

Rapid Identification of *Staphylococcus aureus* in Blood Cultures by Thermonuclease Testing

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The detection of thermonuclease activity in 86 blood culture samples containing gram-positive cocci showed 100% correlation with the subsequent identification of the isolate as *Staphylococcus aureus* by the coagulase test. No positive thermonuclease results were found with 66 samples containing coagulase-negative staphylococci and 56 samples containing other gram-positive organisms. The thermonuclease test provides a rapid, reliable method to identify *S. aureus* in blood cultures.

The clinical usefulness of rapid differentiation of *Staphylococcus aureus* from coagulase-negative staphylococci in blood cultures is clearly evident in view of the high association of *S. aureus* isolation with clinically significant bacteremia (10). Several publications have described methods for the rapid identification of *S. aureus* in samples of growing blood cultures. These methods include coagulase tests (21) and commercial latex agglutination tests (3) on bacterial pellets and lysostaphin susceptibility (6, 18). Thermonuclease (TN) activity is another specific diagnostic parameter which can be used to identify *S. aureus* isolates (2, 12, 16, 20) and has been used for the rapid, direct detection of *S. aureus* in foods (4, 7, 9, 14). We present here the use of direct TN testing of blood culture samples growing gram-positive cocci as a method for rapid identification of *S. aureus*.

Blood cultures submitted to the City of Memphis Hospital Clinical Microbiology Laboratory in BACTEC 6B and 7C media (Johnston Laboratories, Inc., Cockeysville, Md.) were examined by recommended procedures (17). From September through December 1982, a 1- to 3-ml sample of broth was removed for TN testing from bottles which demonstrated gram-positive cocci resembling staphylococci. Initially, samples were centrifuged to remove erythrocytes before testing; however, this step was subsequently found to be nonessential and was discontinued. TN tests were performed as described by Zarzour and Belle (22). One-milliliter broth samples in sterile glass tubes were placed in a boiling-water bath for 15 min and then cooled to room temperature. Toluidine blue-DNA plates, prepared by the formula of Lachica et al. (8), were purchased commercially (Edge Diagnostics, Memphis, Tenn.). Wells 6 mm in

diameter were cut out of the DNA plates, with a maximum of 12 wells per plate. Approximately 0.1 ml of a heat-treated sample was added to each well, and the plates were placed at 35°C. Positive and negative controls, prepared from brain heart infusion broth cultures, were included with each series of samples. Reactions were observed at 1, 2, 3, 4, and 24 h. A positive reaction consisted of a light-pink zone of clearing at the edge of the well with a darker-blue ring at the outer periphery of the zone. Negative reactions showed no zone or a small zone of clearing without pink or the hyperpigmented peripheral ring (the latter is seen particularly with hemolyzed samples).

The results of the TN tests on 152 blood culture samples which subsequently yielded a *Staphylococcus* sp. are shown in Table 1. A total of 86 samples from 33 patients yielded *S. aureus* (8 samples from 3 patients had methicillin-resistant strains, and 5 samples were mixed with other gram-positive organisms), and 66 samples from 56 patients grew other *Staphylococcus* species (1 was mixed with *Escherichia coli*). The agreement between direct TN tests on blood culture samples and subsequent tube coagulase tests on the isolates by standard procedures (13) was 100%, a finding which parallels the excellent correlation between TN tests and coagulase tests on clinical staphylococcal isolates in previous studies (2, 12, 16, 20). It is of note that *Staphylococcus epidermidis*, the most frequently encountered coagulase-negative species in blood cultures (11, 19), has not been identified as a species which may give positive TN results (5).

All positive reactions were detected by 2 h after inoculation, although they generally intensified over the 24-h period. This is a shorter incubation time than the four-hour time previ-

ously noted (20) and renders the test more clinically useful as a rapid method. The positive samples were taken from both 6B (aerobic) and 7C (anaerobic) BACTEC bottles (59 from 6B bottles and 27 from 7C bottles), although a previous study had noted an inhibitory effect of anaerobiosis on TN production (4). Time studies in simulated blood cultures, using a clinical isolate of *S. aureus*, showed that the aerobic bottle samples became positive, as determined by growth index, Gram stain, and TN activity, earlier than the anaerobic bottle samples (Table 2). This is probably related to a stimulatory effect of aeration on growth and TN production (4), particularly since the aerobic bottles were shaken during the 24-h incubation, as are routine cultures. In both bottles, the Gram stain and TN test became positive at the same sample time, which was immediately before the growth index was positive. In food samples, as well, large numbers of organisms ($>10^6$ /ml) must be present to give detectable TN activity (7, 14). In practical terms, these studies indicate that TN activity should be detectable in blood cultures with *S. aureus* when the Gram stain is positive. We have identified *S. aureus* in aerobic and anaerobic clinical blood culture bottles by TN testing as early as 12 h after receipt of the samples in the laboratory.

Table 1 also shows the results of TN tests on 55 blood culture samples which yielded other gram-positive cocci. Although staphylococci and streptococci are generally easily presumptively identified by cellular morphology in blood cultures (1), the morphology may occasionally be indistinct. Because some streptococci, particularly group D, may produce thermonuclease activity (15), these samples were tested to detect possible false-positive reactions. In the 55 samples tested, which included 18 group B streptococci, and 11 viridans streptococci, 8 enterococci (1 mixed with *Serratia* sp. and 3 mixed with group B streptococci), 7 pneumococci, 2 group F streptococci, 4 anaerobic gram-positive cocci, and 5 *Micrococcus* sp., no positive reactions were noted.

These data indicate that the direct detection of TN activity in blood culture samples growing

TABLE 1. Results of thermonuclease tests on samples of blood cultures growing gram-positive cocci

Organism isolated	No. of samples	% of samples TN positive
<i>S. aureus</i>	86	100
Coagulase-negative staphylococci	66	0
Other gram-positive cocci	55	0

TABLE 2. Time required to detect a positive reaction in seeded blood cultures^a

BACTEC Bottle	Time (h) ^b to positive		
	Growth index ^c	Gram stain	TN ^d
6B (aerobic)	18	12	12
7C (anaerobic)	24	18	18

^a Each BACTEC bottle was inoculated with 3 ml of blood containing 30 CFU of an *S. aureus* clinical isolate per ml.

^b Individual bottles were sampled at 0, 3, 6, 12, 18, and 24 h.

^c Positive, >35 in aerobic bottle and >20 in anaerobic bottle.

^d TN reactions were observed at 1, 2, 3, 4, and 24 h.

gram-positive cocci provides a reliable method for the rapid identification or exclusion of *S. aureus*. In addition, we have found that the procedure is technically easy to perform and interpret and easily incorporated into the work flow for processing positive blood cultures.

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