False-Negative Thermonuclease Tests with New BacTAlert Blood Culture Bottles

The Microbiology Unit of Southern Cross Pathology Australia, Monash Medical Centre, has used the Organon Technika BacTAlert automated blood culture instrument since 1995 for the detection of causative organisms of septicemia and bacteremia. When a blood culture bottle is flagged as positive, it is removed from the instrument and subculturing and Gram staining are performed. Gram-positive cocci, which morphologically resemble staphylococci, are tested by the thermostable nuclease (TSN) method (1) for rapid differentiation of *Staphylococcus aureus* from coagulase-negative staphylococci. The results are available after 2, 4, or 24 h of incubation at 35°C. We have used this test for the past 6 years and have found it to be reliable and rapid in the determination of significant isolates from blood cultures. Clinicians use the test results to decide whether to commence immediate antibiotic therapy.

Organon Technika recently replaced its blood culture bottles with a new line of ventless bottles. From March of this year until now, we have had five types of this new series of bottles in use at our hospital. These are the pediatric PF bottle, the aerobic FA and anaerobic FN bottles, and the standard aerobic and anaerobic bottles.

Since these new bottles have been in circulation, it has been noted that a number of false-negative TSN results have been obtained. That is, the TSN reaction was negative at 2, 4, and 24 h while the *S. aureus* latex test (SSI Systems) and later DNase testing of the cultured organism yielded positive results. In June and July 2001, 23.5% (4 of 17 isolates) and 14% (5 of 36 isolates) of isolates, respectively, produced false-negative TSN results, which was evident when *S. aureus* was seen upon Gram staining and subsequently cultured. On two of these occasions, repeat testing by the TSN method was performed and the results remained the same. This is disturbing, given that until recently the TSN method was very reliable. No false-negative or false-positive results had previously been reported at Southern Cross Pathology Australia. In the article by Forward et al. (1), the sensitivity of the TSN test was reported to be 92.6% and the negative predictive value was reported to be 97.9%.

All of the recent false-negative results were obtained with the new ventless bottles, including the pediatric PF bottle, the aerobic and anaerobic (FA and FN) bottles, and a standard (vent-less) aerobic bottle. The error was further highlighted in one case when a standard (vented) bottle and a FA (ventless) bottle from the same patient were flagged as positive on the same day. The results of testing by the TSN method were positive for the vented bottle and negative for the ventless bottle concurrently.

We have also observed that the zone sizes of samples positive for TSN are noticeably smaller than those of a given positive control. Previously, the TSN zone size under test was similar in diameter to that of the positive control, ranging from 26 to 28 mm. A survey of the new ventless bottles revealed the average zone size was 14 mm compared to 26 mm for the positive control.

One isolate from a continuous ambulatory peritoneal dialysis patient was thought to have a false-positive result. The *S. aureus* latex test and the DNase test yielded negative results, and subsequent identification confirmed that the isolate was *Staphylococcus schleiferi*. This organism is known to be thermonuclease positive.

In conclusion, we have been unable to determine the cause of the observed false-negative reaction. It appears to be unpredictable: some strains of *S. aureus* from the new bottles are TSN positive while others are TSN negative. However, until further research is done, it is evident that some caution should be taken when interpreting the results of the TSN test for isolates of gram-positive cocci morphologically resembling staphylococci from these new ventless bottles from Organon Technika.

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