genus and of *S. aureus*.

The genus *Staphylococcus* currently consists of 38 species (14, 15). Among staphylococci, *Staphylococcus aureus* is the most virulent species and the most important pathogen, but the incidence of infections caused by coagulase-negative staphylococci (CoNS) has increased throughout the world (2, 28, 29). *S. epidermidis* and other CoNS are common members of the normal flora of skin and mucous membranes and are therefore common contaminants in culture, particularly in blood cultures. A conundrum often exists in the interpretation of cultures that contain these microorganisms, since CoNS have also become an important cause of hospital-acquired infections (5, 9, 21). It is most important to distinguish between *S. aureus* and CoNS in clinical samples, given the differences in virulence between these organisms (2, 28, 29). It is also important, however, to confirm the presence of CoNS given the increasing importance of CoNS infections (27, 33).

Phenotypic methods have been the standard means of differentiating *S. aureus* from CoNS and for differentiating the members of CoNS. These methods all require growth of the organisms followed by biochemical testing, are time-consuming, and may still occasionally produce inaccurate results (7, 8, 23, 26). A variety of genotypic methods have been explored for the rapid detection of *S. aureus* and/or for the detection and differentiation of CoNS. These methods include the use of traditional in situ hybridization, peptide-nucleic acid in situ hybridization, and a variety of nucleic acid amplification-based methods (1, 3, 11, 13, 17, 18, 22).

We designed a single broad-range PCR primer set directed toward the *tuf* gene to amplify the most clinically relevant staphylococci. For this study, PCR products were detected by use of a LightCycler instrument (Roche Diagnostics, Indianapolis, Ind.) and two sets of fluorescence resonance energy transfer (FRET) hybridization probes. The first set of FRET hybridization probes was designed to detect a signature sequence of the *Staphylococcus* genus, whereas the second set of FRET hybridization probes was designed to specifically detect *S. aureus*. This assay can detect *S. aureus* and CoNS simultaneously and can differentiate *S. aureus* by a postamplification melt curve analysis using the F3 channel of the LightCycler system.

We validated this dual-FRET-probe real-time PCR assay for *Staphylococcus* with lysates from 138 cultivated bacterial reference strains and well-characterized clinical isolates. In addition, we examined the sediments from 100 positive BacT/ALERT blood bottles that contained gram-positive cocci in clusters by using this PCR assay according to a previously described simple centrifugation protocol (31).

MATERIALS AND METHODS

Bacterial strains and DNA preparation. One hundred thirty-eight bacterial strains, most of which are commonly encountered in the clinical microbiology laboratory, were cultivated for 24 to 48 h on 5% sheep blood agar, unless chocolate or another enriched agar was needed for growth. Lysates to obtain template bacterial DNAs were extracted by a rapid boiling procedure as previously described (25).

The isolates consisted of 41 strains of *Staphylococcus*, representing at least nine species, and 97 strains of nonstaphylococcal bacteria (Table 1). The bacteria tested were predominantly clinical isolates that were recovered from our facility, although some were type isolates obtained from the American Type Culture Collection (Manassas, Va.). The clinical isolates were identified by routine biochemical methods, many of which included evaluation by use of the Vitek and API systems.

Positive blood culture bottles and DNA preparation. Using our PCR method, we examined sediments from FAN BacT/ALERT blood culture bottles that signaled positive and contained gram-positive cocci in clusters after DNA preparation by a simple centrifugation protocol (31). The person who performed the PCRs was blinded to the results of the conventional cultures until after the PCR results were obtained.

Primer and probe design. The *tuf* gene sequences of *Staphylococcus* species (*S. aureus* [AF298796], *S. epidermidis* [AF298800], *S. saprophyticus* [AF298804], *S.*

* Corresponding author. Mailing address: Clinical Microbiology/L40, The Cleveland Clinic Foundation, 9500 Euclid Ave., Cleveland, OH 44195. Phone: (216) 444-5879. Fax: (216) 445-6984. E-mail: procopg@ccf.org.

TABLE 1. Bacterial validation battery

Organism	No. of positive samples		
	n	Pan-Staph probes	*S. aureus* probes
Staphylococcus isolates			
S. aureus (includes ATCC 25923, 29213, 33592, and 43300)	7	7	7
S. epidermidis (includes ATCC 12228, 35547, 700578, and 700579)	8	8	0
S. saprophyticus	2	2	0
CoNS, not further identified	18	18	0
S. cohnii, *S. haemolyticus*, *S. hominis*, *S. lugdunensis*, *S. xylosus*, and *S. wareni* (one each)	6	6	0
Non-*Staphylococcus* isolates			
Gram-positive bacteria	33	0	0
Micrococcus (2), *Stomatococcus* (2), *Lactobacillus* (2), *Enterococcus* (3), milleri group streptococci (2), viridans group streptococci, not milleri group (1), *Streptococcus pneumoniae* (3), *Streptococcus pyogenes* (3), *Streptococcus agalactiae* (3), *Aerococcus urinae* (2), *Aerococcus viridans* (1), *Listeria* (3), *Bacillus* (3), and *Corynebacterium* (3)			
Gram-negative bacteria	64	0	0
Salmonella (4), *Providencia* (2), *Shigella sonnei* (2), *Burkholderia cepacia* (2), *Yersinia* (3), *Citrobacter* (3), *Escherichia coli* (3), *Proteus* (3), *Klebsiella* (3), *Enterobacter* (3), *Serratia* (3), *Pseudomonas* (3), *Acinetobacter* (3), *Haemophilus* (3), *Neisseria* (9), *Moraxella* (3), *Bacteroides* (3), *Afipia felis* (1), *Vibrio cholerae* (1), *Eikenella corrodens* (1), *Pasteurella multocida* (1), *Campylobacter jejuni* (1), *Mesorhizobium* (1), *Rhizobium* (1), and *Bartonella* (2)			

haemolyticus [AF298801], *S. hominis* [AF298802], *S. lugdunensis* [AF298803], *S. simulans* [AF208805], *S. warneri* [AF298806], and *S. capitis* [AF298798]) that were available from GenBank were analyzed by use of the ClustalW multiple sequence alignment program provided by the European Bioinformatics Institute (http://www.ebi.ac.uk/clustalw/). The sequences of the primers and *Staphylococcus*-specific hybridization probes were designed to be perfect matches (i.e., 100% homologous) to the *S. aureus*, *S. epidermidis*, *S. saprophyticus*, *S. haemolyticus*, *S. hominis*, *S. lugdunensis*, *S. warneri*, and *S. capitis* sequences. The sequences of *S. aureus*-specific hybridization probes contained a single mismatch compared with the *S. aureus* sequence, which was purposefully introduced to diminish probe-probe and probe-primer interactions. There were six or more base pair mismatches between the *S. aureus*-specific hybridization probes and the non-*S. aureus* sequences tested. The 3′ end of the first FRET hybridization probe in each set was labeled with fluorescein isothiocyanate (FITC). The 5′ end of the acceptor FRET hybridization probe in the set used for the detection of the *Staphylococcus* genus was labeled with LCRed640, whereas the 5′ end of the acceptor FRET hybridization probe in the set used for the specific detection of *S. aureus* was labeled with LCRed705. The positions of the primers and probes given below were derived from the *S. aureus* sequence (GenBank accession no. AF298796). The sequences of the primers were as follows: forward primer (PanStaphF), 5′-CAATGCCACAAACTCG-3′ (positions 33 to 48); and reverse primer (PanStaphR), 5′-GCTTCAGCGTAGTCTA-3′ (positions 510 to 495). The broad-range *Staphylococcus*-specific FRET hybridization probe sequences were as follows: PanStaphHP1, 5′-ACGGCCTGTAGCAACAGTAC-FITC-3′ (positions 391 to 372); and PanStaphHP2, 5′-LCRed640-CGACCAGTGATTG AGAATACGTCC-phosphate-3′ (positions 369 to 346). The *S. aureus*-specific FRET hybridization probe sequences were as follows: SAtufHP1, 5′-GGCGAT GCTCAATACGAAGAAAAAATC-FITC-3′ (positions 239 to 265); and SAtufHP2, 5′-LCRed705-AGAATCAATGGAAGCTGTAGATAC-3′ (positions 268 to 291). All oligonucleotide primers and probes were obtained from BioChem (Salt Lake City, Utah).

LightCycler assays. PCR mixtures consisted of 3.0 mM $MgCl_2$, a 1.0 μM concentration of each primer, a 0.2 μM concentration of each hybridization probe, and 2 μl of 10× LightCycler FastStart DNA master hybridization probe mixture (Roche) in a volume of 18 μl. Two microliters of template DNA extract was added to the reaction mixture for a final reaction volume of 20 μl in each capillary tube. The cycling parameters consisted of a single 95°C incubation for 10 min for enzyme activation and DNA denaturation, followed by 45 PCR amplification cycles consisting of 95°C for 10 s, 61°C for 8 s, and 72°C for 22 s. The FRET-generated fluorescent signals were acquired in the F2 channel of the instrument after the primer annealing phase of 61°C for 1 s. PCR cycling was followed by a postamplification melt curve analysis, wherein the temperature ramped from 40 to 75°C, with a temperature transition rate of 0.35°C/s, and by continuous fluorescence acquisition.

Interpretative criteria. Interpretative criteria were established after assessments of the DNA lysates from the battery of bacteria tested. These criteria were used for the interpretation of PCR results for the positive blood cultures that contained gram-positive cocci in clusters. The presence of an amplification or quantification curve for the LC640 signal captured in the F2 channel of the LightCycler, in conjunction with a melt curve with a melting temperature (T_m) of >58°C, was considered a positive result for the *Staphylococcus* genus; the absence of a quantification curve or the presence of a quantification curve but the absence of a corresponding melt curve with a T_m of >58°C was considered a negative result for the *Staphylococcus* genus. The presence of a melt curve for the LC705 signal captured in the F3 channel of the LightCycler was considered a positive result for *S. aureus*. The specimen was considered to be negative for *S. aureus* if this melt curve was absent.

Evaluation of detection limit of LightCycler assays. The minimum detection limit of each assay was evaluated by use of a 10-fold dilution series of a DNA extract from *S. aureus*. Dilutions were prepared according to spectrophotometric readings taken with a GenQuant Pro instrument (Biochrom Ltd., St. Albans, United Kingdom) to contain 1.0, 1.0×10^{-1}, 1.0×10^{-2}, 1.0×10^{-3}, 1.0×10^{-4}, and 1.0×10^{-5} μg of DNA extract/ml of *S. aureus* (ATCC 25923). The LightCycler assay was performed with these six dilutions, along with a negative control, according to the same protocol.

RESULTS

An analysis of the bacterial battery demonstrated the specificity of our primers and FRET probes. The broad-range *Staphylococcus* FRET probes designed to detect that genus generated positive quantification curves for all of the staphylococci tested (Fig. 1a). The T_ms of the staphylococci were all >58°C (60.50 ± 0.92°C [mean ± standard deviation]) (Fig. 1b). The *S. aureus*-specific assay detected only the *S. aureus* isolates in the battery. The melt curve T_m for the *S. aureus*-specific FRET probes was 55.83 ± 0.11°C (mean ± standard deviation) (Fig. 2).

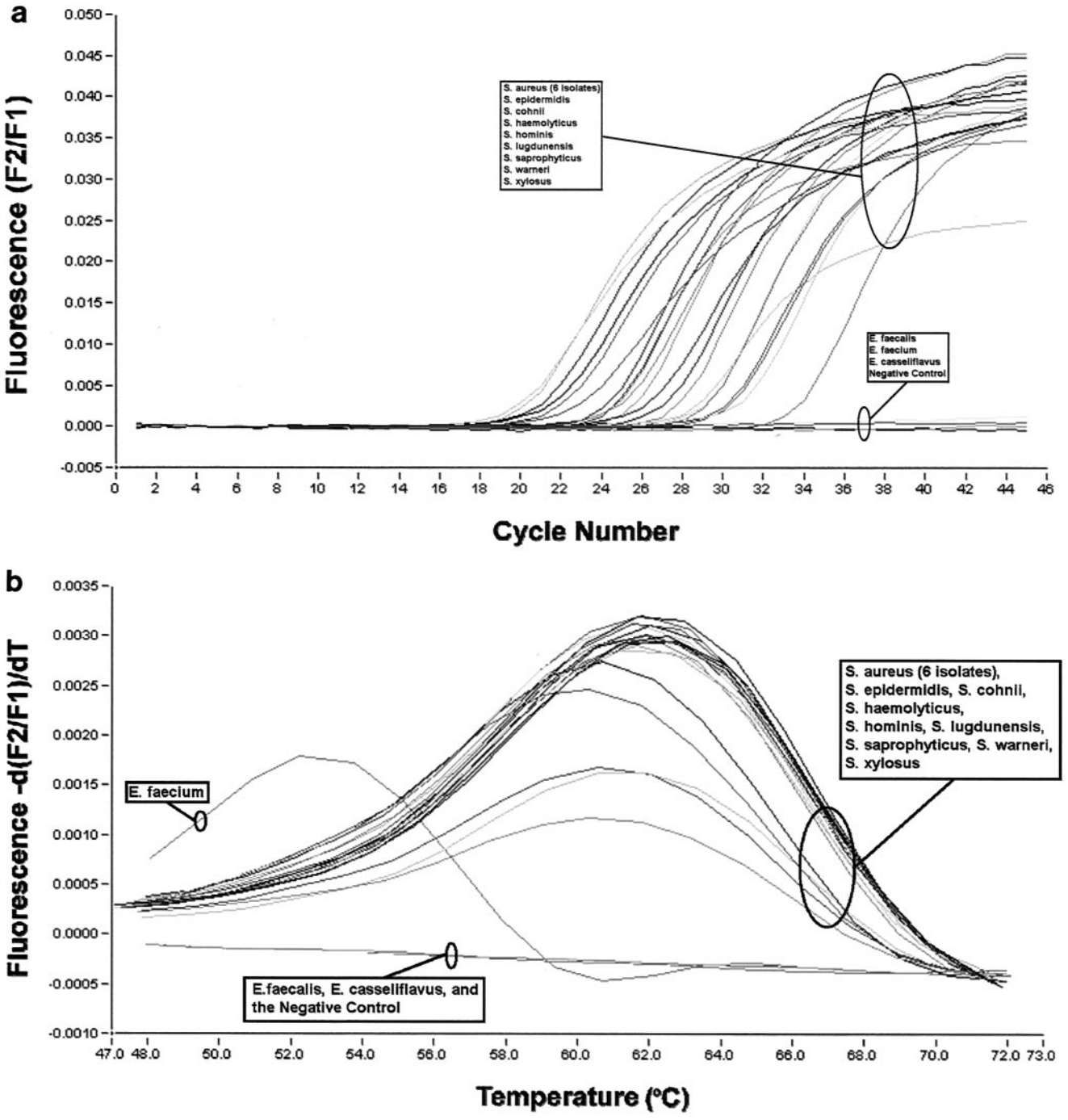

FIG. 1. (a) Amplification curves with broad-range *Staphylococcus* FRET probes. The broad-range *Staphylococcus* FRET probes generated positive quantification curves for all of the staphylococci tested. Three strains of *Enterococcus* were distinctly negative in this study. (b) Melt peak analysis of broad-range *Staphylococcus* assay. *Enterococcus faecium* was not detected in the quantification mode while the melting curve was generated by melt peak analysis. The T_ms of the staphylococci were all >58°C. The T_m of *E. faecium* was distinctively lower than those of the other CoNS.

The detection curve was linear over 5 log, and the slope (r^2) value was 1.00. This assay detected the lowest concentration tested, 20 fg of *S. aureus* DNA template. This detection limit was converted to 6.5 genome copies, as deduced by using the genome size and known GC content of *S. aureus*.

One hundred consecutive positive blood culture bottle specimens that were tested by this real-time PCR method were shown by conventional culture to consist of 34 bottles that contained *S. aureus*, 64 that contained a CoNS, and 2 that contained a *Micrococcus* species. The *S. aureus*-specific real-

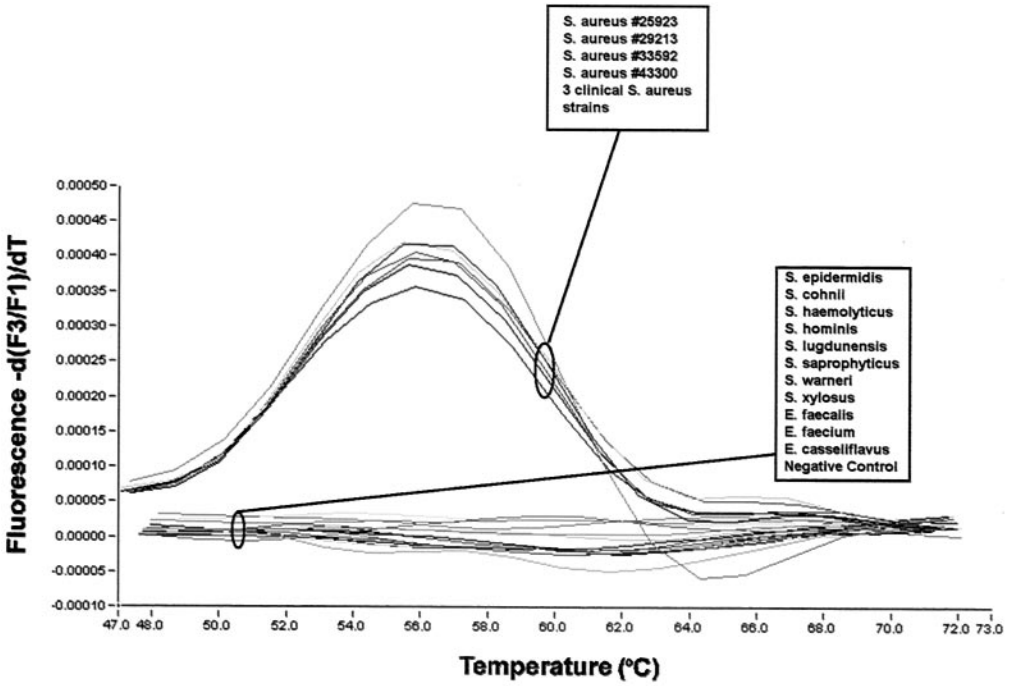

FIG. 2. Melt curves of *S. aureus*-specific assay. The melt curves were generated from *S. aureus* samples only.

time PCR assay correctly identified all 34 specimens that contained *S. aureus*. The *Staphylococcus* genus-specific reaction correctly characterized all 98 specimens that contained staphylococci. The combination of these results afforded the differentiation of *S. aureus* and CoNS in these specimens. Two specimens that contained a *Micrococcus* species were appropriately negative in this assay. The sensitivity and specificity of this assay for the detection of *S. aureus* and CoNS in positive blood culture bottles that contained gram-positive cocci in clusters were 100% and 100%, respectively. There were no blood cultures in this study that contained a mixture of *S. aureus* and CoNS.

DISCUSSION

Staphylococci are among the most commonly recovered bacteria in the clinical microbiology laboratory and because of skin colonization are the most frequently isolated bacteria in blood cultures. The staphylococci are generally categorized as *S. aureus* and non-*S. aureus* or CoNS. The distinction is not merely taxonomic. *S. aureus* is a more virulent, more clinically significant bacterium than CoNS and is more likely to be associated with abscess formation and more rapidly progressive infections. Although they are less virulent, CoNS may also cause infections, some of which may be life-threatening. For example, *S. lugdunensis* is a particularly virulent CoNS that in some scenarios may rival *S. aureus* in its aggressive nature. Practically, however, CoNS are the most common blood culture contaminants in most laboratories, since these organisms colonize the skin.

The Gram stain is commonly used to evaluate blood culture bottles that signal positive for staphylococci, but it cannot distinguish *S. aureus* from CoNS, both of which appear as gram-positive cocci in clusters. Therefore, genotypic methods have been employed to differentiate *S. aureus* from CoNS. Most of the genotypic methods described for the detection of *S. aureus* are specific only for that species of staphylococci and include both in situ hybridization and nucleic acid amplification reactions (22, 31). For example, a real-time PCR assay used by Shrestha et al. for the rapid detection of *S. aureus* in blood cultures that signaled positive and contained gram-positive cocci in clusters was 100% accurate in their study but could only categorize PCR-negative blood cultures as negative for *S. aureus*; they were unable to determine if a CoNS was present (31). In their study, it could only be assumed that the non-*S. aureus* reactions were due to CoNS. However, both *Micrococcus* and *Stomatococcus* species are occasionally recovered from blood cultures, and a negative PCR may be due to inhibitors of the PCR.

Others have devised more complex assays for the detection of both the *Staphylococcus* genus and the species *S. aureus*. For example, Mason et al. developed a multiplex PCR assay that detects a *Staphylococcus*-specific portion of the 16S rRNA gene, the *S. aureus*-specific *clfA* gene, and the *mecA* gene for methicillin resistance (20). The interpretation of data from this assay, however, may be complicated by closely related species that have similar or identical 16S rRNA sequences or due to divergent 16S rRNA sequences that may exist within a single organism (10, 32). Therefore, assays directed toward highly conserved regions of ubiquitous genes, such as the *hsp60*, *sodA*, and *tuf* genes, have been used as alternate targets for PCR (6, 16, 19). Goh et al. and Kwok et al. used the *hsp60* gene and Poyart et al. used the *sodA* gene to identify CoNS, but these assays required postamplification analysis, which is undesirable in the clinical laboratory (6, 23a).

The *tuf* gene encodes the elongation factor Tu, which is an essential constituent of the bacterial genome. One to three *tuf* genes per genome are present, depending on the bacterial species. Most gram-positive bacteria that have a low GC content possess only a single *tuf* gene (12). Martineau et al. developed a PCR-based assay that targets this gene for the genus- and species-specific identification of *S. aureus* and a variety of CoNS (19). This assay was highly sensitive and provided specific identification of *S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, and *S. saprophyticus* by the use of five hybridization probes. Unfortunately, postamplification analysis was also needed for this assay, and DNA from *Enterococcus faecalis* gave a weakly positive amplification signal in a 40-cycle PCR in their study. Edwards et al. also described a multiplex real-time PCR assay for the detection and differentiation of CoNS, using multiple primers and four biprobes for identification (4). Their assay is more complicated than the assay described in the present report and was clearly designed to differentiate CoNS species, not just to detect this group in its entirety. Although it is useful, the species differentiation of the CoNS was not the goal of our assay. In fact, in our clinical microbiology laboratory we rarely identify CoNS to the species level in routine practice.

We aligned the *tuf* gene sequences available in GenBank (provided by Martineau et al.) and designed broad-range staphylococcal primers that were expected to amplify the members of this genus, particularly the members that are most commonly encountered in the clinical microbiology laboratory. We tested this dual-FRET-probe real-time PCR assay against a battery of DNA preparations from a wide variety of bacteria, most of which are commonly encountered in the clinical setting. Forty-one of the DNA preparations tested were from cultured isolates of staphylococci that represented at least nine species. The remaining DNA preparations were from non-staphylococcal bacteria, some of which, like *Micrococcus*, were phylogenetically related to *Staphylococcus*.

The PCR assay performed well, amplifying all staphylococci. The *S. aureus* isolates were detected by both the *S. aureus*-specific FRET hybridization probes and the *Staphylococcus* genus-specific hybridization probes. The *S. aureus* component of this assay is considered a "melt-only" assay, since the primer annealing temperature of the PCR is higher than the annealing temperature of the *S. aureus*-specific hybridization probes. This was done to increase the specificity of the broad-range *Staphylococcus* primer set. CoNS were detected only with the *Staphylococcus* genus-specific hybridization probes. As in Martineau et al.'s study, *Enterococcus* may be weakly amplified with the *Staphylococcus* genus-specific probes; the melting temperature for *Enterococcus*, however, was considerably less than 58°C, which allowed for the easy differentiation of *Enterococcus* from *Staphylococcus*. Interpretative criteria, which we generated by using a battery of well-characterized bacterial lysates, were applied to 100 positive blood cultures that contained gram-positive cocci in clusters.

All 100 of the blood cultures examined were appropriately categorized as expected. The 34 blood cultures that contained *S. aureus* were detected with both the *S. aureus*-specific hybridization probes and the *Staphylococcus* genus-specific hybridization probes. The 64 blood cultures that contained CoNS were only detected with the *Staphylococcus* genus-specific hybridization probes. The two blood cultures that contained *Micrococcus* were appropriately negative in this assay.

We believe that this dual-FRET-probe real-time PCR assay provides several advantages over other PCR assays for *S. aureus* as well as over those that detect both *S. aureus* and CoNS. First, this assay not only specifically detects *S. aureus* but also generates a signal based on the presence of a signature sequence for the genus *Staphylococcus*. Therefore, in the absence of an *S. aureus*-specific signal, CoNS can be identified. One limitation would occur if there was a mixture of both *S. aureus* and a CoNS in the same blood culture bottle, for which the *Staphylococcus* genus signal would be assumed to be only from the *S. aureus* that was detected. We believe that this would not represent a significant limitation, since *S. aureus* would represent the most important pathogen in such a mixture. Second, the presence of both CoNS and *S. aureus* accounts for the vast majority, 98% in this study, of bacteria that occur in positive blood cultures and appear as gram-positive cocci in clusters. Although this assay does not contain a true internal control, the presence of the *Staphylococcus* genus signal in the absence of the *S. aureus* signal helps to assure the user that the amplification reaction occurred and was not inhibited. If the PCR from a blood culture bottle that contained gram-positive cocci in clusters was negative, although inhibition might responsible for such a reaction, the possibility of the presence of bacteria other than *Staphylococcus* must be considered. This finding could then be considered in conjunction with routine bacteriological findings for a workup of the cultivated bacterium. Finally, unlike some other assays that detect both *S. aureus* and CoNS, this assay does not require a complex postamplification analysis.

Shrestha et al. calculated the cost-effectiveness of a PCR assay for the preoperative detection of nasal carriage of *S. aureus* (30). The high sensitivity of this assay may allow it to be used not just to detect colonization and determine the agent responsible for positive blood cultures that contain gram-positive cocci in clusters, but also for the direct detection of staphylococci from surgical specimens. The use of such technologies for the detection of infections of orthopedic devices, for which CoNS and *S. aureus* are common pathogens, is of particular interest.

In conclusion, we described here a sensitive and specific dual-FRET-probe real-time PCR assay that employs a single broad-range primer set for the detection and differentiation of *S. aureus* and CoNS. This assay has been shown to be applicable for the analysis of cultivated bacteria as well as the direct analysis of positive blood culture bottles. Further analysis should include the use of this assay for the direct detection of staphylococci from clinical specimens, such as infections associated with medical devices.

REFERENCES

1. **Anthony, R. M., T. J. Brown, and G. L. French.** 2000. Rapid diagnosis of bacteremia by universal amplification of 23S ribosomal DNA followed by hybridization to an oligonucleotide array. J. Clin. Microbiol. **38:**781–788.
2. **Brumfitt, W., and J. Hamilton-Miller.** 1989. Methicillin-resistant Staphylococcus aureus. N. Engl. J. Med. **320:**1188–1196.
3. **Chapin, K., and M. Musgnug.** 2003. Evaluation of three rapid methods for the direct identification of *Staphylococcus aureus* from positive blood cultures. J. Clin. Microbiol. **41:**4324–4327.
4. **Edwards, K. J., M. E. Kaufmann, and N. A. Saunders.** 2001. Rapid and accurate identification of coagulase-negative staphylococci by real-time PCR. J. Clin. Microbiol. **39:**3047–3051.

5. **Gastmeier, P., C. Geffers, D. Sohr, F. Schwab, M. Behnke, and H. Ruden.** 2003. Surveillance of nosocomial infections in intensive care units. Current data and interpretations. Wien Klin. Wochenschr. **115:**99–103.
6. **Goh, S. H., S. Potter, J. O. Wood, S. M. Hemmingsen, R. P. Reynolds, and A. W. Chow.** 1996. HSP60 gene sequences as universal targets for microbial species identification: studies with coagulase-negative staphylococci. J. Clin. Microbiol. **34:**818–823.
7. **Grant, C. E., D. L. Sewell, M. Pfaller, R. V. Bumgardner, and J. A. Williams.** 1994. Evaluation of two commercial systems for identification of coagulase-negative staphylococci to species level. Diagn. Microbiol. Infect. Dis. **18:**1–5.
8. **Ieven, M., J. Verhoeven, S. R. Pattyn, and H. Goossens.** 1995. Rapid and economical method for species identification of clinically significant coagulase-negative staphylococci. J. Clin. Microbiol. **33:**1060–1063.
9. **Isaacs, D.** 2003. A ten year, multicentre study of coagulase negative staphylococcal infections in Australasian neonatal units. Arch. Dis. Child **88:**F89–F93.
10. **Jacobs, J. A., L. M. Schouls, and R. A. Whiley.** 2000. DNA-DNA reassociation studies of Streptococcus constellatus with unusual 16S rRNA sequences. Int. J. Syst. Evol. Microbiol. **50:**247–249.
11. **Jansen, G. J., M. Mooibroek, J. Idema, H. J. Harmsen, G. W. Welling, and J. E. Degener.** 2000. Rapid identification of bacteria in blood cultures by using fluorescently labeled oligonucleotide probes. J. Clin. Microbiol. **38:** 814–817.
12. **Ke, D., M. Boissinot, A. Huletsky, F. J. Picard, J. Frenette, M. Ouellette, P. H. Roy, and M. G. Bergeron.** 2000. Evidence for horizontal gene transfer in evolution of elongation factor Tu in enterococci. J. Bacteriol. **182:**6913–6920.
13. **Kempf, V. A., K. Trebesius, and I. B. Autenrieth.** 2000. Fluorescent in situ hybridization allows rapid identification of microorganisms in blood cultures. J. Clin. Microbiol. **38:**830–838.
14. **Kleeman, K. T., T. L. Bannerman, and W. E. Kloos.** 1993. Species distribution of coagulase-negative staphylococcal isolates at a community hospital and implications for selection of staphylococcal identification procedures. J. Clin. Microbiol. **31:**1318–1321.
15. **Kloos, W. E., and T. L. Bannerman.** 1994. Update on clinical significance of coagulase-negative staphylococci. Clin. Microbiol. Rev. **7:**117–140.
16. **Kwok, A. Y., S. C. Su, R. P. Reynolds, S. J. Bay, Y. Av-Gay, N. J. Dovichi, and A. W. Chow.** 1999. Species identification and phylogenetic relationships based on partial HSP60 gene sequences within the genus Staphylococcus. Int. J. Syst. Bacteriol. **49:**1181–1192.
17. **Louie, L., S. O. Matsumura, E. Choi, M. Louie, and A. E. Simor.** 2000. Evaluation of three rapid methods for detection of methicillin resistance in *Staphylococcus aureus*. J. Clin. Microbiol. **38:**2170–2173.
18. **Lu, J. J., C. L. Perng, S. Y. Lee, and C. C. Wan.** 2000. Use of PCR with universal primers and restriction endonuclease digestions for detection and identification of common bacterial pathogens in cerebrospinal fluid. J. Clin. Microbiol. **38:**2076–2080.
19. **Martineau, F., F. J. Picard, D. Ke, S. Paradis, P. H. Roy, M. Ouellette, and M. G. Bergeron.** 2001. Development of a PCR assay for identification of staphylococci at genus and species levels. J. Clin. Microbiol. **39:**2541–2547.
20. **Mason, W. J., J. S. Blevins, K. Beenken, N. Wibowo, N. Ojha, and M. S. Smeltzer.** 2001. Multiplex PCR protocol for the diagnosis of staphylococcal infection. J. Clin. Microbiol. **39:**3332–3338.
21. **Mekontso-Dessap, A., M. Kirsch, E. Vermes, C. Brun-Buisson, D. Loisance, and R. Houel.** 2002. Nosocomial infections occurring during receipt of circulatory support with the paracorporeal ventricular assist system. Clin. Infect. Dis. **35:**1308–1315.
22. **Oliveira, K., G. W. Procop, D. Wilson, J. Coull, and H. Stender.** 2002. Rapid identification of *Staphylococcus aureus* directly from blood cultures by fluorescence in situ hybridization with peptide nucleic acid probes. J. Clin. Microbiol. **40:**247–251.
23. **Perl, T. M., P. R. Rhomberg, M. J. Bale, P. C. Fuchs, R. N. Jones, F. P. Koontz, and M. A. Pfaller.** 1994. Comparison of identification systems for Staphylococcus epidermidis and other coagulase-negative Staphylococcus species. Diagn. Microbiol. Infect. Dis. **18:**151–155.

23a. **Poyart, C., G. Quesne, C. Boumaila, and P. Trieu-Cuot.** 2001. Rapid and accurate species-level identification of coagulase-negative staphylocci by using the *sodA* gene as a target. J. Clin. Microbiol. **39:**4296–4301.

24. **Reischl, U., H. J. Linde, M. Metz, B. Leppmeier, and N. Lehn.** 2000. Rapid identification of methicillin-resistant *Staphylococcus aureus* and simultaneous species confirmation using real-time fluorescence PCR. J. Clin. Microbiol. **38:**2429–2433.
25. **Reischl, U., M. Pulz, W. Ehret, and H. Wolf.** 1994. PCR-based detection of mycobacteria in sputum samples using a simple and reliable DNA extraction protocol. BioTechniques **17:**844–845.
26. **Renneberg, J., K. Rieneck, and E. Gutschik.** 1995. Evaluation of Staph ID 32 system and Staph-Zym system for identification of coagulase-negative staphylococci. J. Clin. Microbiol. **33:**1150–1153.
27. **Sewell, C. M., J. E. Clarridge, E. J. Young, and R. K. Guthrie.** 1982. Clinical significance of coagulase-negative staphylococci. J. Clin. Microbiol. **16:**236–239.
28. **Sheagren, J. N.** 1984. Staphylococcus aureus. The persistent pathogen (first of two parts). N. Engl. J. Med. **310:**1368–1373.
29. **Sheagren, J. N.** 1984. Staphylococcus aureus. The persistent pathogen (second of two parts). N. Engl. J. Med. **310:**1437–1442.
30. **Shrestha, N. K., K. M. Shermock, S. M. Gordon, M. J. Tuohy, D. A. Wilson, R. E. Cwynar, M. K. Banbury, D. L. Longworth, C. M. Isada, S. D. Mawhorter, and G. W. Procop.** 2003. Predictive value and cost-effectiveness analysis of a rapid polymerase chain reaction for preoperative detection of nasal carriage of Staphylococcus aureus. Infect. Control Hosp. Epidemiol. **24:**327–333.
31. **Shrestha, N. K., M. J. Tuohy, G. S. Hall, C. M. Isada, and G. W. Procop.** 2002. Rapid identification of *Staphylococcus aureus* and the *mecA* gene from BacT/ALERT blood culture bottles by using the LightCycler system. J. Clin. Microbiol. **40:**2659–2661.
32. **Staclebramdt, E., and B. M. Goebel.** 1994. A place for DNA-DNA reassociation and 16s rRNA sequence analysis in the present species definition in bacteriology. Int. J. Syst. Bacteriol. **44:**846–849.
33. **Weinstein, M. P., S. Mirrett, L. Van Pelt, M. McKinnon, B. L. Zimmer, W. Kloos, and L. B. Reller.** 1998. Clinical importance of identifying coagulase-negative staphylococci isolated from blood cultures: evaluation of MicroScan rapid and dried overnight gram-positive panels versus a conventional reference method. J. Clin. Microbiol. **36:**2089–2092.
34. **Wittwer, C. T., M. G. Herrmann, A. A. Moss, and R. P. Rasmussen.** 1997. Continuous fluorescence monitoring of rapid cycle DNA amplification. BioTechniques **22:**130–131