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Evaluation of Several Commercial Biochemical and Immunologic Methods for Rapid Identification of Gram-Positive Cocci Directly from Blood Cultures

TESSA RAPPAPORT,[1,2] KAREN P. SAWYER,[1] AND IRVING NACHAMKIN[1,3]*

Department of Microbiology, Thomas Jefferson University,[2] and Clinical Microbiology Laboratory,[1] Department of Pathology and Laboratory Medicine,[3] Hospital of the University of Pennsylvania, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-4283

Received 12 November 1987/Accepted 12 April 1988

To develop laboratory methods for the rapid identification of gram-positive cocci from blood cultures, several commercial immunological and biochemical tests for identifying staphylococci and streptococci from two different blood culture systems (Thiol/TSB, Difco Laboratories; BACTEC, Johnston Laboratories, Inc.) were evaluated and compared with conventional identification methods. A total of 44 cultures contained *Staphylococcus aureus* as determined by conventional methods. Commercial immunological methods (six tested) ranged in overall sensitivity from 38.6 (Staphyloslide; BBL Microbiology Systems) to 77.3% (Staphaurex; Wellcome Diagnostics). All methods tested had 100% specificity. A total of 30 isolates of group D streptococci were tested with immunological and biochemical identification systems. The overall sensitivity ranged from 14.2% (Phadebact Group D; Pharmacia Diagnostics) to 100% with Streptex (Wellcome; immunological) and Identicult A-E (Scott Laboratories, Inc.; biochemical). The results of this study suggest that some reagents can be used to provide rapid identification of gram-positive cocci from blood cultures 24 h earlier than standard methods.

Gram-positive cocci are frequently encountered bacteria isolated from blood cultures (13). The gram-positive cocci most likely to be found in bacteremia include *Staphylococcus aureus*, *Streptococcus pneumoniae*, alpha- and beta-hemolytic streptococci, viridans group streptococci, and the enterococcal and nonenterococcal group D streptococci (7). Coagulase-negative staphylococci, although commonly seen as contaminants, have increasingly been seen as pathogenic in certain patients (3). Since bacteremias caused by these organisms can cause life-threatening infections, prompt detection and identification are essential for proper patient management. Several rapid commercial immunologic and biochemical reagents have been developed for isolate identification which can also be applied to the identification of gram-positive cocci directly from blood cultures (4, 14, 16, 17). A systematic study of these systems applied in a dichotomous scheme has not been reported.

To develop a dichotomous scheme for the identification of gram-positive cocci from blood cultures, we evaluated several commercial immunological and biochemical tests for identifying staphylococci and streptococci and compared these results with results of conventional identification methods.

MATERIALS AND METHODS

Identification of isolates. Staphylococci and streptococci isolated from blood cultures were identified by using standard methods (5, 8).

For immunologic identification of staphylococci, the following systems were used: Bacto Staph Latex (Difco Laboratories, Detroit, Mich.), Staphaurex (Wellcome Diagnostics, Research Triangle Park, N.C.), Accu-Staph (Carr-Scarborough Microbiologicals Inc., Decatur, Ga.), Staphyloslide (BBL Microbiology Systems, Cockeysville, Md.), Staphylatex (American MicroScan, Mahwah, N.J.), and Veri-Staph (Zeus Technologies, Raritan, N.J.). Immunologic reagents used for identification of streptococci were Streptex Group D (Wellcome Diagnostics), Meritec Streptococcus (Meridian Diagnostics, Cincinnati, Ohio), Serostat Streptococcus and Serostat Group D (Scott Laboratories, Fiskeville, R.I.), Phadebact Streptococcus and Phadebact Group D (Pharmacia Diagnostics, Piscataway, N.J.), Pneumoslide (BBL Microbiology Systems), and Wellcogen Strep Pneumo (Wellcome Diagnostics). Biochemical tests used for identifying streptococci were Identicult A-E (Scott Laboratories) and Strep-A-Chek (E-Y Laboratories, San Mateo, Calif.).

Processing clinical samples. Blood cultures, collected from patients at the Hospital of the University of Pennsylvania, were analyzed from November 1986 through April 1987. Blood was inoculated into a Difco 2 bottle blood culture system (from November 1986 to December 1986) consisting of tryptic soy broth and Thiol broth under vacuum with CO_2 and 0.025% sodium polyanetholsulfonate. BACTEC 6A (NR) and 7A (NR) blood culture bottles (Johnston Laboratories, Inc., Towson, Md.) were also used (January 1987 to April 1987). Positive cultures detected by either system were Gram stained and observed for the presence of gram-positive cocci.

If gram-positive cocci were seen, 10 ml of the appropriate medium was removed and centrifuged at 150 × *g* for 10 min to sediment the erythrocytes. The supernatant was centrifuged at 1,000 × *g* for 15 min to pellet the bacteria, which was then suspended in 1 ml of 0.85% NaCl. This pellet was sued for all subsequent testing.

A catalase test was performed on the pellet material; 1 drop of the pellet was mixed with 1 drop of 3% hydrogen peroxide and observed for bubble formation (15). Results of the catalase test determined whether staphylococcal or streptococcal reagents were to be used for further testing.

* Corresponding author.

TABLE 1. Identification of catalase- and gram-positive cocci directly from blood cultures[a]

Kit	No. of correct identifications of:		Sensitivity (%)	Negative predictive value (%)
	S. aureus (n = 44)	Coagulase-negative staphylococci (n = 33)		
Staphaurex	34	33	77.3	76.7
Bacto Staph	26	33	59.0	64.7
Staphylatex	30	33	68.2	70.2
Accu-Staph	23	33	52.2	61.1
Staphyloslide	17	33	38.6	55
Veri-Staph	19/30[b]	28/28[b]	63.3	71.8

[a] Data from Difco and BACTEC blood bottle systems are combined. For all kits, specificity and positive predictive value were 100%.
[b] With this kit, 30 *S. aureus* isolates and 28 coagulase-negative staphylococci were tested.

A modified rapid bile solubility test for the identification of *Streptococcus pneumoniae* (12) was performed on all catalase-negative specimens.

For immunologic identification of staphylococci and streptococci, 1 drop of the pellet was transferred to a well on a test card (supplied with each reagent) and instructions of the manufacturers were followed. The direct results were compared with results of conventional identification methods.

Seeded blood cultures. To determine the sensitivities of the individual reagents, blood bottles were seeded with clinical isolates of staphylococci and streptococci. Uninoculated bottles (Difco and BACTEC) were seeded with different amounts of organism, and after incubation, a suspension equivalent to a 0.5 McFarland standard was prepared from the seeded bottles. Ten-fold serial dilutions were prepared from the corresponding blood culture broth and checked quantitatively by plate counts to determine the concentration of organism present at the time of detection.

RESULTS

A total of 136 blood cultures were tested during this study, yielding either staphylococci or streptococci by conventional methods. The distribution of isolates obtained was as follows. Of the 87 staphylococci detected, 44 were *S. aureus* and 43 were identified as coagulase-negative staphylococci. Of the 49 streptococci, 28 were group D enterococci, 2 were group D nonenterococci, 15 were viridans group streptococci, and 4 were *Streptococcus pneumoniae*.

Catalase testing. The evaluation of the catalase test directly from blood cultures yielded the following results. Of 87 staphylococci tested, 77 were catalase positive; the remaining 10 isolates were catalase negative, and therefore were classified as streptococci for further testing. These 10 isolates were identified as coagulase-negative staphylococci by conventional methods. All 49 isolates of streptococci were catalase negative. This evaluation resulted in the following values: sensitivity, 88.5%; specificity, 100%; positive predictive value, 100%; and negative predictive value, 83.1%.

Direct testing of staphylococci with immunological reagents. A total of 77 of the classified staphylococci were tested with a variety of immunological reagents (Table 1). Combined data from both blood culture systems revealed 100% specificity with all reagents; however, sensitivities ranged from 38.6% (Staphyloslide) to 77.3% (Staphaurex).

Blood cultures seeded with staphylococci. When testing blood cultures seeded with *S. aureus*, Staphaurex detected 2×10^6 CFU/ml as compared with 2×10^9 CFU/ml for the other reagents.

Testing of *Streptococcus pneumoniae* from blood cultures. All 59 catalase-negative specimens were first tested with a rapid slide bile solubility test. Four blood cultures were positive and were identified as *Streptococcus pneumoniae* (100% sensitivity). Some 55 specimens were negative by bile solubility (100% specificity). Pneumoslide correctly identified all four blood cultures of *Streptococcus pneumoniae*. Only 19 of 55 nonpneumococcal samples were tested with Pneumoslide, because this reagent was incorporated late in the study. Eight of nine enterococci were negative, and the remaining isolate showed nonspecific agglutination with the negative control reagent. None of the three staphylococcal strains, five viridans group streptococcal strains, or two group D nonenterococcal strains reacted with Pneumoslide. Wellcogen Strep Pneumo identified two of four *Streptococcus pneumoniae* isolates; two isolates exhibited nonspecific agglutination with the negative latex control.

Direct testing of streptococci with group D immunologic reagents. All bile solubility-negative specimens were tested with group D immunologic tests (Table 2). Streptex Group D correctly identified all 30 isolates of group D streptococci. None of the 15 viridans group streptococcal isolates or the 10 staphylococcal isolates reacted. Serostat Group D identified 13 of 30 isolates, for a sensitivity of 43.3%. One enterococcal isolate failed to react, and 16 isolates exhibited nonspecific agglutination with the negative control reagent. None of the viridans group streptococci or staphylococci reacted. Phadebact Group D detected 2 of 14 (14.2%) group D streptococcal isolates. The remaining 12 isolates showed nonspecific agglutination with the negative reagent control. Since all viridans group streptococcal and staphylococcal isolates agglutinated in the negative control reagent, the specificity of this reagent could not be determined.

TABLE 2. Evaluation of group D reagents for identification of catalase-negative and gram-positive cocci from blood cultures[a]

Organism	No. tested (n) and no. of reactions[b] with reagent:											
	Streptex Group D				Serostat Group D				Phadebact Group D			
	n	+	−	NS	n	+	−	NS	n	+	−	NS
Enterococcus	28	28	0	0	28	11	1	16	12	2	0	10
Group D nonenterococcus	2	2	0	0	2	2	0	0	2	0	0	2
Viridans group streptococcus	15	0	15	0	15	0	15	0	15	0	0	15
Staphylococcus	10	0	10	0	10	0	10	0	10	0	0	10

[a] Sensitivities and specificities were as follows (respectively): Streptex Group D, 100% and 100%; Serostat Group D, 43.3% and 100%; Phadebact Group D, 14.2% and undeterminable.
[b] Reactions: +, positive; −, negative; NS, nonspecific.

TABLE 3. PYR testing directly from blood cultures[a]

Organism	No. tested (*n*) and no. of reactions[b] with reagent:						
	Identicult A-E				Strep-A-Chek		
	n	+	−	NT[c]	*n*	+	−
Enterococcus	28	24	0	4	28	21	7
Group D nonenterococcus	2	0	2		2	0	2
Viridans group streptococcus	15	0	11	4	15	0	15
Staphylococcus	10	0	8	2	10	0	10

[a] Sensitivities and specificities were as follows (respectively): Identicult A-E, 100% and 100%; Strep-A-Chek, 75% and 100%.
[b] Reactions: +, positive; −, negative.
[c] NT, Not tested. Organisms were isolated from Difco Thiol broth, and uninoculated Thiol broth exhibited false-positive reactions with Identicult A-E.

Direct testing of blood culture for detecting beta-hemolytic streptococci. All specimens that failed to react directly with the group D immunologic reagents were tested with various immunologic reagents for beta-hemolytic streptococcal groups A, B, C, F, and G. Of the 10 staphylococcal isolates (pellet, catalase negative), 8 showed multiple reactions with each of these reagents; the remaining 2 specimens were negative. Of the 15 viridans group streptococcal isolates, 13 showed multiple reactions with each of these reagents; the remaining 2 were negative for all streptococcal groups.

PYR testing directly from blood culture pellets. All catalase-negative specimens (except for four identified as *Streptococcus pneumoniae*) were tested with biochemical reagents for hydrolysis of L-pyrrolidonyl-β-naphthylamide (PYR) (Table 3). Identicult A-E correctly identified 24 of 28 enterococcal isolates; 11 of 15 viridans group streptococcal isolates, both group D nonenterococcal isolates, and 8 of 10 staphylococcal isolates were negative. Four enterococcal isolates, four viridans group streptococcal isolates, and two staphylococcal isolates were isolated from Difco Thiol bottles; uninoculated Thiol broth reacted with Identicult A-E reagent. Therefore, these isolates were not evaluated. Strep-A-Chek identified 21 of 28 enterococcal isolates, for a sensitivity of 75%. There were no false-positives.

Blood cultures seeded with streptococci. When testing blood cultures seeded with group D enterococci, Streptex and Serostat detected at 2×10^7 CFU/ml, compared with 2×10^8 CFU/ml for the biochemical tests. All concentrations tested with Phadebact Group D exhibited nonspecific reactions; therefore, an endpoint could not be determined.

DISCUSSION

The results of this study show that certain immunologic and biochemical tests are feasible to use for accurate identification of staphylococci and streptococci from blood cultures. We evaluated several commercial reagents that have not previously been evaluated for direct testing in blood culture systems.

When data from test and seeded blood cultures from the two blood culture systems are combined, Staphaurex was the most sensitive reagent for detection of *S. aureus*. Positive reactions with Staphaurex were stronger and clearer to interpret than with the other reagents. Negative reactions were smoother and more homogeneous than those with Bacto Staph, Veri-Staph, and Staphylatex, which tended to show slightly granular reactions.

Since Staphyloslide had the lowest sensitivity (38.6%) directly from the bottle, we cannot recommend this reagent for direct testing of blood cultures.

Compared with other reagents, Staphyloslide requires an additional step of setting up a negative control with each isolate (1). Since Staphyloslide detects only clumping factor, this could result in the lower sensitivity of this reagent compared with the sensitivities of other reagents which detect clumping factor or protein A or both. On the basis of these observations, we conclude that Staphaurex is the preferred method for identifying *S. aureus* directly from blood cultures. Since a direct negative result is not conclusive as to whether the specimen contains *S. aureus*, we recommend that all negative specimens be tested by conventional methods after subculture on solid media.

Although not evaluated in our study, SeroStat Staph, another immunologic reagent, has been evaluated by Doern and Robbie (4) for direct detection of staphylococci in blood cultures. Using seeded blood cultures and clinical isolates, they found this reagent to be very good, yielding 100 and 94.4% sensitivity, respectively.

Even though there was 100% sensitivity with the rapid slide bile solubility test, this test has its limitations. This test is a combination of the bile solubility test and the Gram stain (12). For an isolate to be identified as *Streptococcus pneumoniae*, the sodium deoxycholate reagent must completely lyse all the cocci. Other streptococci could give a false-positive reaction, especially if the cells are old or if they wash off during the staining process (12). The latex reagents may give more reliable results than the bile solubility test for identification of *Streptococcus pneumoniae* from blood cultures.

There have been several reports (9, 14, 17) on the cross-reactivity between the beta-hemolytic streptococcal group C reagent with *Streptococcus pneumoniae* when tests were performed directly on blood cultures. We did not encounter this problem, since blood cultures were tested first in the identification scheme with the bile solubility test and latex reagents directed against *Streptococcus pneumoniae*.

Several immunologic group D reagents were evaluated for reliability in replacing the bile esculin test for identifying group D streptococci. Our results with Streptex are in contrast to the results of a study reported by Shales et al. (14) in which only 61.5% of isolates were correctly identified with this reagent. In their study, they tested blood culture broth rather than the concentrated pellet. Because the group-specific antigen of group D streptococci is cell membrane associated, the sensitivity of the test may be higher when the culture pellet is used, as our results showed.

In comparison to Serostat and Phadebact reactions, Streptex reactions were clearer and easier to interpret (16). Since Serostat and Phadebact group D reagents exhibited cross-reactions with both blood culture systems, we do not recommend their use for identification of group D streptococci from blood cultures. We did not attempt to eliminate the nonspecific reactions, since these methods would increase the turnaround time and complexity of the procedure (16).

Group D enterococci need to be differentiated from group D nonenterococci, since infections with nonenterococcal strains do not require combination therapy for a successful therapeutic response (10). The hydrolysis of PYR can be used as an alternative to the 6.5% salt tolerance test for identification of the enterococci (2, 11). Several studies (6, 13) have evaluated a 4-h tube test for PYR from isolated colonies. Recently, two 10-min rapid tests (Identicult A-E and Strep-A-Chek) have replaced the tube test. We have adapted these procedures to the direct testing of blood cultures.

The only recommendations which we make regarding

Identicult A-E is not to use this reagent when testing organisms directly from Difco Thiol bottles, since uninoculated Thiol broth produces false-positive results with this reagent. Overall, these studies indicate that Streptex and Identicult A-E are the preferred methods for identifying group D enterococci and nonenterococci directly from blood cultures. Compared with Serostat and Phadebact reactions, Streptex reactions were clearer and easier to interpret (16).

Since a majority of viridans group streptococcal isolates showed multiple reactions when tested with the beta-hemolytic streptococcus kits for groups A, B, C, F, and G, the methods proposed in this study should not be considered when testing organisms other than group D streptococci. We agree with the comments of the manufacturer that bacteria other than beta-hemolytic streptococci may give erroneous results (17). In our study, we did not know whether the organisms were beta-hemolytic, since hemolysis was not demonstrable before direct testing was performed. However, a study by Wetkowski et al. (17) revealed no major cross-reactions with the non-beta-hemolytic streptococci when the Phadebact Streptococcus kit was used, and they concluded that there was no need for hemolysis determination before testing.

In conclusion, some of the immunologic and biochemical methods evaluated in our study can be very useful in identifying gram-positive cocci from blood cultures. Within 45 min of initiation of the test, the physician can receive a preliminary identification and proper antibiotic therapy can be initiated. We used Difco and BACTEC blood culture media in our study; however, we suggest that laboratories evaluating direct testing with their own blood culture systems should run preliminary tests with the media to ensure that the uninoculated broth does not interfere with the interpretation of results.

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