Evaluation of Staf-Sistem 18-R for identification of staphylococcal clinical isolates to the species level.

R Piccolomini, G Catamo, C Picciani and D D'Antonio
J. Clin. Microbiol. **1994, 32(3):649.**

JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1994, p. 649–653
0095-1137/94/$04.00+0

Vol. 32, No. 3

Evaluation of Staf-Sistem 18-R for Identification of Staphylococcal Clinical Isolates to the Species Level

RAFFAELE PICCOLOMINI,[1]* GIOVANNI CATAMO,[1] CARLA PICCIANI,[1] AND DOMENICO D'ANTONIO[2]

Cattedra di Microbiologia, Istituto di Medicina Sperimentale, Università "G. D'Annunzio," I-66100 Chieti,[1] and Servizio di Microbiologia della Divisione di Ematologia, Ospedale "Santo Spirito," Pescara,[2] Italy

Received 5 August 1993/Returned for modification 10 November 1993/Accepted 3 December 1993

The accuracy and efficiency of Staf-Sistem 18-R (Liofilchem s.r.l., Roseto degli Abruzzi, Teramo, Italy) were compared with those of conventional biochemical methods to identify 523 strains belonging to 16 different human *Staphylococcus* species. Overall, 491 strains (93.9%) were correctly identified (percentage of identification, ≥90.0), with 28 (5.4%) requiring supplementary tests for complete identification. For 14 isolates (2.8%), the strains did not correspond to any key in the codebook and could not be identified by the manufacturer's computer service. Only 18 isolates (3.4%) were misidentified. The system is simple to use, is easy to handle, gives highly reproducible results, and is inexpensive. With the inclusion of more discriminating tests and adjustment in supplementary code numbers for some species, such as *Staphylococcus lugdunensis* and *Staphylococcus schleiferi*, Staf-Sistem 18-R is a suitable alternative for identification of human coagulase-positive and coagulase-negative *Staphylococcus* species in microbiological laboratories.

In the past few years, a number of changes have been made to the genus *Staphylococcus*. Within this genus, 27 species have been delineated by DNA-DNA hybridization (19, 21) or by rRNA gene restriction site polymorphism (5, 6, 10). However, many *Staphylococcus* species besides the coagulase-positive *Staphylococcus aureus* and new coagulase-negative staphylococci have emerged as increasingly important nosocomial pathogens (8, 20, 24, 28). Although some studies have suggested that the exact identification of coagulase-negative staphylococci to the species level may have limited utility (25), many investigators realize the need to discriminate between pathogenic and saprophytic coagulase-negative staphylococci (1, 12, 20). The clinical microbiologist is thus faced with the problem of identifying these new *Staphylococcus* species.

Kloos and Schleifer (16) published a simplified scheme for the identification of a variety of species of coagulase-negative staphylococci and *S. aureus*. This scheme was recently revised by Kloos and Lambe (14). However, because conventional methods are labor intensive and require long incubation periods, they are impractical in the routine clinical laboratory.

With increased government attention to health costs (4), today the clinical microbiologist is also more interested than ever in rapid reporting and reductions in laboratory costs (2). Consequently, it seemed essential to develop new, simple, and economical systems for rapid and accurate identification of *Staphylococcus* species, and many commercial multitest systems are available for this purpose (3, 26, 27).

Staf-Sistem 18-R (Liofilchem s.r.l., Roseto degli Abruzzi, Teramo, Italy) is a new system designed to identify coagulase-negative staphylococci and *S. aureus* in 18 h. The system at present is available only in Europe, at a cost of $3.95 (U.S. currency) per determination. The kit, which should be marketed in the United States and Canada in 1994, consists of a disposable tray with 18 wells containing the dehydrated biochemical substrates. With inoculation of a bacterial suspension in each well, a six-digit code number can be generated from 18 different biochemical reactions. From this code number, an identification is derived from a codebook furnished to laboratories by the manufacturer.

The present study attempted to evaluate the accuracy and utility of this new system to identify several hundred clinically significant coagulase-positive and coagulase-negative staphylococci isolated from a variety of infections. The identifications by Staf-Sistem 18-R were compared with those by conventional methods.

MATERIALS AND METHODS

Bacterial strains. A total of 523 *Staphylococcus* strains were tested. Of these, 156 were obtained as significant blood cultures from immunocompromised patients with hematological malignancies admitted to the Hematology Division, Presidio Ospedaliero "S. Spirito," Pescara, Italy; a total of 183 isolates obtained from a variety of clinical sources and miscellaneous infections were sent from the Presidio Ospedaliero "S.S. Trinità," Microbiology Section, ULSS No. 12, Popoli, Pescara, Italy (courtesy of E. Ricci), and from the Division of Infection Diseases, University of Chieti (courtesy of E. Pizzigallo). Another 168 clinical isolates and the 16 American Type Culture Collection (ATCC) strains were stock cultures from the collection of the Clinical Microbiology Laboratory, Cattedra di Microbiologia, University of Chieti, that have been kept frozen (−75°C) in 50% glycerol. Species names, number of isolates included in the study, and ATCC strains are listed in Table 1.

Presumptive identifications of isolates as coagulase-positive or coagulase-negative *Staphylococcus* species were verified by Gram stain, catalase production, acid production from glycerol in the presence of erythromycin (0.4 μg/ml) (23), and tube coagulase (14) tests.

Before the experiment, the 184 stock cultures were subcultured three times into Tryptone Soya agar supplemented with 5% sheep blood (BTSA) (Unipath S.p.A.; Garbagnate Milanese, Milan, Italy) at 35°C for 48 h to raise their levels of enzymatic activity and to ensure purity and viability prior to

* Corresponding author. Mailing address: Istituto di Medicina Sperimentale, Università "G. D'Annunzio," Via dei Vestini, 31, I-66100 Chieti, Italy. Fax: 0039 871 355282.

TABLE 1. Comparison of Staf-Sistem 18-R with conventional methods for the identification of staphylococcal clinical isolates

Staphylococcus species	No. or designation of strains tested				No. (%) of strains:			
					Correctly identified[a]			
	Total	Fresh clinical isolates	Stock cultures	ATCC type strain	Without supplementary tests	With supplementary tests	Not identified[b]	Misidentified
S. aureus	181	149	31	25923	177 (97.8)		4 (2.2)	
S. auricularis	10	5	4	33753		10 (100)		
S. capitis	34	13	20	27840	32 (94.2)	1 (2.9)		1 (2.9)
S. cohnii	12	6	5	29974	11 (91.7)	1 (8.3)		
S. epidermidis	84	66	17	14990	74 (88.1)	3 (3.6)		7 (8.3)
S. haemolyticus	51	33	17	29970	48 (94.1)			3 (5.9)
S. hominis	45	24	20	27844	43 (95.6)	2 (4.4)		
S. hyicus	2		1	11249	1 (50)			1 (50)
S. intermedius	7	2	4	29663	6 (85.7)	1 (14.3)		
S. lugdunensis	8	2	5	43809			8 (100)	
S. saprophyticus	26	15	10	35552	26 (100)			
S. schleiferi	2		1	43808			2 (100)	
S. sciuri	4	1	2	29062	3 (75)	1 (25)		
S. simulans	14	6	7	27848	9 (64.3)	4 (28.6)		1 (7.1)
S. warneri	27	11	15	27836	22 (81.5)	2 (7.4)		3 (11.1)
S. xylosus	16	6	9	29971	11 (68.8)	3 (18.7)		2 (12.5)
Total (%)	523 (100)	339 (64.8)	168 (32.1)	16 (3.1)	463 (88.5)	28 (5.4)	14 (2.8)	18 (3.4)
					491 (93.9)			

[a] The correct identification of the species was the only option, and the % ID was ≥90.0.
[b] The profile did not correspond to any key in the Staf-Sistem 18-R code book furnished to laboratories.

use. The remaining 339 fresh clinical isolates were grown on BTSA at 35°C for 3 days, after which the plates were kept for an additional 2 days at room temperature. To determine whether the cultures were pure or mixed, colony morphology was carefully observed. These procedures were necessary for correct preparation of the inoculum for the systems.

The acceptability of reactions from the conventional methods was checked by testing the ATCC strains with each run. The results of each run were considered valid when the ATCC strains were correctly identified. To ascertain the reproducibility of results from Staf-Sistem 18-R, growth of the 16 ATCC strains from BTSA was employed as an inoculum on three separate occasions.

Staf-Sistem 18-R identification method. The Staf-Sistem 18-R identification system consists of a plastic tray containing 18 different reaction wells covered with a transparent plastic cover (Fig. 1). The 18 biochemical tests included in the system are listed in the legend to Fig. 1.

The kit was used according to the manufacturer's instructions with some substantial modifications, such as procedures to obtain distinguishable colony morphologies of most of the species and strains of coagulase-negative staphylococci on inoculum plates and the preparation of the tray inoculum. Two or three identical, well-developed colonies isolated from the original BTSA plate used for the inoculum were taken and emulsified in 4.0 ml of sterile physiological solution. The final turbidity was adjusted to that of a 1.0 McFarland barium sulfate standard.

With sterile tweezers, xylose and ribose disks were placed in wells 10 and 15, respectively. The reaction wells then were inoculated with 200 μl of the vortexed bacterial suspension by using a multichannel pipette (Titertek; Flow Laboratories, Milan, Italy). Wells for arginine dihydrolase and urease tests were covered with sterile mineral oil. The tray was closed with the plastic cover and then incubated in a moist chamber for 18 h at 35°C in an aerobic atmosphere. The time required to manipulate the system was 4 to 5 min.

After incubation, 3 drops of α-naphthol plus 1 drop of 40% NaOH solution (Voges-Proskauer [VP] reagent) and 2 drops of sulfanilic acid plus 2 drops of dimethyl-1-naphthylamine (nitrate reduction reagent) were added, respectively, to the VP and nitrate reduction reaction wells. The VP and nitrate

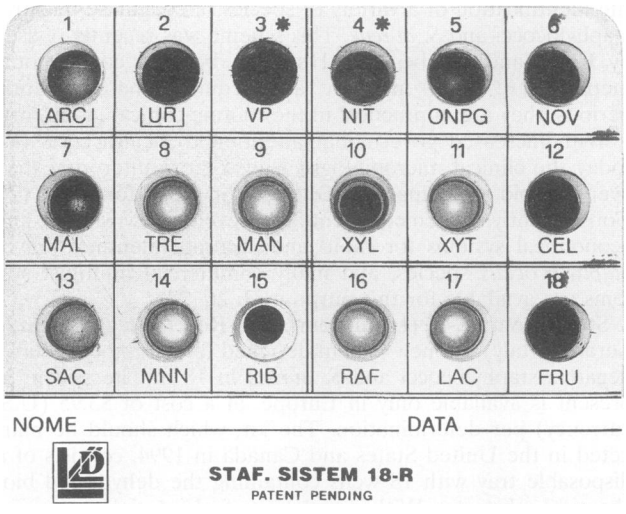

FIG. 1. Staf-Sistem 18-R. Test wells: 1, Arginine dihydrolization; 2, urea hydrolysis; 3, acetoin production (VP test); 4, Nitrate reduction; 5, *o*-nitrophenyl-β-D-galactopyranosidase; 6, novobiocin resistance; 7 through 18, fermentation with acid formation of maltose, D-trehalose, D-mannitol, D-xylose, xylitol, D-cellobiose, sucrose, D-mannose, D-ribose, Raffinose, α-lactose, and β-D-fructose, respectively.

reduction reactions were evident before 12 to 15 min and within 5 min, respectively.

Results of all of the reactions were read by use of a color chart provided with the kit. The biochemical reactions could be clearly interpreted and were recorded on data sheets provided with the kit; a six-digit code number was generated for each microorganism, which was then identified as a single species or as one of several possible species by using the Staf-Sistem 18-R codebook index.

In the Staf-Sistem 18-R code book, identifications are classified according to the percentage of identification (% ID) (18) as follows: excellent (% ID, 99.9), good (% ID, ≥90.0), acceptable (% ID, ≥80.0), and low (% ID, <80.0) confidence. In this work we have considered a correct identification as a % ID of ≥90.0.

Conventional identification system. The 523 *Staphylococcus* strains employed in this study were tested with a microdilution modification (11) of the simplified scheme of Kloos and Schleifer (16). Other species, which could not be accurately identified with this classic procedure, were fully characterized by the consolidated conventional methods with partitioned quadrant plates as outlined previously (7, 9, 14, 15, 17, 22). For the purpose of this evaluation, the results of the conventional methods were considered to be correct.

RESULTS

The results obtained with Staf-Sistem 18-R and conventional methods in identifying the 523 strains belonging to 16 different human *Staphylococcus* species are shown in Table 1. Staf-Sistem 18-R agreed with the conventional methods in the identification of 491 of the 523 isolates (93.9%) at the species level. Among these 491 strains, the system provided a good identification (% ID, ≥90.0) for most species, such as *S. aureus* (177 of 181 strains tested), *S. capitis* (32 of 34), *S. epidermidis* (74 of 84), *S. haemolyticus* (48 of 51), *S. hominis* (43 of 45), *S. intermedius* (6 of 7), *S. saprophyticus* (26 of 26), and *S. warneri* (22 of 27). Most of the uncommon described coagulase-negative staphylococci, such as *S. cohnii* (11 of 12), *S. sciuri* (3 of 4), *S. simulans* (9 of 14), and *S. xylosus* (11 of 16), were correctly identified (% ID, ≥90.0) by the system.

Of the above-mentioned 491 strains, 28 were not directly identified and needed different growth conditions or additional tests, such as an incubation temperature of 37°C for 36 h (*S. auricularis*), ornithine and lysine decarboxylase activities (*S. capitis*), alkaline phosphatase activity (*S. cohnii*, *S. epidermidis*, *S. intermedius*, and *S. sciuri*), β-glucoronidase activity (*S. hominis* and *S. xylosus*), β-glucosidase activity (*S. xylosus*), esculin hydrolysis (*S. intermedius*), pyrrolidonyl arylamidase activity (*S. simulans*), and susceptibility to 300 U of polymyxin B (*S. simulans* and *S. warneri*).

Fourteen microorganisms (2.8%), including all of the newly described coagulase-negative staphylococci, such as *S. lugdunensis* and *S. schleiferi*, could not be identified because the generated six-digit code numbers were included neither in the codebook furnished to laboratories nor in the data base available in the manufacturer's computer. However, these 14 isolates, including the four urease-negative *S. aureus* strains, produced biochemical reaction patterns identical to those obtained with the conventional methods.

Staf-Sistem 18-R disagreed with conventional methods for 18 of the 523 isolates (3.4%) at the species level (complete disagreement). One *S. capitis* strain was identified as VP-negative *S. auricularis*; seven atypical ribose-negative strains of *S. epidermidis* were called *S. hominis*; three *S. haemolyticus* strains were identified as *S. warneri* because of negative galactose and lactose reactions; one β-galactosidase-positive *S. hyicus* isolate was recognized as *S. intermedius*; one trehalose-negative *S. simulans* isolate was identified as *S. epidermidis*; of three *S. warneri* strains that showed unusual features, two mannitol-negative strains were diagnosed as *S. saprophyticus* and *S. epidermidis*, respectively, while the other, which was unable to utilize arginine and to reduce nitrates, was identified as *S. auricularis*; and two *S. xylosus* strains (one VP negative and the other unable to reduce nitrates) were identified as *S. gallinarum* and *S. saprophyticus*, respectively.

When species identification was performed with 16 ATCC type strains, Staf-Sistem 18-R showed 87.5% agreement with the conventional methods because of the above-mentioned lack of code numbers for *S. lugdunensis* and *S. schleiferi.*

The reproducibility of the tests with the quality control strains was 100%.

DISCUSSION

Our results demonstrate that the Staf-Sistem 18-R identification kit produced a good level of identification accuracy (% ID, ≥90.0) for common and several uncommon human *Staphylococcus* species as compared with conventional methods.

Of the 491 correctly identified isolates that were recognized at the species level, 28 needed further tests or the use of different growth conditions. Among these strains were all of the *S. auricularis* strains. This might be due to the incubation time (18 h) that was used, which is not the optimum growth time for these species (14). At present, this is not mentioned in the user's instructions for Staf-Sistem 18-R.

Although Staf-Sistem 18-R correctly identified 93.9% of the 523 isolates tested, the profiles of 14 isolates, including the more recently described species (*S. lugdunensis* and *S. schleiferi*), did not correspond to any key in the computer data base or in the codebook furnished to laboratories. With regard to *S. ludgunensis* species, we presume that because of the lack of the ornithine decarboxylase test in Staf-Sistem 18-R, it is difficult to correctly identify these species with this commercial kit. In fact, to date ornithine decarboxylase-negative strains of *S. lugdunensis* have not been reported, and this positive biochemical activity can identify *S. lugdunensis* with considerable accuracy. Both *S. lugdunensis* and *S. schleiferi* were suitably identified by the conventional methods, even though the original scheme by Kloos and Schleifer (16) did not include either of these species (but a later scheme by Kloos and Lambe [14] does). Since the Staf-Sistem 18-R data base lists *S. aureus* as 100% urease positive, the system failed to identify four isolates as urease-negative *S. aureus*, thus contributing to the lack of the six-digit code number for these atypical microorganisms. If these 14 unlisted strains and respective code numbers had been incorporated in the codebook or in the manufacturer's data base, the percentage of microorganisms correctly identified by the system would have risen from 93.9% to 96.5%. This justifies the above-mentioned considerations and suggestions.

Only 18 strains were really misidentified by the Staf-Sistem 18-R method because they were atypical in a certain characteristic or gave one or two negative results with Staf-Sistem 18-R and positive results when tested conventionally (especially by ribose and mannitol fermentation, acetoin production, nitrate reduction, and arginine utilization).

Although they were misidentified, *S. hyicus* and *S. warneri* strains were easily differentiated from *S. intermedius* and *S. epidermidis*, respectively, by the evaluation of resistance (*S.*

hyicus) or susceptibility (*S. warneri*) to 300 U of polymyxin B. This differentiation test was neither provided nor indicated by the manufacturer.

In a few cases, some of the results of certain biochemical tests (e.g., acid production from several of the carbohydrates and estimation of novobiocin susceptibility) were different from those obtained by conventional methods. These disagreements may be due, in part, to differences in incubation time, substrate concentration, indicator sensitivity, or all of these factors.

It is well known that most *Staphylococcus* species should be allowed to grow for at least 48 h before the primary isolation plate is used for determination of species or strain composition (15). In particular, the identification of coagulase-negative staphylococci to the species level and the differentiation of strains of coagulase-negative staphylococci are facilitated by cultural testing, such as the examination of colony morphology after 3 days of incubation at 35°C followed by 2 days of growth at room temperature (13). This growth time is particularly important if it is necessary to sample more than one colony from a pure culture to obtain sufficient inocula. In fact, even though the inoculum should always be prepared from a single colony, sometimes this procedure is not practicable because of insufficient colony development. Contrary to the manufacturer's instructions for the preparation of the tray inoculum, we suggest that on BTSA plates, the total incubation time should be extended to at least 5 days for distinguishable colony differentiation. From these plates, a bacterial suspension is prepared to obtain a turbidity equivalent to that of a 1.0 McFarland standard. This is especially important because for some strains with an inoculum density not carefully controlled, identification by Staf-Sistem 18-R is difficult because of the appearance of false-negative or false-positive biochemical reactions.

In conclusion, we have evidence that Staf-Sistem 18-R, in its present form, accurately identifies common and several uncommon species of coagulase-positive and coagulase-negative staphylococci isolated from human specimens in microbiological laboratories. However, the overall accuracy of the Staf-Sistem 18-R was not sufficient to recommend this commercial kit for the identification of newly described *Staphylococcus* species without substantial expansion of the data base and inclusion of more discriminating tests in the system. From a practical point of view, the system was simple to use, was easy to handle, gave highly reproducible results, and was inexpensive.

ACKNOWLEDGMENTS

R.P. was supported by MURST grant 40%.

We thank colleagues who contributed clinical isolates. The technical assistance of Gianfranco Del Sasso, Carmelita Della Pelle, and Stefania Lupo was most appreciated.

REFERENCES

1. **Baddor, L. M., D. L. Smalley, A. P. Kraus, Jr., W. J. Lamoreaux, and G. D. Chritensen.** 1986. Comparison of microbiologic characteristics of pathogenic and saprophytic coagulase-negative staphylococci from patients on continuous ambulatory peritoneal dialysis. Diagn. Microbiol. Infect. Dis. **5**:197–205.
2. **Bale, M. J., and J. M. Matsen.** 1981. Time, motion, and cost comparison study of Micro-ID, Api 20E, and conventional biochemical testing in identification of *Enterobacteriaceae*. J. Clin. Microbiol. **14**:665–670.
3. **Bannerman, T. L., K. T. Kleeman, and W. E. Kloos.** 1993. Evaluation of the Vitek System gram-positive identification card for species identification of coagulase-negative staphylococci. J. Clin. Microbiol. **31**:1322–1325.
4. **Bartlett, R. C.** 1974. Medical microbiology: quality, cost and clinical relevance, p. 9–13. John Wiley & Sons, Inc., New York.
5. **Chesneau, O., S. Aubert, A. Morvan, J. L. Guesdon, and N. El Solh.** 1992. Usefulness of the ID32 Staph System and a method based on rRNA gene restriction site polymorphism analysis for species and subspecies identification of staphylococcal clinical isolates. J. Clin. Microbiol. **30**:2346–2352.
6. **De Buyser, M. L., A. Morvan, S. Aubert, F. Dilasser, and N. El Solh.** 1992. Evaluation of a ribosomal RNA gene probe for the identification of species and subspecies within the genus *Staphylococcus*. J. Gen. Microbiol. **138**:889–899.
7. **Devriese, L. A., V. Hajek, P. Oeding, S. A. Meyer, and K. H. Schleifer.** 1978. *Staphylococcus hyicus* (Sompolinsky 1953) comb. nov. and *Staphylococcus hyicus* subsp. *chromogenes* subsp. nov. Int. J. Syst. Bacteriol. **28**:482–490.
8. **Fidalgo, S., F. Vazquez, M. Mendoza, F. Perez, and J. Mendez.** 1990. Bacteremia due to *Staphylococcus epidermidis*: microbiologic, epidemiologic, clinical and prognostic features. Rev. Infect. Dis. **12**:520–528.
9. **Freney, J., Y. Brun, M. Bes, H. Meugneir, F. Grimont, P. A. D. Grimont, C. Nervi, and J. Fleurette.** 1988. *Staphylococcus lugdunensis* sp. nov. and *Staphylococcus schleiferi* sp. nov., two species from human clinical specimens. Int. J. Syst. Bacteriol. **38**:168–172.
10. **Gattard, F., J. Etienne, B. Pozzetto, F. Tardy, O. G. Gaudin, and J. Fleurette.** 1993. Characterization of unrelated strains of *Staphylococcus schleiferi* by using ribosomal DNA fingerprinting, DNA restriction patterns, and plasmid profiles. J. Clin. Microbiol. **31**:812–818.
11. **Grosjean, S., and E. L. Rank.** 1985. Identifying species of coagulase-negative staphylococci using a microtiter plate panel. Lab. Med. **16**:688–692.
12. **Kamath, U., C. Singer, and H. D. Isenberg.** 1992. Clinical significance of *Staphylococcus warneri* bacteremia. J. Clin. Microbiol. **30**:261–264.
13. **Kleeman, K. T., T. L. Bannerman, and W. E. Kloos.** 1993. Species distribution of coagulase-negative staphylococcal isolates at a community hospital and implications for selection of staphylococcal identification procedures. J. Clin. Microbiol. **31**:1318–1321.
14. **Kloos, W. E., and D. W. Lambe, Jr.** 1991. *Staphylococcus*, p. 222–237. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
15. **Kloos, W. E., and K. H. Schleifer.** 1975. Isolation and characterization of staphylococci from human skin. II. Description of four new species: *Staphylococcus warneri*, *Staphylococcus capitis*, *Staphylococcus hominis*, and *Staphylococcus simulans*. Int. J. Syst. Bacteriol. **25**:62–79.
16. **Kloos, W. E., and K. H. Schleifer.** 1975. Simplified scheme for routine identification of human *Staphylococcus* species. J. Clin. Microbiol. **1**:82–88.
17. **Kloos, W. E., and K. H. Schleifer.** 1983. *Staphylococcus auricularis* sp. nov.: an inhabitant of the human external ear. Int. J. Syst. Bacteriol. **33**:9–14.
18. **Lapage, S. P., S. Bascomb, W. R. Willcox, and M. A. Curtis.** 1973. Identification of bacteria by computer: general aspects and perspectives. J. Gen. Microbiol. **77**:273–290.
19. **Meyer, S. A., and K. H. Schleifer.** 1978. Deoxyribonucleic acid reassociation in the classification of coagulase-positive staphylococci. Arch. Microbiol. **117**:183–188.
20. **Rupp, M. E., D. E. Soper, and G. L. Archer.** 1992. Colonization of the female genital tract with *Staphylococcus saprophyticus*. J. Clin. Microbiol. **30**:2975–2979.
21. **Schleifer, K. H., W. Heise, and S. A. Meyer.** 1979. Deoxyribonucleic acid hybridization studies among micrococci. FEMS Microbiol. Lett. **6**:33–36.
22. **Schleifer, K. H., and W. E. Kloos.** 1975. Isolation and characterization of staphylococci from human skin. I. Amended descriptions of *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* and descriptions of three new species: *Staphylococcus cohnii*, *Staphylococcus haemolyticus*, and *Staphylococcus xylosus*. Int. J. Syst. Bacteriol. **25**:50–61.

23. **Schleifer, K. H., and W. E. Kloos.** 1975. A simple test system for the separation of staphylococci from micrococci. J. Clin. Microbiol. **1:**337–338.
24. **Schumacher-Perdreau, F.** 1991. Clinical significance and laboratory diagnosis of coagulase-negative staphylococci. Clin. Microbiol. Newsl. **13:**97–101.
25. **Sewell, C., J. Clarridge, E. Young, and R. Guthrie.** 1982. Clinical significance of coagulase-negative staphylococci. J. Clin. Microbiol. **16:**236–239.
26. **Skulnick, M., M. P. Patel, and D. E. Low.** 1989. Evaluation of five commercial systems for identification of coagulase-negative staphylococci to species level. Eur. J. Clin. Microbiol. Infect. Dis. **8:**1001–1003.
27. **Stager, C. E., and J. R. Davis.** 1992. Automated systems for the identification of microorganisms. Clin. Microbiol. Rev. **5:**302–327.
28. **Wenzel, R. P.** 1986. Reemergence of gram-positive cocci as major nosocomial pathogens. Infect. Control **7:**118–119.