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Automated Identification of Gram-Positive Bacteria

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A total of 451 strains of gram-positive bacteria were identified with a prototype of the Gram-Positive Identification card used in conjunction with the AutoMicrobic system (Vitek Systems, Inc., Hazelwood, Mo.). Of the species that the Gram-Positive Identification card is capable of identifying, 85% of staphylococcal, 50% of beta-hemolytic group A, B, C, F, and G streptococcal, 91% of group D streptococcal, 100% of pneumococcal, 63% of viridans streptococcal, and 100% of *Listeria monocytogenes* strains tested displayed Gram-Positive Identification card identifications that were in agreement with identifications obtained by conventional methods.

Gram-positive bacteria account for a large portion of the organisms which are routinely isolated and identified in clinical laboratories. Many of these organisms (e.g., *Staphylococcus aureus* and groupable beta-hemolytic streptococci) can be identified or presumptively identified with simple and fairly rapid tests. However, identification at the species level of other gram-positive organisms, such as coagulase-negative staphylococci and viridans streptococci, requires numerous time-consuming physiological tests. The scheme of Kloos and Schleifer (4) for the identification of human staphylococci uses 13 biochemical tests, and that of Facklam (2) for viridans streptococci requires the performance of 12 key tests.

Interest in simple, rapid identification methods for these organisms as well as for the more firmly established gram-positive pathogens led us to investigate the identification of these bacteria with an automated computerized bacteriological system, the AutoMicrobic system (AMS) (Vitek Systems, Inc., Hazelwood, Mo.). The general features of the AMS have been previously described by Aldridge et al. (1). The Gram-Positive Identification (GPI) card, designed for use with the AMS, allows for the performance of miniaturized biochemical tests used in the identification of strains of streptococci, staphylococci, certain corynebacteria, *Listeria monocytogenes*, and *Erysipelothrix rhusiopathiae*. Our objective in this study was to evaluate the performance of a prototype of the card with strains of staphylococci, streptococci, and *L. monocytogenes*.

MATERIALS AND METHODS

Bacterial strains. Most of the organisms examined in this study were isolated from clinical specimens in the bacteriology laboratory at the Massachusetts General Hospital, Boston. Stock strains of coagulase-negative staphylococci were supplied by W. E. Kloos, North Carolina State University, Raleigh. Strains of *L. monocytogenes* have been previously described (6). Most of the streptococci were stored frozen in horse blood at −70°C, whereas most of the staphylococci and all of the *L. monocytogenes* strains were stored in a mixture of broth and horse blood on sterile glass beads at −70°C (8). Strains of *S. aureus, Streptococcus pneumoniae*, and group A, B, C, and G streptococci were fresh clinical isolates that had not previously been frozen. For use in this study, the strains were propagated on horse blood agar (GIBCO Diagnostics, Madison, Wis.) or sheep blood agar plates (Remel Regional Media Laboratories, Inc., Lenexa, Kans.) incubated at 35°C in the presence of 3 to 5% CO_2.

Identification methods. Staphylococci were subjected to a tube coagulase test with rabbit plasma (GIBCO Diagnostics). Coagulase-negative staphylococci were differentiated with the simplified scheme described by Kloos and Schleifer (4). Streptococci were tested for the presence of Lancefield group antigens by the lysozyme-*Streptomyces albus* extraction method of Watson et al. (12) followed by microprecipitin testing or with Streptex latex agglutination testing (Wellcome Research Laboratories, Beckenham, England). Group D streptococci were differentiated with tests described by Facklam (3). Pneumococci were presumptively identified by colonial morphology, hemolytic reaction, and inhibition by optochin (Taxo P disks; BBL Microbiology Systems, Cockeysville, Md.). Viridans streptococci were identified with the modification of the scheme of Facklam (2) described by Ruoff and Kunz (9).

In most cases when the identification with the GPI card disagreed with the identification arrived at by the methods described above, the strains were retested with the card and subjected to the standard identification tests recommended by Vitek Systems, Inc. These recommended methods included Streptex latex agglutination testing for beta-hemolytic streptococci, the methods of Facklam (3) for nonhemolytic group D

TABLE 1. Agreement between identification of gram-positive organisms by the GPI card and identification by conventional methods

Species	No. tested	No. agreeing at the following probability:			Overall % agreement
		90–100%	80–89%	<80%	
Staphylococci					
S. aureus	50	43[a]	5[b]	1[c]	98
S. epidermidis	49	34	6	3	88
S. saprophyticus	23	18	2	3	100
S. hominis	7	3	1	1	71
S. haemolyticus	19	13[c]			68
Streptococci					
Group A	10	10			100
Group B	10	8[d]		2	100
Group C	10	3[e]			30
Group F	9	1[f]			11 (20)[g]
Group G	10	7[e]			70
S. faecium	12	12			100
S. faecalis	13	13			100
S. bovis	5	1	1		40
S. durans	2	2			100
S. pneumoniae	15	14	1		100
S. mutans	14	6	2		57
S. uberis	2	2			100
S. sanguis I	28		5	7	43
S. salivarius	9	2	1	1	44
S. sanguis II	27	15	1	3	70
S. mitis	14	5	5	1	79
S. MG-intermedius	35	22	2	3	77
S. anginosus-constellatus	9	5			56
S. morbillorum	2				0 (50)[g]
L. monocytogenes	30	30			100

[a] *S. aureus*/*S. simulans*, 34 strains; *S. aureus*/*S. haemolyticus*, 9 strains. See text for explanations of this and the following footnotes.
[b] *S. aureus*/*S. simulans*, three strains; *S. aureus*/*S. haemolyticus*, two strains.
[c] *S. aureus*/*S. haemolyticus*.
[d] *S. agalactiae*, four strains; *S. agalactiae*/*S. morbillorum*, four strains.
[e] *S. equisimilis*/group G.
[f] *S. anginosus*/*S. anginosus-constellatus*.
[g] Number in parentheses indicates the overall percent agreement when strains displaying insufficient growth are deleted.

streptococci, pneumococci, and viridans streptococci, and the following tests for differentiation of coagulase-negative staphylococci: hemolysis on sheep blood agar, resistance to novobiocin, phosphatase assay, growth and acid production on mannitol salt agar, acid production in purple broths containing trehalose, mannitol, and maltose, and ability to grow anaerobically in thioglycolate broth. All media for these tests were manufactured by Remel Regional Media Laboratories, Inc.

GPI card. The GPI card was used according to the instructions of the manufacturer. Growth from blood agar plates was used to make suspensions (0.5 McFarland standard, or 1.0 McFarland standard for slow-growing strains) in 0.45% sterile saline. The AMS filling module was then used to fill cards with portions of each suspension. The cards were subsequently placed in the AMS reader-incubator module for processing. The GPI card contains 27 differential test media and one positive and two negative control broths. The substrates or differential substances used in the test wells are bacitracin, optochin, hemicellulose, NaCl (5%), bile (10% and 40%), esculin, arginine, urea, tetrazolium red, glucose, lactose, mannitol, raffinose, salicin, sorbitol, sucrose, trehalose, arabinose, pyruvate, pullulan, inulin, melibiose, melezitose, cellobiose, D-ribose, and potassium thiocyanate.

RESULTS

The levels of agreement between identifications based on conventional methods and those based on the GPI card are shown in Table 1. Among the staphylococci, *S. aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus* were identified with greatest accuracy. In its present form, the computer program provides two staphylococcal identifications that are dependent on the coagulase reaction. The "*S. aureus*/*S. simulans*" and "*S. aureus*/*S. haemolyticus*" identifications stipulate that if the iso-

late is coagulase positive it should be identified as *S. aureus*. If the strain is coagulase negative, its physiological properties suggest an identification of either *Staphylococcus simulans* or *Staphylococcus haemolyticus*, respectively.

Three additional species of coagulase-negative staphylococci, not identified by the GPI card, were also tested. Seven strains of *Staphylococcus warneri* were all identified as *Staphylococcus* spp. by the card. Of six strains identified by conventional methods as *Staphylococcus cohnii*, three were identified as *S. saprophyticus*, one as *S. aureus/S. simulans*, one as *S. aureus/S. haemolyticus*, and one as *Staphylococcus* spp. by the card. Eight of twenty-four strains of *Staphylococcus capitis* were identified as *Staphylococcus hominis*, seven as *Staphylococcus* spp., five as *S. aureus/S. simulans*, two as *S. aureus/S. haemolyticus*, and one as *Corynebacterium xerosis*, and one was unidentified by the GPI card.

Among the groupable beta-hemolytic streptococci, good agreement between serological and GPI card identifications was observed with group A and B streptococci. Some of the group B strain identifications were based on the hemolytic reaction of the isolate. The "*S. agalactiae*/*S. morbillorum*" identification advised that if beta-hemolytic, the isolate resembled a group B streptococcus, but if nonhemolytic, it should be identified as the viridans species *Streptococcus morbillorum*. The GPI card identification of group C, F, and G streptococci agreed with serological identifications at levels of 30, 11, and 70%, respectively. The correctly identified group C and G streptococci were identified with a preliminary call ("*S. equisimilis*/group G") with advice that the identification be confirmed serologically. The group F streptococci that were correctly identified by the card were called *Streptococcus anginosus* if beta-hemolytic or *Streptococcus anginosus-constellatus* if nonhemolytic.

Table 1 shows that all nonhemolytic group D streptococcal species except *Streptococcus bovis* gave GPI card identifications that agreed with those obtained by conventional methods. *S. pneumoniae* strains were also identified with a high degree of accuracy by the GPI card. Identifications of four of the nine viridans streptococcal species tested (*Streptococcus uberis*, *Streptococcus sanguis* II, *Streptococcus mitis*, and *Streptococcus MG-intermedius*) displayed only fair agreement (70% or higher) with identification by conventional methods (Table 1).

All strains of *L. monocytogenes* were correctly identified at high probabilities by the GPI card.

Table 2 presents the nature of the discrepancies between identifications by conventional methods and identifications by the GPI card. With the exception of one staphylococcal strain identified as *C. xerosis* and one unidentified isolate, all of the misidentified staphylococci were identified as other species of the genus *Staphylococcus*. It should be noted that one strain of *S. aureus* was not identified by the card and that strains of questionable clinical importance (*S. hominis*, *S. haemolyticus*) were misidentified as *S. saprophyticus*, a species which is considered to be clinically significant (5).

Table 2 shows a number of instances in which the card misidentified groupable beta-hemolytic streptococci as viridans species. The inability of four of nine group F strains to grow in the wells of the card precluded their identification.

Misidentified strains of viridans streptococci were usually identified as other viridans species by the card. However, Table 2 shows misidentifications as *S. pneumoniae* for strains of *S. sanguis* II and one strain of *S. anginosus-constellatus*, and as groupable streptococci for strains of *Streptococcus mutans* and one strain of *S. anginosus-constellatus*.

DISCUSSION

Among the staphylococci tested with the GPI card, correct identifications were most prominent among the three clinically significant (5) species *S. aureus*, *S. epidermidis*, and *S. saprophyticus*. Of some concern is the misidentification of organisms of "probable" clinical significance, *S. hominis* and *S. haemolyticus* (5), as the known pathogen *S. saprophyticus*. The examination of 37 strains of coagulase-negative staphylococcal species not identified by the card revealed that 21 of the strains were given definite species identifications instead of the "unidentified" or "*Staphylococcus* spp." designation. Incorporation into the card of additional substrates such as fructose, maltose, or mannose might be helpful in improving the accuracy of staphylococcal identification (4). Although attempts have been made to simplify the identification of coagulase-negative staphylococci, it appears that there is some degree of physiological heterogeneity among these species and that a large battery of biochemical tests is needed to identify certain strains accurately (4). Perhaps this situation is reflected by the seemingly decreased accuracy of the GPI card in the identification of these organisms.

The results obtained with group C, F, and G beta-hemolytic streptococci attest to the superiority of serological methods for the identification of these organisms. The apparent physiological unreactiveness of group F streptococci in the test wells of the card accounted for its failure to identify about half of the group F strains tested.

TABLE 2. Nature of discrepant identifications produced by the GPI card

Species	No. tested	No. of discrepancies	GPI card identification [(no. of strains)/ % probability of identifications]
Staphylococci			
S. aureus	50	1	*Staphylococcus* spp. (1)/89
S. epidermidis	49	6	*Staphylococcus* spp. (4)/94, 87, 71, 59 *C. xerosis* (1)/89 Unidentified (1)
S. hominis	7	2	*S. saprophyticus* (2)/69, 58
S. haemolyticus	19	6	*S. simulans* (1)/99 *S. hominis* (2)/98, 61 *S. saprophyticus* (1)/72 *Staphylococcus* spp. (2)/99, 87
Streptococci			
Group C	10	7	*S. pyogenes* (1)/87 *S. MG-intermedius* (4)/97, 97, 71, 56 *S. mitis* (1)/92 *S. anginosus* (1)/57
Group F	9	8	*S. pneumoniae* (1)/67 *S. equi* (1)/77 *S. acidominimus* (2)/98, 98 Insufficient growth (4)
Group G	10	3	*S. pneumoniae* (1)/56 *S. MG-intermedius* (1)/80 Unidentified (1)
S. bovis	5	3	*S. MG-intermedius* (1)/81 Unidentified (2)
S. mutans	14	6	*S. pyogenes* (1)/50 *S. avium* (1)/99 *S. uberis* (1)/98 *S. MG-intermedius* (1)/78 *S. sanguis* I (1)/58 Unidentified (1)
S. sanguis I	28	16	*S. sanguis* II (3)/99, 99, 81 *S. salivarius* (1)/71 *S. uberis* (1)/55 *S. MG-intermedius* (1)/95 *S. mutans* (1)/50 *S. mitis* (2)/92, 85 Unidentified (7)
S. salivarius	9	5	*S. bovis* subsp. (1)/76 *S. mutans* (2)/99, 54 *S. MG-intermedius* (1)/58 Unidentified (1)
S. sanguis II	27	8	*S. pneumoniae* (3)/98, 93, 56 *S. mitis* (2)/95, 86 *S. salivarius* (1)/78 *S. MG-intermedius* (1)/73 Unidentified (1)
S. mitis	14	3	*S. MG-intermedius* (2)/65, 65 Unidentified (1)
S. MG-intermedius	35	8	*S. bovis* subsp. (1)/94

TABLE 2—*Continued*

Species	No. tested	No. of discrepancies	GPI card identification [(no. of strains)/ % probability of identifications]
			S. uberis (1)/99 *S. mutans* (1)/92 *S. salivarius* (2)/81, 71 *S. sanguis* I (2)/73, 65 Unidentified (1)
S. anginosus-constellatus	9	4	*S. pneumoniae* (1)/98 *S. equinus* (1)/88 *S. morbillorum* (1)/98 Unidentified (1)
S. morbillorum	2	2	*S. uberis* (1)/99 Insufficient growth (1)

The taxonomical enigma reprsented by the viridans streptococci seemed to be an almost insurmountable challenge for the GPI card. The misidentification of one viridans species as another viridans species by the card is almost understandable in light of the confused taxonomic state of this group. It would appear that the appropriate substrates for identification of the viridans group have been included in the card; expansion of the data base may improve the identification of this group of organisms.

The time required for automated identification of the various gram-positive organisms tested ranged from 4 to 13 h. This clearly represents a reduction in the amount of time required to perform conventional identification tests for coagulase-negative staphylococci, viridans streptococci, *L. monocytogenes*, and species of group D streptococci. The advantage of the GPI card for identification of *S. aureus* or beta-hemolytic streptococci is questionable, since convenient rapid methods exist for serogrouping or identification of these organisms (7, 10, 11).

In spite of the deficiencies of the prototype GPI card noted above, a high proportion of accurate identifications were noted among group A, B, and D streptococci, pneumococci, *S. aureus*, *S. saprophyticus*, and *L. monocytogenes*. Perhaps further testing and expansion of the data base of the system will improve the performance of the GPI card in other areas.

ADDENDUM IN PROOF

Vitek Systems, Inc., has recently informed us of changes in the GPI card which may affect its overall performance. Changes include alteration of substrate concentrations in four wells of the card, expansion of the data base, an increase in the minimum acceptable initial raw value reading for the positive control well, and a safeguard against processing results when readings have been interrupted by mechanical malfunctions. The critical value for an "unidentified" organism call was raised from a probability of 45 to 50%.

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