

Evaluation of Rapid Identification of Gram-Positive Cocci in Positive Blood Cultures by Use of the AutoMicrobic System Gram-Positive Identification Card

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Because rapid identification of gram-positive organisms from blood cultures may provide valuable information for patient care and because the AutoMicrobic system Gram-Positive Identification (AMS-GPI) Card (Vitek Systems, Inc., Hazelwood, Mo.) is designed for the identification of these organisms in 4 to 13 h, we designed this study to evaluate the performance of the AMS-GPI Card in the direct identification of gram-positive organisms upon detection of growth in blood culture bottles. We compared direct identification by the AMS-GPI Card with the final AMS-GPI Card identification and with our standard identification methods. We evaluated 51 gram-positive organisms from clinical blood cultures as well as 49 simulated blood cultures. The isolates included *Streptococcus pneumoniae* (17), *Streptococcus pyogenes* (13), group D enterococci (12), *Streptococcus agalactiae* (11), viridans streptococci (10), coagulase-negative staphylococci (21), *Staphylococcus aureus* (15), and *Listeria monocytogenes* (1). The AMS-GPI Card identified all of the group D enterococci, viridans streptococci, and coagulase-negative staphylococci and all but one each of the *Streptococcus pyogenes* and *Streptococcus agalactiae* isolates. *L. monocytogenes* was also correctly identified. However, the AMS-GPI Card identified only 12 of 17 *Streptococcus pneumoniae* and 9 of 15 *Staphylococcus aureus* isolates by direct inoculation. We therefore conclude that the results of direct identification of gram-positive organisms by the AMS-GPI Card may be used cautiously for rapid direct identification of gram-positive organisms from positive blood cultures.

Blood cultures are one of the most critical types of patient specimens processed by the microbiology laboratory. They are second in importance only to cerebrospinal fluid cultures. It is therefore not surprising to see the number of developmental-research reports related to the rapid identification of isolates from blood cultures by biochemical and serological tests on broth or centrifuged and pelleted organisms from blood culture bottles.

Although there is an abundance of information related to rapid direct identification of gram-negative organisms from positive blood cultures, methods for rapid identification of gram-positive organisms have not been thoroughly evaluated. Such organisms as *Staphylococcus aureus*, *Streptococcus pneumoniae*, and groupable beta-hemolytic streptococci can be identified by straightforward, simple tests (11, 13, 16); however, species identification of viridans streptococci and coagulase-negative staphylococci is more complex and requires the performance of time-consuming, complex procedures. These procedures are often not suitable for the routine microbiology laboratory because of the necessity for a large number of tests, highly specialized media, and long incubation times (1). In addition, viridans streptococci and coagulase-negative staphylococci have been considered by many to be mere skin contaminants that are not worthy of rapid identification. However, with the increasing realization that these two groups of organisms may, under the appropriate circumstances, be potentially pathogenic (6, 8, 15) and with the introduction of commercial systems for their identification, we found it tempting to evaluate the performance of one commercially available product for direct identification of gram-positive organisms in a manner analogous to

that of the tests for identification of gram-negative organisms that we use (H. J. Cannon, Jr., and A. C. Hamoudi, Abstr. Annu. Meet. Am. Soc. Microbiol., 1981, C266, p. 307).

Recently, Vitek Systems, Inc., Hazelwood, Mo., introduced the Gram-Positive Identification (GPI) Card to be used with the AutoMicrobic system (AMS). The performance of the AMS-GPI Card has been evaluated in several studies for the identification of both streptococci (3, 14) and coagulase-negative staphylococci (2, 7, 14) from isolated colonies on plated media.

The purpose of this study was to evaluate the performance of the GPI Card in the direct identification of gram-positive organisms from blood culture bottles (BACTEC system; Johnston Laboratories, Inc., Cockeysville, Md.); upon the detection of growth of a single gram-positive organism, and to compare these direct results with the final identification of the organisms (from isolated colonies) by the recommended procedure for the AMS-GPI Card and by conventional methods.

It is important to note that the conventional methods we use do not allow for the identification of viridans streptococci, group D enterococci, or coagulase-negative staphylococci to species levels. It is not our intention to evaluate the performance of the AMS-GPI Card in the species identification of these aforementioned organisms.

MATERIALS AND METHODS

The routine processing of blood cultures in our laboratory is performed with two BACTEC bottles, a 6B aerobic and a 7D anaerobic bottle. We recommend the inoculation of 2 to 5 ml of blood into each bottle.

All positive blood cultures (detected by a high BACTEC growth index [30 for aerobic and 20 for anaerobic blood cultures] or by visible turbidity) were allowed to settle while

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TABLE 1. Identification of clinical isolates by direct inoculation of AMS-GPI Card

Organism (no. of isolates)	No. correctly identified ^a (% probability) by AMS-GPI Card
<i>Streptococcus pneumoniae</i> (12) ^b	7 (≥93) 1 (83) 1 (53)
Group D enterococci (6)	6 (99)
Viridans streptococci (4) ^c	4 (≥85)
Coagulase-negative staphylococci (21)	14 (≥85) 7 (63–84)
<i>Staphylococcus aureus</i> (5) ^d	3 (≥89)
Other (3)	3 (99)

^a Correct identifications: for viridans streptococci, 2 *Streptococcus mitis* and 2 *Streptococcus sanguis* II isolates; for group D enterococci, 6 *Streptococcus faecalis* isolates; for coagulase-negative staphylococci, 19 *Staphylococcus epidermidis* and 2 *Staphylococcus hominis* isolates; for other, 1 *Streptococcus pyogenes*, 1 *Streptococcus agalactiae*, and 1 *L. monocytogenes* isolate.

^b Incorrect identification, one isolate (as *C. hemolyticum*, 97% probability); no identification, two isolates.

^c Not group D.

^d Incomplete identification, two isolates (*Staphylococcus* spp.).

we prepared and interpreted a Gram-stained smear and decided on the course of action. Only positive cultures with gram-positive organisms of a single morphotype were included in the study. If the organisms were in short chains or pairs, a direct sodium deoxycholate test was set up (12). All gram-positive organisms were then processed (after settling for at least 1 h) by adding 1 to 2 drops of the supernatant fluid to the AMS saline to achieve a turbidity equal to a 0.5 McFarland standard, were mixed well, and were used to set up a GPI Card. In addition, all gram-positive organisms were inoculated onto blood and chocolate agar plates and incubated for conventional processing (13). On day 2, another GPI Card was set up from isolated colonies on solid media according to the recommendations of the manufacturer. The GPI Card gives identification results in 4 to 13 h. Identification procedures for all organisms of the simulated blood cultures that were misidentified by direct inoculation (with our conventional methods as the reference methods) were repeated in duplicate, once by the centrifugation pellet method as previously described (11, 16) and once by direct inoculation as described above. The purpose of repeating the experiments was to determine whether the centrifugation method (which required more technologist time for handling) was more accurate than the settling method described above.

The final conventional identification of the organisms was performed by latex agglutination for grouping beta-hemolytic and nonhemolytic streptococci. Growth in 6.5% NaCl and bile-esculin hydrolysis were used for identification of group D streptococci and viridans streptococci that are not group D (5). *Streptococcus pneumoniae* was conventionally identified by its morphological characteristics and by the sodium deoxycholate test on isolated colonies for solid media. If the results were questionable, the organism was typed by the use of Phadebact pneumococcal reagents (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.). Staphylococci were

divided into *Staphylococcus aureus* and coagulase-negative staphylococci based on slide and tube coagulase reactions (9).

If the direct AMS identification was different from the conventional identification, the organism was considered misidentified by direct inoculation into the AMS-GPI Card.

Simulated positive blood cultures were prepared in the following manner. BACTEC aerobic bottles were each inoculated with 1 ml of a suspension that contained 2×10^2 CFU/ml and that had been prepared from an overnight growth of stock cultures on blood agar plates. Blood (2 ml per bottle) was then added from an outdated blood unit, kindly supplied by the Transfusion Service. The bottles were then incubated overnight at 35°C. On the next morning, the bottles were allowed to settle for at least 1 h, and a GPI Card was inoculated from each bottle, as described above.

The exact probability that should be used as a cutoff point for definitive identification with the GPI Card is not yet established with certainty, although the manufacturer suggests 60% probability as an acceptable cutoff point for all gram-positive organisms.

RESULTS

A total of 51 gram-positive organisms were isolated from clinical specimens during the period of the study (Table 1). Of 12 *Streptococcus pneumoniae* isolates, 8 were correctly identified with acceptable probabilities by direct testing, whereas 1 was identified with only 53% probability. One organism was identified as *Corynebacterium hemolyticum*. Two other isolates did not grow in the system and were reported as insufficient growth.

All six of the group D enterococci were identified by direct testing as *Streptococcus faecalis* with a probability of 99%. Similarly, two viridans streptococci were identified as *Streptococcus mitis*, and two were identified as *Streptococcus sanguis* II, with a probability of ≥85% for each.

There were five isolates of *Staphylococcus aureus*; three were identified correctly with a probability of ≥89%, and two were incompletely identified as *Staphylococcus* spp., with probabilities of 58 and 90%, and as *Staphylococcus aureus* for a second choice, with probabilities of 37 and 8%. Three other organisms, one each of *Streptococcus pyogenes*, *Streptococcus agalactiae*, and *L. monocytogenes*, were correctly identified by direct GPI inoculation. Both correct and incorrect direct results were reproduced when a GPI Card from isolated colonies was inoculated according to the recommendations of the manufacturer.

The results for simulated blood cultures are shown in Table 2.

Of 12 *Streptococcus pyogenes* isolates, 10 were correctly identified with high probabilities by direct testing. An additional isolate was identified with a lower-than-acceptable probability, and one was misidentified as *Streptococcus salivarius*. Of 10 *Streptococcus agalactiae* isolates, 9 were directly identified as such, whereas 1 was repeatedly identified as *Streptococcus faecalis* with a consistent 99% probability. There were five simulated positive blood cultures inoculated with *Streptococcus pneumoniae*. Of these, three were correctly identified with a high probability, but one was misidentified as *Streptococcus faecalis* and one was misidentified as *Streptococcus mutans*, with 99 and 76% probabilities, respectively. All six isolates of group D enterococci were identified as *Streptococcus faecalis* by direct inoculation. Six isolates of viridans streptococci were identified with high probabilities; four were *Streptococcus mitis*, and

two were *Streptococcus sanguis* II. Of 10 isolates of *Staphylococcus aureus*, 6 were correctly identified with $\geq 90\%$ probability, 2 were not identified, and 2 conventionally identified *Staphylococcus aureus* isolates were misidentified by the AMS as *Corynebacterium* spp. and *Streptococcus faecalis* with high probabilities. To determine whether the centrifugation method is more accurate, all procedures leading to misidentifications were repeated in duplicate as described above (once after centrifugation and once after settling). Repeating the experiment gave the correct identification by both methods in three of six instances (Table 2).

DISCUSSION

The clinical importance of blood culture isolates demands that their rapid, correct identification be one of the top priorities of microbiology laboratories. However, rapid identification procedures from positive blood culture bottles are not standardized and, in most instances, should be repeated from isolated colonies (13). Such tests are naturally invalid if more than one organism is found in the blood culture; therefore, direct identification schemes were not attempted if organisms with different morphologies were seen in the Gram-stained smear of the positive blood culture.

A variety of tests are suggested in the literature for direct identification of gram-positive and gram-negative organisms from blood cultures (11, 16). Furthermore, there are several commercially available systems which have greatly simplified the identification of many gram-positive organisms to species level. The AMS-GPI Card is one such system and is designed for the identification of gram-positive cocci and a select group of gram-positive rods.

Several studies have evaluated the GPI Cards for the identification of gram-positive organisms from solid media (2, 3, 7, 14). Morello et al. found the AMS-GPI Cards to be 95% accurate with *Streptococcus sanguis* II and *Streptococcus mitis*. These organisms were the most common viridans group isolate tested in that study (J. A. Morello, L. H. Knipp, and E. Horton, Proc. Fifth Annu. Vitek AMS Symp. 1983, p. 1-6). Almeida et al. (2) report that the greatest accuracy with the AMS-GPI Card for the identification of coagulase-negative staphylococci is achieved with *Staphylococcus epidermidis* isolates (95.7%), which is also the *Staphy-*

lococcus species most frequently isolated from blood cultures (4).

Others have utilized techniques for the identification of gram-negative organisms from positive blood cultures by direct inoculation of commercially available biochemical systems (10, 11). It only seemed logical for us to utilize a similar technique to inoculate the GPI Cards. The preliminary results of our study indicate that direct inoculation may give reproducible results when repeated on isolated colonies as recommended by the manufacturer.

Direct inoculation of positive blood cultures into GPI Cards resulted in reproducible identification of 21 of 21 coagulase-negative staphylococci, 12 of 12 group D enterococci, and 10 of 10 viridans streptococci. Only 9 of 15 *Staphylococcus aureus* isolates (60%) and 12 of 17 *Streptococcus pneumoniae* isolates (71%) were identified as such. Of 13 *Streptococcus pyogenes* and 11 *Streptococcus agalactiae* isolates tested, the GPI Card missed 1 isolate from each species by the method outlined here.

Others have suggested that rapid, direct, and definitive identification of *Streptococcus pneumoniae* is so simple, rapid, and cost effective that there would be no point in attempting to identify these organisms by the more expensive GPI Card (Morello et al., Proc. Fifth Annu. Vitek AMS Symp.). We concur, and we add that in our modified use of the GPI Card, by directly inoculating the card, GPI Card was found to be less accurate than the above-mentioned simple, conventional tests. We found these simple, inexpensive tests highly dependable based on our own evaluation of direct sodium deoxycholate lysis and serological testing (M. J. Marcon and A. C. Hamoudi, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 128, 1983). Direct serological testing on groupable beta-hemolytic streptococci also appears to be simple, rapid, and accurate. However, the hemolysis characteristics are not determined at the time of the initial visualization of gram-positive cocci in the Gram-stained smear.

Our study shows promise for the use of direct inoculation of GPI Cards. The need for additional tests, such as simple biochemical and serological tests, is indicated. In our opinion, coagulase testing for staphylococci should be performed. In addition, correlation with colonial morphology

TABLE 2. Identification of simulated blood cultures by direct inoculation of AMS-GPI Cards

Organism (no. of isolates)	No. (% probability) identified by AMS-GPI Card		Incorrect identification
	Correct	Incorrect	
<i>Streptococcus pyogenes</i> (12)	10 (≥ 93) 1 (59)	1 (91)	<i>Streptococcus salivarius</i>
<i>Streptococcus agalactiae</i> (10)	7 (≥ 90) 2 (80-90)	1 (99)	<i>Streptococcus faecalis</i> ^c
<i>Streptococcus pneumoniae</i> (5)	3 (≥ 92)	1 (99) 1 (76)	<i>Streptococcus faecalis</i> ^c <i>Streptococcus mutans</i>
Group D enterococci (6)	6 (99)	0	
Viridans streptococci (6) ^a	5 (≥ 92) 1 (88)	0	
<i>Staphylococcus aureus</i> (10) ^b	6 (≥ 90)	1 (75) 1 (99)	<i>Corynebacterium</i> sp. ^c <i>Streptococcus faecalis</i> ^c

^a Includes four *Streptococcus mitis* isolates and two *Streptococcus sanguis* II isolates.

^b Not identified, two isolates.

^c Repeat experiments gave the correct identification. (All other experiments gave consistent results when repeated.)

and serological typing of beta-hemolytic streptococci are very important for the final identification of gram-positive organisms. In select cases, additional tests such as growth in 6.5% NaCl and esculin hydrolysis may prove essential.

Based on the results of the study, we are evaluating the following protocol. When a Gram-stained smear suggests a *Streptococcus* sp., we suggest that a direct sodium deoxycholate lysis test be performed. A positive direct test is reported as presumptive *Streptococcus pneumoniae*, and no GPI Card is set up. A direct GPI Card should be set up on streptococci which are negative by deoxycholate lysis test. In our own pediatric setting, <10% of streptococcal blood culture isolates are considered contaminants (unpublished data); thus, rapid methods for identification of streptococci will yield clinically useful information in the majority of cases in our laboratory. Additionally, a direct serological test for group B streptococcal antigens is performed when streptococci are suspected in neonatal blood cultures (<1 month of age).

Coagulase-negative staphylococci are still major contaminants in our institution, thus precluding the routine direct testing of these isolates by AMS-GPI Cards because of the expenses involved in such testing. Discretion should be used in deciding which isolates are to be tested by direct inoculation of GPI Cards, depending on the clinical setting and the number of positive blood culture bottles.

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