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Comparison of Phenotypic Characteristics, DNA-DNA Hybridization Results, and Results with a Commercial Rapid Biochemical and Enzymatic Reaction System for Identification of Viridans Group Streptococci

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The rapid ID 32 Strep system (bioMérieux, Marcy l'Etoile, France) was evaluated for its ability to identify 21 species of viridans group streptococci; results were compared with DNA-DNA hybridization results and results of conventional physiological tests. A total of 171 strains of the 21 species including 147 clinical strains was analyzed. Of the 156 strains of species included in the database of this system, 136 strains (87%) were correctly identified. Incorrect identification occurred for 13 strains (8%), and no identification was given for 7 strains (5%). It was difficult to differentiate *S. mitis* and *S. oralis* accurately with this system. Of the 17 strains identified as *S. mitis* by the rapid ID 32 Strep system, the results of DNA-DNA hybridization were in agreement for only 3 strains. *S. crista* and *S. parasanguis*, which are not included in the database, were identified as *S. mitis* or *S. sanguis* or were not identified, but *S. parasanguis* could probably be identified by using the rapid ID 32 Strep system because the biochemical profile is well characterized for this species. The rapid ID 32 Strep system can be used to differentiate most species for which phenotypic characteristics have been described if the database is revised according to recently reported amended criteria for the identification of viridans group streptococci. However, identification of a few species such as *S. mitis* and *S. oralis* is problematic with this system.

Viridans group streptococci constitute part of the normal flora of the human oral cavity (10) and are causative organisms of serious infectious diseases such as infective endocarditis (13, 31, 32, 36), septicemia (4, 5, 8, 14), and meningitis (29). *S. sanguis*, *S. gordonii*, and *S. oralis* are the species most frequently isolated from patients with infective endocarditis (13), and *S. oralis* and *S. mitis* are the species most commonly isolated from the blood of patients with neutropenic cancer (5). Since the antimicrobial susceptibilities (e.g., penicillin tolerance) of viridans group streptococci differ between several species, it is important to identify the individual species associated with diseases and to recognize their pathogenic traits (22, 23).

Molecular biology-based analyses such as DNA-DNA hybridization (1, 9, 15, 20, 24, 27, 34, 35, 37–41), DNA-rRNA hybridization (27, 34), rRNA sequencing (7, 39), and restriction fragment length polymorphism analysis of rRNA genes (ribotyping) (33) have helped to clarify the taxonomy of viridans group streptococci. Emended descriptions of *S. sanguis*, *S. oralis*, *S. mitis*, *S. anginosus*, *S. constellatus*, and *S. intermedius* have been accepted (25–27, 38), and new species have been described (9, 20, 25, 39, 40, 41). The characteristics of these new or emended species are not incorporated into commercial kits for the identification of viridans group streptococci (6). New taxonomic descriptions have been developed for *S. sanguis* and related species (*S. sanguis* group) such as *S. sanguis*, *S. oralis*, *S. mitis*, *S. gordonii*, *S. parasanguis*, and *S. crista*. We have shown that 42 of 45 strains (93%) of viridans group streptococci isolated from patients with infective endocarditis belong to these six species (23). Moreover, all of the nutritionally variant streptococci that we isolated from clinical specimens were from patients with infective endocarditis (24). Therefore, we considered that it was important to investigate the precise taxonomic positions of these eight species to investigate correlations between the species and their pathogenicities.

Rapid ID 32 Strep (bioMérieux, Marcy l'Etoile, France) is a new system which allows for the rapid identification (4 h) of streptococci and related genera. It is designed for use with a semiautomated system that simplifies the identification process. We evaluated the rapid ID 32 Strep system for its ability to identify species of viridans group streptococci that were isolated primarily from patients with infective endocarditis.

The identification kits were evaluated with reference strains that were usually identified by physiological reactions (2, 3, 16, 18, 19, 21). However, we considered that physiological identification only was not sufficient for the evaluation of kits for viridans group streptococci because there have been some variations among physiological reactions within the same species (6, 25), and some problems with phenotypic shifts have been reported (33). Therefore, we used DNA-DNA hybridization as the "gold standard" and conventional physiological tests to confirm the reference identification.

MATERIALS AND METHODS

Organisms. The organisms used in the study are listed in Table 1. Of the 171 strains, 147 were isolated from patients with various infectious diseases including infective endocarditis (70 strains) or from the oral flora of healthy volunteers. These strains were stored in 10% skim milk (Difco, Detroit, Mich.) at −85°C and were grown anaerobically (AnaeroPack; Mitsubishi Gas Chemical, Tokyo, Japan) for 24 h at 37°C on Columbia agar plates (Oxoid, Basingstoke, United Kingdom) with 5% defibrinated horse blood (Nihon Bio-test, Tokyo, Japan) (BA plates). Anaerobic cultures were used because some strains such as *S. cricetus*, *S. downei*, *S. ferus*, *S. intermedius*, and *S. macacae* would not grow aerobically. Ten

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TABLE 1. Viridans group streptococci

Organism	No. tested	Identifiable with the rapid ID 32 Strep system[a]	Type strain[b]	Reference strain	No. of clinical strains from:			
					Patients with infective endocarditis	Patients with bacteremia	Oral flora	Others
Streptococcus adjacens	5	Yes	ATCC 49175^T		4			
Streptococcus anginosus	1	Yes	GIFU 8787^T (NCTC 10713)					
Streptococcus constellatus	1	Yes	GIFU 8332^T (ATCC 27823)					
Streptococcus cricetus	1	No	GIFU 8635^T (ATCC 19642)					
Streptococcus crista	4	No	NCTC 12479^T		2		1	
Streptococcus defectivus	4	Yes	ATCC 49176^T		3			
Streptococcus downei	1	Yes	NCTC 11391^T					
Streptococcus ferus	1	No	GIFU 8820^T (ATCC 33477)					
Streptococcus gordonii	17	Yes	ATCC 10558^T		14		1	1
Streptococcus intermedius	1	Yes	GIFU 8327^T (ATCC 27335)					
Streptococcus macacae	1	No	GIFU 12704^T (NCTC 11558)					
Streptococcus mitis	6	Yes	NCTC 12261^T		1			4
Streptococcus mutans	2	Yes	RIMD 3125001^T (ATCC 25175)	ATCC 35668				
Streptococcus oralis	67	Yes	NCTC 11427^T	ATCC 10557	18	18	4	25
Streptococcus parasanguis	7	No	ATCC 15912^T		1	3		2
Streptococcus rattus	1	No	GIFU 8641^T (ATCC 19645)					
Streptococcus salivarius subsp. *salivarius*	2	Yes	GIFU 8326^T (ATCC 7073)	ATCC 13419				
Streptococcus salivarius subsp. *thermophilus*	1	Yes	GIFU 8593^T (NCDO 573)					
Streptococcus sanguis	46	Yes	ATCC 10556^T		27	4	12	2
Streptococcus sobrinus	1	Yes	GIFU 8819^T (ATCC 33478)					
Streptococcus vestibularis	1	Yes	GIFU 12457^T (NCTC 12166)					
Total	171				70	25	18	34

[a] A total of 156 strains was identifiable with the rapid ID 32 Strep system.
[b] ATCC, American Type Culture Collection, Rockville, Md.; GIFU, Department of Microbiology, Gifu University School of Medicine, Gifu, Japan; NCDO, National Collection of Dairy Organisms, Reading, England; NCTC, National Collection of Type Cultures, London, England; RIMD, Culture Collection, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan.

mg of pyridoxal hydrochloride (Sigma Chemical Co., St. Louis, Mo.) per liter was added to all of the media for *S. adjacens* and *S. defectivus.*

Conventional physiological methods. The strains were identified by using physiological tests as described previously (6, 9, 17, 25). The following tests were used: Gram staining; colonial morphology on mitis-salivarius agar (Difco); sheep blood hemolysis; production of catalase and oxidase; hydrolysis of esculin, hippurate, and arginine; fermentation in purple broth base (24 g/liter; Difco) and thioglycolate medium without glucose or indicator (12 g/liter; Difco) with 0.5% (wt/vol) amygdalin, D-arabinose, L-arabinose, arbutin, cellobiose, cyclodextrin, dulcitol, esculin, fructose, galactose, glucose, glycerol, inulin, lactose, lyxose, maltose, mannitol, mannose, melezitose, melibiose, *N*-acetylglucosamine, raffinose, rhamnose, ribose, salicin, soluble starch, sorbitol, sorbose, sucrose, trehalose, or xylose (Sigma); susceptibilities to optochin and bacitracin; bile solubility; growth in 6.5% NaCl or at 45°C; production of dextran or levan; chromophore test; and satellitism. All strains were also tested on the API 20 STREP system (bioMérieux). The API ZYM kit (bioMérieux) was used to test strains suspected of being *S. parasanguis* and *S. crista.*

DNA-DNA hybridization. The microplate hybridization method described previously (15) was performed, but a few minor changes were integrated. DNAs of 11 type strains (*S. adjacens*, *S. crista*, *S. defectivus*, *S. gordonii*, *S. mitis*, *S. oralis*, *S. parasanguis*, and *S. sanguis* [Table 1] and *S. pneumoniae* NCTC 7465^T, *Gemella morbillorum* GIFU 8337^T, *Gemella haemolysans* ATCC 27824^T) were extracted and purified by a large-scale method (15). DNAs of clinical isolates of the *S. sanguis* group (*S. sanguis*, *S. crista*, *S. gordonii*, *S. mitis*, *S. oralis*, and *S. parasanguis*), *S. adjacens*, and *S. defectivus* identified by conventional methods were extracted by a small-scale method (15). The DNAs of the type strains or salmon sperm DNA (Boehringer Mannheim, Mannheim, Germany), which was used as a negative control, were coated onto the sides of a microdilution well (15). A 100-μl volume of heat-denatured DNA of a type strain or salmon sperm DNA (10 mg/liter) in phosphate-buffered saline (pH 7.2) containing 0.1 M $MgCl_2$ was incubated for 12 h at 30°C in a microdilution well (Immuno Plates Maxisorp; Nunc, Rockilde, Denmark). Photobiotin-labeled DNA was prepared as described previously (15). A total of 10 ng of labeled DNA of a test strain was distributed in each well in the microdilution plate. Hybridization of the plate-bound DNA of the type strain and the labeled DNA of the test strain was carried out at 55°C for 2 h in the hybridization mixture containing 50% formamide (1). After hybridization, the hybridized DNAs were detected with streptavidin-horseradish peroxidase, tetramethylbenzidine, and hydrogen peroxide as described previously (24, 28). The optical density at 650 nm of each well was measured. When the ratio of the maximal color intensity and the color intensity of the negative control (value for salmon sperm DNA) was greater than 1.9, the data were processed as follows (28). The value of the maximal color intensity was taken to be 100%, and that of the negative control well was taken to be 0%. When there were no other wells in which color intensity was greater than 70% of the maximal color intensity, the test strain was identified as the plate-bound species of the well with the maximal color intensity.

The rapid ID 32 Strep system. Growth from BA plates was suspended in sterile distilled water to obtain a final turbidity equivalent to that of a no. 4 McFarland standard. Fifty-five microliters of this suspension was dispensed into each well of the strip. The strip was incubated and interpreted according to the manufacturer's instructions without performing additional tests. The strip was read either by the ATB 1525 reader (bioMérieux) or visually, and the results were interpreted with ATB 1545 Plus software (bioMérieux), which includes the rapid ID 32 Strep database (version 1.0). If the automated reader could not determine some reactions (determined as "questionable"), retests were performed at least twice. If the retests showed the same results, the visual results were interpreted according to the manufacturer's instruction. If the identification obtained with the rapid ID 32 Strep system contained an atypical reaction, the tests were repeated at least twice. If, after retesting, a correct identification was obtained, it was included in the correct tally and the first identification was discarded. If a strain of *S. downei* or *S. sobrinus* was identified as *S. downei*/*S. sobrinus*, which requires an additional test for species determination, its identification was included in the "correct identification." Identifications were classified as (i) species level (excellent, very good, good, or acceptable identification), (ii) group level (excellent, very good, good, or acceptable genus), (iii) low discrimination, (iv) incorrect (incorrect compared with the identification by DNA-DNA hybridization), and (v) unidentified (doubtful or unacceptable profile). The categories from (i) to (iii) were assigned to the correct identification.

RESULTS

Of the 171 strains of viridans group streptococci, only 156 strains corresponded to species included in the rapid ID 32 Strep database (Table 1). Identification rates for the rapid ID 32 Strep system are presented in Table 2. Of these 156 strains, 136 (87%) were correctly identified, including 100% of the *S.*

TABLE 2. Identification of 171 strains of viridans group streptococci by the rapid ID 32 Strep system

Organism[a]	No. tested	No. of strains identified by category (% of strains identified)						Misidentification (no.)
		Correct				Incorrect	Unidentified	
		Species level	Group level	Low discrimination	Total correct			
Identifiable with the rapid ID 32 Strep system								
S. adjacens	5	5	0	0	5	0	0	
S. anginosus	1	1	0	0	1	0	0	
S. constellatus	1	1	0	0	1	0	0	
S. defectivus	4	4	0	0	4	0	0	
S. downei	1	1	0	0	1	0	0	
S. gordonii	17	14	1	0	15	2	0	*S. mitis* (2)
S. intermedius	1	1	0	0	1	0	0	
S. mitis	6	1	2	0	3	3	0	*S. oralis* (3)
S. mutans	2	2	0	0	2	0	0	
S. oralis	67	44	9	0	53	8	6	*S. mitis* (7), *S. pneumoniae* (1)
S. salivarius subsp. *salivarius*	2	2	0	0	2	0	0	
S. salivarius subsp. *thermophilus*	1	1	0	0	1	0	0	
S. sanguis	46	38	7	0	45	0	1	
S. sobrinus	1	1	0	0	1	0	0	
S. vestibularis	1	1	0	0	1	0	0	
Total	156	117 (75)	19 (12)	0 (0)	136 (87)	13 (8)	7 (5)	*S. mitis* (9), *S. oralis* (3), *S. pneumoniae* (1)
Not identifiable with the rapid ID 32 Strep system								
S. cricetus	1					1	0	*S. mutans*
S. crista	4					4	0	*S. mitis* (3), *S. sanguis* (1)
S. ferus	1					0	1	
S. macacae	1					0	1	
S. parasanguis	7					3	4	*S. sanguis* (2), *S. mitis* (1)
S. rattus	1					0	1	
Total	15					8 (53)	7 (47)	*S. mitis* (4), *S. sanguis* (3), *S. mutans* (1)

[a] Clinical strains identified by DNA-DNA hybridization and type and reference strains.

djacens strains ($n = 5$) and *S. defectivus* strains ($n = 4$), 98% of the *S. sanguis* strains ($n = 46$), 88% of the *S. gordonii* strains ($n = 17$), 79% of the *S. oralis* strains ($n = 67$), 50% of the *S. mitis* strains ($n = 6$), and 100% of the other type and reference strains ($n = 11$). One hundred seventeen strains (75%) were identified to the species level, and 19 strains (12%) were identified to the group level. Misidentification occurred for 13 strains, including 9 strains of *S. oralis* ($n = 7$) and *S. gordonii* ($n = 2$) which were identified as *S. mitis*, 3 strains of *S. mitis* identified as *S. oralis*, and 1 strain of *S. oralis* identified as *S. pneumoniae*. Seven strains (5%), including six *S. oralis* strains and one *S. sanguis* strain, were not identified. Fifteen strains of six species (precise species) were not included in the rapid ID 32 Strep database. Of these strains, eight (53%) were misidentified as *S. mitis*, *S. sanguis*, or *S. mutans* and seven (47%) were not identified. From only the first results before the retests, 126 of the 156 strains (81%) included in the database were correctly identified (data not shown). For 15 strains not included in the database, the same results were obtained on retesting. Identical results were obtained by the conventional and the rapid ID 32 Strep system tests for most of reactions. However, there were some critical discrepancies between the two methods in tests for arginine dihydrolase (Table 3) and β-*N*-acetylglucosaminidase (βNAG). There were only five discrepancies in tests for arginine dihydrolase, but all of the strains that tested false positive belonged to *S. oralis*. There were no strains that tested false negative in the rapid ID 32 Strep system tests, while there were nine strains that tested false negative in the API-20 STREP system tests. βNAG in the rapid ID 32 Strep system was undetected in the cases of *S. crista* and *S. parasanguis*, but was detected in six of seven cases involving strains of *S. parasanguis* and in three of four cases involving strains of *S. crista* by the API ZYM kit (data not shown).

The biochemical profiles of the rapid ID 32 Strep system that we obtained during our study of eight species, including stock collection and clinical isolates, are presented in Table 4. With the 32 reactions, these 156 strains were positive for alanine-phenylalanine-proline-arylamidase (APPA) and fermentation of maltose (MAL) and sucrose (SAC) and were negative by the Voges-Proskauer test, negative for the hydrolysis of

TABLE 3. Comparison among ADH test results produced by the conventional method, the API-20 STREP system, and the rapid ID 32 Strep system[a]

Test	No. of strains	
	Con negative	Con positive
Rapid positive, API positive	3[b]	69
Rapid positive, API negative	2[c]	9[d]
Rapid negative, API positive	1[e]	0
Rapid negative, API negative	87	0
Discrepancy	6	9
Total	93	78

[a] Con, conventional method; API, API 20 STREP system; Rapid, rapid ID 32 Strep system.
[b] *S. oralis.*
[c] *S. oralis.*
[d] *S. gordonii*; ($n = 5$) and *S. sanguis* ($n = 4$).
[e] *S. oralis.*

TABLE 4. Physiological reactions of strains of eight species of viridans group streptococci in the rapid ID 32 Strep system

Organism[a]	No. of positive reactions/percentage of positive reactions[b]																						
	ADH	βGLU	βGAR	βGUR	αGAL	PAL	RIB	MAN	SOR	LAC	TRE	RAF	βGAL	PyrA	βNAG	GTA	GLYG	PUL	MEL	MβDG	TAG	βMAN	CDEX
S. adjacens																							
ATCC 49175^T (n = 1)	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
Clinical strains (n = 4)	0	0	0	3	0	0	0	0	0	0	0	0	0	4	1	0	0	0	0	0	4	0	0
Percentage of positive reactions (total, n = 5)	0	0	0	80	0	0	0	0	0	0	0	0	0	100	20	0	0	0	0	0	100	0	0
S. crista																							
NCTC 12479^T (n = 1)	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	1	0	0
Clinical strains (n = 3)	3	0	2	0	1	0	0	0	0	2	1	1	1	0	0	3	0	2	1	0	1	0	0
Percentage of positive reactions (total, n = 4)	100	0	75	0	25	0	0	0	0	75	50	25	25	0	0	100	0	50	25	0	50	0	0
S. defectivus																							
ATCC 49176^T (n = 1)	0	0	1	0	1	0	0	0	0	1	1	0	1	1	0	0	0	1	0	0	0	0	0
Clinical strains (n = 3)	0	0	3	0	3	0	0	0	0	2	3	0	3	3	0	0	0	3	0	0	0	0	0
Percentage of positive reactions (total, n = 4)	0	0	100	0	100	0	0	0	0	75	100	0	100	100	0	0	0	100	0	0	0	0	0
S. gordonii																							
ATCC 10558^T (n = 1)	1	1	1	0	0	1	0	0	0	1	1	0	0	0	0	1	0	0	0	1	0	1	0
Clinical strains (n = 16)	16	14	16	0	1	16	0	0	0	16	16	0	0	0	0	15	0	0	0	14	8	13	0
Percentage of positive reactions (total, n = 17)	100	88	100	0	6	100	0	0	0	100	100	0	0	0	0	94	0	0	0	88	47	82	0
S. mitis																							
NCTC 12261^T (n = 1)	0	0	1	0	1	0	1	0	0	1	0	1	0	0	0	1	0	1	1	0	0	0	0
Clinical strains (n = 5)	0	0	4	0	1	3	1	0	0	5	0	1	0	0	0	5	0	4	1	0	0	0	0
Percentage of positive reactions (total, n = 6)	0	0	83	0	33	50	33	0	0	100	0	33	0	0	0	100	0	83	33	0	0	0	0
S. oralis																							
NCTC 11427^T (n = 1)	0	0	1	0	0	1	0	0	0	1	1	0	1	0	1	1	0	1	0	0	1	0	0
ATCC 10557 (n = 1)	0	0	1	0	1	1	0	0	0	1	0	1	0	0	1	1	0	1	1	0	0	0	0
Clinical strains (n = 65)	5	1	60	0	42	50	0	0	0	63	22	40	21	1	16	65	1	61	40	0	26	0	1
Percentage of positive reactions (total, n = 67)	7	1	93	0	64	78	0	0	0	97	34	61	33	1	27	97	1	94	61	0	40	0	1
S. parasanguis																							
ATCC 15912^T (n = 1)	1	1	1	0	1	1	0	0	0	1	1	1	1	0	1	1	0	0	1	1	0	1	0
Clinical strains (n = 6)	6	2	6	0	5	6	0	0	0	6	1	4	1	0	0	6	0	0	4	1	4	0	0
Percentage of positive reactions (total, n = 7)	100	43	100	0	86	100	0	0	0	100	29	71	29	0	14	100	0	0	71	29	57	14	0
S. sanguis																							
ATCC 10556^T (n = 1)	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	1	0	0	0	0	0
Clinical strains (n = 45)	45	22	9	0	32	0	0	1	25	36	44	24	0	0	0	45	0	43	23	10	14	0	0
Percentage of positive reactions (total, n = 46)	100	50	20	0	70	0	0	2	54	80	98	52	0	0	0	100	0	96	50	22	30	0	0

[a] Clinical and reference strains were identified by DNA-DNA hybridization.

[b] Reactions of the rapid ID 32 Strep system: ADH, arginine dihydrolase; βGLU, β-glucosidase; βGAR, β-galactosidase detected by *p*-nitrophenyl-β-D-galactopyranoside as substrate; βGUR, β-glucuronidase; αGAL, α-galactosidase; PAL, alkaline phosphatase; βGAL, β-galactosidase detected by 2-naphthyl-β-galactopyranoside as substrate; PyrA, pyrrolidonyl arylamidase; βNAG, *N*-acetyl-β-glucosaminidase; GTA, glycyl-tryptophane arylamidase; βMAN, β-mannosidase, RIB, MAN, SOR, LAC, TRE, RAF, GLYG, PUL, MEL, TAG, and CDEX, fermentation of ribose, mannitol, sorbitol, lactose, trehalose, raffinose, glycogen, pullulan, melibiose, tagatose, and cyclodextrin, respectively.

TABLE 5. Correlation between the identification of the *S. sanguis* group by the rapid ID 32 Strep system and that by DNA-DNA hybridization

Type strains and strains identified by DNA-DNA hybridization	No. tested	No. of strains identified by the rapid ID 32 Strep system (% correct identification)							% Correct identification
		S. gordonii	*S. mitis* 1	*S. mitis* 2	*S. oralis*	*S. sanguis*	Others	Unidentified	
S. crista	4	0	0	3	0	1	0	0	0
S. gordonii	17	15	0	2	0	0	0	0	88
S. mitis	6	0	3	0	3	0	0	0	50
S. oralis	67	0	6	1	53	0	1	6	79
S. parasanguis	7	0	0	2	0	1	0	4	0
S. sanguis	46	0	0	0	0	45	0	1	98
Total	147	15 (100)	9 (33)	8 (0)	56 (95)	47 (96)	1	11	79

hippurate and urease, and negative for the fermentation of melezitose, L-arabinose, and D-arabitol.

Four of five *S. adjacens* strains including the type strain, ATCC 49175, had the same profiles, while one strain had a similar profile in that it was negative for β-glucuronidase (βGUR) and positive for βNAG. Three of four *S. defectivus* strains including the type strain, ATCC 49176, had the same profile, and one strain had a similar profile in that it was negative for lactose (LAC) fermentation.

Five strains of *S. gordonii* including the type strain, ATCC 10558, had the same profile and seven strains had similar profiles in that they were positive for tagatose (TAG) fermentation. Two strains had some atypical profiles, as a result of a positive α-galactosidase (αGAL) test in one case and negative glycyl-tryptophane arylamidase (GTA) and β-mannosidase (βMAN) tests in the other. Two strains with negative β-glucosidase (βGLU), βMAN, and methyl-β-D-glucopyranoside (MβDG) fermentation were identified as *S. mitis* (*S. mitis* 2).

Six strains of *S. mitis* including the type strain, NCTC 12261, had negative arginine dihydrolase (ADH), fermentation of trehalose (TRE), βNAG, and β-galactosidase reactions when 2-naphthyl-β-galactopyranoside (βGAL) was used as a substrate. Of the five strains that had a positive β-galactosidase reaction detected by using *p*-nitrophenyl-β-D-galactopyranoside (βGAR) as a substrate, two strains with positive fermentation of melibiose (MEL) and one strain with a positive alkaline phosphatase (PAL) reaction were identified as *S. oralis.*

Sixty-seven strains of *S. oralis* including the type strain, NCTC 11427, and the reference strain, ATCC 10557, had positive GTA, LAC (except one strain), βGAR, and pullulan (PUL) fermentations (except three strains). However, it was difficult to differentiate between *S. oralis* and *S. mitis* except when there were certain profiles such as positive fermentation for TAG and TRE (only for *S. oralis*) and positive fermentation for ribose (only for *S. mitis*). Consequently, seven strains of *S. oralis* were identified as *S. mitis*. One strain with a negative PUL fermentation was identified as *S. pneumoniae*. Of five strains of *S. oralis* with a positive ADH reaction, four were assigned a doubtful profile and one was identified as *S. mitis* (*S. mitis* 2). These strains, however, did not hydrolyze arginine by a conventional method (Table 3). Of the two strains not identified, one strain had positive fermentations for cyclodextrin and glycogen (GLYG), giving a doubtful profile for *S. oralis*, and the other one assigned to *G. morbillorum* with a doubtful profile was afermentative except for MAL and SAC.

Forty-six strains of *S. sanguis* including the type strain, ATCC 10556, had positive ADH, GTA, TRE (except one strain), and PUL (except two strains) reactions and negative PAL, βNAG, and βMAN reactions. There were variable profiles for βGLU, αGAL, MβDG, fermentation of sorbitol (SOR), raffinose (RAF), and MEL. Forty-five strains were correctly identified. One strain with mannitol (MAN) fermentation was assigned to *S. sanguis* with a doubtful profile.

The other 11 type and reference strains representing species present in the rapid ID 32 Strep database were identified correctly to the species level.

Of the species not present in the rapid ID 32 Strep database, four strains of *S. crista* including the type strain, NCTC 12479, had a positive ADH reaction and negative βGLU, βNAG, and PAL reactions. Three strains with a positive βGAR reaction were identified as *S. mitis* (*S. mitis* 2), and one strain with a negative βGAR reaction was identified as *S. sanguis.*

All seven strains of *S. parasanguis* including the type strain, ATCC 15912, had positive ADH, βGAR, and PAL reactions and a negative PUL reaction. Six strains including the type strain had a positive αGAL reaction. Four strains including the type strain were assigned an unacceptable profile or a doubtful profile. Two strains were identified as *S. mitis* (*S. mitis* 2) and one strain was identified as *S. sanguis.*

Table 5 shows the correlation between the identification of the *S. sanguis* group by the rapid ID 32 Strep system and that by DNA-DNA hybridization. The identification of *S. gordonii*, *S. sanguis*, and *S. oralis* by the rapid ID 32 Strep system corresponded well with the identification of these species by DNA-DNA hybridization. However, of the 17 strains identified as *S. mitis* by the rapid ID 32 Strep system, only 3 strains were designated *S. mitis* by DNA-DNA hybridization. Of the six *S. mitis* strains evaluated (by DNA-DNA homology), the rapid ID 32 Strep system correctly identified only three of them, two to the group level only. Among the eight strains identified as *S. mitis* 2 by the rapid ID 32 Strep system, DNA-DNA hybridization did not identify any of them as *S. mitis.*

Of the strains in the *S. mutans* group closely related to *S. mutans* (*S. mutans*, *S. cricetus*, *S. downei*, *S. ferus*, *S. macacae*, *S. rattus*, and *S. sobrinus*), the type strain of *S. cricetus* was identified as *S. mutans*. The type strain of *S. rattus* was assigned to *S. mutans* with a doubtful profile because of a positive ADH result. The type strains of *S. ferus* and *S. macacae* were assigned doubtful profiles.

Table 6 summarizes the identification of the *S. sanguis* group according to selected rapid ID 32 Strep reactions. *S. parasanguis*, whose profile is not included in the database, could be differentiated from *S. gordonii* by the αGAL reaction. *S. crista* and *S. sanguis* could not be differentiated by the rapid ID 32 Strep system profile. No reactions satisfactorily separated *S. mitis* from *S. oralis.*

DISCUSSION

Species identification protocols for viridans group streptococci use a series of physiological reactions (6, 25) on which commercial identification kits are based (2, 3, 16, 18, 21).

TABLE 6. Identification results for the *S. sanguis* group according to the selected rapid ID 32 Strep system

Organism	Reactions with the rapid ID 32 Strep system[a]													
	ADH	αGAL	βGAR	βGAL	βGLU	βNAG	PAL	RIB	SOR	TRE	RAF	PUL	MEL	TAG
S. crista	+	d	d	d	−	−	−	−	−	d	d	d	d	d
S. gordonii	+	−	+	−	+	−	+	−	−	+	−	−	−	d
S. mitis	−	d	+	−	−	−	d	d	−	−	d	+	d	−
S. oralis	−	d	+	d	−	d	d	−	−	d	d	+	d	d
S. parasanguis	+	+	+	d	d	−	+	−	−	d	d	−	d	d
S. sanguis	+	d	−	−	d	−	−	−	d	+	d	+	d	d

[a] +, ≥80% of strains positive; −, ≤20% of strains positive; d, 21 to 79% of strains positive. See footnote *b* of Table 4 for definitions of the abbreviations for the reactions.

However, there has been some confusion concerning the taxonomy of viridans group streptococci (11), and therefore, it is difficult to revise the databases in these kits. The rapid ID 32 Strep database takes into account the new descriptions of viridans group streptococci (9, 25, 38). With this kit, *S. gordonii* and *S. sanguis* can be separated from the *S. sanguis* group, *S. mutans* and *S. downei*/*S. sobrinus* can be distinguished from the *S. mutans* group, *S. anginosus*, *S. intermedius*, and *S. constellatus* can be differentiated from "*S. milleri*," and new species (*S. adjacens*, *S. defectivus*, and *S. vestibularis*) can be identified. Freney and colleagues (19) reported that this system correctly identified 76 of 135 (56%) strains of viridans group streptococci without extra tests. According to our results, 136 of 156 (87%) strains representing species included in the rapid ID 32 Strep system database were correctly identified without any additional tests. The previous studies used test strains that were identified by physiological reactions. However, we considered that physiological identification was insufficient for evaluation of kits for viridans group streptococci because there were some variations among physiological reactions in the same species (6, 25) and some phenotypic shifts (33). Therefore, we used strains identified by DNA-DNA hybridization as the gold standard of identification. The strains that could not be identified by DNA-DNA hybridization were eliminated from our test panels, even if their physiological reactions were typical.

Moreover, *S. parasanguis*, whose profile is not included in the database, could be differentiated from the other species in the *S. sanguis* group (Table 6). Our results indicate that the rapid ID 32 Strep system is a more suitable system for the species identification of viridans group streptococci than some other conventional kits such as the API 20 STREP system (2, 16, 18, 21).

However, there were some problems with this system. There were some critical discrepancies between the rapid ID 32 Strep system and the conventional tests. There were only five discrepancies of ADH between the two methods, but all of the strains testing false positive belonged to *S. oralis* (Table 3). Similar results were shown with the API 20 STREP system and were described in another report (18). In Table 6, the *S. sanguis* group was separated into two groups by ADH. This is a critical problem because ADH is a key reaction for the physiological differentiation of viridans group streptococci (6, 17, 18). The reason for this discrepancy is unclear; however, the medium components (Maeller's decarboxylase medium with 1% L-arginine [Difco]) and incubation time (7 days) of the conventional method were quite different from those of the rapid ID 32 Strep system. In the rapid ID 32 Strep system, the ADH cupule contained the substrate arginine, tryptone, yeast extract, and some kind of buffer to increase the sensitivity of the system and to allow determination of species within 4 h of incubation (9a). The differences in both medium components might affect this discrepancy.

Beighton et al. (6) have shown that the βNAG reaction with the 4-methylumbelliferyl substrate is helpful in differentiating the *S. sanguis* group, since *S. oralis*, *S. parasanguis*, and *S. crista* produced this enzyme but *S. mitis* did not. Although the rapid ID 32 Strep system included the reaction of this enzyme, it was undetected in the case of *S. crista* and *S. parasanguis* and was detected in only 16 of 67 strains (27%) of *S. oralis* tested. It was detected by the API ZYM kit in six of seven cases involving strains of *S. parasanguis* and in three of four cases involving strains of *S. crista*. This low percentage might affect the differentiation of *S. oralis* and *S. mitis.*

The database of the rapid ID 32 Strep system did not include profiles for some new species such as *S. crista*, *S. parasanguis*, *S. cricetus*, *S. ferus*, *S. macacae*, and *S. rattus*. The database for the *S. sanguis* group was based on description of Kilian et al. (25). Some *S. parasanguis* and *S. crista* strains were identified as *S. mitis* 2 by using physiological tests by their classification. The problems of this database were due to the discrepancies between the results of the rapid ID 32 Strep system and DNA-DNA hybridization results. The rapid ID 32 Strep system can distinguish only three species of the *S. mutans* group as *S. mutans* and *S. downei*/*S. sobrinus*, which can be separated further with one additional test (salicin fermentation), as recommended in the manufacturer's instructions. The *S. mutans* group can be divided into seven species by certain physiological tests such as ADH, fermentation of RAF, inulin, GLYG, SOR, starch and MEL; susceptibility to bacitracin; production of hydrogen peroxide; and aerobic growth (11, 41). The rapid ID 32 Strep system contains a reaction for ADH and fermentation of RAF, GLYG, SOR, and MEL. According to these characteristics, *S. rattus*, *S. ferus*, and *S. macacae* can be differentiated from other species in the *S. mutans* group. Differentiation of *S. cricetus* requires tests such as susceptibility to bacitracin, determination of cell wall sugar components, or peptidoglycan typing (11). Because only the type strains were tested, by our tests, *S. rattus* was assigned to *S. mutans* with one atypical positive ADH reaction and *S. ferus* had a doubtful profile because of a positive GLYG fermentation. Our results suggest that members of the *S. mutans* group can be differentiated by the rapid ID 32 Strep system with the aid of some additional tests. However, these species are very rarely isolated, and the number of strains isolated throughout the world up to now is so limited that it is difficult to include these species in commercial systems.

Even with the additional physiological tests recommended by the manufacturer, some strains such as *S. mitis* and *S. oralis* could not be differentiated except by DNA-DNA hybridization. Freney et al. (19) reported that for 11 of 17 strains of *S. mitis* and 14 of 22 strains of *S. oralis* additional tests were

required for identification by the rapid ID 32 Strep system. Ohkuni et al. (30) reported that of the 68 strains that they tested, there were only 25 cases of agreement (37%) between strains assigned to these two species by conventional methods and their identifications by DNA-DNA hybridization. None of the conventional physiological reactions that we tested could differentiate these two species completely (data not shown). Some physiological tests such as βNAG and β-*N*-acetyl-galactosaminidase that use 4-methylumbelliferyl substrates (6) and amylase binding (12) or genetic methods such as DNA-DNA hybridization (1, 15, 20, 27, 35, 39) and ribotyping (33) might be required for the accurate differentiation of these two species. The rapid ID 32 Strep system also could not differentiate *S. sanguis* and *S. crista*. According to our results, 4 strains of *S. crista* did not produce dextran, whereas 44 of 46 strains of *S. sanguis* did (data not shown). However, Handley et al. (20) showed that dextran production by *S. crista* was variable. Beighton et al. (6) reported that *S. crista* produced α-L-fucosidase and β-*N*-acetyl-galactosaminidase with 4-methylumbelliferyl substrates but that *S. sanguis* did not. Rudney and Larson (33) reported that the ribotype of *S. crista* was different from that of *S. sanguis*. These additional tests might be required for the differentiation of *S. sanguis* and *S. crista*.

We investigated only eight species that were mainly isolated from patients with infective endocarditis, except for type and reference strains. We did not test some important pathogens such as *S. anginosus*, *S. constellatus*, and *S. intermedius*. Further studies are proposed to evaluate this kit for its ability to identify these species.

In conclusion, the rapid ID 32 Strep system can be used to differentiate most species for which phenotypic characteristics have been described, but the revision of the database and the improvement of some reactions are needed.

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