JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1984, p. 164–168
0095-1137/84/020164-05$02.00/0
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Vol. 19, No. 2

Comparative Evaluation of the API 20S System and the AutoMicrobic System Gram-Positive Identification Card for Species Identification of Streptococci

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Received 28 July 1983/Accepted 21 October 1983

Two commercial methods, the API 20S system (API; Analytab Products, Inc., Plainview, N.Y.) and the Gram-Positive Identification Card (GPI; Vitek Systems, Inc., Hazelwood, Mo.), were evaluated without additional tests for the identification of 241 streptococcus strains. Organisms included 60 beta-hemolytic strains, 36 group D strains, 26 *Streptococcus pneumoniae* strains, and 119 viridans streptococcus strains. API correctly identified to species 68.3% of beta-hemolytic strains, 86.1% of group D strains, 53.9% of *S. pneumoniae* strains, and 12.6% of viridans streptococci. This method provided excellent identification of group A and B and *S. faecalis* strains. Overall, API correctly identified 41.9% of strains to species, with 41.1% good likelihood but low selectivity, 15.8% incorrect, and 1.2% not identified. GPI correctly identified to species 58.3% of beta-hemolytic strains, 97.2% of group D strains, 80.8% of *S. pneumoniae* strains, and 57.2% of viridans streptococci. Group A, B, and D strains were all accurately identified by this system. Overall, GPI correctly identified to species 66.0% of strains, with 8.7% correct preliminary identification, 20.8% incorrect, and 4.6% not identified. Both methods represent a worthwhile advance in streptococcal identification. Neither system, however, can be recommended for species identification of the viridans group at this time.

Streptococci comprise a large portion of gram-positive cocci isolated and identified in clinical microbiology laboratories. Although many of these organisms (e.g., groupable beta-hemolytic streptococci and pneumococci) can be presumptively or definitively identified by relatively rapid and simple tests, this is not the case for species identification of the viridans group. Although enterococcal and nonenterococcal group D strains can be differentiated by relatively simple tests, accurate species identification of group D organisms requires additional testing. Viridans streptococci can cause a variety of human infections, including endocarditis, cerebral, orofacial, and visceral abscesses, and dental caries (11). Unfortunately, clearer delineation of the precise pathogenic potential of individual viridans species has been hampered by difficulties in taxonomy and the necessity of time-consuming conventional tests for accurate species identification (2, 3). Species identification of viridans streptococci, especially from deep suppurative infections and normally sterile sources, will help delineate the spectrum of disease caused by specific species (e.g., bacteremia with *Streptococcus milleri* [*S. MG-intermedius* and *S. anginosus-constellatus* as described by Facklam] [2] has been associated with deep visceral abscesses). In a patient with recurrent viridans streptococcal endocarditis, streptococcal species identification may provide information necessary to differentiate between a therapeutic failure and reinfection. Additionally, species identification would permit monitoring for the emergence of potential antibiotic resistance. Accurate differentiation of group D species (especially from the blood) may also be important with regard to differing bacterial susceptibilities (e.g., between *S. faecium* and *S. faecalis*) (7) and early detection of colon malignancy in patients with *S. bovis* septicemia (6).

Commercial methods which have recently been developed for the identification of streptococci include the Minitek disk method (4, 10, 12) (BBL Microbiology Systems, Cockeysville, Md.) and the API 20S system (API) (5, 8) (Analytab Products, Inc., Plainview, N.Y.). Recently, Vitek Systems (Hazelwood, Mo.) (1) developed an automated computerized Gram-Positive Identification Card (GPI) for the identification of streptococci, staphylococci, and certain coryneforms with the AutoMicrobic system (9).

For reasons outlined above, microbiology laboratories without facilities for extended conventional testing require an accurate and reliable method for streptococcal species identification. The aim of the current study was to compare the abilities of API and GPI to accurately identify a spectrum of clinically significant streptococci without the aid of additional tests.

MATERIALS AND METHODS

Bacteria. The organisms used (see Table 2) were isolates chosen to represent a spectrum of clinically significant streptococci isolated from human infections. Of the 241 cultures, 227 were clinical isolates obtained from Cleveland (University Hospitals and Veterans Administration Hospital). Six isolates (two *S. faecalis*, two *S. mitis*, one *S. sanguis* I, and one *S. sanguis* II) were obtained from the Centers for Disease Control, Atlanta, Ga., four isolates (one group B, two group G, and one *S. faecalis*) were obtained from the College of American Pathologists, Skokie, Ill. (proficiency samples), and four isolates (one *S. pneumoniae*, one *S. durans*, and two *S. faecalis*) were obtained from the American Type Culture Collection, Rockville, Md. Strains

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were stored at −40°C in thioglycolate-glycerol broth (85%:15%). Before use, organisms were subcultured twice on sheep blood agar (BBL) and incubated at 35°C in the presence of 5 to 10% CO_2. Cultures were checked for purity throughout the study by Gram stain and colonial morphology.

Identification methods. Beta-hemolytic streptococci of groups A, B, C, and G were tested for the presence of Lancefield group antigens by the Phadebact coagglutination method (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) and for the presence of group F organisms by the precipitation technique with specific antiserum (Wellcome Reagents, Research Triangle Park, N.C.). *S. pneumoniae* strains were identified by colonial morphology, optochin susceptibility (Taxo P disks, BBL), and bile solubility. Group D and viridans streptococci were identified by modified procedures with modified tests based on the methodology described by Facklam (2, 3). Group D streptococci were isolated and differentiated by growth on bile-esculin agar, growth in 6.5% NaCl, starch hydrolysis, and fermentation of sorbitol, mannitol, and arabinose (3). Viridans streptococci were identified by a modification of the method of Facklam (2, 3) with key reactions as described by Sands and co-workers (11). Tests included colonial morphology, growth on bile-esculin agar, hemolysis, hydrolysis of esculin and hippurate, litmus milk reduction, growth morphology on 5% sucrose agar, and fermentation of lactose, mannitol, raffinose, and inulin. Additionally, growth in 6.5% NaCl, starch hydrolysis, and fermentation of sorbitol, arabinose, and sucrose were also tested, and identification was checked against standard tables (2, 3). The Minitek disk method (4, 10, 12) was used for lactose, mannitol, raffinose, sucrose, sorbitol, and arabinose fermentation: tests were incubated anaerobically for 48 h and read by the method of Setterstrom and colleagues (12). Conventional methodology (Remel, Inc., Lenexa, Kans.) was used for the fermentation of inulin (2, 10). Additionally, 35 strains (representative of all streptococcal groups tested) were sent to the Centers for Disease Control for identification.

API. Growth from a blood agar plate was suspended in 2.5 ml of 0.85% saline to yield a final turbidity equivalent to that of a no. 1 McFarland standard. Viability plates were included with each run. Strips were inoculated, incubated, and interpreted according to instructions of the manufacturer. The computer facilities of the firm were consulted for organisms whose codes did not appear in the book. Identifications were compared with those obtained by primary methods and classified as (i) correct to species (corresponding to excellent, very good, good, or acceptable identifications, as listed in the code book), (ii) good likelihood but low selectivity (GLLS), which included the correct identification among a spectrum of possibilities (the correct identification occurring as the first of a list of likelihoods was still taken as GLLS), (iii) incorrect, or (iv) no identification. For the purpose of this study, no additional tests were performed to pinpoint a correct identification in category ii. If inocula did not grow, initial results were discarded and tests were repeated with viable cultures. All cultures yielding unacceptable profiles were repeated.

GPI. Growth from a blood agar plate was used to make suspensions (0.5 McFarland, or 1.0 McFarland for slower growers) in 1.8 ml of sterile saline. When clumping occurred (usually with beta-hemolytic or viridans streptococci), a cotton swab was used to obtain uniformly turbid inocula by vigorous mixing of the inocula in saline. Cards were filled, processed, and incubated according to instructions of the manufacturer. Cards were read by the system, and results were obtained in 4 to 13 h. All cultures yielding insufficient growth results were repeated, as were those yielding no identification. The current GPI data base supplies two identification possibilities, one with >50% probability and the other with <50% probability. According to instructions of the firm at this time, the first identification, with >50% probability, should be taken as definitive. Results with GPI were therefore classified as (i) correct to species (>50% probability), (ii) correct preliminary identification (PI) but requiring additional testing to pinpoint the correct choice from a result with >50% probability, (iii) incorrect, or (iv) no identification. For the purpose of this study, no supplemental tests were performed.

RESULTS

The 241 isolates used included 60 beta-hemolytic, 36 group D, 26 *S. pneumoniae*, and 119 viridans strains (Tables 1 and 2). Species identification of all strains sent to the Centers for Disease Control agreed with our initial results in all cases.

Problems were encountered in interpretation of API reactions: tests for β-glucosidase, *N*-acetylglucosaminidase, β-galactosidase, and phosphatase contain chromogenic substrates, and reactions are detected by yellow *ortho*- and *para*-nitrophenol. Yellow reactions are read as positive, and colorless or light-yellow reactions are read as negative. In many cases, equivocal light-yellow color changes made these reactions difficult to read. Carbohydrate reactions which yielded yellow-orange or orange color changes also sometimes presented problems, and aminopeptidase tests (especially serine) were often difficult to read.

Identification rates by API and GPI are presented in Table 1. As can be seen, 41.9% of the strains were correctly identified to species by API, with 41.1% identified as GLLS, 15.8% identified as incorrect, and 1.2% not identified. Among the beta-hemolytic strains, 68.3% were correctly identified, with 28.3% identified as GLLS and 3.3% identified as incorrect. Good identification rates of group D strains were observed with API (86.1% correct to species, 8.3% GLLS, 2.8% incorrect, and 2.8% not identified); in contrast, only 53.9% of *S. pneumoniae* strains were correctly identified, with 38.5% GLLS and 7.7% incorrect. API identification rates of viridans streptococci were disappointing: 12.6% correct to species, 58.0% GLLS, 27.7% incorrect, and 1.7% not identified. In comparison, 66.0% of strains were correctly identified by GPI, with 8.7% correct PI, 20.8% incorrect, and 4.6% not identified. Among beta-hemolytic strains, 58.3% were correctly identified by GPI, with 26.7% correct PI, 13.3% incorrect, and 1.7% not identified. Excellent GPI identification of group D strains (97.2% correct to species and 2.8% incorrect) was observed, with good identification of *S. pneumoniae* (80.8% correct to species, 15.4% correct PI, and 3.8% incorrect). Identification rates of viridans streptococci were less satisfactory, with 57.2% correct to species, 0.8% correct PI, 33.6% incorrect, and 8.4% not identified.

The level of identification of specific streptococcal organisms by API and GPI is presented in Table 2. Among beta-hemolytic organisms, all strains of groups A and B were correctly identified by API; less satisfactory results were obtained with group C, F, and G organisms, the majority of which required serogrouping to pinpoint the correct identification from a range of beta-hemolytic organisms. Excellent identification rates of enterococcal group D organisms were observed with API (96.7% correct to species and 3.3%

TABLE 1. Percentages of streptococcal strains identified by API and GPI

Group (no. of strains)	% of strains identified							
	Correct		One of a spectrum of possibilities		Incorrect		Unidentified	
	API	GPI	API (GLLS)	GPI (PI)	API	GPI	API	GPI
Beta-hemolytic (60)	68.3	58.3	28.3	26.7	3.3	13.3		1.7
Group D (36)	86.1	97.2	8.3		2.8	2.8	2.8	
S. pneumoniae (26)	53.9	80.8	38.5	15.4	7.7	3.8		
Viridans (119)	12.6	57.2	58.0	0.8	27.7	33.6	1.7	8.4
Total (241)	41.9	66.0	41.1	8.7	15.8	20.8	1.2	4.6

incorrect); rates for nonenterococcal organisms were less satisfactory, with 33.3% correct to species, 50.0% GLLS, and 16.7% incorrect (Table 2). No strains yielded identifications as viridans streptococcus with no species given. Twenty-seven percent of the codes (especially with the viridans group) had to be referred to the computer facilities of the firm, necessitating delays of up to several hours before identifications were obtained. GPI accurately identified group A and B beta-hemolytic strains; in contrast, no strain of group C, F, or G was correctly identified to species. Of the 16 beta-hemolytic strains identified as correct PI (Table 2), 1 *S. agalactiae* strain yielded a statement advising that if the organism was beta-hemolytic, then the identification should be as *S. agalactiae*, but if it was alpha-hemolytic, the organism would resemble *S. morbillorum*. One group C and 14 group G strains yielded identifications as *S. equisimilis* group G, with serogrouping necessary for more accurate identification. Excellent identification of enterococcal as well as nonenterococcal group D strains was observed with GPI. The four *S. pneumoniae* strains with correct PI yielded statements advising that optochin testing was necessary for accurate identification. Among the viridans strains, only *S. mitis* and *S. sanguis* II strains yielded correct identification of most organisms. According to the current recommendations of the manufacturer (see above), identification by GPI of all organisms with probability rates of >50% was regarded as definitive. In an attempt to clarify the accuracy of these identifications, rates of organisms correctly identified by GPI were broken down into 90 to 100, 80 to 89, and 51 to 80%, respectively (Table 2). Percentage probabilities for correctly identified beta-hemolytic, group D, and pneumococcal organisms were mostly 90 to 100%, with several group A and *S. pneumoniae* strains in the 80 to 89% category. Probability rates for correctly identified viridans streptococci were generally lower, with fewer in the 90 to 100% category and more in the 80 to 89% group. Seventeen correctly identified strains (7.1%) (6 beta-hemolytic, 1 *S. pneumoniae*, and 10 viridans strains) yielded percent probabilities of 51 to 80% (Table 2).

Additional tests devised to pinpoint an accurate API

TABLE 2. Level of identification of specific streptococcal organisms by API and GPI

Organism (no. of strains)	Level of identification (no.) by:										
	API				GPI						
					Correct at % probability of:						
	Correct	GLLS	Incorrect	Unidentified	90–100	80–89	51–80	>51 (total)	Correct PI	Incorrect	Unidentified
Group A (18)	18	0	0	0	9	7	1	17	0	1	0
Group B (21)	21	0	0	0	12	1	5	18	1[a]	2	0
Group C[b] (3)	0	1	2	0	0	0	0	0	1[c]	2	0
Group F (2)	0	2	0	0	0	0	0	0	0	2	0
Group G (16)	2	14	0	0	0	0	0	0	14[c]	1	1
S. faecalis (25)	25	0	0	0	24	0	0	24	0	1	0
S. faecium (4)	3	0	0	1	4	0	0	4	0	0	0
S. durans (1)	1	0	0	0	1	0	0	1	0	0	0
S. bovis I (6)	2	3	1	0	6	0	0	6	0	0	0
S. pneumoniae (26)	14	10	2	0	16	4	1	21	4[d]	1	0
S. mitis (33)	3	22	8	0	19	3	1	23	0	8	2
S. salivarius (30)	5	24	1	0	7	8	5	20	0	7	3
S. sanguis I (17)	0	8	9	0	1	3	1	5	0	10	2
S. sanguis II (16)	1	11	4	0	10	2	0	12	0	4	0
S. MG-*intermedius* (12)	4	2	5	1	5	0	2	7	0	4	1
S. morbillorum (4)	1	2	1	0	0	0	0	0	1[a]	3	0
S. uberis (1)	0	0	1	0	0	0	0	0	0	1	0
S. mutans (3)	1	0	2	0	0	0	1	1	0	1	1
S. anginosus-constellatus (3)	0	0	2	1	0	0	0	0	0	2	1

[a] Identified as *S. agalactiae* or *S. morbillorum*, depending on hemolysis.
[b] Identification as to species of group C strains was not performed.
[c] Identified as *S. equisimilis* group G, with serogrouping necessary for accurate identification.
[d] Identified preliminarily as *S. pneumoniae*, to be confirmed by optochin testing.

identification from a range of GLLS possibilities included serogrouping (beta-hemolytic strains), optochin susceptibility, bile solubility (*S. pneumoniae*), esculin hydrolysis, starch hydrolysis, and fermentation of inulin, melibiose, and glucan formation (*S. bovis* and viridans streptococci). No such information, however, is provided by GPI at this time.

Major discrepancies between API or GPI and primary identifications centered mainly around the viridans group. Of particular note was the misidentification of six of eight *S. mitis* strains as *S. pneumoniae*. Most incorrect identifications of the viridans group by API were read as other viridans streptococci. Only three organisms yielded codes which could not be identified with the current API data base. With the exception of two *S. mitis* strains identified as *Corynebacterium haemolyticum*, all other misidentifications by GPI were read as other species of the genus *Streptococcus*. Some beta-hemolytic strains were misidentified by GPI as viridans species; misidentified strains were usually identified as other viridans species. Eleven strains could not be identified by GPI, even after repeat testing.

DISCUSSION

Previous reports (5, 8) of the capability of API to accurately identify streptococci utilized data bases which have subsequently been enlarged and updated. Keville and Doern (5) reported excellent identification rates of group A strains, with less satisfactory classification of group B strains; other beta-hemolytic streptococci were identified with various degrees of accuracy as not group A, B, or D. In the current study, identification rates of group A and B organisms were excellent; however, despite an updated data base, most non-A or B beta-hemolytic strains still required serogrouping for accurate identification. Nachamkin et al. (8) evaluated API for species identification of alpha- and nonhemolytic streptococci (including group D and *S. pneumoniae*). Excellent identification rates of *S. faecalis* were observed; however, when these were excluded from the data, only 19.7% of strains yielded correct identifications. Our results parallel those of Nachamkin et al. (8) in excellent identification of group D strains, fair identification of *S. pneumoniae*, and unsatisfactory identification of the viridans group (the latter despite a further updated data base). More than 50% of viridans strains required additional tests for accurate species identification, and many strains yielded codes not in the current book. Improvements in API which could lead to improved identification of alpha-hemolytic streptococci include the modification of biochemical tests to obviate the current subjective interpretive variability and the enlargement of the data base.

Results of GPI streptococcal identification were generally similar to those reported by Ruoff et al. (9), with good to excellent identification of groups A, B, and D and *S. pneumoniae*, but lower rates of identification of the viridans group. The importance of a uniformly turbid inoculum with which to inoculate GPI is stressed. In the current study, spontaneous clumping occurred in ca. 10% of strains. If vortexing alone is carried out instead of vigorous mixing of the suspension with a cotton swab, clumping may be observed, which could yield falsely incorrect results. Several beta- and alpha-hemolytic strains yielded incorrect identifications with clumped suspensions but correct identifications when uniformly turbid inocula were used. Approximately 5% of the strains could not be adequately suspended; all yielded incorrect results. An easy and reliable method for obtaining uniformly turbid inocula from organisms with a clumping tendency will probably improve the identification capacity of GPI. Clumping was not a major factor with API; identification rates of organisms with a clumping tendency were similar to those yielding uniform suspensions. Eleven organisms yielded no identification with GPI, pointing to the need for an enlarged data base. More accurate statistical data for organisms with probability cutoffs of 50 to 90% should be provided by the manufacturer. Perhaps the concomitant provision of frequency rates in conjunction with probability statistics may clarify this problem. Additionally, in cases in which percentages are low, a printout of additional tests may be useful. At present, the use of ≥80% probability is suggested rather than the use of >50% as currently recommended.

Although the data bases of both API and GPI are predicated upon the conventional sugar methodology of Facklam (2, 3), we believe that the Minitek disk method employed by us for sugar fermentation has been amply shown to be accurate, reproducible, and reliable. Holloway et al. (4) reported excellent correlations between Minitek and the conventional sugar methodology, with only minor differences which did not affect the accuracy of identifications. Setterstrom and colleagues (12) reported that, by incubating Minitek sugar fermentation disks anaerobically for 48 h with the utilization of 2 to 3 drops of a phenol red indicator, an overall agreement of 98.9% between Minitek and the conventional methodology was observed. By applying the Facklam key (2) to results obtained with either conventional media or Minitek, 100% of oral streptococci were correctly identified. Under conditions described above, the substitution of conventional sugars with bromcresol purple indicator (2) by Minitek disks with phenol red indicator did not alter the reliability of the Facklam key for accurate streptococcal species identification (12). Ruoff and Kunz (10) reported agreement rates of between 98 and 100% for sugar fermentation tests with Minitek compared with conventional methodology, using the method of Setterstrom et al. (12), as described above. Similarly, we tested 45 strains utilized in the current study for fermentation of lactose, mannitol, and raffinose in duplicate with Minitek and the conventional methodology; correlation rates of 98, 98, and 100% were observed for each of the three sugars, respectively. Although key reactions as described by Sands et al. (11) were used for primary identification of viridans streptococci, all tests used for identification of group D organisms (growth in 6.5% NaCl, starch hydrolysis, and fermentation of sorbitol and arabinose) as well as sucrose fermentation were also used in viridans streptococcal testing, and the results were checked against standard tables (2, 3). In all cases, the additional tests corroborated the identification according to Sands et al. (11). The method of Sands et al. (11) is not really a modification of that of Facklam (2) in that reactions were not changed or modified, but key reactions with a high degree of discrimination were selected for the identification of viridans strains. For reasons stated above, we are therefore confident of our identification of the viridans strains.

Both API and GPI yield same-day streptococcal identification; API provides results in 4 h, and GPI provides results in 4 to 13 h (average, ca. 8 h). Both methods clearly represent a reduction in the amount of time required to perform conventional identification tests for viridans and group D streptococci (2 to 4 days). The advantages of API and GPI for the identification of beta-hemolytic strains and pneumococci are questionable, since convenient, cheaper, and more rapid methods already exist for the identification of these strains. Neither system accurately identifies non-A or B beta-hemolytic strains.

In summary, both API and GPI have potential use for the rapid identification of streptococci. Both methods provide good identification of group A, B, and D strains and reasonable identification of *S. pneumoniae* without additional tests. Both techniques, however, are currently unsatisfactory for accurate species identification of viridans strains. Modifications of these systems as discussed above may well improve their performance for accurate, rapid differentiation of the viridans group.

ACKNOWLEDGMENTS

This study was supported in part by grants-in-aid from Analytab Products and Vitek Systems, Inc.

We thank David Shlaes for providing some of the cultures and for helpful discussion and Kathryn Ruoff for helpful discussion.

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