JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1982, p. 1048–1051
0095-1137/82/121048-04$02.00/0

Vol. 16, No. 6

Direct Identification of *Staphylococcus aureus* in Blood Culture Fluid with a Commercial Latex Agglutination Test

GARY V. DOERN[1,2]* AND LOIS I. ROBBIE[1]

Department of Clinical Microbiology[1] and Division of Infectious Disease,[2] University of Massachusetts Medical Center, Worcester, Massachusetts 01605

Received 24 March 1982/Accepted 18 June 1982

A commercial latex agglutination slide test (SeroSTAT Staph, Scott Laboratories, Inc., Fiskeville, R.I.) accurately identified *Staphylococcus aureus* when applied directly to blood culture fluid containing staphylococci. This latex agglutination test exhibited 100% accuracy when 30 seeded aerobic and anaerobic radiometric blood cultures (15 strains of *S. aureus*, 15 strains of other staphylococcal species) were tested blindly. In 36 actual clinical specimens yielding 16 isolates of *S. aureus* and 20 isolates of *Staphylococcus epidermidis*, 94.4% accuracy was achieved. The latex agglutination test provided positive test results before objective criteria of blood culture positivity such as radiometric growth indices and Gram stains became positive.

Staphylococcal bacteremia is one of the most frequent causes of positive blood cultures (2, 5, 7, 8). *Staphylococcus aureus*, when found in blood cultures is usually representative of significant clinical disease. In contrast, non-*S. aureus* species of *Staphylococcus*, in particular *S. epidermidis*, although potentially of clinical importance, are usually found to be contaminants. For this reason, the rapid differentiation of these two groups, when present in blood cultures, should be an important goal of the clinical microbiology laboratory.

In 1980, Essers and Radebold described a rapid, latex agglutination slide test (LAT) which permitted the differentiation of *S. aureus* from *Micrococcus* spp. and other non-*S. aureus* species of *Staphylococcus* (4). Latex particles coated with human plasma were found to agglutinate in the presence of *S. aureus* whole cells. The agglutination reaction was thought to be a product primarily of interaction between protein A on the bacterial surface and immunoglobulins adsorbed to the latex particles. Interaction between cell-associated clumping factor and latex particle-bound clotting factors was thought to augment the agglutination reaction. A commercially available modification of this test has recently been shown in two studies to identify *S. aureus* at least as accurately as the tube coagulase test when staphylococci or micrococci are cultivated on solid media before testing (3, 6). The LAT procedure requires approximately 1 min to obtain a test result.

The intent of the present study was to determine whether this LAT could be used to directly identify *S. aureus* growing in blood culture fluid, thus permitting a definitive identification of this organism within minutes rather than days of its detection.

MATERIALS AND METHODS

LAT. A 10-ml sample of blood culture fluid was aseptically transferred to a sterile screw-capped glass test tube (16 by 125 mm) and centrifuged at 2,000 × *g* for 10 min at room temperature. The supernatant was discarded, and the pellet was suspended in sterile distilled water. This suspension was agitated vigorously and then centrifuged under the same conditions. The resultant pellet was again suspended in sterile distilled water, and the centrifugation procedure was repeated. The final pellet was suspended in 1.0 ml of 0.85% NaCl (pH 7.2) (saline), and 2 drops were transferred to an etched circle on an opaque blue glass slide. One drop of latex reagent (SeroSTAT Staph, Scott Laboratories, Inc., Fiskeville, R.I.) was mixed with the bacterial suspension, and the slide was rotated manually on a level surface for 1 min. The slide was examined macroscopically for the presence of obvious clumping.

Seeded blood cultures. Test strains of staphylococci, identified according to conventional criteria, were obtained from various clinical specimens processed in the clinical microbiology laboratories of the University of Massachusetts Medical Center. Organisms were propagated on 5.0% sheep blood agar plates overnight at 35°C in 5 to 7% CO_2. A suspension equivalent to a 0.5 McFarland standard was prepared in sterile saline, and a 10^{-6} dilution was made in sterile Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.). Aerobic (8b medium) and anaerobic (7c medium) blood culture bottles (Johnston Laboratories, Inc., Cockeysville, Md.) to which 4.0 ml of human blood had been added were then inoculated with 0.1 ml of Trypticase soy broth suspension. The concentration of organisms present in individual Trypticase soy

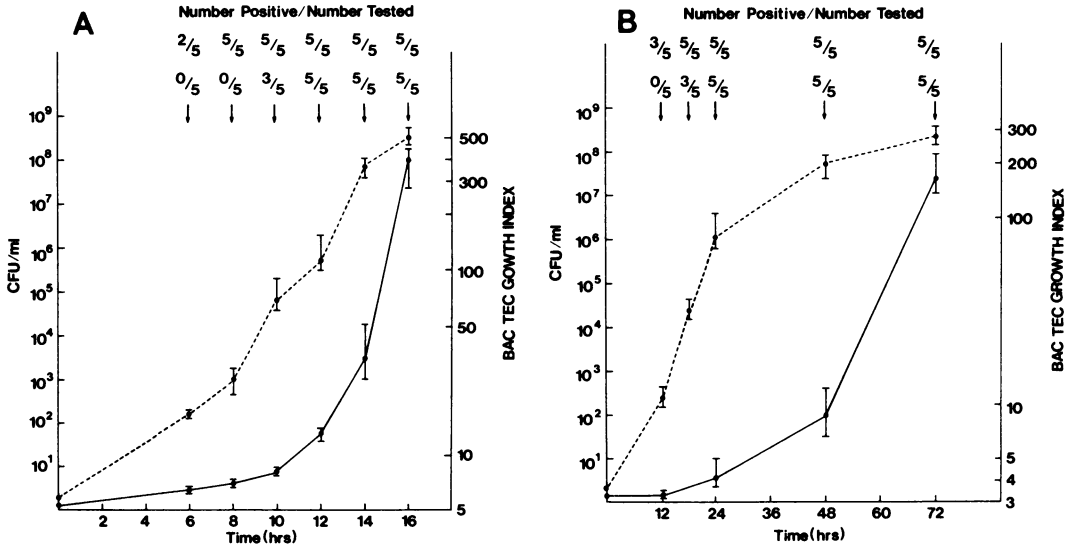

FIG. 1. Results of LAT and Gram stain applied at different time intervals to aerobic (A) or anaerobic (B) blood cultures seeded with five strains of *S. aureus*. Arrows indicate times at which the LAT (top row) and Gram stains (bottom row) were performed. ------, CFU per milliliter; —, radiometric growth index. Standard deviations are shown.

broth suspensions was determined by colony counts on 5.0% sheep blood agar surface-spread plates. This procedure achieved an inoculum size of 3 to 15 colony-forming units (CFU) per bottle. Aerobic bottles were incubated on a shaker at 35°C; anaerobic bottles were incubated on a stationary rack at the same temperature. At selected time intervals (see below), the LAT was performed, radiometric growth indices were measured with a model 460 BACTEC blood culture instrument (Johnston Laboratories, Inc.), colony counts were determined, and Gram stains were performed on uncentrifuged blood blood culture fluid. Colony counts, expressed as CFU per milliliter, were performed by streaking 0.1 ml of serial dilutions of blood culture fluid in Trypticase soy broth onto the surface of 5.0% sheep blood agar plates, incubating overnight at 35°C in 5 to 7% CO_2, counting the colonies, and multiplying by the appropriate dilution factor.

Clinical blood cultures. Blood from patients was distributed into aerobic and anaerobic blood culture bottles in equal 3- to 5-ml volumes by aseptic techniques. If less than 6 ml of blood was obtained, only an aerobic bottle was inoculated. Aerobic bottles were incubated on a shaker at 35°C for the first 48 h and on a stationary rack thereafter; anaerobic bottles were incubated on a stationary rack at the same temperature. The bottles were examined for macroscopic evidence of growth and radiometric growth indices were determined three times (spaced approximately 8 h apart) during days 1 and 2 of incubation and once daily thereafter through day 7 of incubation. Macroscopic evidence of growth, a growth index of ≥20 U, or an incremental increase of ≥5 U over the previous reading prompted a Gram smear of uncentrifuged blood culture fluid. When gram-positive cocci were observed, the LAT was performed in addition to other tests routinely applied to positive blood cultures.

RESULTS

Five known strains of *S. aureus* were used to seed radiometric blood culture bottles incubated aerobically and anaerobically. At various time intervals, the radiometric growth index was determined, and colony counts, Gram stains of uncentrifuged blood culture fluid, and the LAT were performed (Fig. 1). After 6 h of incubation, two of five strains grown aerobically yielded positive results with the LAT (Fig. 1A). Mean colony counts at 6 h were 2×10^2 CFU/ml, Gram stains were uniformly negative, and growth indices were less than 10. By 8 h, all five strains were LAT positive; however, Gram stains were still negative, and growth indices remained below 10. Colony counts now yielded an average of 10^3 CFU/ml. At 10 h, as colony counts approached 10^5 CFU/ml, three of five cultures had become Gram stain positive, but the growth indices were still below 10. All strains were LAT positive. At 12 h, the growth indices were greater than 10, and all strains were both LAT and Gram stain positive. Mean colony counts were 7×10^5 CFU/ml. At 14 and 16 h, as colony counts and growth indices continued to rise, all strains remained positive by the LAT and by Gram stain. In all cases in which the LAT was positive, Gram stains of the pellet used to perform the LAT revealed gram-positive cocci in clusters. In all cases in which the LAT was negative, Gram stains of the pellet were also negative.

When the same five strains were grown anaer-

obically (Fig. 1B), a similar pattern was observed. Under these conditions, however, the development of all parameters was delayed due to slow growth of *S. aureus* anaerobically.

Fifteen known strains of *S. aureus*, thirteen strains of *S. epidermidis*, and one strain each of *Staphylococcus haemolyticus* and *Staphylococcus simulans* were used to seed both aerobic and anaerobic blood culture bottles. The LAT was performed blindly after 16 h of incubation with aerobic cultures and after 40 h of incubation with anaerobic cultures. All 15 strains of *S. aureus* yielded positive LAT results when tested after aerobic and anaerobic incubation. All non-*S. aureus* species of *Staphylococcus* were negative by the LAT under the same conditions.

The LAT was also applied to actual clinical specimens. A total of 36 unique episodes of bacteremia with blood cultures revealing gram-positive cocci in clusters were investigated over a 3-month period. In all 36 cases, staphylococci were recovered from aerobic blood culture bottles. Sixteen isolates were identified as *S. aureus*, twenty as *S. epidermidis*. The mean length of time to detection was 32 h. Companion anaerobic blood cultures yielded the same *Staphylococcus* strain, as judged by species identity and antibiogram, in 20 of the 36 cases. The mean length of time until detection was 64 h. In eight cases, staphylococci were not recovered from companion anaerobic bottles, and in another eight cases, anerobic bottles were not processed.

Of the 16 aerobic blood cultures which yielded *S. aureus*, 15 were LAT positive. Companion anaerobic bottles were received in 13 cases; all yielded *S. aureus*, and all were LAT positive. The single false-negative LAT result was with an aerobic bottle for which no anaerobic companion bottle was cultured. Testing of this isolate after subculture to solid medium produced equivocal LAT results. Of 20 aerobic cultures yielding *S. epidermidis*, 19 were LAT negative. One equivocal result was obtained. Companion anaerobic bottles yielded the same *Staphylococcus* species in seven cases, all of which were LAT negative. The single questionable LAT result was obtained with an aerobic culture in which the anaerobic companion bottle failed to yield staphylococci. The isolate of *S. epidermidis* recovered from this aerobic culture was LAT negative when tested after subculture to solid medium.

DISCUSSION

In several studies, the staphylococci have been shown to be the genus of bacteria most commonly isolated from positive blood cultures (2, 5, 7, 8). *S. aureus* is usually of clinical significance. Other species of staphylococci, in particular *S. epidermidis*, although potentially associated with diseases such as prosthetic valve endocarditis, bacteremia secondary to vascular canulae, and central nervous system shunt infections, are usually found to be contaminants. Conventional microbiological techniques require, in effect, 2 days from the time a blood culture is first detected as being positive for definitive isolation and species identification of the staphylococci. When the indicator of positivity is either macroscopic evidence of growth or blind smears, 1 day is required for isolation of the organism onto solid medium, and a second day is necessary for identification of species. When staphylococci are recovered from early blind subcultures, i.e., blind subcultures performed on the day after receipt of the specimen as recommended by Bartlett et al. (1), the average length of time until definitive identification would be approximately 60 h. Procedures capable of shortening the length of time required for definitive identification of staphylococci in positive blood cultures would clearly serve a useful clinical purpose.

The LAT examined in this study is at least as accurate as the tube coagulase test for identifying *S. aureus* grown on solid culture media (3, 6). In the present study, when organisms were separated from cellular material and concentrated by three 10-min centrifugation steps, the LAT rapidly and accurately identified *S. aureus* growing either aerobically or anaerobically in radiometric blood culture media. The entire procedure required approximately 35 min.

A total of 30 known strains of staphylococci, 15 recent clinical isolates of *S. aureus* and 15 strains of other species of *Staphylococcus*, all yielded the expected LAT result when tested blindly in seeded aerobic and anaerobic radiometric blood cultures. Of 36 actual clinical specimens which were found to contain gram-positive cocci in clusters (16 isolates of *S. aureus*, 20 isolates of *S. epidermidis*), the LAT yielded the correct result in 34 cases (overall accuracy, 94.4%). One false-negative result was obtained with a culture containing a strain of *S. aureus* which produced an equivocal LAT reaction when tested after isolation in pure culture on solid medium. One questionable result, considered uninterpretable, was obtained with a blood culture which yielded *S. epidermidis*. Testing of this strain after isolation on solid medium produced a negative LAT result. The exclusion of this equivocal test result from the data analysis would yield an overall accuracy of 97.1%.

Time interval studies of both aerobic and anaerobic culture bottles seeded with five different strains of *S. aureus* demonstrated that the LAT became positive before other objective

criteria of blood culture positivity, i.e., radiometric growth index or Gram stain of uncentrifuged culture fluid, became positive. Colony counts obtained at times when only the LAT was positive demonstrated the presence of 10^2 to 10^3 organisms per ml of blood culture fluid. Since the LAT was applied to a 1.0-ml suspension of organisms concentrated from 10 ml of blood culture fluid by centrifugation, assuming insignificant loss of organisms during processing, it can be inferred that the LAT is capable of yielding positive results with suspensions of *S. aureus* containing as little as 10^3 to 10^4 organisms per ml. These observations also imply that the LAT might reasonably be applied to blood culture fluid before conventional objective criteria indicate positivity in situations in which a high clinical suspicion of *S. aureus* bacteremia exists.

In summary, the LAT was found to be an accurate, rapid tool for directly identifying *S. aureus* in radiometric blood cultures.

LITERATURE CITED

1. **Bartlett, R. C., P. D. Ellner, and J. A. Washington II.** 1974. Cumitech 1, Blood cultures. Coordinating ed., J. C. Sherris. American Society for Microbiology, Washington, D.C.
2. **Blazevic, D. J., J. E. Stemper, and J. M. Matsen.** 1975. Effect of aerobic and anaerobic atmospheres on isolation of organisms from blood cultures. J. Clin. Microbiol. **1**:154–156.
3. **Doern, G. V.** 1982. Evaluation of a commercial latex agglutination test for identification of *Staphylococcus aureus*. J. Clin. Microbiol. **15**:416–418.
4. **Essers, L., and K. Radebold.** 1980. Rapid and reliable identification of *Staphylococcus aureus* by a latex agglutination test. J. Clin. Microbiol. **12**:641–643.
5. **Harkness, J. L., M. Hall, D. M. Ilstrup, and J. A. Washington II.** 1975. Effects of atmosphere of incubation and of routine subcultures on detection of bacteremia in vacuum blood culture bottles. J. Clin. Microbiol. **2**:296–299.
6. **Myrick, B. A., and P. D. Ellner.** 1982. Evaluation of the latex slide agglutination test for identification of *Staphylococcus aureus*. J. Clin. Microbiol. **15**:275–277.
7. **Thiemke, W. A., and K. Wicher.** 1975. Laboratory experience with a radiometric method for detecting bacteremia. J. Clin. Microbiol. **1**:302–308.
8. **Washington, J. A., II, M. M. Hall, and E. Warren.** 1975. Evaluation of blood culture media supplemented with sucrose or with cysteine. J. Clin. Microbiol. **1**:79–81.