Direct Identification of *Staphylococcus aureus* from Positive Blood Culture Bottles

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Received 15 May 2002/Returned for modification 26 September 2002/Accepted 12 November 2002

Fluorescence in situ hybridization (FISH) using peptide nucleic acid (PNA) probes targeting *Staphylococcus aureus* 16S rRNA is a novel method for direct identification of *S. aureus* from positive blood culture bottles. The test (*S. aureus* PNA FISH) is performed on smears made directly from positive blood culture bottles with gram-positive cocci in clusters (GPCC) and provides results within 2.5 h. A blinded comparison of *S. aureus* PNA FISH with standard identification methods was performed in collaboration with eight clinical microbiology laboratories. A total of 564 routine blood culture bottles positive for GPCC recovered from both aerobic and anaerobic media from three different manufacturers (ESP, BACTEC, and BacT/Alert) were included in the study. The sensitivity and specificity of *S. aureus* PNA FISH were 100% (57 of 57) and 99.2% (116 of 117), respectively, with 174 GPCC-positive ESP blood culture bottles, 98.5% (67 of 68) and 98.5% (129 of 131), respectively, with 200 GPCC-positive BACTEC blood culture bottles, and 100% (74 of 74) and 99.1% (115 of 116), respectively, with 190 GPCC-positive BacT/Alert blood culture bottles. It is concluded that *S. aureus* PNA FISH performs well with commonly used continuously monitoring blood culture systems.

Identification of *Staphylococcus aureus* in blood cultures begins with presumptive identification of gram-positive cocci in clusters (GPCC) in the Gram-stained smear of blood culture bottles that signal a positive result, whereas final identification must await subculture and overnight incubation (1, 8). This delayed identification often results in empirical antibiotic therapy administered to patients with GPCC-positive blood cultures, although the majority of GPCC-positive blood cultures are later identified as coagulase-negative staphylococci (CoNS), such as *Staphylococcus epidermidis*, a common blood culture contaminant. Direct identification of *S. aureus* in GPCC-positive blood culture bottles may provide important diagnostic information, which would allow the selection of an appropriate course of treatment in a timely manner.

Immunological, tube coagulase, and stable-endonuclease methods routinely used for identification of *S. aureus* following subculture have been applied directly to GPCC-positive blood culture bottles, but with variable sensitivities and specificities (6, 9, 10). In addition, molecular techniques, such as hybridization protection (3), fluorescence in situ hybridization (FISH) (5), and PCR (2) have been described for identification of *S. aureus* directly from positive blood cultures. In general, all studies have been performed on just a single blood culture medium and, therefore, do not address the potential for interference from different blood culture media, which in fact might

explain the variable results. Furthermore, none of the studies involve blood culture media supplemented with charcoal, such as that used in the FAN BacT/Alert medium (bioMérieux, Hazelwood, Mo.), or resins, such as that in the BACTEC Plus medium (Becton Dickinson, Sparks, Md.). Those supplements may interfere with assays for direct identification of positive blood culture organisms.

FISH with a peptide nucleic acid (PNA) probe to target 16S rRNA of *S. aureus* is a novel method for the rapid and specific identification of *S. aureus* directly from GPCC-positive blood cultures. *S. aureus* PNA FISH had a 97% sensitivity and 100% specificity compared to conventional methods when tested with the BacT/Alert blood culture system and FAN medium (7). The aim of the present study was to perform a blinded comparison of *S. aureus* PNA FISH with conventional methodology on blood cultures representing the ESP medium (Trek Diagnostic Systems, Inc., Westlake, Ohio), BACTEC medium (Becton Dickinson), and BacT/Alert medium (bioMérieux). Routine positive blood culture bottles in which gram-positive cocci in clusters were observed in Gram-stained smears were randomly collected at each of eight clinical microbiology laboratories. For the study, one smear for future testing was prepared from each blood culture shortly after the Gram-staining results were known. The smears are stable at room temperature for several weeks and were collected over a 1- to 2-week period. For the most part, smears were transported to Boston Probes (now Applied Biosystems) for blinded analysis by *S. aureus* PNA FISH, although the majority of smears from two clinical microbiology laboratories were tested on site. *S.*

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TABLE 1. Reaction of *S. aureus* PNA FISH with 174 GPCC-positive blood culture bottles from the ESP system compared to identification by standard methods^a

Identification	<i>S. aureus</i> PNA FISH result	
	Positive (n)	Negative (n)
<i>S. aureus</i>	57 ^b	0
Yeast	0	1
Micrococci	0	1
Enterococci	0	2
CoNS	1 ^c	112 ^d

^a The blood culture bottles consisted of 80A and 80N bottles (Trek Diagnostics).

^b Two samples were identified as mixed culture by both standard methods and *S. aureus* PNA FISH. One sample was identified as a mixed culture by *S. aureus* PNA FISH only.

^c Mixed culture by *S. aureus* PNA FISH.

^d Includes two mixed cultures of CoNS with nonstaphylococcal organisms.

aureus PNA FISH was performed as previously reported (7). Briefly, one drop of hybridization solution with a fluorescein-labeled PNA probe targeting *S. aureus* 16S rRNA was added to each smear. Coverslips were placed on the smears to ensure even coverage with hybridization solution, and the slides were subsequently incubated for 90 min at 55°C. Following hybridization, the coverslips were removed by submerging the slides into prewarmed wash solution in a water bath at 55°C and washed for 30 min. Each smear was finally mounted by using one drop of mounting fluid and covered with a coverslip. Microscopic examination was conducted by using a fluorescence microscope with a fluorescein isothiocyanate-Texas Red dual band filter set and a 60× or 100× objective. Multiple clusters of bright fluorescent cocci were usually apparent immediately upon examination of the slides. When just a few fluorescent organisms morphologically consistent with *S. aureus* were observed, bright field was applied to examine the smears for organisms morphologically consistent with staphylococci. The presence of fluorescent and nonfluorescent cocci was reported as positive for *S. aureus*, but the possibility of a mixed culture was noted.

The results were compared to those obtained at the eight clinical microbiology laboratories as part of the routine identification of positive blood culture bottles by their respective standard methods.

The comparative results for each blood culture system are presented in Tables 1 to 3. Table 1 summarizes data for ESP blood culture bottles from two different clinical microbiology laboratories. The data in Table 2 are based on BACTEC blood culture bottles from three clinical microbiology laboratories and include three bottles with resin and two bottles with Myco/F Lytic which all gave results in agreement with conventional identification. Table 3 summarizes data on BacT/Alert blood culture bottles obtained from three clinical microbiology laboratories. Two of these sites used the charcoal-containing FAN medium, and one site used the standard medium without charcoal. Data on the FAN medium alone showed 100% sensitivity (52 of 52) and 98.9% specificity (89 of 90). All bottles represent both aerobic and anaerobic media.

In summary, results obtained with four blood culture samples (0.7%) by *S. aureus* PNA FISH disagreed with conventional identification. Two of the isolates were not available for

TABLE 2. Reaction of *S. aureus* PNA FISH with 200 GPCC-positive blood culture bottles from the BACTEC system compared to identification by standard methods^a

Identification	<i>S. aureus</i> PNA FISH result	
	Positive (n)	Negative (n)
<i>S. aureus</i>	64	3 ^c
CoNS	4 ^b	123
<i>Staphylococcus warneri</i>	0	1
<i>Pseudomonas aeruginosa</i>	0	1
<i>Candida albicans</i>	0	1
Streptococci	1	2

^a The blood culture bottles consisted of BACTEC Standard 10 Aerobic/F, BACTEC Lytic/10 Anaerobic/F, Pediatrics Plus (n = 3) and BACTEC Myco/F Lytic (n = 2) (Becton Dickinson).

^b All samples were identified as mixed cultures by *S. aureus* PNA FISH. Two samples showed very few *S. aureus* clusters and mostly non-*S. aureus* clusters by *S. aureus* PNA FISH. Multiple blood culture samples from two patients were identified as *S. aureus* by standard methods.

^c One sample was identified as a mixed culture of *S. aureus* and *Staphylococcus haemolyticus* by standard methods. One isolate was positive by Staphaurex but negative by DNase test. One isolate was positive by *S. aureus* PNA FISH by retest.

retest and one false-negative isolate was positive by retest with *S. aureus* PNA FISH, whereas another false-negative isolate remained negative by *S. aureus* PNA FISH. Interestingly, this isolate was positive by Staphaurex (Remel, Lenexa, Kans.) but negative by DNase test and therefore may not be *S. aureus*. Six samples (1.1%) were reported as mixed culture by *S. aureus* PNA FISH, but *S. aureus* was not found by conventional methods. However, in two of these cases, multiple blood cultures from the same patients were positive for *S. aureus*, indicating that at least some of these "false positives" are true positive samples that also contained CoNS. One GPCC-positive FAN BacT/Alert sample gave a very weak and variable fluorescent

TABLE 3. Reaction of *S. aureus* PNA FISH with 190 GPCC-positive blood culture bottles from the BacT/Alert system compared to identification by conventional methods^a

Identification	<i>S. aureus</i> PNA FISH result	
	Positive (n)	Negative (n)
<i>S. aureus</i>	74 ^b	0
CoNS	1 ^c	55 ^{d,e}
<i>Staphylococcus epidermidis</i>	0	37
<i>Staphylococcus haemolyticus</i>	0	4
<i>Staphylococcus lugdunensis</i>	0	2
<i>Staphylococcus capitis</i>	0	2 ^f
<i>Staphylococcus marcescens</i>	0	8 ^g
Enterococci	0	3
<i>Enterococcus cloacae</i>	0	2
<i>Pseudomonas aeruginosa</i>	0	2

^a The blood culture bottles consisted of BacT/Alert SA, BacT/Alert SN, BacT/Alert FA, and BacT/Alert FN (bioMérieux).

^b One sample was identified as a mixed culture of *S. aureus* and *Enterococcus faecalis* by standard methods.

^c Few *S. aureus* clusters and mostly CoNS by *S. aureus* PNA FISH. Another sample from the same patient grew CoNS and was negative by *S. aureus* PNA FISH.

^d One sample gave an inconclusive result (weak fluorescence) by *S. aureus* PNA FISH but was negative by repeat testing of the isolate.

^e One sample from pleural fluid collected in a BacT/Alert FA bottle.

^f One sample was identified as a mixed culture of *Staphylococcus capitis* and *Staphylococcus epidermidis* by standard methods.

^g Two samples were identified as mixed cultures of *Staphylococcus marcescens* and *Pseudomonas aeruginosa* by standard methods.

TABLE 4. Performance characteristics for *S. aureus* PNA FISH on three types of blood culture medium

Blood culture medium	% Sensitivity ^a	% Specificity ^b	Positive predictive value ^c	Negative predictive value ^d
ESP	100 (57/57)	99.2 (116/117)	98.3 (57/58)	100 (116/116)
BACTEC	98.5 (67/68)	98.5 (129/131)	97.1 (67/69)	99.2 (129/130)
BacT/Alert	100 (74/74)	99.1 (115/116)	98.7 (74/75)	100 (115/115)
Total	99.5 (198/199)	98.9 (360/364)	98.0 (198/202)	99.7 (360/361)

^a Values in parentheses are numbers of correct positive test results/numbers of positive samples.

^b Values in parentheses are numbers of correct negative test results/numbers of negative samples.

^c Values in parentheses are numbers of correct positive test results/numbers of positive test results.

^d Values in parentheses are numbers of correct negative test results/numbers of negative test results.

signal by *S. aureus* PNA FISH and was reported as inconclusive. The blood culture bottle was not available for retesting, but the isolate yielded a clear negative result by PNA FISH, which was in agreement with the routine identification. The remaining 558 (98.2%) GPCC-positive blood cultures gave clear test results in agreement with identification by conventional methods.

A summary of the performance data for *S. aureus* PNA FISH with blood culture media from the three different manufacturers is listed in Table 4. The two mixed cultures that were supported by multiple other *S. aureus*-positive blood cultures and the two false negatives that were resolved by retesting and DNase testing are resolved in the data listed in Table 4. In general, high sensitivity and specificity is associated with molecular techniques and in particular with PNA probes (4). The high predictive values obtained by *S. aureus* PNA FISH enables GPCC-positive blood cultures to be definitively reported as *S. aureus* or non-*S. aureus* in less than 3 h after the initial Gram stain results become available.

As indicated above, some of the false-positive results were likely a result of mixed cultures where true *S. aureus*-positive blood samples also contained CoNS, but they may have been missed on culture plates because of similar appearance or mixed growth with CoNS or because *S. aureus* may be overlooked following subculture if the number of CoNS colonies is much greater than the number of *S. aureus* colonies. To support this hypothesis, we prepared artificially spiked blood culture bottles with various ratios of *S. aureus* to *Staphylococcus epidermidis*. Results by *S. aureus* PNA FISH showed that *S. aureus* could be detected down to a ratio of 1 to 10,000.

This study has shown that *S. aureus* PNA FISH is compatible with commonly used blood culture media, including ESP,

BACTEC, and BacT/Alert media, where GPCC-positive blood cultures were identified as *S. aureus* or non-*S. aureus* with 98.5 to 100% sensitivity and 98.5 to 99.2% specificity.

We thank Bruce Hanna, NYU School of Medicine, Bellevue Hospital, for his helpful comments in the review of the manuscript.

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