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Accuracy and Reproducibility of the IDS RapID STR System for Species Identification of Streptococci

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The RapID STR system (Innovative Diagnostic Systems, Inc., Atlanta, Ga.) was evaluated in the identification of 266 streptococci. Organisms included 60 beta-hemolytic streptococci, 71 group D strains (48 enterococci and 23 nonenterococci), 26 *Streptococcus pneumoniae*, and 109 viridans group strains. With concomitant optochin testing, as is currently recommended by the manufacturer for all alpha-hemolytic strains, the RapID STR system correctly identified 100% of beta-hemolytic strains, 87.3% of group D strains (93.7% of enterococci, 73.9% of nonenterococci), 88.5% of *S. pneumoniae*, and 72.5% of viridans strains. Without the use of optochin, the correct identification of *S. pneumoniae* and the viridans group was 26.9 and 52.3%, respectively. The RapID STR system incorrectly identified 3.0% of strains, including four group D streptococci, three pneumococci, and one viridans isolate. Reproducibility was excellent, with 95% of strains tested in triplicate yielding identical results on each of the three occasions. The RapID STR system represents a worthwhile advance in streptococcal species identification, especially for group D and viridans strains.

Streptococci make up a large proportion of gram-positive cocci isolated and identified in the clinical microbiology laboratory. Although many of these organisms (e.g., groupable beta-hemolytic strains, pneumococci) can be presumptively or definitively identified by rapid and simple tests, this does not apply to species identification of group D and viridans strains, which require time-consuming conventional tests for accurate species identification (2, 8, 10). Although enterococcal and nonenterococcal group D strains can be differentiated with relatively simple tests, accurate species identification of enterococci and nonenterococci requires additional testing (10). Species identification of group D and viridans streptococci is important clinically and epidemiologically and will help delineate the spectrum of diseases caused by these species (2).

Commercial methods which are available in the United States for the identification of streptococci include the API 20S (Analytab Products, Plainview, N.Y.) (2, 7, 10, 12, 14), the Gram-Positive Identification Card (Vitek Systems, Inc., Hazelwood, Mo.) (2, 7, 10, 17), and the Rapid Strep system (Analytab Products) (1, 10, 11, 18). All three systems yield comparably good identifications of commonly encountered beta-hemolytic and group D strains as well as *Streptococcus pneumoniae* but display varying degrees of accuracy in identification of viridans strains (1, 2, 7, 10–12, 14, 17, 18).

Microbiology laboratories without facilities for extended conventional testing require a rapid, reliable, accurate method for streptococcal species identification. The aim of the current study was to evaluate the capability of the RapID STR method (Innovative Diagnostic Systems, Inc., Atlanta, Ga.) to accurately identify a spectrum of clinically significant streptococci, with and without additional tests. The method is based on detection of preformed enzymes in 4 h and the principles used were developed by the same firm for anaerobes (3) and *Haemophilus* (6) and *Neisseria* (15) spp.

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MATERIALS AND METHODS

Bacteria and identification methods. Organisms (Table 1) were chosen to represent a spectrum of clinically significant streptococci isolated from human infections. Of 266 cultures, 237 were clinical isolates in Cleveland (University Hospitals of Cleveland and Cleveland Veterans Administration Medical Center); 13 isolates were obtained from the Centers for Disease Control, Atlanta, Ga.; 7 isolates were obtained from the College of American Pathologists, Skokie, Ill. (proficiency samples); and 9 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 staining and examination of colonial morphology. Isolates were identified by conventional biochemical and serological tests as described in previous studies (1, 2). Optochin testing was done by standard methodology (10). *Enterococcus casseliflavus* strains were identified and differentiated from *Enterococcus gallinarum* and other enterococci by additional testing (5). Identification of all group C, F, and G strains identified by RapID STR as *Streptococcus milleri* (*Streptococcus anginosus*) was confirmed by conventional tests (9).

RapID STR system. The RapID STR system is a qualitative micromethod in which chromogenic substrates are used for the identification of clinically isolated streptococci. Tests are based upon microbial degradation of specific substrates by preformed enzymes, and detection by indicator systems or addition of reagents. Tests include L-arginine utilization; esculin hydrolysis; fermentation of mannitol, sorbitol, raf-

TABLE 1. Level of streptococcal identification with the RapID STR system

Organism (no. of strains)	No. of strains giving the following result:		
	Correct without extra tests	Corect with extra tests	Incorrect
Group A (18)	18	0	0
Group B (21)	21	0	0
Group C (3)	3[a]	0	0
Group F (1)	1[b]	0	0
Group G (17)	17[c]	0	0
E. faecalis (28)	28	0	0
E. faecium (9)	9	0	0
E. durans (9)	8	1	0
E. casseliflavus (2)	0	0	2
S. bovis (14)	9	4	1
S. bovis var (9)	8	0	1
S. pneumoniae (26)	7	16[d]	3
S. mitis (26)	12	14[e]	0
S. sanguis I (8)	5	3[f]	0
S. sanguis II (17)	4	12[g]	1
S. salivarius (35)	23	12	0
S. intermedius (2)	1	1	0
S. constellatus (10)	2	8	0
S. mutans (11)	10	1	0

[a] Two strains were identified as group C or G; one was identified as *S. anginosus*.
[b] Identified as *S. anginosus*.
[c] Sixteen strains were identified as group C or G; one was identified as *S. anginosus*.
[d] All strains were correctly identified with optochin testing.
[e] Nine strains were correctly identified with optochin testing.
[f] Two strains were correctly identified with optochin testing.
[g] Eleven strains were correctly identified with optochin testing.

finose, and inulin; hydrolysis of *p*-nitrophenyl α,D-galactoside, *p*-nitrophenyl-α,D-glucoside, *p*-nitrophenyl-β,D-*N*-acetyl glucosaminide, *p*-nitrophenyl phosphate, tyrosine-β-naphthylamide, hydroxyproline-β-naphthylamide, lysine-β-naphthylamide, and pyrrolidine-β-naphthylamide. Inocula were prepared by suspending growth from blood agar plates into RapID STR inoculation fluid to a turbidity approximately equal to a no. 1 McFarland standard. Panels were inoculated as recommended in the instructions of the manufacturer and incubated aerobically at 35°C for 4 h. Reactions were read before and after the addition of reagent to the last four cavities. Tests were assigned numerical values from which an STR microcode was generated. Identifications were obtained by using the RapID STR Code Compendium, and results were compared against the Innovative Diagnostic Systems current computer database. Codes which did not appear in the compendium were referred to the computer facilities of the firm. All identifications used in data analysis in the current study were those from the current database. In those cases where more than one organism choice presented a significant probability (probability overlap), supplemental tests recommended in the code compendium were performed. Identifications were classified as (i) correct without additional tests (≥95% probability), corresponding to implicit, satisfactory, and adequate; (ii) correct with additional test procedures recommended to resolve probability overlaps; or (iii) incorrect. Tests in triplicate were set up by one person but read independently by three persons within 1 h of each other.

RESULTS

Reactions in the RapID STR system were relatively clear-cut and easy to interpret. Contrary to the package insert included with the system, however, we determined that only yellow sorbitol fermentation reactions should be read as positive, and all other color variations (including yellow-orange) should be read as negative. Additionally, it was important that, as recommended in the insert, only distinct yellow colors in the four *p*-nitrophenol reactions be read as positive: underreading of these tests could have led to erroneous results.

Identification rates by RapID STR are presented in Table 1. All β-hemolytic strains were correctly identified by this system; 1 group F organism, 1 of 3 group C organisms, and 1 of 17 group G organisms were identified as *S. anginosus* (beta-hemolytic *S. milleri*) (8), and the remaining groups C and G strains were identified as group C/G, with the code compendium recommending serogrouping for accurate differentiation. Because the latter is the case for all commercial systems currently available (1, 2), these identifications were taken as correct. Very good identification rates of group D strains were obtained (87.3% correct without extra tests, 7.0% correct with extra tests, 5.6% incorrect). All enterococci, with the exception of two members of a newly described species, *E. casseliflavus*, were correctly identified by the system. The two *E. casseliflavus* strains were isolated from blood and from the sputum of a cardiac transplant patient. These strains were differentiated from *E. gallinarum*, an organism with similar biochemical properties, by their failure to ferment D-raffinose and sorbitol and by their lack of arginine dehydrolase activity (5). Initially, poor identification rates for *S. pneumoniae* (approximately 80% incorrect) were obtained. However, when microcodes were interpreted with the aid of the computer service of the firm and also optochin testing, results were greatly improved. With optochin testing regarded as a primary test together with the microcode, 88.5% of pneumococci were correctly identified, with 11.5% incorrect.

Overall identification rates of viridans streptococci were 52.3% correct without extra tests, 46.8% correct with extra tests, and 0.9% incorrect. Among the viridans group, 46.1% of *Streptococcus mitis* strains were correctly identified without extra tests, and 53.9% were correctly identified with extra tests. Corresponding rates for *Streptococcus sanguis* I were 62.5 and 37.5%, respectively. Of the *Streptococcus sanguis* II strains, 23.5% were correctly identified without extra tests, and 70.6% were correctly identified with extra tests, with 5.9% incorrect, while 65.7% of *Streptococcus salivarius* organisms were correctly identified without extra tests and 34.3% required extra tests for accurate identification. Of 12 *Streptococcus intermedius* or *Streptococcus constellatus* strains, 25.0% were correctly identified without extra tests, and 75.0% were correctly identified with extra tests; corresponding rates for *Streptococcus mutans* were 90.9 and 9.1%, respectively. When results of optochin testing (Table 1) were interpreted together with microcode numbers, 72.5% of viridans strains were correctly identified without extra tests, and 26.6% were correctly identified with extra tests: correct identification rates for *S. mitis*, *S. sanguis* I, and *S. sanguis* II would then have risen to 80.8, 87.5, and 88.2%, respectively. One strain of *S. salivarius*, misidentified as *Streptococcus equinus* by the compendium, yielded the correct identification when the microcode was referred to the computer service. When code compendium and computer service identifications were compared for viridans strains, a few *S. mitis*, *S. sanguis* I, *S. sanguis* II, and *S. salivarius* results were different. These discrepancies were a result of updating, extending, and improving the technical service database of the firm.

Additional tests recommended by the compendium to resolve probability overlaps and provide correct identification included tests for bile esculin, starch hydrolysis, and sucrose fermentation for group D isolates; optochin susceptibility for pneumococci; and optochin, esculin and starch hydrolysis, lactose fermentation, and production of glucans for viridans strains. In all cases in which correct identification depended on optochin testing, the compendium instructed the user that if the strain was optochin positive it was *S. pneumoniae*.

Eight organisms were misidentified by RapID STR. These included three *S. pneumoniae* strains (one was misidentified as *S. intermedius*, two were misidentified as *S. sanguis* II), two *E. casseliflavus* (both misidentified as *E. gallinarum*), one *Streptoccocus bovis* (misidentified as *S. intermedius*), one *S. bovis* var (misidentified as *S. bovis*), and one *S. sanguis* II (misidentified as *S. pneumoniae*).

To test the reproducibility of RapID STR, 20 streptococci were tested in triplicate, each on the same day. In 19 of 20 cases (95%), results of all three identifications were identical, with 16 correct identifications and three probability overlaps. One *S. pneumoniae* strain with discrepant results on the third testing yielded two correct identifications and a third as *S. sanguis* II or *S. pneumoniae*; optochin testing was necessary to resolve the identification. All probability overlap codes yielded correct results with additional testing.

DISCUSSION

Identification rates of beta-hemolytic and group D organisms with the RapID STR system were comparable to those reported with other systems (1, 2, 7, 10–12, 14, 17, 18). Excellent identification rates of groups A and B were observed, but serogrouping was necessary to differentiate between groups C and G. No commercial system is currently capable of differentiating between the latter two at this time. The RapID STR system has the advantage of addressing *S. anginosus* (beta-hemolytic *S. milleri*) strains (8). Very good identification rates of group D strains were observed, with satisfactory differentiation between *S. bovis* and *S. bovis* var. This distinction is important, since *S. bovis*, but not *S. bovis* var as yet, from the blood has been linked to colon malignancy (7). Accurate identification of pneumococcal strains required inclusion of optochin testing: with the latter reaction, very good identification rates could be obtained. In our laboratory, the RapID STR system demonstrated excellent ability to identify viridans streptococci. Differentiation of the viridans group, especially *S. mitis*, *S. sanguis* I, and *S. sanguis* II from pneumococci, was dependent on optochin testing. The manufacturer recommends that primary optochin testing be performed on all alpha-hemolytic streptococci during preparation of plates used for inoculation of panels. In our experiments, when results of optochin testing were included the RapID STR system represented a marked improvement over API 20S, the Gram-Positive Identification Card, and Rapid Strep, with significantly fewer misidentifications (0.9% versus 27.7, 33.6, and 8.9%, respectively [1, 2]).

An advantage of the RapID STR system is use of current U.S. taxonomy in combination with British nomenclature: this is especially important in the case of *S. intermedius* and *S. constellatus*, both of which are included in British nomenclature as *S. milleri* (4, 9, 13). Additionally, in contrast to the RapID STR system, differentiation between *E. casseliflavus*, *Enterococcus avium*, and *E. gallinarum* (5) is not possible with other commercial kits at this time. Standardization of nomenclature by different commercial methods will facilitate delineation of the disease processes associated with different streptococcal strains, especially group D and viridans organisms. In our laboratory, we experienced difficulty in interpreting sorbitol fermentation reactions. As currently recommended in the package insert, any significant yellow-orange color is to be interpreted as positive: if these instructions had been followed, >80% of the *S. bovis* strains tested would have been identified as *S. mutans*. In our experiments, accurate identification of these two groups was achieved only when clear yellow color reactions were scored as positive. The package insert is under revision by the manufacturer to reflect sorbitol interpretation changes and to include optochin testing. The current code compendium non-beta-hemolytic section should be interpreted together with optochin results, especially for *S. pneumoniae*, *S. mitis*, *S. sanguis* I, and *S. sanguis* II. An update of the code compendium which will accommodate additional data for these taxa is also currently under development. The Innovative Diagnostic Systems computer service will process microcodes through an extended database via the telephone.

All *S. bovis* strains and 11 of 12 *S. salivarius* strains requiring extra tests for accurate identification were identified as *S. salivarius* or *S. bovis* or as *S. salivarius* or *S. bovis* var. Differentiation between these two groups by conventional biochemical and serological means is sometimes difficult, and may require determination of cellular fatty acid content to help resolve the species identification (16). Negative α-glucosidase reactions led to requirement for lactose fermentation and starch hydrolysis for accurate identification of probability overlap profiles (most commonly *S. constellatus* or *S. sanguis* I).

As with other commercial systems, the advantage of RapID STR for the identification of typical group A and B beta-hemolytic streptococci is questionable, since convenient rapid methods already exist for grouping of these strains. However, the differentiation of *S. anginosus* (beta-hemolytic *S. milleri*) strains, by the RapID STR system, from other group A, C, F, and G isolates (13) is important clinically, since *S. milleri* is considered an important cause of deep suppurative infections (4). The need for additional optochin testing detracts from the usefulness of the system in the identification of pneumococci: we would not recommend testing of optochin-susceptible strains in the RapID STR system.

In summary, the major use of the RapID STR system is in species identification of group D and viridans streptococci. The method is convenient and reproducible and, if coupled with optochin testing, yields very accurate identification of both groups of organisms.

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LITERATURE CITED

1. **Appelbaum, 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. **Appelbaum, P. C., M. R. Jacobs, J. I. Heald, W. M. Palko, A. Duffett, R. Crist, and 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. **Appelbaum, P. C., C. S. Kaufmann, and J. W. Depenbusch.** 1985. Accuracy and reproducibility of a four-hour method for anaerobe identification. J. Clin. Microbiol. **21**:894–898.

4. **Ball, L. C., and M. T. Parker.** 1979. The cultural and biochemical characters of *Streptococcus milleri* isolated from human sources. J. Hyg. **82:**63–78.
5. **Collins, M. D., D. Jones, J. A. E. Farrow, R. Kilpper-Bälz, and K. H. Schleifer.** 1984. *Enteroboccus avium* nom. rev., comb. nov.; *E. casseliflavus* nom. rev., comb. nov.; *E. durans* nom. rev., comb. nov.; *E. gallinarum* comb. nov.; and *E. malodoratus* sp. nov. Int. J. Syst. Bacteriol. **34:**220–223.
6. **Doern, G. V., and K. C. Chapin.** 1984. Laboratory identification of *Haemophilus influenzae*: effects of basal media on the results of the satellitism test and evaluation of the RapID NH system. J. Clin. Microbiol. **20:**599–601.
7. **Facklam, R., G. S. Bosley, D. Rhoden, A. R. Franklin, N. Weaver, and R. Schulman.** 1985. Comparative evaluation of the API 20S and AutoMicrobic Gram-Positive Identification systems for non-beta-hemolytic streptococci and aerococci. J. Clin. Microbiol. **21:**535–541.
8. **Facklam, R. R.** 1977. Physiological differentiation of viridans streptococci. J. Clin. Microbiol. **5:**184–201.
9. **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.
10. **Facklam, R. R., and R. B. Carey.** 1985. Streptococci and aerococci, p. 154–175. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
11. **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.
12. **Keville, 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.
13. **Lawrence, J., D. M. Yajko, and W. K. Hadley.** 1985. Incidence and characterization of beta-hemolytic *Streptococcus milleri* and differentiation from *S. pyogenes* (group A), *S. equisimilis* (group C), and large-colony group G streptococci. J. Clin. Microbiol. **22:**772–777.
14. **Nachamkin, I., J. R. Lynch, and H. P. Dalton.** 1982. Evaluation of a rapid system for species identification of alpha-hemolytic streptococci. J. Clin. Microbiol. **16:**521–524.
15. **Robinson, M. J., and T. R. Oberhofer.** 1983. Identification of pathogenic *Neisseria* species with the RapID NH system. J. Clin. Microbiol. **17:**400–404.
16. **Ruoff, K. L., M. J. Ferraro, J. Holden, and L. J. Kunz.** 1984. Identification of *Streptococcus bovis* and *Streptococcus salivarius* in clinical laboratories. J. Clin. Microbiol. **20:**223–226.
17. **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.
18. **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.