

## Thermonuclease Test for Same-Day Identification of *Staphylococcus aureus* in Blood Cultures

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**We used a thermonuclease test to presumptively identify *Staphylococcus aureus* directly in blood cultures on the same day that a smear of the broth showed gram-positive cocci in clusters. There were no discrepancies between the identification of *S. aureus* directly from 250 blood cultures and identification by the tube coagulase test which was performed on the 18- to 24-h subculture isolates. These rapid results were clinically relevant and enabled physicians to make more timely and cost-effective decisions about antibiotic therapy.**

A report of "gram-positive cocci in clusters" is a common preliminary report issued by the microbiology laboratory for positive blood cultures. This report is important because it suggests that the isolate may be *Staphylococcus aureus*. *S. aureus* continues to be a common, serious cause of sepsis in both immunocompromised and noncompromised patients and requires prompt and appropriate antimicrobial therapy (7). For the physician treating the patient, the knowledge that the culture is positive for a possible staphylococcus is complicated by the additional knowledge that the gram-positive coccus may be a member of the coagulase-negative group of staphylococci which are often skin contaminants. Weinstein et al. (7), in an analysis of 500 episodes of bacteremia and fungemia in adults, showed that of 163 blood cultures positive for *S. epidermidis* (i.e., coagulase-negative staphylococci), only 6% represented true septicemia and 94% were considered to be contaminants. The coagulase-negative staphylococci, moreover, made up the most commonly isolated group of organisms (32%) from the 500 positive cultures. In contrast, for the 91 blood cultures positive for *S. aureus*, 75% represented cases of true septicemia and 25% represented skin contaminants.

Our experience at Vanderbilt University Hospital has revealed that 30 to 50% of all isolates from positive blood cultures are coagulase-negative staphylococci (6). The isolates were predominantly false-positives. A telephone survey of five other medical centers confirmed that the phenomenon of endemic staphylococcal pseudobacteremia is widespread, especially in hospitals in which members of the house staff draw the blood cultures.

Physicians are therefore often faced with the dilemma of whether to treat their patients for a possible *S. aureus* septicemia or to wait until the following day to rule out the possibility that the isolate is a coagulase-negative staphylococcus and represents a skin contaminant. A rapid, direct test that gives dependable, same-day information directly from the blood culture broth would therefore be extremely useful to physicians.

In November 1983, we started using the thermonuclease test as described by Madison and Baselski (4) for rapid identification of *S. aureus* strains in blood cultures. The principle of this test involves the elaboration of the enzyme DNase by *S. aureus* cells. DNase production is a routine diagnostic procedure used in some laboratories to distin-

guish between *S. aureus* and other members of the *Micrococcaceae*. Studies have shown, however, that some strains of coagulase-negative staphylococci also elaborate this enzyme and that the DNase test is not reliable as the sole criterion for identification of *S. aureus* (2). Other studies (1, 5, 8) have shown that the nuclease produced by *S. aureus* is uniquely and consistently thermostable, whereas the nucleases produced by coagulase-negative staphylococci are thermolabile when tested by the method of Lachica et al. (3).

The procedure used for performing this direct test consists of removing 2 to 3 ml of blood broth from a blood culture bottle showing gram-positive cocci in clusters on direct Gram stain. The blood broth (including erythrocytes) is put into a sterile screw-cap tube, which is placed in a boiling water bath for 15 min and allowed to cool to room temperature. Wells of 6 mm in diameter are cut in an appropriate (3) toluidine blue DNA plate (Thermal agar; Edge Diagnostics, Memphis, Tenn.). The end of a 6-mm capillary pipette works well for cutting these wells. Two or three drops of the cooled broth are put into a well with a capillary pipette, and the plate is incubated, right side up, for 2 h at 35°C. A positive reaction, indicating thermonuclease activity, will show a pink zone of clearing at the edge of the well with a darker blue ring at the outer periphery of the zone. This reaction can be reported as presumptive for *S. aureus*. Each plate can have 12 wells, and the plate can be used on successive days as long as appropriate positive and negative controls are used each day; this reduces the maximum cost per test to approximately \$0.20 for materials. The controls used must be from blood culture bottles containing blood and a known *S. aureus* strain for the positive control and a known coagulase-negative staphylococcus strain for the negative control. These are processed in the same manner as the specimen from the patient. Control bottles can be used for a week or more and can be kept at room temperature.

We performed the direct thermonuclease test on 250 blood cultures showing gram-positive cocci in clusters on Gram stain. There were no discrepancies when the results were compared with the results of the tube coagulase test, which was performed on the organism isolated the following day from the subculture of the blood culture bottle. The positive reaction was clear-cut and easy to read, and many tests were positive as early as 1 h after inoculation. A total of 60 cultures were positive and 190 were negative on the thermonuclease plate. Included in the 190 negative results were those from six organisms that were not staphylococci (en-

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terococcus, pneumococcus, or group B streptococcus). Because a negative result is therefore not definitive for coagulase-negative staphylococci, only positive results should be reported as indicating *S. aureus*, whereas negative results should be reported as "gram-positive cocci in clusters; not *S. aureus*."

This study confirms that the thermonuclease test described by Madison and Baselski (4) for same-day identification of *S. aureus* from blood cultures is a rapid, accurate, and easily performed procedure. The same-day information provided by this test enables physicians to make more timely and cost-effective decisions about antibiotic therapy.

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