JOURNAL OF CLINICAL MICROBIOLOGY, May 1984, p. 588–591
0095-1137/84/050588-04$02.00/0

Vol. 19, No. 5

Evaluation of the Rapid Strep System for Species Identification of Streptococci

PETER C. APPELBAUM,[1]* PURNIMA S. CHAURUSHIYA,[1] MICHAEL R. JACOBS,[2] AND ANNELIESE DUFFETT[3]

Departments of Pathology (Clinical Microbiology), Hershey Medical Center, Hershey, Pennsylvania 17033,[1] and Case Western Reserve University[2] and University Hospitals of Cleveland,[3] Cleveland, Ohio 44106

Received 7 December 1983/Accepted 26 January 1984

The Rapid Strep system (API System S.A., Montalieu-Vercieu, France) was evaluated, without additional tests, in the identification of 209 streptococci. Organisms included 59 beta-hemolytic, 36 group D, 24 *Streptococcus pneumoniae*, and 90 viridans group streptococci. The Rapid Strep system correctly identified to species level 69.5% of the beta-hemolytic strains, 100% of the group D strains, none of the *S. pneumoniae* strains, and 84.5% of the viridans group streptococci. The method provided excellent identification rates of groups A, B, and D but failed to differentiate between groups C and G. The method for preparation of suspensions for the Rapid Strep system, as initially recommended by the manufacturer, was responsible for the failure to identify *S. pneumoniae*. This method was subsequently modified to yield a heavier inoculum; all 10 pneumococcal strains tested with the revised inoculum method were correctly identified. Good identification rates of commonly encountered viridans strains were found. The Rapid Strep system represents a worthwhile advance in streptococcal species identification, especially for group D and viridans strains.

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 strains and pneumococci) can be presumptively or definitively identified by relatively simple and rapid tests, this is not the case for species identification of the group D and viridans group streptococci, which require time-consuming conventional tests for accurate species identification (4, 5). Although enterococcal and non-enterococcal group D strains can be differentiated with the aid of relatively simple tests, accurate species identification of enterococcal and non-enterococcal group D organisms requires additional testing (5). Species identification of group D and viridans group streptococci is important clinically and epidemiologically and will help delineate the spectrum of disease caused by particular species (1).

Commercial methods which have been developed for the identification of streptococci include the API 20S system (1, 6, 7) (Analytab Products, Inc., Plainview, N.Y.), API ZYM (12) (API Laboratory Products, Farnborough, United Kingdom), and the Gram-Positive Identification Card (1, 8) (Vitek Systems, Inc., Hazelwood, Mo.). All of these systems are fairly inaccurate in the identification of viridans group streptococci and display various degrees of accuracy in the identification of other streptococcal groups (1, 6–8, 12).

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 evaluate the capability of the Rapid Strep system (API System S.A., Montalieu-Vercieu, France) to accurately identify a spectrum of clinically significant streptococci without the aid of additional tests. Previous reports have noted excellent agreement between results obtained with a prototype of this system and those obtained by conventional methods (11); good identification rates of viridans group streptococci have also been reported with this technique (9).

* Corresponding author.

(This study was presented in part at the 84th Annual Meeting of the American Society for Microbiology [P. C. Appelbaum, P. S. Chaurushiya, and M. R. Jacobs, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, C126, p. 257].)

MATERIALS AND METHODS

Bacteria. Organisms (Table 1) were chosen to represent a spectrum of clinically significant streptococci isolated from human infections. Of the 209 cultures, 196 were clinical isolates in Cleveland (University Hospitals and Veterans Administration Hospital); 5 (1 *S. faecalis*, 2 *S. mitis*, 1 *S. sanguis* I, and 1 *S. sanguis* II) isolates were obtained from the Centers for Disease Control, Atlanta, Ga.; 4 (1 group B, 2 group G, and 1 *S. faecalis*) isolates were obtained from the College of American Pathologists, Skokie, Ill. (proficiency samples), and 4 (1 *S. pneumoniae*, 1 *S. durans*, and 2 *S. faecalis*) isolates were obtained from the American Type Culture Collection, Rockville, Md. Strains were stored at −40°C in thioglycolate-glycerol broth (85:15, vol/vol). Before use, organisms were subcultured twice on sheep blood agar (BBL Microbiology Systems, Cockeysville, Md.) 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 antigen by the Phadebact coagglutination test (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.), and group F organisms were tested by the precipitation technique with a 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 streptococci were identified by growth on bile-esculin agar (Difco Laboratories, Detroit, Mich.); growth in 6.5% NaCl (Difco); starch hydrolysis (Difco); and fermentation of sorbitol, mannitol, and arabinose (Remel, Inc., Lenexa, Kans.). Viridans group streptococci were characterized (1, 10) by colonial morphology; growth on bile-esculin agar (Difco); hemolysis;

hydrolysis of esculin (Difco) and hippurate (Difco); litmus milk reduction (Oxoid, Inc., Columbia, Md.); growth morphology on 5% sucrose agar (Difco); and fermentation of lactose, mannitol, inulin, and raffinose (Remel) (4).

Rapid Strep system. The Rapid Strep system consists of a strip with 20 cupules containing dehydrated substances for the determination of the following reactions: Voges-Proskauer; production of the enzymes pyrrolidonyl arylamidase, α-galactosidase, β-glucuronidase, β-galactosidase, alkaline phosphatase, leucine arylamidase; hydrolysis of esculin and hippurate; arginine dihydrolase; and fermentation of ribose, L-arabinose, mannitol, sorbitol, lactose, trehalose, inulin, raffinose, starch, and glycogen. Growth from one sheep blood agar plate (tryptic soy agar base) incubated for 24 to 48 h was suspended in 0.85% sterile saline to yield sufficient inocula for the strips (≥4 McFarland turbidity standard). Viability plates were included with each run. Strips were inoculated, incubated, and interpreted according to the instructions of the manufacturer. After incubation for 4 h, reagents were added to the Voges-Proskauer, hippurate, and enzyme wells, and results for all 20 reactions were recorded. A seven-digit profile number was generated and compared with those provided in a code book. When called for in the code book or when 4-h profiles did not appear in the database, strips were reincubated overnight; esculin, arginine dihydrolase, and sugar fermentation reactions were then reread, and a second seven-digit profile was constructed and interpreted as described above. For organisms requiring overnight incubation of strips, the second profile was taken as definitive. When organisms yielded codes which did not appear in the book, the firm was contacted by telephone and profiles were referred to their extended database. In cases in which codes did not appear in the extended database, profiles were telexed through to the parent company in France. Identifications were compared with those obtained by primary methods and classified as (i) correct to species level (corresponding to excellent, very good, good, acceptable, or species identification, as listed in the code book); (ii) low discrimination (LD) (including identifications as *S. equisimilis*/group G), which included the correct identification among a spectrum of two or more possibilities; where the correct identification occurred as first of a list of likelihoods, this was still taken as LD; (iii) incorrect; or (iv) doubtful profile (extenced database). Identifications as *Streptococcus milleri* biotypes 1, 2, and 3 were taken to be synonymous with *S. MG-intermedius* in the scheme of Facklam (2, 4, 9). For the purpose of this study, no additional tests were performed to pinpoint a correct identification in category (ii).

TABLE 1. Level of identification of specific streptococci by the Rapid Strep system

Organism (no. of strains)	No. of strains giving following result:			
	Correct	One of a spectrum of ≥2 possibilities	Incorrect	Doubtful profile[a]
Group A (18)	18	0	0	0
Group B (22)	21	0	0	1
Group C (3)	0	2[b]	0	1
Group F (2)[c]	2	0	0	0
Group G (14)	0	11[b]	0	3
S. faecalis (23)	23	0	0	0
S. faecium (5)	5	0	0	0
S. durans (1)	1	0	0	0
S. bovis I (7)	7	0	0	0
S. pneumoniae (24)	0	7	13	4
S. mitis (26)	23	0	3	0
S. sanguis I (9)	7	0	2	0
S. sanguis II (11)	9	2	0	0
S. salivarius (32)	29	3	0	0
S. MG intermedius (8)	6[d]	0	1	1
S. mutans (2)	1	0	1	0
S. morbillorum[e] (1)	1	0	0	0
S. anginosus-constellatus[f] (1)	0	0	1	0

[a] Extended database (see text).
[b] Identified as *S. equisimilis*/group G, with serogrouping necessary for accurate identification.
[c] Identified as *S. milleri* biotype 1; group F is currently not included in database (see text).
[d] Identified as *S. milleri* biotype 1 or 2 (see text).
[e] Not currently included in code book, but correctly identified with extended database.
[f] Not currently listed as such in database (see text).

RESULTS

Reactions from the Rapid Strep system were clear-cut and easily read. The few esculin, arginine dihydrolase, and sugar fermentation reactions (ca. 5%) which yielded equivocal results after 4 h were easily interpretable after overnight incubation. A total of 77% of the beta-hemolytic strains and 78% of the group D strains yielded profiles with definitive results after 4 h of incubation; corresponding figures for *S. pneumoniae* and viridans group streptococci were 0 and 7%, respectively. Overall, 38% of the strains yielded definitive results after 4 h.

Identification rates by the Rapid Strep system are shown in Table 1. Of the beta-hemolytic organisms, all of the group A and 96% of the group B strains were correctly identified. Group F is not included in the current database, but further conventional biochemical testing of these two strains yielded biochemical results consistent with *S. MG-intermedius*, corresponding to Rapid Strep identification as *S. milleri* biotype 1 (2, 9). Less satisfactory results were obtained with groups C and G organisms, which required serogrouping to differentiate *S. equisimilis* (group C) from group G strains. Excellent identification rates of enterococcal and non-enterococcal group D strains were obtained (100% correct to species level). Identification rates of *S. pneumoniae*, by the method of inoculum preparation recommended by the manufacturer at the time this study was performed, were unsatisfactory (29.2% LD, 54.2% incorrect, 16.7% doubtful profile). Overall, good identification rates of viridans group streptococci were observed (84.5% correct to species level, 5.6% LD, 8.9% incorrect, and 1.1% doubtful profile). Of the viridans group streptococci, 88.5% of 26 *S. mitis* isolates were correctly identified to species level, with 11.5% incorrect identifications. Corresponding rates for nine *S. sanguis* I strains were 77.8 and 22.2%, respectively. A total of 81.8% of 11 *S. sanguis* II strains were correctly identified, with 18.2% LD; corresponding rates for 32 *S. salivarius* strains were 90.6 and 9.4%, respectively. Of eight *S. MG-intermedius* strains, 75% were correctly identified (as *S. milleri* biotype 1 or 2), with 12.5% incorrect and 12.5% doubtful profile.

Additional tests required to pinpoint the correct identification in LD profiles included serotyping (groups C and G); optochin inhibition (*S. pneumoniae*); growth morphology on 5% sucrose agar, bile-esculin, urease, serogrouping, fermentation of arbutin, and melibiose (viridans group streptococci). Seventeen organisms (8.1%) yielded profiles which were not in the code book and had to be referred to the extended database of API (see Materials and Methods). Of these isolates, 7 yielded definitive identifications (5 correct:

2 *S. faecium* ribose-negative, 3 *S. salivarius*; 2 LD: *S. pneumoniae*) and 10 doubtful identifications. Of the 10 doubtful profiles, 6 (groups C and G; *S. MG-intermedius*), included the correct identification in a range of two or more possibilities; the remaining 4 (all *S. pneumoniae*) yielded incorrect results.

Misidentifications by the Rapid Strep system are shown in Table 2. A total of 12 *S. pneumoniae* strains were misidentified as *S. sanguis* II, and 1 was misidentified as *S. salivarius-S. bovis* II. Three *S. mitis* strains were misidentified as *S. sanguis* I. Two *S. sanguis* I strains were misidentified, one as *S. salivarius-S. bovis* II, the other as *S. sanguis* II. One *S. MG-intermedius* strain was misidentified as *S. sanguis* I, one *S. mutans* as *S. milleri*, and one *S. anginosus-constellatus* as *S. pneumoniae-S. sanguis* II.

TABLE 2. Misidentifications with the Rapid Strep system

Organism (no. of strains)	Rapid Strep system identification
S. pneumoniae (12)	*S. sanguis* II
S. pneumoniae (1)	*S. salivarius-S. bovis* II
S. mitis (3)	*S. sanguis* I
S. sanguis I (1)	*S. salivarius-S. bovis* II
S. sanguis I (1)	*S. sanguis* II
S. MG intermedius (1)	*S. sanguis* I
S. mutans (1)	*S. milleri*
S. anginosus-constellatus (1)	*S. pneumoniae-S. sanguis* II

DISCUSSION

Identification rates of beta-hemolytic and group D organisms by the Rapid Strep system are comparable to those reported with other systems (1, 6–8); excellent identification rates of groups A, B, and D were observed, but serogrouping was necessary to differentiate between groups C and G. Unsatisfactory results with *S. pneumoniae* may be explained by insufficient inocula, leading to negative key sugar reactions. After completion of the study, we were informed by the manufacturer that inocula of *S. pneumoniae* should be prepared from two blood agar plates incubated anaerobically. In our experiment, inocula from one plate incubated under CO_2 yielded sufficiently turbid inocula, equivalent to those used for organisms yielding correct identifications. Retesting of 10 pneumococcal strains with the modified inoculum preparation technique yielded correct results in every case. Since the Rapid Strep system is only likely to be used for exceptional pneumococcal isolates that do not have the typical morphology of *S. pneumoniae*, use of the heavier inoculum may be advisable for all isolates that the system identifies as *S. sanguis* II (see Table 2). Results of testing viridans group streptococci in our study were similar to those obtained by Ruoff and Kunz (9). If additional tests would have been performed for LD profiles, correct species identification rates would have reached 90.0%, a rate higher than those reported for the API 20S system (1, 7), the Gram-Positive Identification Card (1, 8), and the API ZYM (12), all of which are below 75%. The Rapid Strep system is relatively easy to use and easy to interpret. Improvement in the packaging of Voges-Proskauer and enzyme reagents would facilitate the use of the product.

The Rapid Strep system permits identification of biotypes within species of *S. pyogenes*, *S. faecalis*, *S. faecium*, *S. durans*, *S. bovis* II, *S. mitis*, *S. milleri*, *S. sanguis I*, *S. sanguis* II, and *S. pneumoniae*. No attempt was made in the current study to correlate these biotypes with those obtained by conventional testing. The three *S. mitis* strains (arginine dihydrolase positive) misidentified as *S. sanguis* I (biotype 3) correspond to arginine dihydrolase-positive *S. mitis* and to dextran-positive *S. mitior* (*S. mitis*) described by Colman and Williams (3); this may be more a taxonomic discrepancy than a misidentification. In the Rapid Strep system, European rather than U.S. nomenclature is used in the identification of *S. MG-intermedius* and *S. anginosus-constellatus*; *S. milleri* biotype 1 corresponds to beta-hemolytic *S. milleri* (including some group F strains), biotype 2 includes strains designated either as *S. MG-intermedius* or as *S. anginosus-constellatus* in the scheme of Facklam (4), and *S. milleri* biotype 3 corresponds to *S. milleri* strains described by Ball and Parker (2) and referred to as unidentified urine isolates in previous studies (9). In the current study, all correctly identified *S. MG-intermedius* strains were identified as either *S. milleri* biotype 1 (beta-hemolytic) or biotype 2. Identification of group F (currently not included as such in the database) as *S. milleri* biotype 1 (beta-hemolytic) has been described previously (2). Although *S. morbillorum* is currently not included in the code book, the one strain tested was correctly identified by the extended database.

A shortcoming of the Rapid Strep system as currently available is the lack of a computerized back-up service for numbers not present in the code book. At present an incomplete database is available in the United States, and problems must be referred by telex to the parent company in France. Five beta-hemolytic strains with doubtful profiles yielded typical reactions, with the exception of negative ribose tests. Additionally, two correctly identified ribose-negative *S. faecium* strains yielded profiles not present in the current code book. A locally available computerized service which could provide rapid information on such biochemically atypical strains would improve the accuracy of the product. Although the Rapid Strep system enables the identification of most beta-hemolytic and group D strains after 4 h, overnight incubation is required for *S. pneumoniae* and viridans group streptococci. The additional incubation period improves the accuracy of the system and is still shorter than those required by simplified conventional schemes (5, 10).

The advantage of the Rapid Strep system for the identification of beta-hemolytic streptococci is questionable, since convenient, rapid methods already exist for identification of these strains. The failure of the system with the initial inoculum method to identify *S. pneumoniae* is unlikely to detract from its usefulness, since the vast majority of pneumococci will be identified on the basis of colony morphology, bile solubility, and optochin susceptibility.

In summary, the principal potential of the Rapid Strep system is in species identification of group D and viridans group streptococci. The system yields excellent identification of group D strains and good identification of viridans group streptococci. The system would be further improved by revisions in taxonomy and enlargement of the database.

ACKNOWLEDGMENTS

This study was supported in part by a grant-in-aid from DMS Laboratories, Flemington, N.J.

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

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