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Comparative Evaluation of the API 20S and AutoMicrobic Gram-Positive Identification Systems for Non-Beta-Hemolytic Streptococci and Aerococci

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The API 20S system (Analytab Products, Plainview, N.Y.) and the AutoMicrobic Gram-Positive Identification system (GPI; Vitek Systems, Hazelwood, Mo.) were evaluated for their capacity to identify the non-beta-hemolytic streptococci and aerococci to the species level. The 20S system identified 86% (six of seven strains) of nonhemolytic group B streptococci, whereas 100% of the same group B streptococcal strains were correctly identified by the GPI system. With both systems 99% (134 of 135 strains) of four species of group D enterococcus strains and 92% (24 of 26 strains) of the *Aerococcus* spp. strains were identified. The 20S system identified 84% (41 of 49 strains) of three species of group D non-enterococcus strains. The GPI system identified 96% of the same group D non-enterococcus strains. The 20S system identified 84% (190 of 226 strains) of 10 species of viridans streptococci; however, supplemental conventional tests were required to identify 49% (110 of the 226 strains) of the viridans strains to the species level. The GPI system identified 79% of the same viridans streptococci without the need for supplemental tests. Both systems identified 84% (161 of 192 strains) of the seven most commonly occurring viridans *Streptococcus* spp. The 20S system identified 82% (75 of 92 strains) and the GPI system identified 84% (54 of 64 strains) of *Streptococcus pneumoniae*.

Serological grouping of beta-hemolytic streptococci has proven to be a very convenient and useful procedure for differentiating these bacteria into pathogenic categories. Armed with the knowledge of the serogroup of the *Streptococcus* sp. the physician can prescribe proper antimicrobial therapy for streptococcal infection. However, serogrouping is not nearly so useful for differentiating the non-beta-hemolytic streptococci. Knowledge of the presence or absence of group D streptococci, which are more often non-beta-hemolytic than beta-hemolytic, is of limited value because six different species may possess the group D antigen. In addition, some group D species are resistant to some antimicrobial agents (*Streptococcus faecalis*, *Streptococcus faecium*), whereas others are not (*Streptococcus bovis*). The Quellung reaction, a serological test for identification of *Streptococcus pneumoniae*, is a useful procedure, but is expensive and time consuming; therefore, most microbiologists have adopted non-serological methods for differentiating the pneumococci from the viridans streptococci. Identification of the aerococci and the non-beta-hemolytic streptococci (group D and viridans streptococci) into species is achieved by determining physiological characteristics of these bacteria.

The schemes for differentiating the aerococci and non-beta-hemolytic streptococci are based on conventional microbiological tests with media contained in test tubes and agar plates (3, 4). These numerous (20 to 30) conventional tests require 3 to 7 days of incubation; thus the identification of species by conventional techniques is time consuming and has little or no influence on the management of acute streptococcal infections. Therefore, it is desirable that more convenient and yet accurate systems for identifying the non-beta-hemolytic streptococcal species be developed.

Several miniaturized or rapid (or both) identification systems for the streptococci are now commercially available. The Minitek system (BBL Microbiology Systems, Cockeysville, Md.) incorporates various substrates for enzyme reactions into paper disks. The disks are placed into wells in plastic plates, and the inoculum is then introduced into each well. The manufacturer suggests that the plate be incubated anaerobically if viridans streptococci are suspected. The reported results have indicated a high degree of correlation between the Minitek and conventional tests when the substrates are essentially the same (7, 15). However, some tests (esculin and arginine) had to be incubated 5 to 7 days. Thus the system is miniaturized, but not necessarily rapid.

The API 20S system (Analytab Products, Ayerst Laboratories, Plainview, N.Y.) and the API Rapid Strep system (DMS Laboratories, Inc., Flemington, N.J.) are similar. Although the tests contained in the two systems are not exactly the same, both have 20 tests and are read in similar fashion. The API 20S system has a 4-h incubation period, and the Rapid Strep system has 4- and 24-h incubation periods. In the latter system, if identification is not achieved at the 4-h reading, the user is instructed to incubate the test overnight. The dehydrated substances for both systems are incorporated into microcupules attached to stiff cardboard holders (strips). The inoculum rehydrates the substances in the tests. Generally, published results have indicated both systems identify the beta-hemolytic streptococci reasonably well, but serological confirmation is necessary for some serogroups (1, 2, 8, 17; R. R. Facklan, D. L. Rhoden, and P. B. Smith, J. Clin. Microbiol., in press). Both systems also identify the group D species with a high degree of accuracy (95 to 98%) (1, 2, 10, 17; Facklam et al., in press). However, not all group D species have been tested with the API 20S system. The rates of identifying the viridans streptococci to the species level vary between 39 and 85% (1, 2, 10, 14, 17; Facklam et al., in press).

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The AutoMicrobic system (AMS) (Vitek Systems, Inc., Hazelwood, Mo.) is also currently available for identifying streptococci. The Gram-Positive Identification (GPI) Card, one component of the system, is designed to identify several other common gram-positive bacteria as well as streptococci. The card contains dehydrated substances for 27 tests and two controls. The inoculum, which is automatically inserted into the test card by the AMS inoculator module, rehydrates the substances for the tests. The GPI cards are then placed in an AMS reader-incubator module; computer-assisted identification is possible after 4 h, but some strains require the full 13-h incubation period. Investigators have reported fairly good identification rates for beta-hemolytic group A and B streptococci, but the GPI identification of the other beta-hemolytic streptococci has been less successful (2, 13). The identification of the group D *Streptococcus* spp. by the GPI system, although limited to four of the seven species, has been reported to be about 95% accurate (2, 13). The identification rates for the differentiation of viridans *Streptococcus* spp. have been much lower; however, some species were quite accurately identified (*Streptococcus mitis*, 100% [2] and 79% [13]), whereas others were very poorly identified (*Streptococcus sanguis* I, 29% [2] and 43% [14]).

This study is a comparative evaluation of the GPI and API 20S systems using representative strains of non-beta-hemolytic streptococci and aerococci found in human infections. We have examined strains not included in other studies (aerococci, nonhemolytic *Streptococcus agalactiae*, *Streptococcus avium*, an *S. bovis* variant, and *Streptococcus acidominimus*) and used more strains of the infrequently occurring streptococci than have other investigators.

MATERIALS AND METHODS

Strains. All bacteria were selected from the culture collection of the Streptococcus Laboratory, Centers for Disease Control, Atlanta, Ga. All strains were clinical isolates from infected persons. None of the strains, however, was from a respiratory source. Strains were reidentified by serological (Lancefield extraction, capillary precipitin testing) and biochemical procedures (5). Strains were tested for susceptibility to vancomycin by a procedure similar to that used for determining susceptibility to bacitracin or optochin (5). A nonstandardized heavy inoculum was spread over one-half of a 5% sheep blood–Trypticase soy agar plate (BBL) with a wire loop or cotton swab to achieve confluent growth. A 30-μg vancomycin disk was placed in the center of the inoculum, and the plates were incubated for 16 to 18 h in a candle extinction jar at 35°C. Only strains that showed zones of inhibition (12 to 30 mm) when tested in this manner were used in the study. Seven nonhemolytic group B, 184 group D, and 226 viridans streptococci and 26 *Aerococcus* sp. strains were tested in both the GPI and 20S systems; 92 and 64 strains of *S. pneumoniae* were tested in the 20S and GPI systems, respectively. The 64 pneumococcal strains used in the GPI portion of the study were not part of the 92 strains used in the 20S part of the study.

Inoculum. Inocula for both the 20S and GPI systems were prepared from the growth on separate sheep blood-Trypticase soy agar plates that had been incubated for 16 to 20 h in a candle extinction jar at 35°C. Five milliliters of Todd-Hewitt broth was inoculated from one of the sheep blood-Trypticase soy agar plates and was incubated for 16 to 18 h at 35°C under a normal atmosphere. The Todd-Hewitt broth was used to inoculate the conventional biochemical media (5). All strains were coded to insure unbiased identification by any system. The results of conventional tests and identification were unknown until after 20S or GPI identifications were determined.

API 20S identification. A suspension of cells equivalent to a McFarland no. 1 was prepared in 0.85% sterile saline from growth on the sheep blood-Trypticase soy agar plate. This suspension was used to inoculate the 20 cupules in the strip. The strips were placed in a tray, covered, and incubated for 4 h at 35°C in a normal atmosphere. The tests and interpretation criteria of tests are described in detail in the manufacturer's package insert and other reports (2, 10). Identification profile numbers were constructed from the test results in the 20S strips according to the manufacturer's instructions. These numbers were decoded for species identification by using the manufacturer's code book. If a number did not appear in the code book, it was sent to the Analytab computer center for analysis. If the 20S identification was correct, i.e., correlated with the conventional identification, at the excellent, very good, good, or acceptable level, no further testing was done. If the 20S identification was incorrect, i.e., failed to correlate with conventional identification, at these same four identification levels, the strains were retested in the 20S system. If the identification errors were repeated, they were counted as errors; if the identification errors did not repeat, the strain was reisolated and reidentified by the conventional test system and then retested in the 20S system. This procedure was repeated until a correct or incorrect identification was duplicated by the 20S system. If the 20S identification was given at the good likelihood, low selectivity level (GL) and the correct identity of the strain was included in one of the first four choices, we constructed a battery of conventional tests that would differentiate all (up to four) of the choices listed in the code book or the Analytab computer center analysis. We do not feel that the conventional tests suggested in the Analytab code book are as useful as those that we used (5). The tests that we chose were based on the species listed as identification choices at the GL level (see below). We used the supplemental conventional test results for all GL identifications even when the same tests were included in the 20S system, since there is not a good correlation between similar tests in the conventional and the 20S systems. If the GL listing did not contain the name of the species tested, the result was treated as an error, and the strain was retested as described above. If the 20S identification was an unacceptable profile number or possible insufficient growth, the 20S test was repeated. If the identification repeated as unacceptable growth, it was counted as an error. If a correct identification resulted from the retest, it was included in the correct tally, and the first identification was discarded. Tests on the pneumococcal strains were not repeated, although tests on strains of all other species were repeated if necessary.

AMS GPI identification. A suspension of cells equivalent to a McFarland no. 0.5 was made in 0.45% saline; a heavier suspension (no. 1 McFarland) was made for the retest. The bacterial suspension was placed in the AMS inoculator module for inoculation of the GPI card. After inoculation the GPI card was placed in the AMS incubator-reader, and results were recorded onto floppy disks by the AMS reader. The floppy disks were then sent to the Vitek computer center for analysis. Identifications with probability levels greater than 50% were considered correct, whereas identifications with probabilities less than 50% were considered incorrect. Three other identification categories were recognized: unidentified, insufficient growth, and overincubation. The tests for all strains with these results were repeated. If

TABLE 1. Number of group B and D *Streptococcus* spp. and *Aerococcus* spp. strains identified at various levels by the API 20S and AMS GPI systems

Species (*n*)	Identification level[a]										Errors	
	E	90 to 99%	VG	80 to 89%	G	70 to 79%	A	60 to 69%	GL	50 to 59%	20S	GPI
S. agalactiae (7)	0	5	1	1	3	1	1	0	1	0	1	0
S. faecalis (63)	58	61	2	1	2	0	0	0	0	1	1	0
S. faecium (53)	19	51	0	0	21	0	2	1	11	0	0	1
S. durans (4)	0	4	0	0	0	0	1	0	3	0	0	0
S. avium (15)	13	14	1	0	0	0	1	0	0	1	0	0
S. bovis I (26)	8	25	0	0	7	0	6	0	1	0	4	1
S. bovis II (22)	0	13	0	5	0	0	8	3	11	0	3	1
S. equinus (1)	0	1	0	0	0	0	0	0	0	0	1	0
Aerococcus spp. (26)	6	19	3	3	3	1	3	0	9	1	2	2

[a] API identifications: E, excellent; VG, very good; G, good; A, acceptable. The GPI identifications are given as confidence levels.

the repeat test resulted in a report of unidentified or insufficient growth, it was counted as an error. A report of overincubation was not counted as an error because this result was only due to technical error. Strains that were incorrectly identified at probabilities greater than 50% were retested in the GPI system. If the same species identification resulted, regardless of probability level, it was counted as an error. If a new species identification, correct or incorrect, was made from the repeat test, the test was repeated the second time after reidentification by the conventional system. In this manner all strains that were incorrectly reported after the first testing were retested until the errors or correct identifications were duplicated. None of the 64 strains of pneumococci was retested, regardless of the first test result.

The tests incorporated in the GPI card are described in the manufacturer's package insert and elsewhere (2, 13).

Nomenclature. Valid bacterial species names (6, 16) will be used in this manuscript rather than those we, as well as the API and AMS systems, have previously used. Only two changes have resulted; strains previously termed *Streptococcus* MG-*intermedius* and *Streptococcus anginosus-constellatus* will be called *S. intermedius* and *S. constellatus*, respectively (6).

RESULTS

Twelve of the bacterial strains selected for study were omitted after the vancomycin testing; these included nine strains (4%) of viridans streptococci, two strains (1%) of group D streptococci, and one *Aerococcus* sp. strain (4%), which were found to be vancomycin resistant.

The identification levels for 7 nonhemolytic *S. agalactiae*, 184 group D streptococci, and 26 *Aerococcus* spp. strains are listed in Table 1. The 20S system identified six of seven strains, whereas the GPI system identified all seven strains of *S. agalactiae*. One strain of *S. agalactiae* required additional testing for correct identification by the 20S system.

Overall 95 and 98% of the 184 strains of group D streptococcal species were correctly identified to the species level by the 20S and GPI systems, respectively. The level of identification varied considerably for the strains identified by the 20S system. By this system 53, 2, 16, 10, and 14% of the group D strains were identified at the excellent, very good, good, acceptable, and GL levels, respectively. Ninety-two percent of the group D strains were identified at the 90 to 99% confidence level by the GPI system. All strains listed in the GL column required two or three supplemental tests for correct identification by the 20S system. None of the strains listed in Table 1 required supplemental testing for identification by the GPI system.

Twenty and three strains of group D streptococci were retested in the 20S and GPI systems, respectively, because the initial identification was erroneous or insufficient growth occurred. Nine of the 20 identifications made on retesting with the 20S systems duplicated the original error, but 11 strains were correctly identified by the retesting procedure. All three strains retested in the GPI system duplicated the original error.

Both systems correctly identified 24 of 26 (92%) of the *Aerococcus* spp. strains; however, 9 strains required supplemental tests for identification by the 20S system.

Eight and two *Aerococcus* spp. strains were retested in the 20S and GPI systems, respectively, because the initial identification was erroneous. Two strains each were erroneously identified (duplicated) by the 20S and GPI systems. However, six of the eight strains retested by the 20S system were correctly identified by the retesting procedure.

The identification levels for 226 viridans streptococcal species and 156 *S. pneumoniae* strains are listed in Table 2. Overall, 84 and 79% of the viridans strains were correctly identified by the 20S and GPI systems, respectively. There were considerable differences in both the level of identification by each system and the correct identification of each species. By the 20S system, 4, <1, 9, 22, and 49% of the viridans strains were identified at the excellent, very good, good, acceptable, and GL levels, respectively. On the other hand, 60, 11, 7, 7, and 4% of the viridans strains were identified at the 90 to 99%, 80 to 89%, 70 to 79%, 60 to 69%, and 50 to 59% confidence levels, respectively, by the GPI system. The 20S system correctly identified 100% of the *Streptococcus mutans* and *Streptococcus salivarius* strains, whereas the GPI system identified 88 and 80%, respectively, of these same species. On the other hand, the GPI system correctly identified 100% of the *S. mitis* and 92% of the *S. sanguis* I strains, whereas the 20S system identified 92 and 76%, respectively, of these same species. Both systems were less accurate in identifying strains of *Streptococcus intermedius* (20S, 74%; GPI, 70%) and *Streptococcus constellatus* (20S, 68%; GPI, 64%). The GPI system was inaccurate in identifying *Streptococcus morbillorum* (52%) and *S. acidominimus* (0 of 5 strains), whereas the 20S system identified 86 and 80%, respectively, of these same species.

Fifty-eight (26%) and 74 (33%) of the viridans streptococci were retested in the 20S and GPI systems, respectively, because the initial identifications was erroneous or insufficient growth occurred. Thirty-six of the 58 (62%) viridans strains gave repeat erroneous 20S identifications upon re-

TABLE 2. Number of viridans species and pneumococcal strains identified at various levels by the API 20S and AMS GPI systems

Species (*n*)	Identification level[a]										Errors	
	E	90 to 99%	VG	80 to 89%	G	70 to 79%	A	60 to 69%	GL	50 to 59%	20S	GPI
S. uberis (8)	4	5	1	0	1	1	0	1	1	0	1	1
S. mutans (25)	0	16	0	1	11	2	6	1	8	2	0	3
S. sanguis I (25)	0	7	0	8	0	1	2	4	17	3	6	2
S. salivarius (25)	0	4	0	9	0	3	3	3	22	1	0	5
S. intermedius (42)	2	25	0	3	5	4	14	0	10	1	11	9
S. sanguis II (25)	0	17	0	1	1	2	2	1	18	1	4	3
S. mitis (25)	0	21	0	0	0	1	1	2	22	1	2	0
S. constellatus (25)	0	11	0	3	0	1	10	1	7	0	8	9
S. morbillorum (21)	0	8	0	0	1	0	12	2	5	1	3	10
S. acidominimus (5)	3	0	0	0	1	0	0	0	0	0	1	5
S. pneumoniae (92/64)	0	47	0	2	32	0	33	1	10	4	17	10

[a] See footnote *a* to Table 1.

testing. The remaining 22 strains gave correct 20S identifications when retested. Forty-seven of the 74 (64%) viridans strains gave the same erroneous identifications when retested, but 27 strains were correctly identified when retested in the GPI system.

The 20S system identified 82% of 92 strains of *S. pneumoniae*, and the GPI system identified 84% of 64 strains of *S. pneumoniae*. Ten and four strains were identified correctly at the GL and 50 to 60% confidence levels by the 20S and GPI systems, respectively; optochin susceptibility was suggested for correct identification by both systems at these levels.

Twenty-nine percent (156) of 535 strains tested in the API 20S system required supplemental testing because the level of identification (GL) was not high enough to be accepted as correct. To achieve correct identification we constructed batteries of conventional tests that differentiated the species listed as choices resulting from the 20S testing. Table 3 is a list of all 156 strains that were correctly identified at the GL level by the 20S system. The majority of correct identifications, 104 of 156 (67%), were first-choice identifications; however, 21% were second choices, 10% were third choices, and 4% were fourth choices. The number of differential tests we selected to run was determined by the number of choices listed. If only two choices were listed, only one test was necessary to differentiate. For example, the choices listed at the GL level for *S. faecium* and *Streptococcus durans* were either *S. faecium* or *S. durans* (first or second). Acid formation in arabinose broth was the only conventional test necessary to differentiate *S. faecium* (arabinose positive) from *S. durans* (arabinose negative). When three choices were listed as possible correct identifications, two or three conventional tests were necessary for differentiation. For example, the *S. bovis* II strains were correctly identified at the GL level, but as the third choice required acid formation in mannitol and inulin broths because the three choices were *S. mutans*, *S. salivarius*, and *S. bovis* II. *S. mutans* is the only strain of the three to form acid in mannitol broth; *S. salivarius* forms acid in inulin broth, but *S. bovis* II strains do not. Glucan formation could have been used because the three strains generally produce different reactions on 5% sucrose agar. *S. mutans* strains produce dextrans, *S. salivarius* strains produce levans, and *S. bovis* II strains do not produce either dextran or levans. The column listing other first-choice identifications is given to provide evidence that the first-choice identifications made by the 20S system at the GL level are not necessarily correct. For example, eight *S. mutans* strains were correctly identified at the GL level (first choice); however, one *Aerococcus* sp., two *S. salivarius*, two *S. sanguis* I, and three *S. sanguis* II strains were also identified as *S. mutans* (first choice). Listing all of the possible batteries of tests is not practical, but the tests to choose for making all the correct differentiations can be obtained from Table 3 in reference 5.

One hundred and twenty-seven errors are listed in Tables 1 and 2. Excluding the 27 errors in identifying the pneumococcal strains, which were not duplicated, 100 errors were made for the remaining 443 streptococcal strains. A total of 48 errors were made by the 20S system, and 52 errors were made by the GPI system. Only 16 strains were misidentified by both rapid systems, and 3 of these were identified as

TABLE 3. Number of strains giving the GL correct identification by the API 20S system

Conventional identification	No. as choice at the GL level				Other first choices (*n*)
	First	Second	Third	Fourth	
S. agalactiae	1	0	0	0	
S. faecium	11	0	0	0	
S. durans	1	2	0	0	*S. faecium* (2)
S. bovis I	1	0	0	0	
S. bovis II	10	0	1	0	
Aerococcus sp.	5	1	3	0	*S. uberis* (2) *S. mutans* *S. salivarius*
S. mutans	8	0	0	0	
S. salivarius	14	7	1	0	*S. bovis* II (3) *S. mutans* (2) *S. sanguis* I *S. intermedius*
S. sanguis I	7	7	1	2	*S. constellatus* (5) *S. intermedius* (3) *S. mutans* (2)
S. intermedius	6	4	0	0	*S. constellatus* 2 *S. sanguis* I *S. sanguis* II
S. sanguis II	6	4	6	2	*S. morbillorum* (3) *S. mutans* (3) *S. pneumoniae* (2) *S. mitis* (2) *S. sanguis* I *S. uberis*
S. mitis	15	3	2	2	*S. morbillorum* (4) *S. pneumoniae* (2) *S. constellatus*
S. constellatus	7	0	0	0	
S. morbillorum	4	1	0	0	*S. mitis*
S. uberis	1	0	0	0	
S. pneumoniae	7	3	0	0	*S. mitis* (2) *S. morbillorum*

unsatisfactory growth. Table 4 lists the identification errors made by both systems. Identification of streptococci and aerococci is categorized to be most informative for patient management because of differences in virulence and antimicrobial susceptibility (see below). The majority of identification errors by both systems were made for the viridans streptococci; 28 strains of viridans species were identified as other viridans species by both systems. More errors in identification of the viridans streptococci were made by the GPI system than the 20S system because of unsatisfactory growth; 13 versus 4. The majority of errors in identification of the pneumococci by both systems was the result of unsatisfactory profile numbers (20S) and insufficient growth (GPI).

DISCUSSION

Partially completed studies in our laboratory suggest that the viridans streptococci are susceptible to the 30-μg vancomycin disk. We believe that the susceptibility to vancomycin can be used to screen for streptococci; i.e., if a resistant bacterial strain is found, the microbiologist should be aware that it is probably not a streptococcus. Additional studies are underway to test this hypothesis.

It is important that the miniaturized rapid test systems be evaluated with all streptococcal strains that may be encountered in a clinical laboratory. In this evaluation we have included many strains of each of the commonly encountered streptococci and atypical strains of the non-beta-hemolytic streptococci. The development of convenient, rapid serogrouping kits for the identification of beta-hemolytic streptococci precludes these rapid miniaturized systems from being useful procedures for identifying the beta-hemolytic streptococci. These systems will be more useful if, in fact, they can differentiate the different species of streptococci possessing common group antigens. We have reported the evaluation of the Rapid Strep system for this capability, but only a few strains of each species were included (Facklam et al., in press).

To our knowledge this is the first report that includes nonhemolytic *S. agalactiae* strains in an evaluation of the 20S and GPI identification systems. Our results indicate that the GPI system is equal to the Rapid Strep system (Facklam et al., in press) for identifying the nonhemolytic *S. agalactiae* strains. The 20S system failed to identify one of seven strains tested.

TABLE 4. Erroneous identification of non-beta-hemolytic streptococci by the API 20S and AMS GPI systems

Correct identification category	No. in the following incorrect identification category[a]				
	Group D entero-cocci	Group D non-entero-cocci	Viri-dans	Pneumo-cocci	Unsatis-factory
S. agalactiae	0/0	0/0	1/0	0/0	0/0
Group D enterococci	1/0	0/1	0/0	0/0	0/0
Group D non-enterococci	0/0	4/0	3/2	0/0	1/0
Aerococci	0/0	0/0	0/0	0/1	2/1
Viridans	1/0	1/4	28/28	2/2	4/13
Pneumococci	0/0	0/0	6/0	0/0	11/10

[a] No incorrect identification calls were made as *S. agalactiae* or aerococci. The data are given as (incorrect identification by 20S)/(incorrect identification by GPI).

Our results with the enterococcal group D streptococci are similar to those reported by others (2, 10, 13). Nearly all strains (95 to 99%) of *S. faecalis*, *S. faecium*, *S. durans*, and *S. avium* are identified to the species level with the API 20S, AMS GPI, and Rapid Strep systems. *S. avium* strains, not included in other studies (2, 8, 10, 13), were identified 100% of the time by the 20S and GPI systems as well as by the Rapid Strep system (Facklam et al., in press).

It is important that any system used to differentiate streptococci provide an accurate identification of *S. bovis* strains, since the isolation of typical *S. bovis* strains (bovis I) from the blood stream is being used as a potential indicator of colonic cancer (9, 12). It is unknown at this time whether *S. bovis* variant strains (*S. bovis* II) can be used for this same association. Other investigators have not reported that they included the *S. bovis* II strains in their studies, and it is difficult to interpret whether they attempted to differentiate *S. bovis* I strains from *S. bovis* II strains by conventional methods. In three studies *S. bovis* I was identified 100% of the time by the Rapid Strep system (1, 7; Facklam et al., in press), but only 21% accuracy was reported from another study (11). In the last study, however, the investigators used strains isolated from cases of bovine mastitis, and human strains were not included in their study. Nonhuman strains of *S. bovis* may be physiologically different from human strains and thus be refractory to identification by the rapid systems. We reported a 91% accurate identification rate for the *S. bovis* II strains by the Rapid Strep system (Facklam et al., in press). Applebaum et al. (2) reported that the 20S system identified five of six strains of *S. bovis*, but Nachamkin et al. (10) reported that neither of the two strains included in his evaluation of the 20S system were identified. In this study, the 20S system identified 85% of the *S. bovis* I and 86% of the *S. bovis* II strains. However, 12 of the 48 strains (25%) needed supplemental testing for final identification. Applebaum et al. (2) also reported that the GPI system identified all six *S. bovis* strains tested. However, Ruoff and co-workers (13) reported that only two of five *S. bovis* strains were identified by the GPI system. The GPI system in our study accurately identified both *S. bovis* I (96% of 26 strains) and *S. bovis* II (95% of 22 strains). We can conclude that all three systems can be used to identify both *S. bovis* I and *S. bovis* II strains, but supplemental tests are necessary with the 20S system for accurate identification, especially for the *S. bovis* II strains.

Other investigators have not included *Aerococcus* spp. strains in their studies. Both the 20S and GPI systems identified 92% of the 26 strains tested. The Rapid Strep system identified only 6 of 10 (60%) of the *Aerococcus* spp. strains tested (Facklam et al., in press).

Our results of testing the API 20S, AMS GPI, and Rapid Strep systems indicate that reporting an overall identification rate for these systems is misleading, because some species are identified more accurately than others by each system. This is especially apparent for the viridans species, since overall accuracy could be influenced by the number of individual species tested. In our studies, we used supplemental tests to achieve final identification of the strains tested in the 20S system, but other investigators have not. This is probably why we achieved an identification rate of 85% for the viridans streptococci, whereas others have reported 12.6% (2) and 16% (10) identifications rates to species level by the 20S system.

It is also important to consider that in this study where the protocol required retesting of all strains that gave erroneous identifications, that nearly 10 and 12% of the correct identi-

fications were achieved by the 20S and GPI systems, respectively, only after retesting. Our results indicate that supplemental testing is necessary for about 50% of the viridans streptococci when the 20S system is used. Only 35% of the viridans strains were correctly identified without additional testing.

Two other investigators have evaluated the GPI system with viridans streptococci. Applebaum et al. (2) reported that 57% of 119 viridans strains and Ruoff et al. (14) reported that 63% of 132 viridans streptococci were correctly identified by the GPI system. We found that 79% of 226 viridans strains were correctly identified by the GPI system. The only difference in the three studies is the use of different conventional identification systems. We used standard microbiological tests (4, 5), whereas both Applebaum et al. and Ruoff et al. used the Minitek system for conventional identification. Although there is good correlation between conventional tests and Minitek (7, 15), it is not 100%. This could lead to misidentification by conventional means and thus lead to erroneous correlations to the GPI identification.

The identification rates for the viridans streptococci by the Rapid Strep system compare favorably with the rates by the 20S and GPI systems. Waitkins et al. (17) reported a 99% identification rate for 97 strains of five different species. Ruoff and Kunz (14) tested 119 strains of seven different viridans species and reported a 92% identification rate. Applebaum et al. (1) tested 90 strains of eight different viridans species and reported an 85% identification rate. Our previous studies with the Rapid Strep system included 96 strains of 10 different viridans streptococcal species (Facklam et al., in press). Overall, 70% of the strains were correctly identified, whereas 85% of the seven most commonly occurring species could be correctly identified, and only 10% of the three less commonly occurring species were identified. As stated earlier, the overall identification rates are different for different species and numbers of each species tested. If we analyze our data from the evaluations of the three systems for just the seven most commonly occurring viridans species (*S. constellatus*, *S. intermedius*, *S. mitis*, *S. mutans*, *S. salivarius*, *S. sanguis* I, and *S. sanguis* II), we find that the API 20S system identified 84% of 192 strains tested, and the AMS GPI system identified exactly the same percentage of the same strains. These figures are comparable to the Rapid Strep system (85% of the same seven species). Thus, under our test conditions, there was no real difference in overall identification rates by any of the three systems. Supplemental testing is required by the 20S system, but it is not required by either the Rapid Strep or the GPI system.

The majority of errors in the identification of pneumococci by both the 20S and GPI systems were due to the failure of strains to cause changes in the tests in the systems. The 20S system identified 6 of the 92 pneumococcal strains as viridans species (three as *S. sanguis* II, two as *S. morbillorum*, and one as *S. mitis*). The GPI systems identified three pneumococcal strains as *S. acidominimus*, but at a very low confidence level (<50%), and instructed the user to perform optochin susceptibility for possible *S. pneumoniae*.

Very few errors by either the 20S or the GPI would have caused a change in patient management if the physician were deciding treatment on identification of the streptococcus strains. The 20S system identified one nonhemolytic group B streptococcus as a viridans species. The GPI identified one enterococcus (*S. faecium*) as a group D non-enterococcus (*Streptococcus equinus*). This could also lead to mismanagement if susceptibility testing was not done because the enterococci are much more resistant to antimicrobial agents than are non-enterococcal streptococci. One *Aerococcus* sp. strain and two viridans strains were identified as *S. pneumoniae* by the GPI system. The 20S system also identified two viridans strains as *S. pneumoniae*. This is not as potentially serious as the six pneumococcal strains identified as viridans species. *S. pneumoniae* identified as viridans streptococci may lead to undertreatment of patients.

We attempted to correlate the errors made by the 20S and GPI identification systems to atypical biochemical characteristics of certain strains included in the study. Ninety-seven of 410 strains of group D and viridans streptococci tested in this study had one or more atypical biochemical characteristics. Thirty-five of the 97 strains (36%) with aberent characteristics and 40 of the 313 typical strains (13%) were misidentified. These results indicate that strains with atypical biochemical characteristics are more likely to be misidentified than are strains with typical features. No pattern of misidentification of strains according to atypical characteristic could be detected. These results imply that the data bank for the rapid systems could be improved.

In conclusion, the results from our comparative study indicate very few differences between the overall streptococcal identification rates with the 20S and GPI systems. Each system has advantages and disadvantages that must be assessed by the potential user. The 20S system is more convenient, but it requires more conventional testing than does the GPI system. The GPI system requires very little supplemental testing, but requires expensive equipment (inoculation and incubator-reader modules).

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LITERATURE CITED

1. **Applebaum, P. C., P. S. Chaurushiya, M. R. Jacobs, and A. Duffett.** 1984. Evaluation of the rapid Strep System for species identification of streptococci. J. Clin. Microbiol. **19**:588–591.
2. **Applebaum, P. C., M. R. Jacobs, J. I. Heald, W. A. Palko, A. Duffett, R. Crist, P. A. Naugle.** 1984. Comparative evaluation of the API 20S System and the AutoMicrobic System Gram-Positive Identification card for species identification of streptococci. J. Clin. Microbiol. **19**:164–168.
3. **Colman, G., and R. E. O. Williams.** 1972. Taxonomy of some human viridans streptococci, p. 281–299. *In* L. W. Wannamaker and J. M. Matsen (ed.), Streptococci and streptococcal diseases, recognition, understanding, and management. Academic Press, Inc., New York.
4. **Facklam, R. R.** 1977. Physiological differentiation of viridans streptococci. J. Clin. Microbiol. **5**:184–201.
5. **Facklam, R. R.** 1980. Streptococci and aerococci, p. 88–110. *In* E. H. Lennett, A. Balows, W. J. Hausler, and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
6. **Facklam, R. R.** 1984. The major differences in the American and British *Streptococcus* taxonomy schemes with special reference to *Streptococcus milleri*. Eur. J. Clin. Microbiol. **3**:91–93.

6a. **Facklam, R. R., D. L. Rhoden, and P. B. Smith.** 1984. Evaluation of the Rapid Strep system for the identification of clinical isolates of *Streptococcus* species. J. Clin. Microbiol. **20**:894–898.

7. **Holloway, Y., M. Schaareman, and J. Dankert.** 1979. Identification of viridans streptococci on the Minitek minaturized differential system. J. Clin. Pathol. **32**:1168–1173.
8. **Keiville, M. W., and G. V. Doern.** 1982. Comparison of the API 20S streptococcus identification system with an immunorheophoresis procedure and two commercial latex agglutination tests

for identifying beta-hemolytic streptococci. J. Clin. Microbiol. **16**:92–95.

9. **Klein, R. S., R. A. Recco, M. T. Catalana, S. C. Edberg, J. I. Casey, and N. H. Steigbigel.** 1977. Association of *Streptococcus bovis* with carcinoma of the colon. N. Engl. J. Med. **297**:800–802.
10. **Nachamkin, I., J. R. Lynch, and J. P. Dalton.** 1982. Evaluation of a rapid system for species identification of alpha-hemolytic streptococci. J. Clin. Microbiol. **16**:521–524.
11. **Poutrel, B., and H. Z. Ryniewicz.** 1984. Evaluation of the API 20 Strep System for species identification of streptococci isolated from bovine mastitis. J. Clin. Microbiol. **19**:213–214.
12. **Reynolds, J. G., E. Silva, and W. M. McCormack.** 1983. Association of *Streptococcus bovis* bacteremia with bowel disease. J. Clin. Microbiol. **17**:696–697.
13. **Ruoff, K. L., M. J. Ferraro, M. E. Jerz, and J. Kissling.** 1982. Automated identification of gram-positive bacteria. J. Clin. Microbiol. **16**:1091–1095.
14. **Ruoff, K. L., and L. J. Kunz.** 1983. Use of the Rapid Strep System for identification of viridans streptococcal species. J. Clin. Microbiol. **18**:1138–1140.
15. **Setterstrom, J. A., A. Gross, and R. S. Stanko.** 1979. Comparison of Minitek and conventional methods for the biochemical characterization of oral streptococci. J. Clin. Microbiol. **10**: 409–414.
16. **Skerman, V. B. D., V. McGowan, and P. H. A. Sneath.** 1980. Approved list of bacteria names. Int. J. Syst. Bacteriol. **30**: 225–420.
17. **Waitkins, S. A., D. R. Anderson, and F. K. Todd.** 1981. An evaluation of the API-Strep identification system. Med. Lab. Sci. **38**:35–39.