

## Rapid PCR Detection of Methicillin-Resistant *Staphylococcus aureus* and Methicillin-Sensitive *S. aureus* Samples from Charcoal-Containing Blood Culture Bottles<sup>▽</sup>

Recent advances in technology have allowed development of automated, rapid, real-time PCR tests that are able to differentiate between methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) in blood cultures. These include the Cepheid Xpert MRSA/SA blood culture assay used with the GeneXpert Dx system (Cepheid, Sunnyvale, CA), which has been found to have very high sensitivity and specificity for the detection of MSSA and MRSA (1, 2). However, these studies did not evaluate its performance in blood culture media containing activated charcoal, as this assay is not validated for use in these media. In common with many clinical microbiology laboratories, we use the BacT/Alert blood culture system (bioMérieux), for which only BacT/Alert SA (standard aerobic) and BacT/Alert SN (standard anaerobic) are validated for the Cepheid assay. The BacT/Alert FA (FAN aerobic) and BacT/Alert PF (pediatric FAN) used in our laboratory are not recommended for use because of charcoal in the medium. Charcoal is considered unsuitable for two reasons. First, it is thought to have an inhibitory effect on PCR, and second, it has the potential to disrupt the flow of reagents within the Cepheid cartridge. Limiting the use of the Cepheid assay in our hospital to the anaerobic bottle only is not practical, so we decided to evaluate this test using the charcoal-containing bottles.

We evaluated 82 blood culture bottles that had signaled as positive with Gram-positive cocci in clusters on Gram stain, of which 74 were BacT/Alert FA (FAN aerobic) and 8 were BacT/Alert PF (pediatric FAN). We followed a protocol supplied by Cepheid, United Kingdom, to remove the charcoal particles before testing the sample (Johanna Tacou, personal communication). Briefly, 0.5 ml from blood culture bottles was transferred to a 1.5-ml Eppendorf tube and centrifuged at 3,000 rpm for 2 min. Without disturbing the pellet, 50  $\mu$ l of the supernatant was transferred into the tube containing the elution reagent, and then the standard procedure for using the

Cepheid Xpert MRSA/SA blood culture was followed. Of the 82 positive blood cultures, 80 (97.5%) gave results that matched the culture results. These comprised 15 MSSA and 2 MRSA isolates. There was one invalid result by PCR, found to be coagulase-negative staphylococcus (CNS) on culture, and one false-positive MRSA result, which was later confirmed as a mixed culture of MSSA and oxacillin-resistant CNS. Therefore, this assay was found to be 93.75% and 100% sensitive and 100% and 98.75% specific for MSSA and MRSA, respectively.

To our knowledge, this is the first evaluation of the Cepheid Xpert MRSA/SA blood culture assay using charcoal-containing bottles. We have shown the assay to have high sensitivity and specificity for detection of MSSA and MRSA in these bottles by following a simple protocol to remove the charcoal. On the basis of these results, we intend including this assay in our MRSA/MSSA testing algorithm.

### REFERENCES

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<sup>▽</sup> Published ahead of print on 30 March 2011.