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Use of Enzyme Tests in Characterization and Identification of Aerobic and Facultatively Anaerobic Gram-Positive Cocci

SHOSHANA BASCOMB[1] AND MAMMAD MANAFI[2]*

31 Bramble Walk, Lymington SO41 9LW, United Kingdom,[1] and Hygiene Institute, University of Vienna, A-1095 Vienna, Austria[2]

INTRODUCTION

Microorganisms have been classified and identified on the basis of a variety of characteristics including morphological, growth, tolerance, metabolic, biochemical, and genetic. Recently there has been a tendency to determine definitive classification and taxonomic assignment by nucleic acid hybridization, 16S rRNA sequence analysis, and other molecular genetic techniques. After classification has been established, characteristics are selected for the identification of unknown isolates. Commercial kits based on such a process are available for the identification of clinically important bacteria. It is essential to realize that for routine identification of isolates from human, food, or veterinary specimens, ease of testing and total completion time are critical, since the added value of identification information to the clinical or processing outcome decreases the later it becomes available. Completion times for the identification of bacteria taken from isolated colonies can vary from 2 h to several days. Completion times for tests performed directly on clinical specimens are also variable.

Most growth-dependent tests require at least an overnight incubation; others, based on the ability to utilize a single carbon or nitrogen source, may require as long as 7 days. The molecular genetic techniques are still time-consuming and less amenable to routine application. Moreover, most of the techniques available now are for the specific detection of a limited number of taxa. Alternatively, determinations of the enzymatic activities of isolates with a variety of synthetic substrates (16, 52, 124) can be used for identification and give similar results to those obtained by other characterization methods. The enzymatic characterization of microorganisms by means of synthetic substrates makes use of the fact that many enzymes are constitutively present or easily induced and rapidly detectable, often after incubation times of seconds to 3 h. Thus, identification of bacteria based on enzyme patterns offers simple and rapid results.

The ability to detect specific enzymes rapidly with synthetic chromogenic or fluorogenic substrates has been studied extensively (17, 35, 53, 54, 110, 117, 118, 150). Tests involving some of these substrates have been included in commercial kits for identification or taxonomic studies of bacterial isolates.

The first commercial kit with tests for specific enzymes con-

* Corresponding author. Mailing address: Department of Food- and Waterhygiene, Hygiene Institute, University of Vienna, Kinderspitalgasse 15, A-1095 Vienna, Austria. Phone: 43-1-40490-250. Fax: 43-1-40490-295. E-mail: Mohammad.manafi@univie.ac.at.

tained Patho-Tec paper strips (172), a method that evolved to the Micro-ID system (15) for the identification of clinically important gram-negative rods, mainly members of the *Enterobacteriaceae*. The Micro-ID kit included tests for β-galactosidase, cytochrome oxidase, lysine and ornithine decarboxylases, tryptophanase, and urease. Other kits in the form of cards, microtiter trays, or multichamber strips are now available for the identification of certain taxonomic classes of bacteria. The largest number is available for the identification of clinically important aerobic and facultatively anaerobic gram-negative bacteria (e.g., API 20E, MicroScan conventional overnight and MicroScan Rapid GN Identification Systems, and Vitek GNI Card). Fewer kits are available for the identification of gram-positive cocci, staphylococci, streptococci, anaerobic cocci, and yeasts. API 20E, MicroScan conventional overnight, and Vitek GNI Card include growth-dependent tests and a few enzyme tests; in general, they do not use the ability of enzyme tests to provide results rapidly. A specific enzyme test for β-galactosidase based on utilization of the synthetic substrate *o*-nitrophenol-β-D-galactopyranoside (ONPG) or substrates with other synthetic moieties is included in most kits for identification of the *Enterobacteriaceae*. Some kits are completely manual, whereas others offer automation with instruments for all or some of the following tasks: inoculation, incubation, determination of test results, and identification. Dade MicroScan Rapid Identification Systems for gram-negative rods and gram-positive cocci are the fastest systems, providing identification in 2 to 2.5 h by measuring enzymatic activities fluorometrically with a high correct identification rate. Thus, overall rates of 98.4 and 92.5% correct identification to the species level, with and without additional tests, respectively, were obtained during the database development phase of the MicroScan gram-negative rapid identification system type 3 (RNID3) for 4,151 isolates comprising 138 fermentative and nonfermentative gram-negative bacteria (1). Clinical studies of the RNID3 system showed 96.8 and 89.5% correct identification for 405 fresh and 247 challenge isolates, respectively (18).

The commercial identification systems also provide databases of expected results, and an unknown isolate is assigned to one of the taxa in the database either by using a code book or by using an automated system and computer-based identification. Most of these kits have been designed for clinically important groups of bacteria, as reflected in the taxa, tests, and expected results included in the databases. Some may be applicable to isolates taken from different environments (143, 240). These kits can also be used for characterization of microorganism groups other than those specified in the database (27). Methods for easy extraction and analysis of enzymatic activities are required for such a purpose.

Commercial identification kits have been optimized for the sets of taxa included in their databases. They require regulatory approval and are expected to provide a high level of accurate identification in comparison with an acceptable reference method. Characterization kits, on the other hand, are designed to provide an easy and reproducible method for testing a variety of unknown isolates. They do not provide expected results and are less rigorously regulated. They are very useful in taxonomic studies, and the results from such studies may be used for the construction of identification kits. Some of these kits have been designed specifically for the characterization of microorganisms with a variety of chromogenic enzyme substrates. These kits are not accompanied by a database, and some may have methods for computer analysis of the data including classification and identification of unknown isolates. They vary in the complement of tests included, the size of the inoculum required, and the incubation period. Generally speaking, tests that require more than 2 h of incubation and/or include a growth-supporting medium in the test compartment allow the detection of inducible enzymes. Tests requiring inoculum densities of ≥2 McFarland standard units frequently necessitate an additional overnight incubation to achieve a sufficient inoculum.

Studies of the clarification of the taxonomic position of individual taxa have used laboratory-prepared tests (117, 119) as well as commercial characterization and identification kits. Only the latter contain information on expected results for specified taxa in each of the kit tests.

The API ZYM system (API System; bioMérieux, Paris, France) is a semiquantitative micromethod designed for the detection of 19 enzymatic activities (106, 232). The use of API ZYM for taxonomic studies of a variety of taxa of bacteria and other prokaryotic as well as eukaryotic organisms has been tabulated (99). The use of API enzyme research kits detecting 20 glycosidases, 10 esterases, 57 arylamidases, alkaline and acid phosphatases, and phosphoamidase has been reported (151, 153, 164, 226).

In addition, kits based on growth-dependent tests to determine the utilization of amino acids, organic acids, and carbohydrates are available. The API 50CH (12, 123, 164), 50AA, and 50OA (125) tests are strip based; the results are indicated by changes in the color of the pH indicators. The Biolog system (Biolog Inc., Hayward, Calif.) is microtiter tray based and is dependent on the detection of substrate-specific dehydrogenases with tetrazolium salt as an indicator (163). The MAST ID system (Mast Laboratories Ltd., Bootle, England) provides a means of determining the metabolic activities of a number of isolates by agar plate and multipoint inoculator techniques (84, 86, 123, 193). Combinations of these systems permit the detection of over 340 biochemical reactions. A number of studies report the use of commercially available characterization kits alone or in combination with test batteries prepared in their own laboratories (25, 31, 63).

This review will survey the current classification of aerobic and facultative anaerobic gram-positive cocci with emphasis on the role of rapid enzyme tests in characterization and identification.

GRAM-POSITIVE COCCI

The aerobic and facultatively anaerobic gram-positive cocci were originally divided into two families, *Micrococcaceae* and *Streptococcaceae*, on the basis of catalase activity and cell morphology and aggregation. However, this classification may change, since genetic studies have indicated that *Staphylococcus* is more closely related to the *Bacillus-Lactobacillus-Streptococcus* cluster than to *Micrococcus* or *Stomatococcus* (129).

Micrococcaceae

The family *Micrococcaceae*, which includes the aerobic and facultatively anaerobic gram-positive cocci giving a positive reaction in the catalase test, is not a phylogenetically coherent group. Four genera, *Micrococcus*, *Planococcus*, *Staphylococcus*, and *Stomatococcus*, are recognized (129). Tests useful for the separation of these taxa include oxygen requirement for growth, oxidase test, motility, NaCl tolerance, and susceptibility to bacitracin, furazolidone, and lysostaphin. Genetic studies have suggested that *Micrococcus* belongs to the actinomycete group, and the isolates originally identified as species of *Micrococcus* have since been placed in five different genera (see below) (218).

Staphylococcus is the only genus showing susceptibility to

TABLE 1. Comparison of systems and schemes for the identification of gram-positive bacteria

Characteristic and test type	Identification system[a]											
	Identifies *Micrococcaceae* and *Streptococcaceae*				Identifies *Micrococcaceae*					Identifies *Streptococcaceae*		
	MS GP	MS Rapid GP	Vitek GPI	Pasco GP ID	API Staph ID	Geary et al. (86)	Kloos and Bannerman (129)	API Staph ID 32	Staph-Zym	RapID STR	Rapid ID 32 Strep	API 20 Strep
Characteristics												
Inoculum density (McFarland)	0.5	0.5	0.5–1	0.5	3			0.5	2	1	4	4
Inoculum vol (ml)	3	6.5	1.8	3	2			2–3	3	1	2–3	2
No. of tests included	26	34	29	18	10	15	36	26	10	14	32	20
No. of additional tests	2	1	3	2	1	1	0	1	4	2	3	2
No. of reagents	5	0	0	3	1	0	11	6	3	1	5	5
No. of taxa included	48	50	44	26	17	12	36	32		26	52	48
Storage temp (°C)[b]	RT	2–8	2–8		2–8			2–8		2–8	2–8	2–8
Medium for inoculum source[b]		TSA + 5% SB	TSA + 5% SB			BHI		V			SBA	
Incubation period (h)	16–42	2	4–15	16–20	5	18–24	18–72	24	18–24	4	4	4–24
Test type (no. of tests)												
Glycosidases/phosphatases	4	12	0	3	4	1	4	3	4	4	8	4
Formation of acid	10	11	18	6	4	10	12	14	2	4	17	10
Peptidases	1	10	0	1	0	0	2	2	1	4	3	2
Hydrolases	1	0	1	1	0	2	5	1	0	1	1	2
Other metabolic tests	4	1	2	3	2	1	7	5	3	1	3	2
Growth and tolerance tests	6	0	8	4	0	1	6	1		0	0	0
Mandatory additional tests[c]	C, H	C	C, Cg, H	C, H	C	C		C	G	C, H	C, H, P	C, H

[a] MS GP, MicroScan Conventional Gram-Positive Panel; MS Rapid GP, MicroScan Rapid Gram-Positive Panel; Vitek GPI, Vitek Gram Positive Identification Card; Pasco GP ID, Pasco Gram-Positive ID; API Staph ID, API STAPH IDENT; Geary et al. (86), based on multipoint inoculation of agar plates.
[b] RT, room temperature; TSA + 5% SB, Trypticase soy agar plus 5% sheep blood; BHI, brain heart infusion broth; V, various media; SBA, sheep blood agar.
[c] C, catalase; Cg, coagulase; H, hemolysis; G, growth and tolerance tests that need to be done separately from the kit; P, pigmentation.

lysostaphin, but some exceptions occur among species of *Micrococcus* and *Staphylococcus*. The methodology and usefulness of the lysostaphin test for differentiation between *Micrococcus* and *Staphylococcus* have been discussed (10, 83, 100, 133, 134, 138, 178, 184, 207, 210, 253). Lysostaphin is a protein preparation derived from culture filtrates of "*Staphylococcus staphylolyticus*," which contains three enzymes capable of affecting the bacterial cell wall: a glycyl-glycine endopeptidase, an endo-β-*N*-acetylglucosaminidase, and an *N*-acetylmuramoyl-L-alanine amidase (32, 215). The endopeptidase is the component that lyses the cell wall of *Staphylococcus aureus* by hydrolyzing the polyglycine of the pentapeptide bridge between glycopeptide chains of the staphylococcal cell wall. Sloan et al. (215) have shown that the endopeptidase is capable of catalyzing both hydrolysis and transpeptidation reactions when acting on glycyl peptides. The usefulness of the test depends on the purity of the enzyme preparation, the storage of the enzyme, the lysostaphin concentration (184), and the test procedure. When susceptibility is tested by observation of growth inhibition zones, it also depends on the medium on which the bacteria are grown (144). Susceptibility also depends on the cell wall amino acids of the bacterial species; species that contain serine in the interpeptide bridge, such as *S. saprophyticus*, *S. haemolyticus*, and *S. hominis*, are less susceptible. Interestingly, lytic agents such as lysostaphin are produced by members of the genus *Staphylococcus* only.

Of the genera included in the family *Micrococcaceae*, *Staphylococcus* is the most clinically important and contains 32 species, most of which live on the skin and other external surfaces of animals (129). A comprehensive review of the genera *Micrococcus* and *Staphylococcus* and their clinical significance has appeared recently (129). Some staphylococci are human and animal opportunistic pathogens that, under certain circumstances, are major causes of mortality and morbidity. Because of the ability of members of the genus *Staphylococcus* to acquire resistance to many antimicrobial agents (e.g., strains of methicillin-resistant *S. aureus* (MRSA), they can cause major clinical and epidemiological problems in hospitals (30), and their presence is monitored carefully. Schemes for the differentiation of members of the family have been published (12, 129, 194). Kits available for the identification of this group include API STAPH-IDENT, Staph-TRAC, API ID 32 Staph, RAPiDEC Staph, and Vitek GPI Card (bioMérieux-Vitek Inc. Hazelwood, Mo., and bioMérieux S.A., Marcy l'Etoile, France); GP Microplate test panel (Biolog); MicroScan Pos ID and Combo, and Rapid Pos ID and Combo panels (Dade International Inc., West Sacramento, Calif.); Pasco Gram-Positive ID (Pasco, Wheat Ridge, Colo.); and Staph-Zym (Rosco, Tastrop, Denmark). The general requirements of the kits and the tests included are listed in Tables 1 to 6, which also contain some published identification schemes for these taxa. In addition to the schemes listed in the tables, Watts et al. (238) have tested the API 20GP (bioMérieux-Vitek) kit, which consists of 20 microcupules containing dehydrated substrates for the identification of staphylococci and group D streptococci. It includes the 10 Staph-Ident tests and 10 tests selected from the API 20S streptococcal identification system, but it has provided only 56.10% correct identification. STAPHYtest (Lachema, Brno, Czech Republic), a microtiter-based system with nine tests and with eight compartments per isolate for identification of *Micrococcus*, *Stomatococcus*, and *Staphylococcus*, has been described (211). A system described by Rhoden et al. (194) is

TABLE 2. Comparison of glycosidase and phosphatase tests used in systems for identification of gram-positive bacteria

Enzyme	Identification system[a]											
	Identifies *Micrococcaceae* and *Streptococcaceae*				Identifies *Micrococcaceae*					Identifies *Streptococcaceae*		
	MS GP	MS Rapid GP	Vitek GPI	Pasco GP ID	API Staph ID	Geary et al. (86)	Kloos and Bannerman (129)	API Staph ID 32	Staph-Zym	RapID STR	Rapid ID 32 Strep	API 20 Strep
α-L-Arabinosidase		MEU										
N-Acetyl-β-D-glucosaminidase		MEU								PNP	+	
N-Acetyl-β-D-galactosaminidase		MEU										
β-D-Cellobiosidase		MEU										
β-D-*N*′,*N*′-Diacetylchitobiosidase		MEU										
α-D-Galactosidase		MEU								PNP	+	6B
β-D-Galactosidase	PNP	MEU			PNP		PNP	+	+		+, +[b]	βN
α-D-Glucosidase		MEU		PNP						PNP		
β-D-Glucosidase		MEU			PNP		PNP		+		+	
β-D-Glucuronidase	PNP	MEU		PNP	PNP		PNP	+	+		+	AS
β-D-Mannosidase		MEU									+	
Phosphatase	PNP, I	MEU		PNP	PNP	PNP	PNP	+	+	PNP	+	βN
No. of tests	4	12	0	3	4	1	4	3	4	4	8	4

[a] For an explanation of the identification systems, see Table 1, footnote *a*. Synthetic moiety of substrates: AS, naphthol AS-BI; 6B, 6-bromo-β-naphthol; I, indoxyl; βN, β-naphthol; PNP, *p*-nitrophenol; +, proprietary synthetic moiety.
[b] Two substrates with different proprietary synthetic moieties.

based on 6 screening, 18 primary, and 11 confirmatory tests; the tests used are similar to those described by Kloos and Bannerman (129). Eight enzyme tests are included in this scheme, which reports results and assigns isolates to a taxon by using a six-digit numerical code. The RAPiDEC Staph system is based on detection of the activities of three enzymes: "aurease", β-galactosidase, and alkaline phosphatase; it requires a very heavy inoculum and can identify *S. aureus*, *S. epidermidis*, *S. saprophyticus*. Additional tests are required to differentiate between *S. intermedius*, *S. xylosus*, and the remaining *Staphylococcus* species.

The number of taxa identified by these kits varies from 5 by RAPiDEC Staph to 50 by MicroScan Rapid Pos ID panels. Some of the kits are specific to the *Micrococcaceae* or just to staphylococci, whereas others are intended for identification of both staphylococci and streptococci. Tables 1—6 show that tests for the formation of acid from a variety of saccharides are used most frequently followed by tests for glycosidases, hydrolases, and peptidases. The number of enzyme tests included in each of these varies from 2 in the Vitek GPI Card to 23 in MicroScan Rapid Pos ID panels. Resistance to certain antimicrobial agents also plays an important role in differentiation of these organisms. The MicroScan Rapid Pos Identification system is the only one relying on enzyme tests and acid formation detected fluorometrically and providing identification of most clinically significant staphylococci, enterococci, and streptococci in 2 h.

Micrococcus. The members of the genus *Micrococcus* differ from those of *Staphylococcus* by being obligate aerobes, with a G+C content of 63 to 73 mol%, containing cytochromes *a*, *b*, *c*, and *d*, lacking teichoic acids in their cell walls, lacking glycine in the interpeptide bridge of their cell walls, being resistant to furazolidone, and being susceptible to bacitracin. Most strains are also resistant to lysostaphin (129); susceptible strains of *M. luteus* have been described. Lytic activity toward staphylococci, demonstrated by members of the genus *Staphylococcus*, is not a characteristic of 98.5% of micrococci (205).

Previously, the genus *Micrococcus* contained nine species, and tests that may help in differentiation between the species have been described (129). Kocur et al. (136) and Baldellon and Mégraud (12) examined strains from members of the *Micrococcaceae* by using a variety of API test strips. Kocur et al. (136) concluded that *Micrococcus* species produce a variety of aminopeptidases. Substrates for separation within the genus *Micrococcus* included hydroxyprolyl, glycyl-prolyl, aspartyl, and tyrosyl-seryl conjugates of naphthylamine. *M. kristinae*, *M. luteus*, *M. varians*, *M. nishinomiyaensis*, *M. sedentarius*, and *M. lylae* were included in the studies by Baldellon and Mégraud (12). They showed that most species did not exhibit production of acid from the 49 carbohydrates included in API 50CH kit. The exceptions were *M. kristinae* and *M. varians*, which showed mostly faint activity after a 24-h incubation with a small number of sugars. Acid phosphatase, phosphoamidase, and α-glucosidase activities were present in most *Micrococcus* species. Arylamidase activities were found in most species and could be used to separate *Micrococcus* from the other genera as well as to differentiate among some *Micrococcus* species.

Recently, studies of fatty acid and mycolic acid patterns, peptidoglycan type, and 16S rDNA analysis of the type strains of species of *Micrococcus*, *Stomatococcus*, *Arthrobacter*, and related actinomycetes but not species of *Staphylococcus* have shown that the genus *Micrococcus* is heterogeneous. The isolates previously identified as species of *Micrococcus* have been placed in five different genera: *Dermacoccus*, *Kocuria*, *Kytococcus*, *Micrococcus*, and *Nesterenkonia* (218). Thus, *M. sedentarius* is now *Dermacoccus sedentarius*; *M. kristinae*, *M. roseus*, and *M. varians* have been removed to the genus *Kocuria*; *M. nishinomiyaensis* has been moved to the genus *Kytococcus*; and *M. halobius* has been moved to the genus *Nesterenkonia*. The genus *Micrococcus* now contains only two species, *M. luteus* and *M. lylae.*

Of the commercial kits available for the identification of gram-positive cocci, the API STAPH-IDENT database includes one entry "*Micrococcus* sp.," with an additional test table for differentiation among *M. luteus*, *M. lylae*, *M. varians*, *M. kristiniae*, and *M. sedentarius*; the database of MicroScan conventional overnight gram-positive aerobic panels includes one entry "*Micrococcus* sp."; the database of MicroScan 2-h

TABLE 3. Comparison of tests detecting acid formation in systems for identification of gram-positive bacteria

Substrate	Identification system[a]											
	Identifies *Micrococcaceae* and *Streptococcaceae*				Identifies *Micrococcaceae*					Identifies *Streptococcaceae*		
	MS GP	MS Rapid GP	Vitek GPI	Pasco GP ID	API Staph ID	Geary et al. (86)	Kloos and Bannerman (129)	API Staph ID 32	Staph-Zym	RapID STR	Rapid ID 32 Strep	API 20 Strep
n-Acetylglucosamine						+	+	+				
Arabinose	+		+	+			+	+			+	+
Arabitol											+	
Cellobiose		+	+	+		+	+	+				
Cyclodextrin											+	
Fructose								+				
Glucose		+	+					+				
Glycerol		+										
Glycogen											+	+
Hemicellulose			+									
Inulin	+		+							+		+
Lactose	+	+	+	+		+	+	+			+	+
Maltose		+				+	+	+	+		+	
Mannitol	+	+	+	+	+	+	+	+		+	+	+
Mannose	+				+	+	+	+				
Melezitose			+								+	
Melibiose		+	+								+	
Methyl-β-D-glucopyranoside											+	
Pullulan			+								+	
Pyruvate	+		+									
Raffinose	+	+	+				+	+		+	+	+
Ribose	+		+	+		+		+			+	+
Salicin		+	+		+							
Sorbitol	+		+							+	+	+
Starch												+
Sucrose		+	+	+		+	+	+			+	
Tagatase											+	
Trehalose	+	+	+		+	+	+	+	+		+	+
Turanose							+	+				
Xylose			+			+	+					
No. of tests	10	11	18	6	4	10	12	14	2	4	17	10

[a] For an explanation of the identification systems, see Table 1, footnote *a*.

Rapid gram-positive aerobic panels contains three entries: *M. kristinae*, *M. roseus*, and "*Micrococcus* sp.," which covers *M. agilis*, *M. luteus*, *M. lylae*, *M. nishinomiyaensis*, *M. sedentarius*, and *M. varians.* A table of additional tests useful for the differentiation of these species is provided. Tests useful for separation in this group include pigmentation, aerobic acid production from glucose, and nitrate reductase. None of these kits reflects the new taxonomy of the genus described by Stackebrandt et al. (218).

Planococcus. Members of the genus *Planococcus* are cocci arranged in pairs or tetrads. They are positive in the catalase and benzidine tests and have a G+C content of 39 to 52 mol%. They resemble *Micrococcus* spp. in being strict aerobes, lacking the ability to produce bacterial lytic agents such as lysostaphin, and showing resistance to lysostaphin (205, 228). They can tolerate 12% NaCl and are motile.

Staphylococcus. The members of the genus *Staphylococcus* differ from those of *Micrococcus* by being facultative anaerobes with a G+C content of 30 to 39 mol%, containing cytochromes *a* and *b*, containing peptidoglycan and teichoic acids in their cell walls with oligoglycine peptides in the interpeptide bridge of their cell walls, and being susceptible to furazolidone and resistant to bacitracin; most strains are susceptible to lysostaphin (129). Lytic activity is produced by 99.5% of staphylococci (205). Lack of lytic activity is confined to a small number of isolates of *S. xylosus* (205, 228).

The taxonomy of the genus has been studied extensively (8, 9, 86, 94, 128, 129, 132). A self-learning scheme combining 35 biochemical tests with whole-cell fatty acid analysis for the identification of staphylococci has been described (21). In this scheme, isolates were compared to the type strain of each of the 35 taxa for acceptance into the database. The results for the acceptable isolates were used to generate a fatty acid profile library and a biochemical test table to provide a practical system for identification. Both are updated when new acceptable isolates are added to the database.

On the basis of the test for coagulase, the enzyme causing coagulation of human and rabbit serum, the genus was originally divided into the coagulase-positive species *S. aureus* and coagulase-negative staphylococci (CoNS). Determination of coagulase activity is still the most frequently used test for the identification of *S. aureus.*

A number of methods are available for detecting coagulase. In the commonly used tube coagulase method for free coagulase, overnight broth cultures are incubated with diluted rabbit plasma for 4 h at 37°C and then incubated overnight at room temperature; any clotting is deemed positive. In the slide coagulase test for bound coagulase, a very heavy suspension of

TABLE 4. Comparison of peptidase/protease tests used in systems for identification of gram-positive bacteria

Substrate specificity	Identification system[a]											
	Identifies *Micrococcaceae* and *Streptococcaceae*				Identifies *Micrococcaceae*					Identifies *Streptococcaceae*		
	MS GP	MS Rapid GP	Vitek GPI	Pasco GP ID	API Staph ID	Geary et al. (86)	Kloos and Bannerman (129)	API Staph ID 32	Staph-Zym	RapID STR	Rapid ID 32 Strep	API 20 Strep
L-Alanine		AMC										
Alanyl-phenylalanine-proline											+	
L-Arginine		AMC					βNA	βNA				
L-Citrulline		AMC										
α-L-Glutamic acid		AMC										
Glycyl-tryptophan											+	
Hydroxyproline										βNA		
Leucine		AMC										βNA
L-Lysine		AMC								βNA		
Methionine		AMC										
L-Phenylalanine		AMC										
L-Pyroglutamic acid		AMC		βNA							+	
L-Pyrrolidone	βNA						βNA	βNA	+	βNA		βNA
L-Tyrosine		AMC								βNA		
No. of tests	1	10	0	1	0	0	2	2	1	4	3	2

[a] For an explanation of the identification systems, see Table 1, footnote *a*. AMC, 7-amido-4-methylcoumarin; βNA, β-naphthylamine; +, proprietary synthetic moiety.

cells is placed on a microscope slide and a loopful of rabbit plasma is added; a positive reaction is indicated by clumping within 10 s. The results of the tube and slide coagulation tests are not always identical. False-negative results in the tube and slide coagulase tests have been reported for 8 and 17 strains, respectively, of 87 strains of *S. aureus*. In the same study, false-positive results in the slide coagulase test were reported for 9 of 10 and 7 of 10 strains of *S. lugdunensis* and *S. schleiferi*, respectively (85). Tests based on rapid latex agglutination and hemagglutination to detect clumping factor or protein A are also used extensively (79, 131); however, Fournier et al. (78) and Mathieu and Picard (156) showed that they are not reli-

TABLE 5. Comparison of miscellaneous tests used in systems for identification of gram-positive bacteria

Characteristic	Identification system[a]											
	Identifies *Micrococcaceae* and *Streptococcaceae*				Identifies *Micrococcaceae*					Identifies *Streptococcaceae*		
	MS GP	MS Rapid GP	Vitek GPI	Pasco GP ID	API Staph ID	Geary et al. (86)	Kloos and Bannerman (129)	API Staph ID 32	Staph-Zym	RapID STR	Rapid ID 32 Strep	API 20 Strep
Other hydrolases												
40% bile esculin	+											
DNase						+						
Heat-stable nuclease							+					
Esculin			+	+			+	+		+		+
Hemolysins				+		+	+					
Hippurate											+	+
Staphylocoagulase							+					
Clumping factor							+					
No. of tests	1	0	1	2	0	2	5	1	0	1	1	1
Other metabolic tests												
Arginine dihydrolase	+		+	+	+		+	+	+	+	+	+
Ornithine decarboxylase							+	+				
Catalase				+			+					
Nitrate reductase	+						+	+	+			
Oxidase							+					
Production of acetoin (VP)	+			+			+	+			+	+
Urease	+	+	+	+	+	+	+	+	+		+	
No. of tests	4	1	2	4	2	1	7	5	3	1	3	2

[a] For an explanation of the identification systems, see Table 1, footnote *a*.

TABLE 6. Comparison of miscellaneous growth and tolerance tests used in systems for identification of gram-positive bacteria

Characteristic	Identification system[a]											
	Identifies *Micrococcaceae* and *Streptococcaceae*				Identifies *Micrococcaceae*					Identifies *Streptococcaceae*		
	MS GP	MS Rapid GP	Vitek GPI	Pasco GP ID	API Staph ID	Geary et al. (86)	Kloos and Bannerman (129)	API Staph ID 32	Staph-Zym	RapID STR	Rapid ID 32 Strep	API 20 Strep
Colony size							+					
Colony pigmentation							+					
Aerobic growth							+					
Anaerobic growth							+					
Growth in presence of:												
Bacitracin	+		+	+								
10% bile			+									
40% bile			+									
Colistin (polymyxin)									+			
Crystal violet	+											
Deferoxamine									+			
Furazolidone									+			
Micrococcus screen (Bacitracin)	+											
Novobiocin	+		+	+		+	+	+	+			
Optochin	+		+	+								
Polymyxin B							+					
Sodium chloride (%)	6.5		6	6.5								
Tetrazolium red			+									
Peptone base			+									
No. of tests	6	0	8	4	0	1	6	1	4	0	0	0

[a] For an explanation of the identification systems, see Table 1, footnote *a*.

able enough for identification of MRSA isolates, because strains possessing capsular serotype 5 antigen did not react and this serotype is predominant among oxacillin-resistant isolates. A new latex reagent, Pastorex Staph-Plus (Sanofi Diagnostic Pasteur), consisting of a mixture of latex particles for the detection of fibrinogen-binding protein (clumping factor), protein A, and *S. aureus* stereotypes 5 and 8, has demonstrated a 95.1% sensitivity for MRSA isolates (79). Comparison of five agglutination tests (247) has shown sensitivities of 98.9 to 99.6% and specificities of 93.9 to 99.9%.

Positive results in the tube or slide coagulase tests have been found for other *Staphylococcus* species, mainly *S. intermedius*, *S. hyicus*, and *S. lugdenensis* (85, 189). Vandenesch et al. (225) demonstrated that both subspecies of *S. schleiferi* can promote clotting of rabbit plasma in the standard tube test for coagulase. Positive coagulase activity was reported for *S. aureus*, *S. delphini*, *S. hyicus*, *S. intermedius*, and *S. schleiferi* (21).

Hemker et al. (101) provided a better understanding of the staphylococcal clotting mechanism. They have shown that staphylocoagulase activates the proenzyme prothrombin in a stoichiometric reaction between one molecule of prothrombin and one molecule of staphylocoagulase. Engels et al. (66) suggested that staphylocoagulase could be detected rapidly and directly with the chromogenic substrate Chromozym-TH (Boehringer) for the routine identification of *S. aureus*. Morita et al. (168) characterized the enzyme staphylocoagulase of *S. aureus* as an extracellular protein that reacts with prothrombin in human plasma to form an active molecular complex which can convert fibrinogen to a fibrin clot and shows amidase activities. They showed that the staphylocoagulase-prothrombin complex can hydrolyze the chromogenic substrates tosyl-Gly-Pro-Arg-*p*-nitroanilide (Chromozym-TH), Z-Gly-Pro-Arg-*p*-nitroanilide, H-D-Phe-Pip-Arg-*p*-nitroanilide, and tosyl-arginine-methyl ester (TAME); it can also hydrolyze the fluorogenic substrate Boc-Val-Pro-Arg-4-methylcoumaryl-7-amide. Bulanda et al. (36) used D-Phe-Pro-Arg-β-naphthylamide for direct detection of *S. aureus*. The substrate profile suggests arginine-specific endopeptidase activity. The identity of the amino acid adjacent to the arginine is also important since Bz-Phe-Val-Arg-*p*-nitroanilide was not hydrolyzed by the complex, suggesting that a proline but not a valine is required in that position. The reaction with Chromozym-TH was done at pH 8.4, and that with the fluorogenic substrate was done at pH 7.5. Bulanda et al. (36) confirmed that the enzyme is liberated into the medium in the early logarithmic phase (3 to 6 h), and that its production depends on aeration and a rich growth medium such as brain heart infusion.

Raus and Love (189) compared the activities of staphylocoagulases from *S. aureus* and *S. intermedius* by using Chromozym-TH and concluded that *S. intermedius* staphylocoagulase resembled *S. aureus* staphylocoagulase in its rate and mode of action on prothrombin but that the enzyme was produced in lesser amounts in the former species. They concluded that Chromozym-TH can be used to detect the enzyme in both species but that accurate detection of the activity of *S. intermedius* requires a longer incubation period or preconcentration of the extracellular proteins. In further studies, *S. aureus* and *S. intermedius* isolates from humans and other mammals showed differences in the affinity of staphylocoagulase toward prothrombin of bovine or human origin and in its activity on human, bovine, and equine fibrinogen. The studies suggested that the enzymes had structural differences (190). Production of acetoin and acid from maltose and the presence of hyaluronidase activity have been recommended as the best tests for differentiation between *S. aureus* and *S. intermedius*, with the former being positive in all three tests (188). A proprietary fluorogenic substrate dried with prothrombin for detection of "aurease" (coagulase activity of *S. aureus*) is used in the com-

mercially available RAPiDEC Staph kit. Enzymatic activity is detected visually after a 2-h incubation of a no. 4 McFarland standard inoculum (85, 112, 165).

Other enzyme tests, e.g., thermostable nuclease (137, 192), have also been used for the separation of *S. aureus* from other groups of staphylococci. Since some CoNS species are involved in human disease and a number of species are also likely to develop resistance to antibiotics, interest in the identification of members of the CoNS group to the species level has increased. A review of the CoNS group has been published by Kloos and Bannerman (128). The taxonomy of this group can be difficult, since some morphological characteristics of the colonies of different species require up to 4 days to appear and since the number of separating tests is small (129). The ability to differentiate between virulent and avirulent isolates of the same species on the basis of any characteristic has been difficult. In this context, the finding of Kedzia (121) that the phosphatase activities of virulent strains of *S. aureus* were higher than those of avirulent strains is interesting.

The enzymes important in identification of the CoNS group are β-galactosidase, β-glucosidase (245), β-glucuronidase, phosphatase, urease, hydrolysis of esculin (involving β-glucosidase), and utilization of arginine. Thus, most staphylococci are positive for the urease test but *S. arlettae*, *S. auricularis*, *S. haemolyticus*, *S. lentus*, *S. schleiferi*, and *S. sciuri* are negative. Only *S. gallinarum*, *S. lentus*, and *S. sciuri* can hydrolyze esculin and are positive for the β-glucosidase test. Further details can be found in the result tables of the various identification systems. Oberhofer (173) found that all 26 strains of *S. haemolyticus*, the single strain of *S. intermedius*, and 2 of 7 *S. warneri* strains tested were positive in the pyrrolidonyl-arylamidase (PYR) test, while 65 isolates of *S. epidermidis*, 7 isolates of *S. hominis*, 8 isolates of *S. saprophyticus*, and 2 isolates of *S. capitis* were negative. A number of authors have suggested the potential usefulness of including tests for lipases for the differentiation of bacteria in general and of staphylococci in particular (17, 110, 201). Newly synthesized chromogenic substrates for the detection of esterases and lipases, including propionate, decanoate, and laurate esters of 5-(4-hydroxy-3,5-dimethyoxyphenylmethylene)-2-thioxothiazoline-3-acetic acid (SRA), available from Melford Laboratories, Chelsworth, Ipswich, United Kingdom, have been proposed for use with bacterial isolates (161).

Stomatococcus. A description of the genus *Stomatococcus*, which contains only one species, *Stomatococcus mucilaginosus*, is available (12, 129, 160, 198, 217). Strains of the species show the morphological characteristics of the family: cocci arranged in pairs, tetrads, and clusters. They exhibit a high G+C content of 56 to 60 mol%, near to that of members of *Micrococcus* (64 to 75 mol%) and unlike that of staphylococci (30 to 39 mol%). Strains of the species show a low catalase activity and a positive benzidine activity. Responses to metabolic and enzymatic tests resemble those of both staphylococci and micrococci, e.g., production of acid from a number of mono-, di-, and trisaccharides (similar to staphylococci) and hydrolysis of mono-, di-, and triamino acid conjugates (similar to *Micrococcus*). Enzyme tests used for the identification of *S. mucilaginosus* include hydrolysis of esculin (β-glucosidase), catalase, leucine-aminopeptidases (LAP), and PYR. Identification of members of this taxon has been reported (12, 194, 195). Cross-infection between patients has been studied by using phenotypic characters, acid production from carbohydrates as determined by API 50CH, and MICs (227).

Identification schemes and kits. Reports on the identification of staphylococci with commercial kits are summarized in Table 7; when meaningful, identification of individual taxa is also listed. Not included in the table are early developments of the API systems that have been described (26, 33), studies of Staph-TRAC (11) when used in conjunction with biochemical and susceptibility tests, and STAPH-IDENT (179) for characterization of taxa. In addition, reports on the identification of gram-positive cocci have shown the following percent correct identifications: Pasco Gram-Positive ID (104), 90%; MS GP panels (104), 87.0%; MS Rapid ID panels (19, 38, 92, 216), 95.8, 96.2, 95.7, and 93.0%. MS Rapid ID panels have shown 90.0 and 87.0% correct identification with isolates taken from sheep and horse blood agar, respectively (159).

The levels of accuracy of identification with the different kits or systems vary from 38% for MS Rapid GP in identification of non-*S. epidermidis* CoNS blood isolates (241) to 100% for the same system in identification of *S. aureus* without additional tests (221) (Table 7). Overall percent accuracies depend as much on the mixture of isolates tested as on the system used; studies quite often contain too many isolates of the common species, *S. aureus* and *S. epidermidis*, and too few isolates of the less common species. It is therefore difficult to assess the ability of a system to identify a taxon on the basis of testing of one or two isolates. Most systems are fairly successful in differentiating *S. aureus*, *S. epidermidis*, and *S. saprophyticus* (13, 130). The identification of the less common species is more variable. Identification of some isolates of *S. hominis* and *S. warneri* has proven to be problematic with most of the above systems (13, 107, 108, 122, 192, 239). The MS Rapid GP system, which requires 2 h for incubation, provides a level of accurate identification similar to MS GP, Vitek GPI, and Staph ID 32, which require 4 to 24 h of incubation (Table 7). Staph ID 32, MicroScan conventional overnight panels, and the Pasco GP ID system generally do not take advantage of the ability of enzyme tests to provide results rapidly. Even the Vitek GPI cards, when requiring >5 h incubation, are not rapid enough, because results issued by the laboratory late in the afternoon may not reach the clinician or influence patient care till the following day. However, it is important to realize that commercial kits evolve and that performance concerns are dealt with by modifications to formulae and/or software updates. It is therefore important to check which version of the system is available and how its performance relates to published data.

The completion times for the different identification schemes vary from 2 h with the MicroScan Rapid Pos ID system to 24 to 72 h with systems that rely on growth-dependent tests. The MicroScan Rapid Pos ID system requires a McFarland 0.5 inoculum and relies on the detection of enzymatic activity with fluorogenic substrates and automated detection of fluorescence by the WalkAway instrument. RAPiDEC Staph, relying on visual observation of fluorescence change, can also provide identification in 2 h but requires a McFarland 4 inoculum and can identify only three species without additional tests.

Streptococcaceae and Related Organisms

The family *Streptococcaceae* and related organisms include the aerobic and facultatively anaerobic gram-positive cocci that generally give a negative result in the catalase test. These organisms are encountered in the mouths and intestinal tracts of humans and animals and play an important role in the food industry as agents of preservation or spoilage of fermentation products (220). Some of the taxa are virulent pathogens, causing pharyngitis, respiratory infections, skin and soft tissue infections, dental caries, infective endocarditis, and septicemia. Others play an important role in fermentation and preservation processes of a variety of food products. Isolates are characterized by homo- or heterofermentative metabolism of car-

TABLE 7. Comparison of the accuracy of identification systems for the *Micrococcaceae*

Identification system	Year (reference)	Group identified	No. of isolates tested	% Correct (% correct with additional tests)	Comments; (additional test); problematic taxa
API 20GP[a]	1994 (181)	CoNS	277	61.0	Bloodstream isolates; *S. epidermidis*, other CoNS species
API ID 32 Staph[a]	1995 (108)	CoNS	440	95.2	Clinical isolates; *S. capitis*, *S. hominis*
API ID 32 Staph	1995 (192)	Staphylococci	89	82.1	Clinical isolates
API ID 32 Staph	1995 (192)	Staphylococci	111	77.4	Culture collection isolates; CoNS species, including *S. hominis*, *S. schleiferi*, *S. warneri*, *S. xylosus*
ATB 32 Staph[a]	1991 (141)	*S. hyicus*	54	83.3	Isolates from pigs and cattle
API Staph[a]	1982 (87)	CoNS	100	90.0	24–48 h of incubation
API Staph[a]	1982 (155)	*Micrococcaceae*	300	30.0	Stock cultures, 24–48 h of incubation
API STAPH-IDENT[a]	1983 (5)	CoNS	300	67.0 (92.7)	Clinical isolates; (mostly novobiocin)
API STAPH-IDENT	1984 (89)	CoNS	120	79.2	Clinical isolates, 5-h incubation; *S. haemolyticus*, *S. hominis*
API STAPH-IDENT	1986 (107)	CoNS	175	88.0	*S. epidermidis*
API STAPH-IDENT	1990 (176)	CoNS	55	100.0	Blood isolates; reproducibility study
API STAPH-IDENT	1984 (10)	*Micrococcaceae*	414	(97.5)	Clinical isolates; (oxidase, susceptibility to furazolidone and lysostaphin)
API STAPH-IDENT	1983 (61)	Staphylococci	188	80.9 (90.4)	Clinical isolates; (24-h incubation)
API STAPH-IDENT	1983 (143)	Staphylococci	581	54.0	Bovine isolates; *S. epidermidis*, *S. hominis*
STAPH-IDENT[a]	1986 (107)	CoNS	175	88.0	Clinical isolates; *S. epidermidis*
STAPH-IDENT	1995 (195)	*Micrococcaceae*	1,106	81.1	Strains submitted to CDC for identification, stock cultures, and a collection of ocular isolates
		S. aureus	162	77.2	
		S. cohnii	9	11.1	
		S. epidermidis	517	97.1	
		S. haemolyticus	61	75.8	
		S. hominis	57	82.5	
Staph-TRAC (API)	1994 (181)	CoNS	277	73.0	Bloodstream isolates; CoNS species other than *S. epidermidis*
Staph-TRAC (API)[a]	1990 (157)	Staphylococci	130	89.8	Bovine isolates
Staph-TRAC (DMS)[a]	1984 (89)	CoNS	120	88.3	Clinical isolates, 24-h incubation; *S. haemolyticus*, *S. warneri*
Biolog[b]	1993 (162)	*Micrococcaceae*	113	69.0–74.3	Stock isolates tested at two separate laboratories
Geary et al. scheme	1989 (86)	Staphylococci	559	94.6	Clinical isolates, multipoint inoculation on agar plates
MS GP[c]	1986 (107)	CoNS	175	86.4	Clinical isolates; *S. hominis*, *S. warneri*, *S. sciuri*
MS GP	1994 (97)	CoNS	224	79.0	Clinical isolates
		S. epidermidis	44	95.0	
		S. haemolyticus	21	95.0	
		S. hominis	50	64.0	
		S. saprophyticus	11	100.0	
		S. warneri	39	79.0	
MS GP	1991 (130)	Staphylococci	896	80.4 (98.1)	Culture collection, clinical and skin isolates; (includes probable identifications when the correct species has not reached required level of identification, but has been included in the four possible taxa)
		S. aureus	58	91.0 (100.0)	
		S. auricularis	22	91.0 (100.0)	
		S. capitis	70	94.2 (98.6)	
		S. cohnii	49	82.4 (100.0)	
		S. epidermidis	152	82.0 (98.0)	
		S. haemolyticus	71	77.5 (100.0)	
		S. hyicus	22	82.0 (96.0)	
		S. intermedius	30	90.0 (96.0)	
		S. kloosii	18	72.0 (100.0)	
		S. lugdunensis	22	64.0 (96.0)	
		S. saprophyticus	50	90.0 (96.0)	
		S. warneri	55	63.6 (90.9)	
		S. xylosus	32	66.0 (94.0)	
MS GP	1992 (50)	Staphylococci	370	73.4 (92.0)	Multicenter study; (requiring additional tests)
MS GP	1995 (241)	*S. epidermidis*	57	84.0 (95.0)	Blood isolates
		Non-*S. epidermidis*	16	62.0 (70.0)	
MS Rapid GP[c]	1990 (91)	CoNS	342	88.3 (96.2)	Stock cultures
MS Rapid GP	1994 (97)	CoNS	224	76.0	Clinical isolates
		S. epidermidis	44	91.0	
		S. haemolyticus	21	90.0	
		S. hominis	50	64.0	
		S. saprophyticus	11	100.0	
		S. warneri	39	77.0	

Continued on following page

TABLE 7—*Continued*

Identification system	Year (reference)	Group identified	No. of isolates tested	% Correct (% correct with additional tests)	Comments; (additional test); problematic taxa
MS Rapid GP	1991 (130)	Staphylococci	918	88.6 (99.1)	Culture collection, clinical and skin isolates; (includes probable identifications when the correct species has not reached required level of identification, but has been included in the four possible taxa)
		S. aureus	58	91.0 (95.0)	
		S. auricularis	22	100.0 (100.0)	
		S. capitis	70	88.6 (100.0)	
		S. caprae	11	91.0 (100.0)	
		S. cohnii	49	89.8 (93.8)	
		S. chromogenes	19	68.0 (94.0)	
		S. epidermidis	152	92.0 (99.0)	
		S. haemolyticus	71	81.6 (94.5)	
		S. hyicus	22	86.0 (95.0)	
		S. intermedius	30	87.0 (90.0)	
		S. kloosii	19	100 (100.0)	
		S. lugdunensis	24	62.0 (70.0)	
		S. saprophyticus	50	90.0 (100.0)	
		S. warneri	55	83.6 (92.7)	
		S. xylosus	32	97.0 (100.0)	
MS Rapid GP	1992 (50)	Staphylococci	380	80.3 (93.2)	Multicenter study; (requiring additional tests)
MS Rapid GP	1992 (221)	Staphylococci	239	91.6 (95.4)	Stock cultures of clinical isolates; (low probability of identification)
		S. aureus	18	100.0 (100.0)	
		S. capitis	21	85.7 (90.5)	
		S. cohnii	10	80.0 (80.0)	
		S. epidermidis	40	95.0 (100.0)	
		S. haemolyticus	20	100.0 (100.0)	
		S. lugdunensis	14	92.9 (100.0)	
		S. saprophyticus	28	96.4 (100.0)	
		S. warneri	25	96.0 (96.0)	
		S. xylosus	12	75.0 (75.0)	
MS Rapid GP	1995 (108a)	Staphylococci	69	82.6 (91.4)	Clinical isolates; (low probability)
MS Rapid GP	1992 (229)	*S. aureus*	31	100.0	Clinical isolates
MS Rapid GP	1995 (182)	*S. aureus*	20	90.0	Direct identification of positive blood culture centrifuged pellets
		CoNS	95	53.7	
MS Rapid GP	1995 (241)	*S. epidermidis*	57	93.0 (97.0)	Blood isolates
		Other CoNS	16	38.0 (49.0)	
RAPiDEC Staph[a]	1991 (85)	Staphylococci	124	60.5	Reference strains
RAPiDEC Staph	1991 (85)	Staphylococci	121	88.4	Urine isolates
RAPiDEC Staph	1991 (165)	Staphylococci			Direct detection in blood cultures, modification from recommended method by predilution of blood broth mixture with water. CoNS species determination not pursued
		CoNS	59	100.0	
		S. aureus	28	96.4	
RAPiDEC Staph	1994 (112)	Staphylococci	303	87.0	Clinical and stock culture isolates
STAPHYtest	1991 (211)	Staphylococci	145	72.0	Stock cultures, 24 h incubation; many CoNS species including *S. epidermidis*, *S. hominis*, *S. warneri*, *S. xylosus*
Staph-Zym[d]	1992 (191)	CoNS	131	95.0	Clinically significant isolates
Staph-Zym	1995 (108)	CoNS	440	97.5	Clinical isolates; *S. warneri*
Staph-Zym	1991 (239)	Staphylococci	148	91.1	Bovine isolates; *S. warneri*, *S. hominis*
Staph-Zym	1995 (192)	Staphylococci	89	82.1	Clinical isolates
Staph-Zym	1995 (192)	Staphylococci	111	99.0	Culture collection isolates; *S. warneri*
Staph-Zym	1989 (139)	*S. hyicus*, *S. intermedius*	85	100.0	Isolates from pigs, bovines, and canines
Vitek GPI	1992 (191)	CoNS	131	95.0	Clinically significant isolates
Vitek GPI	1993 (13)	CoNS	500	77.0 (86.0)	Clinical isolates; (good confidence-marginal separation)
		S. capitis subsp. *captis*	8	88.0 (88.0)	
		S. epidermidis	322	89.0 (92.0)	*S. capitis* subsp. *ureolyticus* and *S. lugdunensis* are not in the database
		S. haemolyticus	68	71.0 (95.0)	
		S. hominis	37	41.0 (63.0)	
		S. saprophyticus	5	80.0 (100.0)	

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TABLE 7—*Continued*

Identification system	Year (reference)	Group identified	No. of isolates tested	% Correct (% correct with additional tests)	Comments; (additional test); problematic taxa
		S. simulans	12	33.0 (83.0)	
		S. warneri	20	65.0 (80.0)	
Vitek GPI[a]	1994(181)	CoNS	277	67.0	Bloodstream isolates; *S. epidermidis*, other CoNS species
Vitek GPI	1996(122)	CoNS	616	53.0 (85.4)	Clinical isolates; (includes identifications of probability <90%); *S. auricularis*
		S. capitis	16	56.3 (75.0)	
		S. epidermidis	274	53.3 (89.8)	
		S. haemolyticus	124	62.9 (96.8)	
		S. hominis	37	13.5 (73.0)	
		S. saprophyticus	95	14.7 (83.1)	
		S. simulans	31	45.2 (90.4)	
		S. warneri	14	35.7 (42.8)	
		Other species	25	32.0 (32.0)	
Vitek GPI	1990(157)	Staphylococci	130	44.6	Bovine origin isolates

[a] bioMérieux-Vitek Inc., Hazelwood, Mo., and bioMérieux S.A., Marcy l'Etoile, France.
[b] Biolog, Hayward, Calif.
[c] Dade International Inc., West Sacramento, Calif.
[d] Rosco, Tastrop, Denmark.

bohydrates. The end product of homofermentative bacteria is lactic acid; the end products of heterofermentative bacteria are lactic and acetic acids as well as CO_2, which can be detected by the production of gas from a glucose medium. Characterization of the streptococci has traditionally been based on cell morphology (cocci or coccobacilli) and aggregation (tetrads or chains), colony size (large or small), appearance on blood agar (alpha-, beta-, or gamma-hemolysis), and antigenic structure (Lancefield grouping A through V). Further classification and identification are obtained by physiological and metabolic tests. In the last two decades, the taxonomy of the group has been actively studied. Applications of genetic methods (24, 41–46, 209, 244, 248), numerical taxonomy (31, 186), and enzymatic techniques (22, 127) have contributed to a further understanding of this complex taxonomic group. In addition, the isolation of vancomycin-resistant bacteria from human infections (37, 48, 115, 200) has necessitated the development of tests and schemes for the identification of clinical isolates of *Leuconostoc* and *Pediococcus*. A number of vancomycin-susceptible genera, including *Alloiococcus*, *Globicatella*, *Helcococcus*, and *Vagococcus*, have also been isolated from clinical specimens (68).

The major changes introduced into the taxonomy include splitting the genus *Streptococcus* into three genera, *Enterococcus*, *Lactococcus*, and *Streptococcus*; changes to classification of the viridans streptococci; increasing the number of species of *Enterococcus*; and addition of the genera *Alloiococcus*, *Globicatella*, *Helcococcus*, *Tetragenococcus*, and *Vagococcus*. Although the newly described genera resemble members of the family *Streptococcaceae*, their taxonomic position has not been clearly established.

The taxonomy and clinical significance of *Streptococcus* (22, 31, 47, 49, 112, 127, 197), *Enterococcus* (73, 112, 170), and the new genera (68, 198) have been reviewed. Numerical taxonomy of lactic acid bacteria including *Streptococcus*, *Lactobacillus*, *Leuconostoc*, and *Pediococcus* spp. has been studied by Priest and Barbour (186).

The usefulness of the catalase test for separating micrococci from streptococci has also been studied. The presence of catalase is usually determined by addition of a drop of 3% H_2O_2 to a heavy bacterial suspension and observation of effervescence due to the release of O_2. However, release of O_2 has been observed in *Aerococcus* and *Alloiococcus* members of the family *Streptococcaceae* and is presumed to be mediated by peroxidases (68). Moreover, isolates of *Stomatococcus*, a member of the family *Micrococcaceae*, can show a negative or a weak catalase activity. Members of the *Micrococcaceae* differ from streptococci in their ability to synthesize the iron-porphyrin compounds of the heme group, an essential component of respiratory systems including catalase, cytochromes, and nitrate reductase; streptococci lack this ability. A number of tests have been used to differentiate between members of the *Micrococcaceae* and *Streptococcaceae*. The catalase test is the most commonly used, but some members of the *Micrococcaceae*, e.g., *Stomatococcus* and some strains of *S. aureus*, can give weak or even negative reactions. The benzidine test (56) detects iron-porphyrin compounds in catalase- and cytochrome-containing bacteria; it is highly sensitive and is positive for all members of the *Micrococcaceae*, even those which are catalase negative. Tests originally designed to detect ability of *Haemophilus* strains to synthesize porphobilinogen and porphyrin from δ-aminolevulinic acid (126) have been applied to gram-positive cocci (250) for differentiation of micrococci from the streptococci. Production of porphobilinogen correlated 100% with membership in the family *Micrococcaceae*, including 22 catalase-negative isolates. A modification of the benzidine test has been described (75) for the detection of oxidase-positive members of the *Micrococcaceae* based on removal of noncovalently linked heme groups prior to reaction with the benzidine reagent making it specific to the covalently protein bound heme of cytochromes *c*. The modified benzidine test was compared with a modified oxidase test for differentiation within the *Micrococcaceae* and shown to be positive for all members of the genus *Micrococcus* and for strains of *S. sciuri*. By using the modified oxidase test, 2.3% of 302 isolates tested were misclassified mainly because of poor growth; however, the oxidase test was preferred because it is much simpler to perform. Moreover, because benzidine is carcinogenic, its use is not recommended.

The heme-negative coccal group now contains 12 genera. The genera whose cells aggregate in chains include *Enterococcus*, *Globicatella*, *Lactococcus*, *Leuconostoc*, *Streptococcus*, and *Vagococcus*; those arranged in pairs or tetrads include *Gemella*, *Helcococcus*, *Pediococcus*, and *Tetragenococcus*; while *Aerococcus* and *Alloiococcus*, also arranged in pairs and tet-

rads, are the two genera that may show a weak pseudo-catalase activity.

The genera within these groups are further separated by the production of PYR and LAP, the ability to hydrolyze esculin in the presence of bile salts, the production of gas from glucose, the ability to grow in the presence of 6.5% NaCl, and at 10 and 45°C, motility, susceptibility to vancomycin, and the type of hemolysis.

Cells arranged in pairs or chains, with no catalase activity. **(i) *Enterococcus*.** The genus *Enterococcus*, which included *E. faecalis* and *E. faecium*, was separated from *Streptococcus* on the basis of DNA hybridization data by Schleifer and Kilpper-Bälz (208). Collins et al. (44) added *E. avium*, *E. casseliflavus*, *E. durans*, *E. gallinarum*, and *E. malodoratus* to the list of *Enterococcus* species. With the exception of *E. malodoratus*, all the above species have been isolated from human sources (73). Since then, 12 new species have been described. *E. dispar*, *E. hirae*, *E. flavescens*, *E. mundtii*, and *E. raffinosus* have been isolated from humans and other sources (73, 111). *E. pseudoavium*, and *E. saccharolyticus* have been isolated from cattle and other mammals. *E. columbae* and *E. cecorum* have been isolated from pigeons and chickens, respectively. *E. sulfureus* has been isolated from plants (111). *E. solitarius*, which has been isolated from an ear exudate (111), from patients admitted to a public hospital (90), and from the rumens of domestic and wild ruminants (145), is now deemed closer to *Tetragenococcus halophilus* (248). Similarly, *E. seriolicida*, which has been isolated from yellow-tail fish (111) and from water buffalo with subclinical mastitis (222), is now deemed closer to *Lactococcus garvieae* (222). It has been recommended that these two species, which fail to react with the AccuProbe *Enterococcus* probe, should not be included in the genus *Enterococcus* at this time (73).

The enterococci are ubiquitous and can be found free-living in soil, on plants, in dairy and milk products, and as part of the normal flora of the gastrointestinal tracts of humans, canines, birds, cattle, pigs, horses, and other animals. They can cause infections at a wide variety of sites, including the urinary tract, bloodstream, endocardium, abdomen, and biliary tract, as well as burn wounds and indwelling devices (114). In the 1970s and 1980s, enterococci became firmly established as major nosocomial pathogens. They are intrinsically resistant to many antimicrobial agents (e.g., β-lactams, clindamycin), MICs of these drugs being higher than for most streptococci. They are also resistant to low levels of aminoglycosides and had acquired resistance to chloramphenicol and erythromycin as early as 1964. In 1973, there were reports of acquired resistance to high levels of aminoglycosides as well as to the synergistic combination of aminoglycosides and cell wall inhibitors. Enterococcal resistance due to the production of β-lactamase has also been reported (170). An inoculum of 10^7 CFU/ml, higher than that used by disk diffusion and broth microdilution methods, is required for the detection of enterococcal β-lactamase activities (170). The acquisition of resistance to vancomycin, used for treating infections caused by gram-positive cocci that are resistant to other drugs, has been on the increase since the late 1980s (170). Three types of resistance, due to VanA, VanB, and VanC, have been recognized (251). Recently, a fourth type of resistance mediated by VanD has been described (146). Acquired resistance of *E. faecalis* and *E. faecium* is due to VanA, VanB, and VanD. Acquired resistance due to VanA has also been found in *E. avium*, *E. durans*, *E. hirae*, *E. mundtii*, and *E. raffinosus*. Resistance of *E. gallinarum*, *E. casseliflavus*, and *E. flavescens* can be intrinsic due to VanC1 and VanC2 or acquired due to VanA (146).

Enterococcus species are facultative anaerobes, with a G+C content of 37 to 45 mol%. They differ from members of the genus *Streptococcus* by being resilient organisms that can survive and multiply in harsh conditions (e.g., pH 9.6, at 10 and 45°C, and in the presence of 6.5% NaCl and 40% bile salts). All species produce LAP, and most also produce PYR, but they do not contain cytochromes and are mostly negative in the catalase test. They are homofermentative; most strains produce the group D antigen, and some also produce the group Q antigen. The AccuProbe Enterococcus DNA culture confirmation probe (Gen-Probe, San Diego, Calif.) has been recommended for positive identification of all enterococci because most species of enterococci react positively with the probe with the exceptions of the type strains of *E. cecorum*, *E. columbae*, and *E. saccharolyticus* (73). The taxonomy, virulence, clinical significance, and antibiotic susceptibility of the genus have been recently reviewed (71, 73, 111, 114, 170). Identification of isolates from foods of animal origin was described by Devriese et al. (57). The type strain of *E. hirae* has complex nutritional requirements and is used for the bioassay of vitamins and amino acids (111). Differentiation within the genus is based on acid production from a number of carbohydrates, motility, pigmentation, hydrolysis of arginine, ability to grow in the presence of tellurite, and production of PYR (73).

(ii) *Globicatella*. The genus *Globicatella* described by Collins et al. (41) contains one species, *Globicatella sanguis*. Strains of this genus resemble viridans streptococci in colony appearance and many other characteristics, but they are PYR positive and LAP negative, can grow in 6.5% NaCl, and are susceptible to vancomycin. Phylogenetically, the genus is related to *Aerococcus*; the G+C content of the type strain is 37 mol% (41). Strains have been isolated from patients with bacteremia, urinary tract infections, and meningitis.

(iii) *Lactococcus*. The genus *Lactococcus* includes the cocci of serogroup N that produce lactic acid as the main fermentation product; it can be confused with *Enterococcus* and differs from it by its antigenic reaction and its lesser ability to grow at 45°C. The G+C content of this genus is 38 to 40 mol%.

The genus contains seven recognized species and subspecies (*L. lactis* subspp. *lactis*, *cremoris*, and *hordinae*; *L. garvieae*; *L. plantarum*; *L. raffinolactis*; and *L. xylosis*), which can be differentiated by tests for acid formation from carbohydrates, hydrolysis of arginine and hippurate, and production of PYR and acetoin (VP reaction). Isolates are commonly present in the environment, food, and vegetation, but several reports of infections attributed to members of this genus, including blood and urinary tract infections (64, 154), have appeared. A majority of cases were due to *L. lactis* subsp. *lactis* or *L. garvieae*. Oligonucleotide probes for the identification of some members of this genus have been described (23, 204), and schemes for the identification of members of this taxon have appeared (68, 80, 81, 198). The difficulties of differentiating the two species isolated from human infections by using the API Rapid Strep identification system and conventional tests have been discussed by Elliott et al. (64); they have found that neither the conventional test system nor the API Rapid Strep identification system could differentiate between the two species and recommended comparison of whole-cell protein patterns for that purpose.

(iv) *Leuconostoc*. The genus *Leuconostoc* includes heterofermentative coccobacilli that are important in the conservation and preparation of several fermented foods (164), while some are involved in human diseases. The G+C content of members of the genus is 38 to 44 mol%.

Members of the genus (such as *L. citreum*, *L. lactis*, *L. mesenteroides*, *L. pseudomesenteroides*, and *L. paramesenteroides*) are generally found on vegetable material, in milk

products, and in other fermentation products such as wine and sausages. Certain isolates are used as starter cultures in dairy and other food industries (220). The above-mentioned *Leuconostoc* species have been isolated from a wide variety of human infections. Infections in the immunocompromised host may be severe, and because of the intrinsic resistance of this genus to vancomycin, identification of *Leuconostoc* is essential for correct antimicrobial treatment. The taxonomy and clinical significance of *Leuconostoc* spp. have been reviewed (45, 68, 164, 186, 198). Colonies resemble those of alpha-hemolytic enterococci and lactococci; they differ from these taxa by being intrinsically highly resistant to vancomycin, with MICs of >2,000 μg/ml (174, 175). The colonies may also resemble those of viridans streptococci (37). *Leuconostoc* isolates are positive for the production of CO_2 from glucose and negative for arginine dihydrolase, PYR, and LAP; 31% of the strains from clinical sources react with the group D antiserum (68). Schemes for the identification of members of this genus have appeared (68, 69, 80, 198).

The taxonomy of the genus has undergone recent alterations. On the basis of numerical taxonomy, cellular fatty acid analysis, and DNA-DNA hybridization studies, two new species, *L. gelidum* and *L. carnosum*, isolated from chilled meat, have been described (213). On the basis of numerical analysis of total soluble cell protein patterns and DNA-DNA hybridization, a new species from raw milk from Argentina, *L. argentinum*, has been described (60). Collins et al. (45) have proposed the removal of *L. paramesenteroides* to a new genus, *Weissella*; it has also been proposed that *L. oenos*, the key organism in the malolactic fermentation of wine, be transferred to a new genus, *Oenococcus* (59).

(v) *Streptococcus*. The classification of the genus *Streptococcus* has been studied and reviewed extensively (5, 31, 47, 111, 116, 186, 197, 212, 232). The genus was previously divided into four groups: enteric, lactic, viridans, and pyogenic streptococci (212). Members of the genus have a G+C content of 34 to 46 mol% and are pathogenic for humans and other animals; some species are found as members of the normal flora of the mouth and gastrointestinal tract. Differentiation within the genus is achieved by colony size, type of hemolysis, and serological, metabolic, and molecular genetic tests. Unfortunately, serological techniques relied upon previously for the identification of pyogenic streptococci identify isolates with group B and F antigens but not individual species of isolates positive for group A, C, and G antigens (197, 224). Isolates carrying the latter antigens may belong to more than one species; they can be subdivided into large- or small-colony-forming strains. Thus, while isolates with the above antigens that grow in large colonies are pyogenic, the small-colony-forming strains may participate in infection but can also be found as commensals. The small-colony-forming strains belong to a number of species which are placed in the "*S. milleri*" group (197).

The former enteric and lactic streptococci are now placed in the separate genera *Enterococcus* and *Lactococcus*, respectively. The remaining species have been divided, based on 16S rRNA sequencing, into six groups by Bentley et al. (24): pyogenic (group I), group D (group II), pneumococcus/viridans group (group III), *S. mutans* group (group IV), *S. salivarius* group (group V), and the unaffiliated species *S. acidominimus* and *S. suis* (group VI).

The taxonomy of the pyogenic streptococci showing beta-hemolysis originally included *S. pyogenes* (group A antigen), *S. agalactiae* (group B), *S. equi*, *S. equisimilis*, and *S. zooepidemicus* (group C). Separation on the basis of biochemical tests between beta-hemolytic isolates of antigenic groups C and G has been somewhat difficult. Recently, a suggestion has been made to include large-colony-forming group C and group G strains of human origin in the same species and subspecies, *S. dysgalactiae* subsp. *equisimilis*, and those of animal origin in *S. dysgalactiae* subsp. *dysgalactiae* (224). The current division, based on 16S rRNA sequencing (24), shows that the pyogenic streptococci cluster in one phylogenetic group (group I), which includes the above taxa except for *S. anginosus* and *S. sanguis*, which are now included in the viridans group (group III). Additional isolates from nonhuman hosts have been described. *S. uberis*, *S. paruberis*, *S. iniae*, *S. canis*, *S. porcinus*, *S. intestinalis*, with reactions to groups L, M, P, U, and V antisera, also cluster with the pyogenic group. Group D streptococci include three species, *S. bovis*, *S. equinus*, and *S. alactolyticus*, and are assigned to phylogenetic group II. The ability to identify *S. bovis* rapidly is important, since this species is associated with endocarditis and colon cancer, as reviewed by Coykendall (49). The taxonomy of the viridans or oral group has been studied extensively and has suffered from differences in classification and nomenclature advocated in Europe and in the United States (22, 49, 127). Bentley, et al. (24) suggested that the organisms belong in three different phylogenetic groups. Group III contains two subgroups, with *S. pneumoniae*, *S. oralis*, and *S. sanguis* in one and the oral isolates of the "*S. milleri*" group including *S. anginosus*, *S. constellatus*, and *S. intermedius* in the other. Group IV contains *S. mutans*, which has been linked to caries, and additional species isolated from nonhuman hosts. Group V contains *S. salivarius*, *S. vestibularis*, and "*S. thermophilus*." The last species is used in mixed starter cultures for the production of yogurt and fermented milks (220). The Beighton et al. (22) scheme for the identification of oral streptococci, based on the determination of glycosidases with 4-methylumbelliferone (MEU) conjugates and other tests, includes *S. gordonii* and *S. mitis* in addition to the above species.

(vi) *Vagococcus*. The genus *Vagococcus* (42) was created for the motile lactococci, reacting with group N antisera, with a G+C content of 33.6 mol%. Two species have been described, *V. fluvialis* and *V. salmoninarum* (40, 42). Isolates of *V. fluvialis* resemble members of the genus *Enterococcus* both phenotypically (68) and genetically (42), reacting positively with the AccuProbe *Enterococcus* test, but unlike *Enterococcus* spp., they grow poorly at 45°C. Very few isolates have been recovered from human patients. The second species, *V. salmoninarum*, has been isolated from fish, is nonmotile, does not grow at 40°C, and produces H_2S. Acid formation from glycerol, galactose, sorbitol, and D-tagatose differentiates between the two species; the former is positive in the first three tests, and the latter is positive only in the last (233). Schemes for differentiating the members of this genus from the other gram-positive cocci have appeared (68).

Cells arranged in pairs or tetrads, with no catalase activity.

(i) *Gemella*. The genus *Gemella* contains two species *G. haemolysans*, previously classified as *Neisseria haemolysans*, and *G. morbillorum*, previously classified as *Micrococcus* sp., *Diplococcus morbillorum*, *Peptostreptococcus morbillorum*, and *Streptococcus morbillorum* (25, 196). The differences and similarities of the two species were discussed by Berger and Pervanidis (25). The phylogenetic relationship between the two species and other gram-positive bacteria was studied by Stackebrandt et al. (219) and Whitney and O'Connor (246). The clinical significance of the two species has been reviewed (68). The genus contains gram-positive cocci, which may require aerobic (*G. haemolysans*) or strict anaerobic (*G. morbillorum*) conditions for isolation and further culturing. It was also shown that 10% CO_2 is optimal for the growth of both species. A G+C content of 30 to 33.5 mol% has been established (149). *G.*

haemolysans may appear gram variable or gram negative. Isolates of both taxa grow poorly with variable hemolysis, which may depend on the source of blood (rabbit, sheep, or horse). Most strains are positive in the LAP and PYR tests but require a heavy inoculum. Discrepancies were observed between the results of these tests when performed with commercial kits or as tube tests (68). The ability to synthesize cytochrome *b* when grown on media supplemented with hemin was reported (219). Distinguishing these two species from nutritionally variant streptococci is difficult. Schemes for separating the members of this genus from the other gram-positive cocci have appeared (68, 80, 198). On the basis of 16S rRNA studies of two *Gemella*-like isolates from clinical sources, a new genus, *Dolosigranulum*, has been proposed (4).

(ii) *Helcococcus*. Only one *Helcococcus* species has been described, *Helcococcus kunzii* (43). The isolates are similar to those of *Aerococcus* spp. but differ from them with respect to growth on blood agar. *Helcococcus* isolates grow slowly and form small nonhemolytic viridans-like colonies; the growth is greatly stimulated by serum or Tween 80. Organisms have been isolated from mixed cultures from wounds, commonly foot wounds, leg ulcers, and a purulent breast mass (43). Peel et al. (180) have reported the isolation of *H. kunzii* in pure culture from an infected sebaceous cyst. Isolates of the species are PYR positive and LAP negative, are susceptible to vancomycin, and have a G+C content of 29.5 to 30 mol%. Members of the genus were phylogentically only remotely related to aerococci, streptococci, and other catalase-negative, facultatively anaerobic gram-positive taxa with a low G+C content (43).

(iii) *Pediococcus*. The genus *Pediococcus*, with a G+C content of 34 to 43 mol%, contains five species associated with lactic acid fermentations of vegetables, grain mashes, and cheese (*P. acidilactici*, *P. damnosus*, *P. dextrinicus*, *P. parvulus*, and *P. pentosaceus*). Isolates are positive for the LAP and bile-esculin tests but negative for PYR. Some strains are used as starter cultures in silage and various fermented sausages (48). One species (*P. damnosus*) is an important spoilage agent of beer (186); two species (*P. acidilactici* and *P. pentosaceus*) have been isolated from human specimens. The phylogenetic interrelationships within the genus have been discussed by Collins et al. (46). The facts that these organisms are intrinsically resistant to vancomycin and that most strains isolated from human infections have the group D antigen add to the need for their correct identification. Differentiation within the genus is based on tests used in the clinical laboratory, such as formation of acid from carbohydrates and the ability to grow in 6.5% NaCl, and in food microbiology, in particular the ability to grow at low pH, in different beers, and in the presence of hops. Schemes for separating the taxon from others and for differentiation within the genus have been provided (68, 69, 198).

(iv) *Tetragenococcus*. The genus *Tetragenococcus* contains one species, *Tetragenococcus halophilus* (46); this species was previously included in the genus *Pediococcus* but is susceptible to vancomycin. It resembles *Pediococcus* in being positive for the LAP and bile-esculin tests but negative for PYR; it has a G+C content of 34 to 36 mol%. The genus represents a distinct line of descent quite separate from aerococci and pediococci (46). It is important in the fermentation of soy moromi to produce soy sauce (220).

Cells arranged in pairs or tetrads, with weak catalase activity. (i) *Aerococcus*. The history, taxonomy, and clinical significance of the genus *Aerococcus* were summarized by Facklam and Elliott (68). Isolates assigned to this genus show a weak reaction in the catalase test but do not contain cytochrome; they have a G+C content of 35 to 40 mol%.

Two species have been described, *A. viridans* (249) and *A. urinae* (2, 39). They differ in their reactions in the LAP and PYR tests. The former is LAP negative and PYR positive, and the latter is LAP positive and PYR negative. Collins et al. (43, 46) placed them as two different entities on an unrooted tree showing the phylogenetic interrelationship of lactic acid bacteria based on 16S rRNA gene sequences. Christensen et al. (39) described an esculin-positive biotype of *A. urinae*. Schemes for separating the taxon from others and for differentiation within the genus have been described (68, 69, 198).

(ii) *Alloiococcus*. The genus *Alloiococcus* contains only one species, *Alloiococcus otitis* (3), renamed *A. otitidis* (231). Isolates assigned to this genus are obligately aerobic, slow-growing cocci that give a weak reaction in the catalase test but do not contain cytochrome. Members of the genus have a G+C content of 44 to 45 mol%. Isolates grow very slowly in 6.5% NaCl, are LAP and PYR positive, are susceptible to vancomycin, do not produce gas from glucose, and do not form acid from carbohydrates. Bosley et al. (29) have described 19 isolates of this species from ear fluid samples. Heavy growth occurred in brain heart infusion broth supplemented with 0.07% lecithin and 0.5% Tween 80. Although the species falls within the low-G+C content of gram-positive bacteria, it is genealogically distinct from aerococci and streptococci (3).

Identification schemes and kits. A variety of schemes for the identification of all or some of the above taxa have been published. There are a number of identification kits, some covering the facultative gram-positive cocci (e.g., MicroScan GP and Rapid GP panels and Vitek GPI Card) and others being specific for streptococci (e.g., API 20 Strep). There are also kits that use enzyme substrates, identification of the antigenic group, or genetic probes for specific detection of individual species or a limited number of species, e.g., *S. pyogenes*, *S. pneumoniae*, and *S. agalactiae.*

Among the enzymes used for the differentiation of these taxa, PYRase is the most common. Mulczyk and Szewczuk (169) first described the synthesis of a β-naphthylamine conjugate of pyrrolidonyl carboxylic acid and its usefulness in a 4-h test for the detection of pyrrolidonyl aminopeptidase, with enzyme activity being detected by the formation of a cherry-red color after the addition of Fast Blue B reagent (tetra-azotized-*o*-dianisidine). They demonstrated the usefulness of the test for differentiation among enteric gram-negative bacteria and also reported positive results for certain isolates of some gram-positive genera including *Streptococcus*, *Staphylococcus*, and *Sarcina*. The usefulness of various aminopeptidases in bacterial identification was reviewed by Watson (235). Godsey et al. (93) suggested the usefulness of a rapid PYR test with a β-naphthylamine conjugate of pyrrolidonyl for the identification of group A streptococci and enterococci. The PYR-positive isolates could be further distinguished by using a bile-esculin test, for which only enterococci will give a positive result. A number of systems incorporating substrates for the PYR test on paper strips (65, 88, 96, 120, 167, 234) and in liquid (28, 93) and agar (74) media have been devised for the detection of PYRase (EC 3.4.11.8) and reviewed (51, 70, 152, 166, 234, 242). Commercially available chromogenic tests based on the pyrrolidonyl carboxylic acid or pyroglutamic acid conjugates of β-naphthylamine, a colorimetric test based on a proprietary substrate (65), and one fluorogenic test based on a proprietary substrate (88, 234), as well as their performances, have been summarized (152). A filter paper strip combining a PYR fluorogenic test involving a 7-amido-4-methyl coumarin conjugate of L-pyroglutamic acid and a chromogenic β-glucosidase test involving 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside (X-Glu) was described (152). The chromogenic PYR test is included in API

20 STREP kit, RapID STR (bioMérieux-Vitek Inc.), and MicroScan Conventional Gram-Positive Panels (Dade Intl. Inc.); a chromogenic test for the same arylamidase, involving a β-naphthylamine conjugate of pyroglutamic acid, is incorporated in the Pasco Gram-Positive ID Panel. The fluorogenic test with a 7-amido-4-methyl coumarin conjugate of L-pyroglutamic acid is in the MicroScan Rapid Gram-Positive Panels (Dade Intl. Inc.) and in the filter paper strip (152).

The inoculum requirements for two commercially available PYR tests were compared by Gordon et al. (95). All the chromogenic tests for PYR require the addition of a reagent after a suitable incubation time to visualize the released β-naphthylamine, whereas the released fluorophore in the above three fluorogenic tests can be detected without any further step. Moreover, amino acid conjugates of β-naphthylamine are listed as carcinogens (167). The great interest in this enzyme test could be attributed to the desire to perform identification tests directly in the clinical specimens without preculture, e.g., to test for group A streptococci in throat specimens. The effectiveness of such tests depends on the specificity of the test for the detection of the particular taxon as well as on the concentration of enzymes in the clinical specimens related to the bacterial concentration.

Kilian et al. (127) have shown that tests for β-galactosidase and β-glucosidase gave different results when conjugates of naphthol or nitrophenol were used as substrates. Beighton, et al. (22) suggest that glycosidase activities obtained with conjugates of MEU may not agree with results obtained with conjugates of other synthetic moieties. Slifkin and Gill (214) used MEU conjugates for the rapid identification of group A, B, C, F, and G streptococci from throat cultures. Littel and Hartman (148) tested 44 fluorogenic substrates for their ability to differentiate between fecal enterococci and streptococci. The substrates were used in liquid medium and incorporated in a selective agar medium. These investigators found that MEU conjugates of α-D- and β-D-galactose, β-D-glucose, and α-L-arabinose were the only useful substrates. They reported that β-naphthylamine substrates inhibited the growth of streptococci. They also found that the presence of certain saccharides could inhibit the hydrolysis of certain fluorogenic substrates and that 1% glucose inhibited the hydrolysis of conjugates of α-D-galactose and α-L-arabinose by *E. faecium*. Schaufuss et al. (206) demonstrated the usefulness of MEU conjugates of β-D-glucuronide, *N*-acetyl-β-D-glucosaminide, and β-D-manoside in the identification of streptococci from cows with mastitis. An MEU conjugate of β-galactoside was used by Lämmler et al. (142) to differentiate between group B streptococci of human and bovine origin, since only the latter are positive in the test.

The esculin and bile-esculin tests are used to differentiate the streptococci from enterococci. Edberg et al. (62) have shown that testing for β-D-glucosidase activity in the presence of 2.5% sodium deoxycholate gives results equivalent to the conventional bile-esculin test. Panosian and Edberg (177) have recommended the detection of β-D-glucosidase activity in the presence of 2.5% sodium deoxycholate, α-D-galactosidase, and PYR for the rapid identification of *S. bovis*, *S. equinus*, *Enterococcus* spp., *S. pneumoniae*, and the viridans streptococci.

Cimolai and Mah (40) recommended the use of an MEU conjugate of β-D-glucuronide for the separation of *S. equisimilis* of antigenic group C and G large-colony types from group C and G "*S. milleri*" isolates; the latter isolates give negative results. Matthews et al. (158) compared liquid (1 to 2 h) and agar (18 to 24 h) tests incorporating MEU conjugates of β-D-glucuronide, β-D-galactoside, and *N*-acetyl-β-D-glucosaminide for the identification of streptococci isolated from bovine mammary glands and found that the agar test gave more positive results than did the liquid test. The difference may be attributed to the longer incubation period used in the agar test. Whiley et al. (243) devised a scheme for differentiation of the "*S. milleri*" group into three species, *S. anginosus*, *S. constellatus*, and *S. intermedius*. The scheme is based on detection of seven glycosidases by using MEU conjugates and a 10^8-CFU/ml inoculum. The enzymes included were β-D-fucosidase, β-*N*-acetylglucosaminidase, β-*N*-acetylgalactosaminidase, β-galactosidase, β-glucosidase, α-glucosidase, and sialidase. The results were observed after 3 h of incubation and were combined with a 48-h test for the determination of hyaluronidase production. Flynn and Ruoff (77) described results obtained with a rapid commercially available system, Fluo-Card Milleri (Key Scientific, Round Rock, Tex.) for the identification of the "*S. milleri*" group, which is based on the determination of three of the above mentioned-glycosidases, namely, α- and β-glucosidase and β-fucosidase, with MEU derivatives. The system requires a heavy inoculum (5 to 10 colonies) and a 15-min incubation.

Dealler et al. (55) described results obtained with a 5-min test strip method (Strep Strip Lab M) for the identification of *S. pyogenes* based on the detection of PYR and β-glucosidase with β-naphthylamide and indoxyl conjugates, respectively. Devriese et al. (58) described the usefulness of a test for acid production from methyl-α-D-glucopyranoside for differentiating *E. gallinarum* and *E. casseliflavus* from *E. faecalis* and *E. faecium*; the first two species are positive, while the last two species are unable to produce acid from this substrate. The authors suggested that an MEU conjugate of this substrate would allow faster reading of the enzyme reaction. Examination of the proteolytic activities of oral streptococci with fluorogenic endopeptidase synthetic substrates and fluorescein isothiocyanate-labeled bovine serum albumin and fluorescein isothiocyanate-labeled casein has shown a correlation between the two activities and the suitability of aminomethylcoumarin derivatives for the sensitive detection of endopeptidase activity (105).

Kits available for the identification of streptococci include some for the identification of facultative gram-positive cocci described above, as well as some kits specific to streptococci including API 20 Strep, RapID STR (formerly RapSTR; Innovative Diagnostics, Atlanta, Ga.), Rapid ID 32 Strep (bioMérieux) (80), and Strep-Zym (Rosco) (140, 240). In the 1980s, different strip kits for the identification of streptococci were available in Europe (API 20 Strep [API Systems S.A., Montalieu-Vercieu, France], sold in the United States as Rapid Strep by DMS Laboratories Inc., Flemington, N.J.) and the United States (API 20S; Analytab Products, Plainview, N.Y.). Both products contained enzymatic and sugar fermentation tests with incubation times of 4 to 24 h; different accuracy rates were reported (14). Comparison of the reactions of the Rapid Strep test with those of modified conventional tests were reported (76). The general requirements of most kits and the tests included are listed in Tables 1 through 6. In addition, Watts and Yancey (240) reported that the Strep-Zym kit includes 23 enzyme tests requiring 4 to 24 h of incubation. The number of taxa identified by these kits varies from 26 by the Pasco Gram-Positive ID Panel to 52 by Rapid ID 32 Strep. The inoculum requirements vary from 0.5 McFarland standard for MicroScan and Pasco systems to 4.0 McFarland standard for the Rapid ID 32 Strep. Incubation periods vary from 2 h, for the MicroScan Rapid Pos ID panels, to 16 to 42 h, for the MicroScan conventional Pos ID panels. The early recognition of the usefulness of the PYRase test for differentiation of streptococci may be responsible for the large number of enzyme tests included in these kits. The number of peptidase

tests varies from 0 in the Vitek GPI card to 10 in the MicroScan Rapid Pos, and the number of glycosidases and phosphatases varies from 0 to 11, respectively. However, acid formation from sugars is still used most frequently.

The performance of MicroScan Rapid Pos ID panels, which rely on enzyme tests and have a very short incubation period (2 h) and a fairly small inoculum (0.5 McFarland standard) was similar to that of systems requiring a longer incubation period or higher inoculum (103, 130, 203, 229). This suggests that judicious selection of enzyme tests, use of synthetic fluorogenic substrates and instrumentation for measurement of fluorescence, both of which enable the detection of a small amount of enzymatic activity, and a comprehensive database can provide rapid identification of this very complex group. However, the equivocal taxonomy of the viridans streptococci has hindered the development of identification kits with the required accuracy for this group.

The MicroScan Rapid Pos ID panel utilizes a 0.5 McFarland standard inoculum and provides identification in 2 h, the RapID STR system is also enzyme based but the results are observed visually; it requires a 1.0 McFarland standard inoculum and provides results in 4 h. Rapid ID 32 Strep provides results in 4 h; however, it requires a 4.0 McFarland standard inoculum, necessitating an overnight subculture before testing.

Reports on the identification of streptococci and related organisms with commercial kits are summarized in Table 8. Not included in the table are comparisons of test results of 21 reference and 88 clinical isolates with the Rapid Strep and modified conventional test (76), showing that some but not all of the Rapid Strep tests could replace conventional ones. Similar studies with API Rapid Strep and MicroScan POS ID panels have also been reported (135). Lämmler (140) has reported that *S. uberis*, *S. agalactiae*, and *S. dysgalactiae* were characterized and differentiated by the Strep-Zym kits (140). Devriese et al. (57) have reported 77% correct identification of enterococci from fresh and prepared foods tested with API 20 Strep; additional tests were required for complete identification.

Reports on the accuracy of identification obtained with the different kits or systems (Table 8) vary from 0% for group C equine isolates with API 20S (14) to 100% for *E. faecalis* (34, 229). The accuracy levels were lower than those obtained for staphylococci (Tables 7 and 8).

In general, many systems performed better when augmented with serological and other tests (14, 252). Identification of beta-hemolytic streptococci, some of the common viridans streptococci, and the well-established species of *Enterococcus* was fairly successful; that of pneumococci depended on the use of additional tests such as optochin (7). Identification of the viridans group streptococci proved to be more difficult with many systems and often required additional tests. Difficulties in the identification of isolates from veterinary sources have been observed with most systems.

Very few of the recently described new taxa are included in the databases or can be identified with the above identification kits. It is important to realize that time is required to produce updates of commercial identification kits as and when new classifications and/or new nomenclatures are published. The time is needed for selection of a sufficient number of isolates for testing, finding tests to differentiate the newly described taxa from existing ones, establishing the manufacturability of required tests, performing appropriate testing of the modified product with further isolates, and obtaining regulatory approval of the modified kit.

Moreover, some of the newly described taxa have originated from phylogenetic studies of as little as one isolate and may exhibit only genetic and no phenotypic differences from existing taxa. Because of regulatory requirements for testing a minimal number of isolates before claiming the ability to identify any taxon, it is likely that more time will be needed before such taxa are included in commercial identification kits.

GENERAL CONSIDERATIONS AND INTERRELATIONSHIP BETWEEN TESTS

Relationships between results obtained by conventional and enzyme tests have been scantily studied, but quite often these sets of results provide related information. Thus, the ONPG test (147) for the detection of β-galactosidase is a rapid method for differentiation between lactose fermenters and nonfermenters, a useful test for recognition of pathogenic gram-negative taxa. However, a number of factors determine whether the two tests will provide identical information. They include the incubation period, the sensitivity of the detection method, the inoculum size, whether the enzyme is constitutive or inducible, whether the growth conditions for preparation of the inoculum were conducive or inhibitory for production of the enzyme, and whether the two tests depend on the same or different processes for a positive result. Thus, both ONPG and acid formation from lactose depend on the presence of an enzyme to transport the substrate into the cytoplasm and a β-galactosidase to hydrolyze the glycoside, and the results obtained are therefore interdependent. However, while the ONPG test requires only these two processes, acid formation from lactose requires the conversion of the glucose and galactose molecules into lactic and acetic acids, etc. Because many more enzymes are required, the results are not identical.

Similar relationships exist between tests for other glycosidases and acid formation from carbohydrates. Thus, the formation of acid from maltose, melezitose, and trehalose depends on the enzyme α-glucosidase; acid formation from cellobiose and salicin, as well as a positive esculin test, depends on β-glucosidase; and acid formation from melibiose and raffinose depends on α-galactosidase. In practice, differences between the test results exist; such differences may also depend on the medium in which the isolates were grown before the enzyme testing. Colman and Ball (47) noticed different percentages of positive results between streptococcal cells taken from blood agar prepared with a Hartley digest and horse blood and those taken from agar prepared with a commercial base (Columbia agar) and horse blood. Although different numbers of isolates were tested (177 and 965, respectively) the results for α-galactosidase were 4 and 31% for the Hartley digest and Columbia-based agars respectively; the results for acid formation from raffinose were 20 and 40%, respectively. With both agar sources, the percentages were higher for acid formation, probably reflecting the longer incubation period used in the conventional test. The cells taken from Columbia agar gave higher percent positive results for both the enzyme and acid formation tests, probably reflecting better conditions for synthesis of the enzyme. Similar results were observed for β-galactosidase and acid formation from lactose. The relationship between acid formation from melibiose and raffinose was discussed by Beighton et al. (22), suggesting that both are substrates for α-galactosidase but that raffinose may also act as a substrate for fructosyl transferase.

Differences between the results for the same nominal enzyme in different API kits have also been observed (127). These could be attributed to the differing pH conditions of the tests and possibly also to the difference in the affinity of the enzymes to the synthetic moiety of the substrate.

Comparing the synthetic moieties of tests used by the dif-

TABLE 8. Comparison of accuracy of identification systems for *Streptococcaceae* and similar organisms

Identification system	Year (reference)	Group identified	No. of isolates tested	% correct (% correct with additional tests, or to group level)	Comments; (additional tests); problematic taxa
API 20S[b]	1982 (171)	α-Hem.[a] streptococci	194	39.0 (56.5)	Clinical isolates; (group level identification)
API 20S	1982 (171)	α-Hem. streptococci	20	20.0	Reference strains
API 20S	1985 (14)	Group C streptococci	85	0.0	Equine isolates
API 20S	1985 (67)	Non-β-hem. streptococci	535	87.8	Stock cultures; (additional tests required for viridans group)
API 20S	1988 (183)	Viridans group streptococci	109	50.0 (78.0)	Clinical isolates, *S. sanguis* II
API 20S	1991 (109)	Viridans group streptococci	101	52.5	Blood isolates
API 20S	1995 (202)	Enterococci	369	81.5	Nosocomial isolates
API 20 Strep[b]	1984 (185)	Streptococci	84	71.4	Bovine mastitis isolates
API Rapid Strep[b]	1983 (199)	Viridans group streptococci	119	81.0 (92.0)	(Includes isolates with identification of <90%)
API Rapid Strep	1984 (6)	Streptococci	209	73.2	Without additional tests
API Rapid Strep	1984 (72)	*Streptococcaceae*	251	70.5	Isolates from humans
API Rapid Strep	1984 (72)	β-hem. streptococci	75	80.0	Isolates from humans
API Rapid Strep	1984 (72)	Group D + aerococci	70	85.7	Isolates from humans
API Rapid Strep	1984 (72)	*S. pneumoniae* + viridans group streptococci	106	53.8	Isolates from humans
API Rapid Strep	1985 (14)	Group C streptococci	85	94.1	Equine isolates, 24-h incubation; (serology)
API Rapid Strep	1986 (98)	Streptococci	270	78.8	Human and veterinary isolates; problems with group B and group C
API Rapid Strep	1989 (236)	Streptococci	199	88.4	Isolates from bovine mastitis; *S. bovis*
API Rapid Strep	1991 (103)	Viridans group streptococci	203	74.0 (83.0)	Reference and blood culture isolates
API Rapid Strep	1991 (113)	Streptococci	144	96.5	Bovine isolates, 4-h incubation
BBL Minitek[c]	1991 (103)	Viridans group streptococci	203	65.0 (85.0)	Reference and blood culture isolates
BBL Minitek	1989 (237)	Streptococci	127	34.6	Isolates from bovine mastitis; *S. bovis*
MS GP[d]	1990 (223)	Enterococci	100	94.0	Clinical isolates
MS Rapid GP[d]	1991 (103)	Viridans group streptococci	203	66.0 (77.0)	Reference and blood culture isolates
MS Rapid GP	1992 (203)	Streptococci and enterococci	267	94.0	Multicenter study
MS Rapid GP	1992 (229)	*E. faecalis*	12	100.0	Clinical isolates
MS Rapid GP	1995 (108a)	Streptococci and enterococci	76	98.7 (100.0)	Clinical isolates; (low probability)
MS Rapid GP	1995 (182)	*Enterococcus* spp.	17	88.2	Direct identification of positive blood culture centrifuged pellets; identification available after 2 h
		S. bovis	14	28.9	
		S. pneumoniae	13	61.5	
Rapid ID 32 Strep[b]	1991 (109)	Viridans group streptococci	101	82.2	Blood isolates
Rapid ID 32 Strep	1992 (80)	Streptococci plus enterococci and similar organisms	433	70.2 (95.3)	Clinical and veterinary isolates; (agreement with additional tests)
RapID STR[b]	1986 (7)	Streptococci and enterococci	266	69.9	Clinically significant culture isolates
		β-hem. streptococci	60	100.0	
		Group D enterococci	48	93.7	
		Group D non-enterococci	23	73.9	
		S. pneumoniae	26	26.9 (88.5)	(Optochin)
		Viridans group streptococci	109	52.3 (72.5)	(Optochin)
RapID STR	1986 (252)	Streptococci and enterococci	243	79.9 (93.8)	Culture collection isolates of human origin; (serological testing, optochin)
		β-hem. streptococci	75	87.0 (89.0)	
		Enterococci	39	90.0 (98.0)	
		Group D non-enterococci	20	90.0 (100.0)	
		S. pneumoniae	10	80.0 (100.0)	
		Viridans group streptococci	81	62.0 (93.0)	
		Aerococcus spp.	9	88.9 (100.0)	
RapID STR	1988 (183)	Viridans group streptococci	109	62.0 (80.0)	Clinical isolates; *S. intermedius*
RapID STR	1991 (103)	Viridans group streptococci	203	50.0 (61.0)	Reference and blood culture isolates
AMS GPI[b]	1985 (67)	Non-β-hem. streptococci	507	87.5	Stock cultures; viridans group streptococci
Vitek GPI[b]	1988 (183)	Viridans group streptococci	109	72.0 (81.0)	Clinical isolates
Vitek GPI	1991 (34)	*E. faecalis*	116	100.0	Clinical isolates from tertiary-care hospitals
Vitek GPI	1991 (34)	Enterococci, not *E. faecalis*	24	61.9	Clinical isolates from tertiary-care hospitals
Vitek GPI	1991 (103)	Viridans group streptococci	203	61.0 (82.0)	Reference and blood culture isolates
Vitek GPI	1991 (113)	Streptococci	144	94.4	Bovine isolates; 8-h incubation
Vitek GPI	1995 (102)	*S. mutans*	160	72.5	Clinical oral isolates
Vitek GPI	1995 (202)	Enterococci	369	(95.3)	(Motility, pigmentation)
Vitek GPI	1992 (229)	*E. faecalis*	12	100.0	Clinical isolates

[a] α-hem., alpha-hemolytic; β-hem., beta-hemolytic.
[b] bioMérieux-Vitek Inc. Hazelwood Mo. and bioMérieux S. A., Marcy l'Etoile, France.
[c] BBL Becton Dickinson Microbiology System, Becton Dickinson & Co., Sparks, Md.
[d] Dade International Inc., West Sacramento, Calif.

ferent systems (Tables 2 and 4), *p*-nitrophenol and MEU derivatives are most commonly used for glycosidases and phosphatases; the use of 5-bromo-4-chloro-3-indolyl derivatives for the detection of glycosidases may be on the increase, particularly when solid media are used. For the detection of peptidase, the chromogenic β-naphthylamine and fluorogenic 7-amido-4-methylcoumarin derivatives are used. Derivatives of β-naphthylamine are known to be carcinogenic (167). Enzyme activities can be detected without the addition of reagents when the noncarcinogenic fluorogenic derivatives are used.

The relationship between classification results obtained by molecular genetic and phenotypic techniques should also be considered. Molecular genetic studies are usually limited to a very small number of isolates per taxon and may therefore fail to detect variability observed with phenotypic tests applied to a considerably larger number of isolates.

The relationship between classification results obtained by different molecular genetic techniques should also be considered. Different techniques may interrogate different aspects of the genetic code and provide different results. Thus, Bentley et al. (24) point out the difference in the taxonomic positions of *S. anginosus*, *S. constellatus*, and *S. intermedius* based on DNA-DNA hybridization experiments and rRNA sequencing, with the latter technique placing them as three phylogentically distinct taxa.

DISCUSSION AND CONCLUSIONS

The availability of synthetic enzyme substrates with synthetic moieties of high absorption and/or fluorescence coefficients has resulted in an increase in the use of enzyme tests for identification. Enzyme tests have the advantage of being easy to perform, providing rapid results and information on the activities of proteins coded for by defined genes. Most of the enzyme tests available are related to catabolic activities of microorganisms, and very few are available for easy detection of synthetic pathways (126). Most successful enzyme tests are those that detect the activities of glycosidases and dipeptidases. The glycosidases are highly specific to the metabolic moiety; the peptidases are less so. Synthetic substrates for the detection of endopeptidases and lipases are available but are more difficult to incorporate into a dried, easily reconstitutable format. In spite of the great interest in DNA and RNA molecules, substrates are not available for easy detection of nuclease activities.

The expected results of each enzyme test for different taxa can be found in package inserts of the different products; for the MicroScan GP and Rapid GP systems, reports of results obtained independently are given by Kloos and George (130).

Identification of staphylococci and streptococci with rapid enzyme tests has proved to be as accurate as that with methods requiring longer incubation periods. The rapid turnaround of results from the microbiology laboratory using enzyme tests raises the interesting possibility of providing clinicians with a useful identification of infectious agents early enough to influence decisions about the diagnosis and initial treatment of the patient from whom the specimen was taken.

ADDENDUM

Recently, two new gram-positive identification systems, BBL Crystal and Vitek II, have been described in Europe. The BBL Crystal Gram-positive ID Panel (Becton Dickinson Microbiology System, Becton Dickinson & Co., Sparks, Md.) includes 121 gram-positive taxa and requires an overnight incubation; the BBL Crystal Rapid Gram-Positive ID Panel requires a 4-h incubation period and identifies only the more frequently isolated gram-positive bacteria. The kit, which requires overnight incubation, consists of a miniaturized panel which contains 29 fluorogenic, chromogenic, and modified conventional substrates plus a fluorogen control. After 18 to 20 h of incubation at 37°C in ambient atmosphere, color and fluorescence changes are recorded manually. The list of taxa includes aerococci, bacilli, lactococci, micrococci, pediococci, staphylococci, streptococci, corynebacteria, species of *Bacillus*, *Gardnerella*, *Leuconostoc*, *Listeria*, and other gram-positive organisms including some of the newly described genera. Evaluation of the panel for identification of 207 isolates has shown 84.5 and 97% correct identification at the species and genus levels, respectively (230). Evaluation of the system for identification of 170 clinical isolates has shown 100% identification to the species level for staphylococci, micrococci, and enterococci and 86 and 79% correct identification to the species level for miscellaneous and streptococcal isolates, respectively. Particular difficulties were observed with some strains of the viridans group streptococci (187).

The second system is the Vitek II system (bioMérieux-Vitek Inc., and bioMérieux S.A.), a new fluorogenic automated system (82). For identification of gram-positive isolates, the system uses a disposable card containing 46 fluorometric substrates. The system enables the measurement of glycosidase, arylamidase, urease, decarboxylase, and phosphatase activities, growth in the presence of inhibitory compounds, and utilization of various carbohydrates. Inoculated panels are incubated in the new instrument for 2 h at 35°C. Fluorescence levels are measured automatically at 15-min intervals over the 2-h incubation period. A computer-assisted algorithm is used to determine final identifications. The system can identify 53 taxa including species of *Aerococcus*, *Enterococcus*, *Gemella*, *Kocuria*, *Micrococcus*, *Staphylococcus*, and *Streptococcus*. Evaluation of its performance with 1,937 isolates showed 86.8 and 98.0% agreement to the species level without and with supplemental testing, respectively; 1.7% of isolates were misidentified, and 0.3% were unidentified (20).

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