

## Evaluation of RapiDEC Staph for Identification of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus*

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Received 22 March 1994/Returned for modification 15 May 1994/Accepted 2 June 1994

**RapiDEC Staph is a test for presumptive identification of the principal human staphylococcal species, *Staphylococcus aureus*, *S. epidermidis*, and *S. saprophyticus*. The test includes control and test cupules for fluorogenic detection of coagulase and chromogenic substrates for alkaline phosphatase and  $\beta$ -galactosidase. These tests identify *S. aureus*, *S. epidermidis*, and *S. saprophyticus*, respectively. Positive results with both chromogenic substrates provide a presumptive identification of *S. xylosus* or *S. intermedius* (*S. xylosus*-*S. intermedius*). Test cupules are inoculated with an organism suspension, and reactions are read after a 2-h incubation. RapiDEC-Staph was evaluated with 303 clinical and stock staphylococcal strains. Identifications were compared with those obtained by the tube coagulase test, a latex slide coagulase test (StaphAUREX), another commercial identification system (Staph-TRAC), and additional conventional tests. RapiDEC-Staph correctly identified 100% of 130 *S. aureus* strains, 70.3% of 74 *S. epidermidis* strains, and 81.3% of 32 *S. saprophyticus* strains. Four of five *S. xylosus* isolates were called *S. xylosus*-*S. intermedius*. Unidentified *S. epidermidis* and *S. saprophyticus* strains were called "*Staphylococcus* spp." Among the 62 other coagulase-negative staphylococci, 4 were misidentified as *S. epidermidis* and 7 were misidentified as *S. saprophyticus*. While the sensitivity and specificity of the fluorogenic coagulase test for *S. aureus* were 100%, failure to detect alkaline phosphatase activity in several *S. epidermidis* isolates resulted in fewer correct identifications by the RapiDEC-Staph test for this species.**

Staphylococci are frequently isolated as etiologic agents of infectious processes, with *Staphylococcus aureus* being the most important human pathogen. *S. aureus* causes superficial and deep skin and soft tissue infections, bacteremia with metastatic abscess formation, and a variety of toxin-mediated diseases, including gastroenteritis, staphylococcal scalded skin syndrome, and toxic shock syndrome (16, 27). In addition, two coagulase-negative staphylococcal species, *S. epidermidis* and *S. saprophyticus*, are also recognized as important agents of human infections. *S. epidermidis* is associated with infections of indwelling devices, osteomyelitis, wound infections, peritoneal dialysis catheter-associated peritonitis, and nosocomial bacteremia (2, 5, 16, 22, 26). *S. saprophyticus* is recognized primarily as a cause of acute urinary tract infections in young women (2, 14, 16, 22). Together, these two coagulase-negative species comprise the greater majority of the clinically significant coagulase-negative staphylococci recovered from human specimens (16, 22).

The laboratory identification of *S. aureus* has traditionally depended on the demonstration of coagulase production by the tube coagulase test (16). This test has been adapted to rapid latex agglutination and hemagglutination procedures that detect clumping factor and/or protein A on the cell surface (1, 3, 15, 16, 21, 28). Susceptibility to novobiocin is a factor widely used in clinical laboratories for the presumptive identification of *S. saprophyticus* (12, 16). While rapid systems or methods for specific identification of *S. epidermidis* are not generally available, this species and other coagulase-negative

staphylococci can be identified by several kit systems that require from 4 to 24 h of incubation (11, 16).

RapiDEC Staph (RD-Staph) (bioMerieux-Vitek, Inc., Hazelwood, Mo.) is a new, commercially available system that provides a 2-h presumptive identification of *S. aureus*, *S. epidermidis*, and *S. saprophyticus* on the basis of fluorogenic coagulase, alkaline phosphatase (PAL), and  $\beta$ -galactosidase (BGAL) tests, respectively. In the present study, RD-Staph was compared with a commercial latex agglutination test and with the tube coagulase method for identifying *S. aureus* and with commercial and conventional methods for identifying *S. epidermidis* and *S. saprophyticus*.

### MATERIALS AND METHODS

**Bacterial strains.** *Staphylococcus* species (total  $n = 303$  isolates) from clinical specimens and from stock cultures were tested in this evaluation. Clinical isolates were obtained from cultures performed at the University of Illinois Hospital, Cook County Hospital, and Grant Hospital clinical microbiology laboratories in Chicago, Ill. Frozen stock strains were subcultured on tryptic soy agar with 5% sheep blood at least three times before being tested. Conventional tests and the RD-Staph test were performed in a blinded fashion. One colony of the test organism was subcultured onto two blood agar plates that were labeled with random study numbers. These plates were incubated at 37°C for 18 to 24 h and were subsequently used to inoculate RD-Staph, the Staph-TRAC strips, and conventional identification tests. Isolates for which discrepant identifications were obtained were retested by both conventional and kit methods in a blinded fashion.

**RD-Staph.** RD-Staph is a new test for the presumptive identification of clinically significant staphylococci from human

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TABLE 1. Identification of staphylococci by RD-Staph

Organism	No. of isolates tested	No. identified as the following species by RD-Staph (%):				
		<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. saprophyticus</i>	<i>S. xylosus-S. intermedius</i>	<i>Staphylococcus</i> spp.
<i>S. aureus</i>	130	130 (100)	NR <sup>a</sup>	NR	NR	0
<i>S. epidermidis</i>	74	0	52 (70.3)	0	0	22 (29.7)
<i>S. saprophyticus</i>	32	0	0	26 (81.3)	0	6 (18.7)
<i>S. xylosus</i>	5	0	0	1 (20)	4 (80)	0
<i>S. haemolyticus</i>	23	0	0	0	0	23 (100)
<i>S. hominis</i>	14	0	0	0	0	14 (100)
<i>S. warneri</i>	4	0	0	0	0	4 (100)
<i>S. lugdunensis</i>	4	0	0	0	0	4 (100)
<i>S. sciuri</i>	4	0	4 (100)	0	0	0
<i>S. cohnii</i> subsp. <i>cohnii</i>	4	0	0	0	0	4 (100)
<i>S. cohnii</i> subsp. <i>ureolyticum</i>	3	0	0	3 (100)	0	0
<i>S. simulans</i>	3	0	0	3 (100)	0	0
<i>S. capitis</i>	2	0	0	0	0	2 (100)
<i>S. kloosii</i>	1	0	0	1 (100)	0	0

<sup>a</sup> NR, test not read because AUR test positive.

specimens. It is manufactured by bioMerieux S.A. (Marcy l'Etoile, France) and distributed by bioMerieux-Vitek Inc. Each test consists of six cupules. The six cupules include one suspension well, two control wells, and three test wells. Cupules C and S are inoculated with 250 µl of suspension medium (sterile distilled water). Cupule C is a turbidity control that, when reconstituted with the suspension medium, appears cloudy and approximates a no. 4 McFarland standard. Cupule S is used to prepare the bacterial suspension, which is adjusted until it is equivalent to the turbidity control (cupule C). Cupules 0, 1, 2, and 3 are then inoculated by transferring 50 µl of the bacterial suspension from cupule S into each of these four cupules. After the fluid is removed from cupule C, the strip is covered and incubated for 2 h at 37°C in a non-CO<sub>2</sub> incubator. Cupule 0 is a negative control for the fluorescent coagulase test and can also be used to perform a catalase test. Cupule 1 is the "aurease" (AUR) test. This test detects the production of an "aurease" enzyme that is specific for *S. aureus* strains. "Aurease" is a proteolytic enzyme of coagulation that reacts with prothrombin to form a complex called staphylo-thrombin. Staphylo-thrombin then cleaves a fluorogenic peptide present in the cupule, thereby releasing a peptide and a fluorescent radical. The fluorescent radical is visible under UV light. Greater fluorescence in cupule 1 than in cupule 0 indicates a positive AUR test. If the AUR test is positive, the organism is identified as *S. aureus* and testing is completed. Cupule 2 is a PAL test based on the hydrolysis of *p*-nitrophenyl phosphate. This colorless compound is hydrolyzed to release nitrophenol, producing a spontaneous yellow color. Cupule 3 is an enzymatic BGAL test based on the hydrolysis of a β-galactopyranoside substrate and the release of a naphthol by-product. This by-product is detected by the addition of a diazonium salt (Fast Violet Blue reagent), which results in the formation of a pink-red azo compound. Fast Violet Blue is the only reagent required for RD-Staph. Test results for each organism are noted and identification of the organism is obtained according to the RD-Staph interpretive chart provided in the package insert. A positive AUR test identifies *S. aureus*. Positive results by the PAL test only and the BGAL test only provide identifications of *S. epidermidis* and *S. saprophyticus*, respectively. Positive results by both PAL and BGAL tests provide a presumptive identification of *S. xylosus-S. intermedius*. Strains that are negative by all three tests are identified only as *Staphylococcus* species.

**Staph-TRAC.** Staph-TRAC (bioMerieux-Vitek, Inc.) consists of a 20-cupule strip and inoculation medium for the identification of staphylococci. The test strip is inoculated with a suspension (0.5 McFarland) prepared in the inoculation medium provided in the kit. Cupules on the strip are inoculated according to the directions of the manufacturer and incubated aerobically at 37°C for 24 h. After this time, reagents are added to develop reactions in certain tests, the results are recorded, and a numerical profile is generated. The organism is identified by using the Staph-TRAC identification codebook or phone-contacted, voice-activated computer. In this study, coagulase-negative staphylococcal isolates that produced identifications on Staph-TRAC with likelihoods of less than 85% (acceptable identification) were identified by conventional biochemical tests.

**Conventional tests.** Conventional identification procedures included Gram stain, catalase, coagulase by latex agglutination (StaphAUREX; Murex Diagnostics, Inc., Norcross, Ga.), and tube methods; pyrrolidonyl beta-naphthylamide hydrolysis; urease production; acetoin production; arginine and ornithine decarboxylase; nitrate reduction; and hydrolysis of *o*-nitrophenyl-β-D-galactopyranoside (ONPG). Susceptibility to novobiocin (5-µg disk), furazolidone (100-µg disk), polymyxin (300-U disk), and bacitracin (10-U disk) were determined by disk diffusion on sheep blood agar (13, 16). Carbohydrate fermentations were performed by using slants of purple agar base with yeast extract (Remel Laboratories, Lenexa, Kans.). Carbohydrates (1% concentration) included glucose, maltose, fructose, sucrose, lactose, mannitol, mannose, xylose, ribose, xylitol, trehalose, turanose, and a carbohydrate-free control. Phosphatase activity was determined using P agar containing *p*-nitrophenylphosphate disodium (0.495 mg/ml) (PNP agar) (9, 17, 20). Plates were heavily spot inoculated with a loopful of each test strain, incubated aerobically at 37°C, and examined after 4 and 24 h. A bright yellow color in, under, and surrounding the inoculum indicated phosphatase activity.

## RESULTS

Identification results obtained by RD-Staph for the 303 staphylococcal isolates tested in this study are shown in Table 1. All 130 *S. aureus* strains were AUR positive by RD-Staph. Of these, 129 (99.0%) were StaphAUREX latex positive. By the tube coagulase procedure, 94 (91.3%) were positive at 4 h

and the remainder were positive after 18 h. None of the coagulase-negative staphylococci were misidentified as *S. aureus* by RD-Staph, StaphAUREX, or the conventional tube test. RD-Staph correctly identified 52 (70.3%) of 74 *S. epidermidis* strains and 26 (81.3%) of 32 *S. saprophyticus* strains on the basis of positive PAL and positive BGAL tests, respectively. *S. epidermidis* strains that were PAL negative after 2 h remained PAL negative even when incubation was prolonged to 4 h, and all of these remained PAL negative on repeat testing. Four (80%) of the five *S. xylosus* isolates tested were both PAL and BGAL positive, thus identifying these isolates as *S. xylosus*-*S. intermedius* (Table 1). Among the remaining 62 coagulase-negative staphylococci, 4 *S. sciuri* stock strains were misidentified as *S. epidermidis* and 7 isolates were misidentified as *S. saprophyticus*. The seven isolates included three isolates each of *S. cohnii* subsp. *ureolyticum* and *S. simulans* and one *S. kloosii* isolate. Other than the four *S. xylosus* strains, no other organisms were both RD-Staph PAL and BGAL positive.

Since RD-Staph relies completely on detection of PAL and BGAL activities for identification of *S. epidermidis* and *S. saprophyticus*, respectively, the reactions of these isolates were checked with conventional PAL (PNP agar) and BGAL (ONPG broth) procedures. Forty-nine (94.2%) of the 52 *S. epidermidis* isolates that were correctly identified by RD-Staph were PNP positive at 4 h, and all 52 strains were PNP positive after overnight incubation. Among the 22 *S. epidermidis* strains that were not identified by RD-Staph, only 3 (13.6%) were weakly PNP positive after 4 h and 16 (72.7%) were PNP positive after 24 h. Among the 26 *S. saprophyticus* strains that were identified by RD-Staph, 24 (92.3%) were ONPG positive after 4 h, and all 26 were ONPG positive after 24 h. The six *S. saprophyticus* strains that were not identified by RD-Staph were ONPG negative after both 4 and 24 h of incubation. All of the *S. saprophyticus* strains and 5 (9.6%) of the 74 *S. epidermidis* strains were resistant to novobiocin by the disk diffusion procedure.

## DISCUSSION

For the 130 strains of *S. aureus* tested, the sensitivity and specificity of the AUR test were 100%. These data corroborate the findings of European investigators regarding the reliability of the RD-Staph AUR test for identifying *S. aureus* (10, 19). A theoretical advantage of this unique fluorogenic mechanism for detection of coagulase activity is that staphylothrombin is tested directly rather than by an indirect mechanism whereby staphylothrombin transforms fibrinogen into insoluble fibrin. In addition, the enzymatic pathway leading to generation of the fluorescent end product is independent from and unaffected by fibrinolysin production. Fibrinolysin production by some strains may actually hydrolyze the fibrin clot formed in the tube coagulase procedure. This can result in a falsely negative coagulase reaction, particularly after overnight incubation (16).

The StaphAUREX latex agglutination test used in this study was 99% sensitive and 100% specific for identifying *S. aureus*. Other workers have reported various degrees of success with rapid latex agglutination and hemagglutination methods (1, 3, 4, 15, 21, 28). False-negative results have been reported more frequently for oxacillin-resistant *S. aureus*. Piper et al. (23) reported sensitivities of 82 to 86% for various latex agglutination and hemagglutination tests when oxacillin-resistant *S. aureus* strains were being examined. Ruane et al. (24) also reported that the StaphAUREX and Staphyloslide tests identified only 77 and 75% of 73 oxacillin-resistant *S. aureus* strains, respectively. At the time these studies were done, the relation-

ship between oxacillin resistance per se and failure to react in rapid agglutination coagulase procedures was not apparent. In 1989, however, Fournier et al. (6) demonstrated that *S. aureus* strains that were not identified by these rapid tests belonged to capsular serotype 5 and that this serotype is a predominant one found among oxacillin-resistant isolates (7). Failure to react in rapid agglutination tests for clumping factor and protein A is, logically, more intimately associated with the presence of a capsule on the cell surface than with intrinsic resistance to the penicillinase-resistant penicillins. Fournier and coworkers (8) recently reported on a new latex reagent that incorporates not only fibrinogen and immunoglobulin G bound to latex for detection of clumping factor and protein A, but also includes latex-bound monoclonal antibodies directed against serotype 5 and serotype 8 capsular polysaccharides. This new reagent demonstrated increased sensitivity for identifying oxacillin-resistant *S. aureus* compared with other latex agglutination methods (8). Isolates tested in the present evaluation included both methicillin-susceptible and methicillin-resistant strains, but no difference was seen in their "aurease" reactivity.

Identification of *S. epidermidis* by RD-Staph relies entirely on the hydrolysis of *p*-nitrophenylphosphate in cupule 2 by PAL activity. In traditional schemes for identifying coagulase-negative staphylococci, phosphatase activity was reported to be positive for *S. epidermidis* and *S. xylosus* strains and negative for other coagulase-negative staphylococci (16, 17, 25). Subsequent studies have shown, however, that other staphylococcal species may also produce phosphatase enzymes. Using four different methods, including the PNP agar method used in our study, Langlois and coworkers (17) found phosphatase activity in all *S. aureus*, coagulase-positive *S. hyicus*, and *S. intermedius* strains and in most strains of *S. epidermidis*, *S. chromogenes*, coagulase-negative *S. hyicus*, *S. sciuri*, *S. simulans*, *S. xylosus*, and *S. hominis*-*S. warneri* after 24 and 48 h of incubation. Production of phosphatase activity is also affected by pH and by the presence of  $P_i$  in the growth medium. Soro and associates (25) found that all strains of various staphylococcal species were phosphatase positive when testing was done at pH 8.0 and when the organisms were grown in the absence of  $P_i$ . When grown on media supplemented with 0.3%  $P_i$ , only *S. aureus*, *S. epidermidis*, and *S. xylosus* strains were phosphatase positive. These workers concluded that phosphatase activity was a more common property among human staphylococcal isolates than was previously thought and that this activity may be constitutive in some species and repressed by phosphates in others (25). While the PAL test on RD-Staph appears to be relatively specific for detection of phosphatase in *S. epidermidis*, strains that are phosphatase negative or that produce low levels of the enzyme will not be detected or identified by RD-Staph. Consequently, the sensitivity and performance of RD-Staph for identifying *S. epidermidis* will depend on the relative prevalence of PAL-positive and -negative strains in a population.

Among the non-*S. epidermidis*, non-*S. saprophyticus* coagulase-negative staphylococci, only 12 isolates were misidentified by RD-Staph; four stock *S. sciuri* strains were called *S. epidermidis*, and 7 isolates—*S. cohnii* subsp. *ureolyticum* (3 isolates), *S. simulans* (3 isolates), and *S. kloosii* (1 isolate)—were misidentified as *S. saprophyticus*. In an evaluation of RD-Staph conducted in the United Kingdom, Geary and Stevens (10) found that all of five *S. sciuri* strains they tested were also misidentified as *S. epidermidis*. Furthermore, four of five *S. cohnii* strains (subspecies not specified) and all five *S. simulans* strains were misidentified as *S. saprophyticus*. The results in the present study mirror those obtained in the British study of the RD-Staph system (10). The single isolate of *S.*

*kloosii* was also misidentified as *S. saprophyticus*. This species is resistant to novobiocin and is variable for BGAL production but should not present a problem, since this species is rarely isolated and is of uncertain clinical significance. Similarly, *S. sciuri* is found in rodents and other small mammals and therefore is not recovered with any great frequency from human clinical specimens (14).

The place of RD-Staph in the clinical laboratory is unclear at present. While the AUR test was 100% accurate for identifying *S. aureus*, less expensive latex and hemagglutination methods produce essentially equivalent results and are truly rapid. RD-Staph may be useful in geographical areas where the presence of certain *S. aureus* capsular serotypes precludes or limits the usefulness of agglutination-based procedures. Similarly, the reliability of the RD-Staph PAL and BGAL tests for identifying *S. epidermidis* and *S. saprophyticus*, respectively, depends upon the prevalence of strains in which these enzymatic activities are present. When these tests are negative, RD-Staph provides no additional information regarding the identity of an isolate, and additional conventional or kit testing would be required for species-level identification. At present, resistance to novobiocin is still used in most laboratories for presumptively identifying *S. saprophyticus*, but other coagulase-negative species, including *S. epidermidis*, may occasionally be novobiocin resistant. Other simple, rapid tests for identifying *S. epidermidis* are not currently available. Recently, Lindsay and Riley (18) reported that 96.4% of 57 *S. epidermidis* strains were susceptible to the iron-chelating agent desferrioxamine in both tube dilution and disk diffusion formats. By this test, *S. epidermidis* and *S. hominis* were the only staphylococcal species that were inhibited by this compound. The utility of RD-Staph in the clinical laboratory would be greatly enhanced if additional rapid tests were included to detect less typical *S. epidermidis* and *S. saprophyticus* strains and to avoid misidentification of non-*S. epidermidis*, non-*S. saprophyticus* coagulase-negative staphylococci.

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