

## Identification of Coagulase-Negative Staphylococci with the API STAPH-IDENT System

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Received 8 February 1983/Accepted 19 April 1983

A group of 300 clinically derived isolates of coagulase-negative staphylococci were tested in parallel with the API STAPH-IDENT system (Analytab Products) and 14 conventional biochemical tests contained in Kloos and Schleifer's simplified scheme for identification of human *Staphylococcus* species. STAPH-IDENT is a miniaturized biochemical test strip that incorporates four synthetic chromogenic substrates, urea, arginine, and four carbohydrates and that requires only a 5-h test period. Use of the STAPH-IDENT system alone allowed correct or partly correct classification of 67% (201 of 300) of the study isolates. However, if a supplemental test was performed (most often novobiocin susceptibility), correct classification of an additional 25.7% (77) was possible, for a total of 92.7% of isolates identified to the species level. Species correctly identified included 94% (116 of 123) of *Staphylococcus epidermidis* isolates, 98% (63 of 64) of *S. saprophyticus*, 71% (34 of 48) of *S. hominis*, 100% (22) of *S. simulans*, 100% (18) of *S. haemolyticus*, 100% (17) of *S. warneri*, and 100% (8) of *S. capitis*. Fourteen percent (42 of 300) of profile codes encountered in this study were not included in the STAPH-IDENT profile register, but were included in Analytab Products' expanded computer data base.

Coagulase-negative staphylococci (C-NS), formerly regarded as contaminants or only occasionally as opportunistic pathogens, are now the subject of increasing interest. C-NS have frequently been implicated as the etiological agents of various types of human infections, including infections of prostheses (7, 12, 14), urinary tract infections in young women (3), and wound infections (8). Kloos and Schleifer have developed a simplified scheme for routine identification of human *Staphylococcus* species (4). Unfortunately, this scheme is cumbersome for routine clinical laboratory use because of the large number of tests needed, use of specialized media, and the relatively long incubation times involved. Interest has arisen recently concerning rapid methods that are commercially available for identification of C-NS (2, 6, 10). The purpose of this study was to evaluate the accuracy and convenience of the API STAPH-IDENT system (Analytab Products [API], Plainview, N.Y.) for identification of various species of C-NS.

### MATERIALS AND METHODS

**Bacteria.** A total of 300 human clinical C-NS isolates were utilized for this study. These were derived from specimens submitted to the Microbial Pathology Laboratory of the Medical Center Hospital and from Virginia Thomas of the Department of Microbiology, The

University of Texas Health Science Center at San Antonio. All isolates were grown on Trypticase soy agar with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.) at 35°C for 18 to 24 h before testing.

**Conventional identification methods.** Micrococci were first differentiated from staphylococci by testing the ability of isolates to produce acid aerobically from glycerol in the presence of erythromycin (11). Coagulase activity of the staphylococci was determined by using citrated rabbit plasma (Difco Laboratories, Detroit, Mich.) in a 4-h tube test for free coagulase (5). Strains reacting as C-NS were then classified into species with the following tests contained in Kloos and Schleifer's simplified scheme for routine identification of human *Staphylococcus* species (4).

(i) **Aerobic acid production from carbohydrates.** Acid production from nine carbohydrates (lactose, maltose, mannitol, mannose, ribose, sucrose, trehalose, xylitol, and xylose) was observed under aerobic conditions by using purple agar base medium (Difco) containing a 1% final carbohydrate concentration. Acid production was seen as development of a yellow color around the area of growth after 3 days of incubation at 35°C.

(ii) **Phosphatase.** Alkaline phosphatase activity was determined by a modification of the method of Penock and Huddy (9) using a 0.005 M solution of phenolphthalein monophosphate (sodium salt) instead of a 0.01 M solution of disodium phenylphosphate, in 0.01 M citric acid-sodium citrate buffer (pH 5.8). Sterile disposable plastic tubes (12 by 75 mm) containing 0.5 ml of the above buffer solution were inoculated

to a density visually comparable to a McFarland no. 4 opacity standard (approximately  $10^8$  bacteria per ml). After incubation at 35°C for 4 h, the reaction was stopped by adding 0.5 ml of 0.5 N sodium hydroxide and 0.5 ml of 0.5 M sodium bicarbonate. The final color was developed by adding 0.5 ml of 4-aminoantipyrine solution (0.6 g/100 ml) and 0.5 ml of potassium ferricyanide solution (2.4 g/100 ml). Color reactions were classified as follows: positive, development of a moderate to intense red color; weak positive, development of a pink color; negative, color remained yellow.

(iii) **Hemolysis.** The ability of isolates to demonstrate hemolysis on Trypticase soy agar with 5% sheep blood after 24, 48, and 72 h of incubation at 35°C without CO<sub>2</sub> was determined.

(iv) **Novobiocin susceptibility.** Four or five well-isolated colonies of each isolate grown overnight on plates of Trypticase soy agar with 5% sheep blood were inoculated into 5 ml of tryptic soy broth (Difco). The broth cultures were incubated at 35°C for 2 to 5 h until a slightly visible turbidity developed. The turbidity of the broth cultures was then adjusted with sterile broth to obtain a density visually comparable to that of a 0.5 McFarland opacity standard. A sterile cotton swab was then used to streak the suspension evenly in three directions over the entire surface of a 100- by 15-mm plastic petri dish containing 25 ml of P agar (13). At 3 to 5 min after swabbing of the plate to obtain confluent growth, a 5-µg novobiocin disk (BBL) was applied. After 16 to 18 h of incubation at 35°C, the plates were examined, and the diameter of the zone of complete inhibition was measured to the nearest whole millimeter with vernier calipers. Isolates having a zone diameter greater than 16 mm were classified as being susceptible to novobiocin, whereas those with a diameter of 16 mm or less were considered resistant. The use of a 16-mm breakpoint is equivalent to Kloos and Schleifer's criteria of classifying strains as resistant when inhibition is not greater than 5 mm from the edge of a 6-mm disk (4).

(v) **Anaerobic growth in thioglycolate.** A 0.1-ml sample of a saline bacterial suspension (approximately  $10^6$  bacteria per ml) was inoculated into test tubes containing 8 ml of steamed and cooled (50°C) thioglycolate semisolid medium (4). This inoculum suspension was obtained by making a 1:100 dilution of a saline bacterial suspension with a density visually comparable to that of 0.5 McFarland opacity standard. Thioglycolate tubes were incubated at 35°C for 5 days, after which growth characteristics were recorded, e.g., uniform growth throughout the medium, a gradient of dense to light growth proceeding down the tube, large or small individual colonies, or absence of visible growth.

**API STAPH-IDENT system.** The API STAPH-IDENT miniaturized biochemical test system, previously described by Kloos and Wolfshohl (6), was used to identify each isolate according to the instructions of the manufacturer. Growth from a sheep blood agar plate was used to prepare a suspension equivalent to a no. 3 McFarland opacity standard in 5 ml of 0.85% saline (API). A Pasteur pipette was used to inoculate 3 drops of the suspension into each of the 10 microcups contained on each strip (phosphatase, urea utilization, β-glucosidase, mannose utilization, mannitol utilization, trehalose utilization, salicin utilization, β-glucuronidase, arginine utilization, and β-galactosidase). The strips were incubated for 5 h at 35°C in a

TABLE 1. Comparison of STAPH-IDENT results with identifications based on the Kloos and Schleifer scheme<sup>a</sup>

C-NS species	No. of isolates	No. correct by STAPH-IDENT <sup>b</sup>	% Correct by STAPH-IDENT
<i>S. epidermidis</i>	123	116	94
<i>S. hominis</i>	48	34	71
<i>S. haemolyticus</i>	18	18	100
<i>S. warneri</i>	17	17	100
<i>S. capitis</i>	8	8	100
<i>S. saprophyticus</i>	64	63	98
<i>S. simulans</i>	22	22	100

<sup>a</sup> With a total of 300 isolates, the overall agreement was 92.7%.

<sup>b</sup> Supplemental tests were sometimes required (see text and Table 2).

non-CO<sub>2</sub> incubator. After incubation, the first nine tests were interpreted, and the results were recorded on API report sheets. Two drops of STAPH-IDENT reagent (0.35% fast blue BB salt in 2-methoxyethanol) were added to the 10th (beta-galactosidase) microcupule, and it was observed for development of a purple color after 30 s. A four-digit profile number was derived for each isolate by tabulating the positive reactions for tests in groups of three. This number was located in API's STAPH-IDENT profile register (provided as a package insert with the STAPH-IDENT strips) to obtain an isolate identification. Strictly for the purpose of this study, API also provided us with a computer printout that represented all possible STAPH-IDENT profiles, a first, second, and third identification choice for each profile number, an "estimated frequency of occurrence" for each choice, and an identification comment. This printout provided information that ordinarily would be available by telephone inquiry to API on a toll-free telephone number.

**Resolution of discrepancies.** When a discrepancy arose between an isolate's classification by Kloos and Schleifer's simplified scheme (4) and its identification by the API STAPH-IDENT system, the isolate was submitted to Wesley Kloos at North Carolina State University for further testing and final arbitration.

## RESULTS

The following seven species of C-NS were encountered in our group of 300 test isolates: *Staphylococcus epidermidis* (123 isolates), *S. saprophyticus* (64), *S. hominis* (48), *S. simulans* (22), *S. haemolyticus* (18), *S. warneri* (17), *S. capitis* (8). The accuracy of the STAPH-IDENT system identifications for each species as compared with classification by Kloos and Schleifer's simplified scheme is shown in Table 1. The use of STAPH-IDENT allowed correct classification of between 71% and 100% of all species tested. This resulted in an overall agreement of 92.7% (278 of 300) when compared with the conventional classification scheme. However, Table 2 shows that, although 63% of the

TABLE 2. Analysis of STAPH-IDENT identification results<sup>a</sup>

Identification outcome	No. of isolates	% of total	Cumulative % of total
Correct (strip alone)	189	63	63
Correct (with supplemental tests) <sup>b</sup>	77	25.7	88.7
Partly correct (GLLS) <sup>c</sup>	12	4	92.7
Incorrect	22	7.3	

<sup>a</sup> A total of 300 isolates were tested.

<sup>b</sup> Novobiocin susceptibility, 62 isolates; xylose acidification, 12 isolates; coagulase production, 3 isolates.

<sup>c</sup> See the text for an explanation.

isolates were identified correctly by the STAPH-IDENT strip alone, 25.7% (77 of 300) of the isolates required supplemental testing, 4% (12 of 300) were considered by us to be only partly correct due to an identification comment of good likelihood, but low selectivity identification, and 7.3% (22 of 300) were incorrectly identified. Novobiocin susceptibility was the supplemental test most often required (80.5% or 62 of 77 of all supplemental tests) to achieve a correct identification, although xylose acidification (15.6% or 12 of 77) and coagulase tests (3.9% or 3 of 77) were required for some isolates. There was never more than one supplemental test required for any isolate. These additional tests were suggested in the analytical profile index to separate profiles with similar likelihoods of occurrence. The species incorrectly identified by STAPH-IDENT consisted of 14 *S. hominis*, 7 *S. epidermidis* (6 of these were phosphatase negative by both API and conventional methods), and 1 *S. saprophyticus* isolate.

Table 3 shows the STAPH-IDENT analytical profile index (computer printout) comments associated with the C-NS identifications. Sixty-five percent of the profiles had an associated comment of either "excellent," "very good," "good," or "acceptable." However, 35% (105 of 300) of the identifications were considered to be good likelihood, but low selectivity identifications (GLLS). The presence of a GLLS identification comment indicated that there was no significant difference between the likelihood of species listed as the first, second, or sometimes third choice for a given profile number in the analytical profile index. Two C-NS species were associated with a disproportionate number of GLLS comments; 87% of all *S. hominis* identifications and 38% of all *S. warneri* identifications had GLLS profiles. The 22 isolates that were misidentified by STAPH-IDENT included 16 GLLS, 4 acceptable, 1 good, and 1 excellent likelihood profiles.

TABLE 3. Tabulation of STAPH-IDENT analytical profile index comments<sup>a</sup>

Profile index comment	No. of isolates	% of isolates	Cumulative % of total
Excellent ID	123	41	41
Very good ID	2	0.7	41.7
Good ID	8	2.7	44.4
Acceptable ID	62	20.6	65
GLLS	105	35	100

<sup>a</sup> A total of 300 isolates were tested. ID, Identification.

Although all of the 300 C-NS isolates in our study had profile numbers which were listed in API's expanded computer data base (analytical profile index), 14% of the profile numbers were not included in the profile register. The register is a one-page list of profile numbers and corresponding identifications that is included in each STAPH-IDENT kit. Thus, 42 of our isolates would have required telephone contact with API to obtain an identification.

## DISCUSSION

We found the STAPH-IDENT system to be extremely convenient to use during the course of this study. Inoculation and interpretation of reactions required a minimum of time and effort. Biochemical reactions, including those involving the chromogenic substrates (alkaline phosphatase,  $\beta$ -glucosidase,  $\beta$ -glucuronidase, and  $\beta$ -galactosidase), were generally clear cut and easy to interpret. The 5-h incubation period was seen as an obvious asset over the 3- to 5-day total incubation required for certain of the conventional tests.

The STAPH-IDENT system was able to achieve a high rate of correct identifications, when compared with conventional methods on the two most common human C-NS pathogens, *S. epidermidis* (94%) and *S. saprophyticus* (98%), especially when novobiocin was included as a supplemental test. Additionally, two other C-NS species tested (*S. haemolyticus* and *S. simulans*) yielded 100% correct identification rates by use of the STAPH-IDENT strip alone. The overall agreement between identifications derived from STAPH-IDENT and conventional methods in our study and that of Kloos and Wolfshohl (6) was greater than 90%.

We initially experienced some difficulties in using Kloos and Schleifer's simplified scheme (4), particularly in the identification of the few phosphatase-negative *S. epidermidis* isolates that were often misclassified as *S. hominis*. However, at Kloos's suggestion, anaerobic growth in thioglycolate was successfully used to differentiate these two species.

Micrococci were very rarely (<1 of 300) isolated from human clinical specimens during the isolate collection phase of our study. However, it should be noted that the STAPH-IDENT data base does not presently include micrococci, and that a *Micrococcus* species could be erroneously identified as a C-NS species. Thus, for complete accuracy, it might be necessary to perform a preliminary test such as acid from glycerol in the presence of erythromycin to effectively exclude *Micrococcus* spp.

Our study was in agreement with that of Kloos and Wolfshohl (6) in finding that addition of a novobiocin susceptibility test to the strip or performance of this procedure as a supplemental test could improve the accuracy of C-NS identifications. We have previously reported on a simple means of determining novobiocin susceptibility on Mueller-Hinton agar (1) which may be performed as part of a routine antimicrobial susceptibility test. In addition to a novobiocin susceptibility test, the performance of a rapid coagulase and xylose utilization test in conjunction with STAPH-IDENT would significantly reduce the relatively high percentage (35%) of GLLS identifications (Table 3).

The majority of profile numbers encountered in this study were listed in the one-page profile register. The profiles included in the profile register are those most commonly encountered and which have the associated comments of either excellent, very good, good, or acceptable identifications. In a few instances, GLLS codes are included in the profile register, and the first and second identification choices are listed. There were 10 of 42 profiles encountered in this study which were not listed on the profile register, but which yielded a correct identification using the STAPH-IDENT strip alone and which had a comment of "acceptable identification" or better in the more complete analytical profile index. Thus, there are certain profiles that should be added to the existing profile register.

Occasionally, the profile register did not correspond exactly to the expanded analytical profile index, e.g., profile no. 2040. The profile register indicated that *S. saprophyticus* could be differentiated from the second choice, *S. hominis*, by novobiocin susceptibility testing. However, the expanded data base listed a GLLS comment and a first identification choice of *S. saprophyticus*, a second choice of *S. epidermidis*, and a third choice of *S. hominis* with corresponding "frequencies of occurrence" of 1 in 13, 1 in 18, and 1 in 24, respectively. Thus, it would seem preferable to make available a more

complete profile index for routine use than is provided by the current single-page listing of common profiles.

#### ACKNOWLEDGMENTS

We are grateful to the Medical Center Hospital Microbial Pathology Laboratory, San Antonio, Tex., and Virginia Thomas of the Department of Microbiology at The University of Texas Health Science Center at San Antonio for the clinical isolates they generously provided. We thank Wesley Kloos of the Department of Genetics, North Carolina State University, Raleigh, for his assistance in providing referee identifications for our study.

Analytab Products provided partial support for this investigation.

#### LITERATURE CITED

1. Almeida, R. J., and J. H. Jorgensen. 1982. Use of Mueller-Hinton agar to determine novobiocin susceptibility of coagulase-negative staphylococci. *J. Clin. Microbiol.* **16**:1155-1156.
2. Gemmell, C. G., and J. E. Dawson. 1982. Identification of coagulase-negative staphylococci with the API Staph System. *J. Clin. Microbiol.* **16**:874-877.
3. Jordan, P. A., A. Irvani, G. Richard, and H. Baer. 1980. Urinary tract infection caused by *Staphylococcus saprophyticus*. *J. Infect. Dis.* **142**:510-515.
4. Kloos, W. E., and K. H. Schleifer. 1975. Simplified scheme for routine identification of human *Staphylococcus* species. *J. Clin. Microbiol.* **1**:82-88.
5. Kloos, W. E., and P. B. Smith. 1980. Staphylococci, p. 83-87. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), *Manual of clinical microbiology*, 3rd ed. American Society for Microbiology, Washington, D.C.
6. Kloos, W. E., and J. F. Wolfshohl. 1982. Identification of *Staphylococcus* species with the API STAPH-IDENT System. *J. Clin. Microbiol.* **16**:509-516.
7. Liekweg, P. D., and L. T. Greenfield. 1977. Vascular prosthetic infections: collected experience and results of treatment. *Surgery* **81**:335-342.
8. Nord, C. E., S. Holta-oie, A. Ljungh, and T. Wadstrom. 1976. Characterization of coagulase-negative staphylococcal species from human infections, p. 105-111. In J. Jeljaszewicz and G. Fischer (ed.), *Staphylococci and staphylococcal infections*. Gustav Fischer Verlag, Stuttgart.
9. Pennock, C. A., and R. B. Huddy. 1967. Phosphatase reaction of coagulase-negative staphylococci and micrococci. *J. Pathol. Bacteriol.* **93**:685-688.
10. Ruoff, K. L., M. J. Ferraro, M. E. Jerz, and J. Kissling. 1982. Automated identification of Gram-positive bacteria. *J. Clin. Microbiol.* **16**:1091-1095.
11. 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.
12. Speller, D. C. E., and G. R. Mitchell. 1973. Coagulase-negative staphylococci causing endocarditis after cardiac surgery. *J. Clin. Pathol.* **26**:517-522.
13. Vera, H. D., and D. A. Power. 1980. Culture media, p. 965-999. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), *Manual of clinical microbiology*, 3rd ed. American Society for Microbiology, Washington, D.C.
14. Wilson, P. D., E. A. Sahati, and P. Aghetti. 1973. The problem of infection in endo-prosthetic surgery of the hip joint, p. 213-232. In J. E. Miller (ed.), *Clinical orthopaedics and related research*. Lippincott, Philadelphia.