

SHORT REPORT

Rapid identification of *Staphylococcus aureus* from BacT/ALERT blood culture bottles by direct Gram stain characteristics

D R Murdoch, R L Greenlees

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The rapid identification of *Staphylococcus aureus* from positive blood cultures provides important clinical and therapeutic information. Using criteria based on direct Gram stain characteristics, an experienced microscopist was able to distinguish *S aureus* from other staphylococci isolated from BacT/ALERT blood culture bottles with an overall sensitivity of 89% and specificity of 98%. Furthermore, this method was readily taught to a clinical microbiologist who had not previously used the method first hand. Laboratories using the BacT/ALERT blood culture system should become familiar with these criteria so that *S aureus* bacteraemia can be identified rapidly.

The use of continuously monitored blood culture systems has reduced the time taken to detect positive blood cultures. Once a positive blood culture is detected, presumptive identification of the organism relies on direct Gram stain of the inoculated blood culture broth. The appearance of Gram positive cocci in clusters on direct Gram stain is suggestive of staphylococcus species, but differentiation of *Staphylococcus aureus* from coagulase negative staphylococci can usually only be made 18–24 hours later, once the organism has been cultured on solid media. This distinction is important given the differences in virulence, and the relatively high frequency that coagulase negative staphylococci are isolated as contaminants.

“The main characteristics are the size of the bacterial cells, the number of cells in a typical cluster, and knowledge about whether the Gram stain was made from an anaerobic or aerobic blood culture bottle”

Over the past decade, technologists in our laboratory have noted that certain morphological characteristics can help distinguish *S aureus* from coagulase negative staphylococci in direct Gram stains from BacT/ALERT blood culture bottles (BioMérieux, Durham, North Carolina, USA). The main characteristics are the size of the bacterial cells, the number of cells in a typical cluster, and knowledge about whether the Gram stain was made from an anaerobic or aerobic blood culture bottle (table 1; fig 1). The purpose of our study was to evaluate these criteria in a blinded prospective fashion.

METHODS

Consecutive routine blood culture bottles (BacT/ALERT FA and BacT/ALERT SN) were incubated in the BacT/ALERT automated continuous monitoring system. Bottles that signalled as positive were removed, and a Gram stain was

Take home messages

- The rapid identification of *Staphylococcus aureus* from positive blood cultures provides important clinical and therapeutic information
- We have developed a series of criteria based on direct Gram stain characteristics that enabled an experienced microscopist to distinguish *S aureus* from other staphylococci isolated from BacT/ALERT blood culture bottles
- The overall sensitivity was 89% and specificity was 98%
- This method was readily taught to a clinical microbiologist who had not previously used the method
- Laboratories using the BacT/ALERT blood culture system should become familiar with these criteria for the rapid identification of *S aureus* bacteraemia

made using standard methods. Broths for which a Gram stain showed Gram positive cocci resembling *Staphylococcus* spp were included in our study. These broths were inoculated on to solid media, and the organisms isolated were identified by standard laboratory methods (colonial morphology, catalase reaction, 24 hour coagulase test, and bacitracin resistance).

Direct Gram stains of blood culture broth made after the bottles signalled positive were examined by an experienced technologist, who classified the organism as *S aureus* or not based on the criteria listed in table 1. At the time the slides were examined, the technologist knew whether the Gram stains were from aerobic or anaerobic blood culture bottles, and all Gram stains were assessed before the culture results were known. To assess how readily this technique can be taught, the same technologist trained a clinical microbiologist in the use of these criteria by showing typical examples of the key distinguishing characteristics. The microbiologist, who had not previously used the diagnostic criteria first hand, then examined the same series of Gram stains in a blinded fashion.

RESULTS

In total, 150 BacT/ALERT blood cultures in which a direct Gram stain showed Gram positive cocci resembling staphylococci were examined. Cultures of the broths revealed 66 positive for *S aureus*, 81 positive for coagulase negative staphylococci, and three positive for species of micrococcus. Table 2 shows the results of the comparison between Gram stain and culture based identifications for both microscopists.

To assess whether the same criteria for identifying *S aureus* can be applied to other blood culture systems, we performed

Table 1 Criteria used to distinguish *Staphylococcus aureus* from coagulase negative staphylococci in direct Gram stains from BacT/ALERT blood culture bottles

Bottle type	Organism	Cell size	Cluster characteristics
Anaerobic	<i>S aureus</i>	Small (<1 µm)	Irregularly clustered in large numbers (typically 200–300 cells)
	Coagulase-negative staphylococci	Large (≥1 µm)	Tetrads or small clusters up to 16 cells
Aerobic	<i>S aureus</i>	Large (≥1 µm)	Very tight clusters (typically 8–32 cells). Individual cells cannot be distinguished
	Coagulase-negative staphylococci	Variable (typically <1 µm)	Tetrads or small clusters up to 16 cells

the same experiment on 100 direct Gram stains from 100 positive BACTEC blood culture bottles (Becton Dickinson, Franklin Lakes, New Jersey, USA) obtained from another laboratory. These Gram stains were from consecutive positive blood cultures showing Gram positive cocci resembling staphylococci, and cultures of the broths revealed

23 *S aureus*, 74 coagulase negative staphylococci, two micrococcus species, and one mixed *S aureus*/coagulase negative staphylococcus. We could not reliably distinguish *S aureus* from other staphylococci using our criteria because of differences in Gram stain morphology of the staphylococci isolated in the two systems.

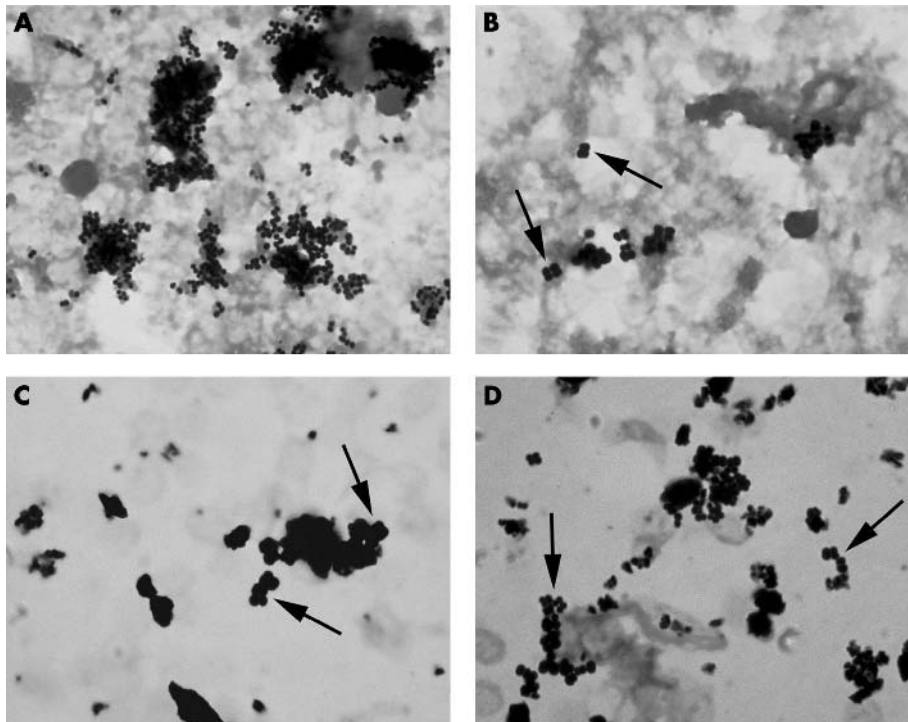


Figure 1 Gram stain smears from positive BacT/ALERT blood culture bottles showing typical appearances of *Staphylococcus aureus* and coagulase negative staphylococci, with key characteristics stated below in parentheses. (A) Anaerobic bottle, *S aureus* (small cells, large clusters); (B) anaerobic bottle, coagulase negative staphylococcus (large cells, tetrads (arrows), and small clusters); (C) aerobic bottle, *S aureus* (large cells, tight clusters (arrows)); (D) aerobic bottle, coagulase negative staphylococcus (tetrads and small clusters (arrows)).

Table 2 Performance characteristics of Gram stain compared with culture for identification of *Staphylococcus aureus* from BacT/ALERT blood culture bottles

Microscopist*	Blood culture bottle type	No. Gram stains positive for <i>S aureus</i> / No. cultures positive for <i>S aureus</i> (% sensitivity)	No. Gram stains negative for <i>S aureus</i> / No. cultures negative for <i>S aureus</i> † (% specificity)	PPV (%)	NPV (%)
Technologist	All	59/66 (89)	82/84 (98)	59/61 (97)	82/89 (92)
	Aerobic	26/31 (84)	49/50 (98)	26/27 (96)	49/54 (91)
	Anaerobic	33/35 (94)	33/34 (97)	33/34 (97)	33/35 (94)
Microbiologist	All	58/66 (88)	77/84 (92)	58/65 (89)	77/85 (91)
	Aerobic	24/31 (77)	47/50 (94)	24/27 (89)	47/54 (87)
	Anaerobic	34/35 (97)	30/34 (88)	34/38 (89)	30/31 (97)

*Technologist experienced with the criteria; clinical microbiologist recently taught the criteria; †includes coagulase negative staphylococci and micrococcus species.
NPV, negative predictive value; PPV, positive predictive value.

DISCUSSION

The ability to identify *S aureus* bacteraemia rapidly and accurately has direct clinical relevance for prompting the initiation of antibiotic treatment or a change to a more appropriate antibiotic regimen. The results of our study suggest that *S aureus* growing in BacT/ALERT blood culture bottles can be identified by direct Gram stain characteristics with a high degree of accuracy. Importantly, we were able to demonstrate that a reasonable level of competence could be achieved after a very brief training period. When the clinical microbiologist read the slides a second time, his readings improved and were similar to those of the experienced technologist.

Staphylococcus aureus was more readily identified in anaerobic than aerobic blood culture bottles. This may be partly explained by the presence of charcoal in the aerobic BacT/ALERT FA bottles, which makes the identification of Gram positive organisms difficult. However, we had noted that Gram stains from aerobic blood culture bottles were more difficult to assess than anaerobic bottles even before charcoal containing bottles were used in our laboratory. Our method has similar or better performance characteristics than some other rapid methods, such as the two hour tube coagulase test, detection of thermostable endonuclease, and immunological tests,^{1,2} but has a lower sensitivity than molecular methods, such as polymerase chain reaction and fluorescence in situ hybridisation.³⁻⁵ However, our approach has the major advantages of being able to provide a result immediately after the detection of a positive blood culture, and at essentially no extra cost.

“We were able to demonstrate that a reasonable level of competence could be achieved after a very brief training period”

Our preliminary observations indicate that the same diagnostic criteria for identifying *S aureus* from BacT/ALERT blood culture bottles cannot be applied to BACTEC blood culture bottles. The differences in direct Gram stain appearance presumably reflect the different growth characteristics in the two media. In one previous evaluation using older blood culture media, it was impossible to differentiate *S aureus* from coagulase negative staphylococci based on Gram stain morphology alone.⁶ Our inability to distinguish *S aureus* from coagulase negative staphylococci in direct Gram stains from BACTEC blood culture bottles does not necessarily mean that differentiating characteristics do not exist for this medium. This merely indicates that the morphological criteria developed for the BacT/ALERT medium were not applicable to the BACTEC medium. Systematic observation

by technologists from laboratories that are experienced with the BACTEC system could potentially produce morphological criteria that could be used to distinguish *S aureus* from coagulase negative staphylococci in this system.

In summary, we describe criteria that can help in the rapid identification of *S aureus* in BacT/ALERT blood culture bottles. We recommend that laboratories using the BacT/ALERT blood culture system become familiar with these criteria. When Gram positive cocci resembling *S aureus* are identified in BacT/ALERT bottles based on these criteria, clinicians can be advised that there is a high likelihood that their patient has *S aureus* bacteraemia. Otherwise, the initial report should only state that Gram positive cocci resembling staphylococci have been seen. We have been informally using this system in our laboratory for several years now, and the findings from our study support this practice.

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Authors' affiliations

D R Murdoch, R L Greenlees, Microbiology Unit, Canterbury Health Laboratories, Christchurch 8001, New Zealand

David R Murdoch, Department of Pathology, Christchurch School of Medicine and Health Sciences, University of Otago, Christchurch 8001, New Zealand

Correspondence to: Professor D R Murdoch, Microbiology Unit, Canterbury Health Laboratories, PO Box 151, Christchurch 8001, New Zealand; david.murdoch@cdhb.govt.nz

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