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Rapid Identification of *Staphylococcus aureus* in Blood Cultures by Use of the Direct Tube Coagulase Test[▽]

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Direct tube coagulase testing for identification of *Staphylococcus aureus* from BACTEC culture broth showed a sensitivity, a specificity, and positive and negative predicative values of 34%, 100%, 100%, and 80.2% with 2 h of incubation and 65%, 98.7, 99.7%, and 88.6% with 4 h of incubation. Anaerobic blood culture contributed significantly to the detection of *S. aureus*.

Staphylococcus aureus is one of the leading causes of bacteremia, with high levels of accompanying morbidity and mortality (7, 10, 11, 18). Complication rates rise steadily with the duration of untreated bacteremia (5). Therefore, methods for the rapid identification of *S. aureus* in blood culture broth have been developed, including peptide nucleic acid fluorescence in situ hybridization, PCR, and the enzymatic RAPIDEC test (8, 12, 15, 16). However, they are expensive and labor intensive and the RAPIDEC test (3, 16) shows suboptimal specificity. In contrast, the direct tube coagulase (DTC) test is rapid, simple, and inexpensive. Previously, the DTC test showed good sensitivity and specificity when applied to the BacT/Alert (17), ESP (3), and Bactec 660 (9) systems. However, only a small study was previously performed with the BACTEC 9240 system with a single 2-h incubation (16). We therefore performed an extensive evaluation of BACTEC 9240-based medium by incorporating it into routine clinical work.

From 2003 to 2005, blood culture broth from Standard/10 Aerobic/F and LYTIC/10 Anaerobic/F bottles was evaluated by DTC test when Gram staining suggested staphylococci (16). Clot formation was assessed after incubation of a mixture of 5 drops of culture broth and 0.5 ml of rabbit plasma at 35°C for 2 and 4 h. Among 1,780 positive blood cultures tested, there were 477 *S. aureus* isolates and 1,303 other bacteria (Table 1). At 2 h (Table 2), 125 *S. aureus* isolates showed obvious gelling and 35 additional isolates showed partial gelling, with reactivity independent of methicillin resistance status (data not shown). At 4 h, an additional 133 and 17 showed strong and weak positivity, respectively. When both complete gelling and partial gelling were considered positive, the sensitivity, specificity, positive predicative value, and negative predicative value for the 2- and 4-h time points were 34%, 100%, 100%, and 80.2% and 65%, 99.7%, 98.7%, and 88.6%, respectively.

The potential for false-positive DTC test results has not been sufficiently studied in actual clinical practice. One previous study evaluated a limited variety of organisms in simulated blood culture and found no false positives after a 2-h incubation (9). Here, we evaluated a much larger number and variety of bacteria initially thought to have a Gram stain compatible with *S. aureus* (Table 1). Four false-positive results (at 4 h) were found, including three isolates identified as coagulase-negative staphylococci (CoNS) and one isolate identified as *Enterococcus faecalis* (Table 2). Unfortunately, the three CoNS isolates were not saved for further analysis but potentially may have been coagulase-producing species (1).

Additionally, we showed for the first time that the DTC test performed equally well with both aerobic broth and anaerobic broth. Among the 32 sets evaluated (both bottles were evaluated since they were flagged simultaneously), 29/32 aerobic and 25/32 anaerobic samples were positive at 2 h ($P = 0.3$; Fisher's exact test) and 31/32 aerobic and 30/32 anaerobic samples were positive at 4 h ($P = 1.0$).

Importantly, during this analysis, we found that anaerobic blood culture contributed significantly to *S. aureus* detection ($P < 0.001$). Among 447 *S. aureus* culture sets analyzed, 299 (62.7%) were positive in both aerobic and anaerobic bottles, 42 (8.8%) were positive in aerobic bottles alone, and 136 (28.5%) were isolated in anaerobic bottles alone. Methicillin resistance was not associated with growth preference (data not shown). Clinical chart review revealed that for 82 (17.2%) patients, *S. aureus* was detected solely on the basis of anaerobic culture (Table 3). Among these, a mean of 3.4 ± 1.3 blood culture sets per patient were drawn within 3 days of the first positive culture and 30% had multiple positive anaerobic cultures. Conversely, aerobic culture was necessary for diagnosis in only 15 (3.1%) patients. Here, a mean of 2.1 ± 0.8 blood culture sets were drawn within 3 days of the first positive culture. Two of these patients had multiple positive aerobic cultures. Our data suggest that some strains grow preferentially in either anaerobic or aerobic bottles, although the lytic component rather than anaerobic conditions might account for preferential growth in the former. Similar results were obtained with the BacT/ALERT system (13). Interestingly, a previous study found the BACTEC PLUS aerobic/F bottle superior to the LYTIC anaerobic/F bottle used here; nevertheless, 13% of the *S. aureus* isolates were still missed in the absence of anaerobic culture (14). Therefore, both bottles need to be used for optimal detection of *S. aureus*, providing an additional rationale for the routine use of anaerobic culture (4, 6, 14).

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TABLE 1. Identification of organisms other than *S. aureus* in blood culture broth tested by DTC test

Organism(s)	No. of isolates tested
CoNS	1,220
Micrococcus spp.	21
Micrococcus spp./*Stomatococcus* spp.	7
Stomatococcus mucilaginosus	1
Peptostreptococcus spp.	14
Corynebacterium spp.	3
Enterococcus faecalis	5
Enterococcus faecium	7
Enterococcus spp.	1
Streptococcus agalactiae	2
Streptococcus pneumoniae	1
Viridans group streptococci	9
Streptococcus mitis	1
Streptococcus anginosus	1
Streptococcus intermedius	1
Streptococcus milleri group	1
Streptococcus vestibularis	1
Propionibacterium acnes	1
Vagococcus	1
Veillonella	1
Peptostreptococcus with *Pseudomonas aeruginosa*	1
CoNS with viridans group streptococci	1
CoNS with gram-negative rods (nonfermenter)	1
CoNS with *Pseudomonas aeruginosa*	1
Total	1,303

Previously, a number of variables have been examined for optimizing the sensitivity of the DTC test. In our study, we found that increasing the incubation period from 2 to 4 h greatly enhanced sensitivity, although this contrasts with a BACTEC 660 study (9) in which no difference was found. The sensitivity (65%) of the DTC test in our study was lower than the sensitivities found in studies performed with the BACTEC 660 (79.5%) (9) and ESP (84.1%) (3) systems and a small study performed with the BACTEC 9240 system (92%) (16). Of note, in our study, the DTC test was performed by a large number of rotating laboratory personnel as a part of the routine workup. When the DTC test was performed by one of us (100 *S. aureus* and 179 CoNS isolates), the sensitivity increased to 68% at 2 h and 90% at 4 h and the specificity was 100%. The higher sensitivity potentially relates to better appreciation of weak positive reactions, as suggested previously (9). A potentially expected decrease in specificity was not observed here because of the smaller number of CoNS isolates examined and/or the high specificity of even weak DTC reactions.

TABLE 2. Characteristics of DTC test-positive samples

Incubation time (h)	No. of isolates with indicated test result of:			
	S. aureus		Organisms other than *S. aureus*	
	Strongly positive	Weakly positive	Strongly positive	Weakly positive
2	125	35	0	0
4	133	17	3[a]	1[b]
Total	258	52	3	1

[a] Includes two isolates of CoNS and one isolate of *E. faecalis*.
[b] CoNS isolate.

TABLE 3. Detection of *S. aureus* bacteremic episodes by aerobic versus anaerobic bottles alone

No. of blood sets drawn/ patient[a]	No. of patients with positive cultures (% with multiple positive cultures)	
	Aerobic bottles only	Anaerobic bottles only
1	3	3
2	5 (40)	21 (24)
3	4 (0)	20 (20)
4	3 (0)	25 (48)
5	0	6 (33)
6	0	6 (33)
7	0	1 (0)
Total	15 (13)	82 (30)

[a] Within 3 days of the first bottle positive for *S. aureus*.

Even with a sensitivity of 65% and the potential for higher sensitivity with further training, we believe the assay is clinically valuable. The DTC test rapidly identifies the majority of *S. aureus* isolates. In cases where clinical suspicion is low, this early identification prevents therapy being withheld for a day pending the identification of a presumed contaminant (i.e., CoNS) to the species level, as documented elsewhere (2). Because of its low cost and simplicity, we believe it will continue to serve an important role in laboratories that can neither afford nor implement around-the-clock testing by more-sensitive methods such as PCR.

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