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Sensitivity and Specificity of an Improved Rapid Latex Agglutination Test for Identification of Methicillin-Sensitive and -Resistant *Staphylococcus aureus* Isolates

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The performance of a second-generation rapid agglutination kit, Slidex Staph Plus (SSP; bioMérieux), was compared to those of the Slidex Staph (SS; bioMérieux), Staphaurex (SRX; Murex Diagnostics), and BBL Staphyloslide (BBL; Becton Dickinson) kits by using 508 clinical isolates composed of 150 methicillin-sensitive *Staphylococcus aureus* (MSSA) organisms, 154 methicillin-resistant *S. aureus* (MRSA) organisms, and 204 non-*S. aureus Staphylococcus* spp. Of the 508 isolates tested, 75% were fresh clinical isolates, with the remainder taken from five different freezer collections. All four agglutination tests had comparable sensitivities for MSSA and MRSA. However, the SS kit was significantly less specific (93.1%) than the three other tests ($P > 0.05$, McNemar test). These results demonstrate that the new rapid latex agglutination kit, SSP, was more specific for the identification of *S. aureus* than the previous version and performed comparably to the SRX and BBL kits.

Staphylococcus aureus remains a pathogen of considerable clinical concern. It is responsible for a multitude of infectious processes as well as for infections of diverse foreign bodies, including intravenous catheters and prostheses (4). Rapid identification is essential because *S. aureus* can cause significant morbidity and mortality and remains a leading cause of nosocomial infection (6, 15). A variety of rapid agglutination kits to facilitate both prompt identification of *S. aureus* isolates and differentiation from non-coagulase-producing staphylococci have been developed and marketed.

The classic criterion for identification of *S. aureus* is that the organism can clump in plasma via the activity of extracellular free coagulase, also termed staphylocoagulase (10). Free coagulase is thought to interact with prothrombin in plasma to produce staphylothrombin, which converts prothrombin into an active form that releases fibrinopeptides from fibrinogen, forming fibrin clots (3). The tube coagulase test using rabbit plasma is based on this reaction and is a straightforward test that is still widely used in the clinical setting as a "gold standard." Its primary limitation is that the tube must be examined after 4 and 24 h of incubation to reliably detect both positive and negative reactions. False-negative results can occur due to rare coagulase-negative *S. aureus* (17, 28), while false-positive results can be produced by some non-*S. aureus* staphylococci which make proteases (called pseudocoagulases) that can also initiate clotting (29). In the slide coagulase test, rabbit plasma is used to agglutinate *S. aureus* organisms. Originally, this agglutination reaction was thought to be mediated by a bound form of staphylocoagulase. However, more recently the agglutination has been shown to be due to a fibrinogen binding cell surface receptor encoded by the *clfA* gene, termed the cell wall polypeptide clumping factor (or clumping factor, fibrinogen receptor, or fibrinogen affinity factor) (19). Although the slide coagulase test may be performed in <1 min, it lacks adequate sensitivity and specificity because some *S. aureus* strains lack clumping factor (28) or mask it with capsular polysaccharides (8), while other species, such as *Staphylococcus lugdunensis* and *Staphylococcus schleiferi* subsp. *schleiferi* (11), produce clumping factor. To circumvent these problems of inadequate sensitivity and specificity for *S. aureus* detection, rapid agglutination kits that bring together two or more properties of *S. aureus* to achieve clinical usefulness have been developed.

The current kits include reagents that react simultaneously with several different surface factors specific to *S. aureus*. These include human fibronectin, which binds to cell wall polypeptide clumping factor; the Fc portion of human immunoglobulin G (IgG), which binds to protein A; and antibodies which bind to specific bacterial surface antigens (1, 20). These reagents may be adsorbed onto the surfaces of erythrocytes or latex particles.

In this study, a second-generation rapid agglutination test, Slidex Staph Plus, was compared to its predecessor, the original Slidex Staph kit, and two other tests, Staphaurex and BBL Staphyloslide. All four tests detect bound coagulase, all tests except the BBL Staphyloslide kit detect protein A, and the Slidex Staph and Staph Plus kits detect *S. aureus* cell wall-specific antigens. Because methicillin-resistant *S. aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA) have demonstrated different sensitivities with some rapid agglutination assays (7, 27), we included equal numbers of MRSA and MSSA organisms, as well as non-*S. aureus* staphylococci representing different species. The sensitivities and specificities of the four rapid agglutination tests in addition to the standard tube coagulase test were determined and compared by using a panel of primarily fresh staphylococcal clinical isolates from the United States.

Bacterial strains. A total of 538 staphylococcal individual patient isolates were tested in the study protocol. Of 508 isolates detailed in the study analysis, 380 (75%) were fresh iso-

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lates collected by the clinical microbiology lab at the Boston Veterans Affairs (VA) Medical Center and the remaining 128 isolates were taken from five frozen collections from diverse sources that have been characterized elsewhere (5, 9, 12, 16). Of the 304 *S. aureus* isolates tested, 229 (75%) were fresh clinical isolates comprising 108 (47%) MRSA and 121 MSSA organisms. Ongoing molecular strain typing of the isolates in our medical center indicates that the MRSA organisms represent three broad genetic lineages plus additional diverse genotypes (18) and that the MSSA organisms are highly diverse (16, 24). The 204 coagulase-negative staphylococci included 151 fresh isolates of which 79 (52%) were methicillin resistant.

***S. aureus* identification tests.** All tests were done on the same day in parallel from a pure subculture of the primary isolate. Fresh clinical isolates streaked on BBL Trypticase soy agar (TSA II) plates with 5% sheep blood (Becton Dickinson Microbiology Systems [BD], Cockeysville, Md.) or frozen glycerol stocks were inoculated into 16- by 150-mm borosilicate glass tubes (VWR Scientific, Boston, Mass.) containing 0.5 ml of Trypticase soy broth (BD). Cultures were grown to a turbidity equal to a 0.5 McFarland standard (bioMérieux) for ~4 h at 37°C and then streaked for isolation on TSA II plates (BD) incubated overnight at 37°C. All subsequent testing was done according to the manufacturers' instructions. Control strains for agglutination reactions included two positive controls, an MSSA strain (ATCC 25923) and an MRSA strain (V 8333), and one negative control, *Staphylococcus epidermidis* (ATCC 14990).

(i) Free-coagulase (tube) test. BBL rabbit coagulase plasma (BD) reconstituted with sterile water (0.5 ml) was dispensed in 10- by 75-mm borosilicate glass tubes (Chasma Scientific, Cambridge, Mass.) and stored at −20°C. For each isolate several isolated colonies were picked with a sterile loop or wooden applicator from the TSA II plate incubated overnight and were inoculated into plasma-containing tubes. Isolates were placed in a 37°C water bath and were observed for clot formation at 4 and 24 h.

(ii) MRSA and MSSA classification. All isolates were inoculated onto a Mueller-Hinton agar plate supplemented with 4% NaCl and 6 mg of oxacillin (BD) per ml as recommended by the National Committee for Clinical Laboratory Standards (21). Plates were incubated at 35°C for a full 24 h and examined for evidence of growth. Controls included an oxacillin-susceptible strain (*S. aureus* ATCC 25923) and an oxacillin-resistant strain (*S. aureus* ATCC 43300).

(iii) Slidex Staph kit (bioMérieux Vitek, Hazelwood, Mo.). The Slidex Staph reagent is a combination agglutination test based on latex and hemagglutination components that detect bound coagulase, protein A, and specific *S. aureus* cell surface antigens. This reagent includes blood cells sensitized with fibrinogen to detect bound coagulase and latex particles sensitized with specific monoclonal antibodies. These monoclonal antibodies detect protein A by the Fc fragment of IgG as well as specific group antigens on the bacterial cell surface.

(iv) Slidex Staph Plus kit (bioMérieux). The Slidex Staph Plus is a latex agglutination kit utilizing latex particles sensitized with human fibrinogen and *S. aureus*-specific monoclonal antibodies. These monoclonal antibodies detect protein A by the Fc fragment of IgG as well as different polysaccharide antigens on the bacterial cell surface.

(v) BBL Staphyloslide kit (BD). The Staphyloslide kit is a hemagglutination test that detects the activity of the cell wall polypeptide clumping factor produced by *S. aureus* strains. This polypeptide binds to fibrinogen-sensitized sheep erythrocytes.

(vi) Staphaurex kit (Murex Diagnostics Limited, Kent, England). The Staphaurex kit is an agglutination test that detects the presence of bound coagulase and protein A. The test reagent is composed of latex particles coated with purified human IgG and fibrinogen.

Species identification. The species of each staphylococcal isolate was determined with the API STAPH kit (bioMérieux). Colonies were picked from TSA II plates and suspended in API STAPH medium according to the manufacturer's instructions. Twenty microwells (19 for biochemical tests and 1 negative control) were inoculated with the suspension and placed at 37°C for 24 h. Results were read and were transformed into a numerical profile from which a species was identified according to the API STAPH identification codebook, based on the Kloos and Schleifer classification system (10a). Isolates with identification percentages of <80% were considered unacceptably identified as per the manufacturer's instructions for interpretation of the numerical profile and were further tested with the ID32 STAPH kit (bioMérieux). Of the 304 *S. aureus* isolates, 4 (1.3%) were not acceptably identified with the API STAPH kit and were subsequently typed with the ID32 STAPH kit. Three coagulase-negative isolates typed as *S. aureus* with the ID32 STAPH kit and a fourth, a coagulase-negative isolate, could not be acceptably identified. These isolates were later verified by DNA hybridization to be *S. aureus*. Of the 204 non-*S. aureus* staphylococcal isolates, 35 (17.2%) were not acceptably identified with the API STAPH kit and were subsequently typed with the ID32 STAPH kit. One-third of these isolates, 11 of 35, remained unacceptably identified. Colonies were picked and suspended to a turbidity equal to a 0.5 McFarland standard (bioMérieux) according to the manufacturer's instructions. The suspension was inoculated into 26 cupules and incubated at 37°C for 24 h. The reactions were recorded and transformed into a numerical profile that was matched to a species according to the ID32 STAPH identification codebook; isolates with identification percentages of <80% were considered unacceptably identified as per the manufacturer's instructions.

Accuprobe DNA probe hybridization. Five coagulase-negative isolates from the completed study, four of which were identified by the API STAPH or ID32 STAPH (bioMérieux) kit as *S. aureus*, were further analyzed to determine species identification by a DNA hybridization method. The Accuprobe *S. aureus*-specific culture identification test (GenProbe, Inc., San Diego, Calif.) detects a unique rRNA sequence specific to *S. aureus* by DNA:RNA hybridization of the chemiluminescently labeled single-stranded DNA probe. A single colony was picked from a TSA II plate incubated overnight and was resuspended in 50 μl of lysis reagent. Samples were processed according to the manufacturer's instructions and the results were read on a PAL luminometer (GenProbe, Inc.). A sample with <600 photometric light units was considered negative, and a sample with >1,500 photometric light units was considered positive. Controls included a positive organism (*S. aureus* ATCC 25923) and a negative organism (*S. epidermidis* ATCC 14990).

Isolates were processed in sets of 15 to 20 such that a tube coagulase test, four rapid agglutination tests (Slidex Staph Plus, Slidex Staph, Staphaurex, and Staphyloslide), and a species identification test (API STAPH or ID32 STAPH) were all performed in parallel with the same subcultures. Isolates for which the coagulase and rapid agglutination test results were concordant, i.e., positive (*S. aureus*) or negative (non-*S. aureus*), were included in the final analysis. Isolates for which the results of one or more tests were discordant were tested again and were included in the final analysis only if the same discor-

TABLE 1. Sensitivities and specificities of four rapid agglutination tests for identification of *S. aureus*

Test method	No. of samples (n = 508) with result				Sensitivity (%)[a]	Specificity (%)
	True positive	False positive	True negative	False negative		
Slidex Staph Plus	300	4	200	4	98.7	98.0
Slidex Staph	300	14	190	4	98.7	93.1[b]
Staphaurex	301	6	198	3	99.0	97.1
Staphyloslide	301	5	199	3	99.0	97.5

[a] There was no statistical difference between the results for any two tests ($P > 0.05$, McNemar test).
[b] The specificity of the Slidex Staph kit was statistically different from those of the Slidex Staph Plus and Staphyloside kits ($P < 0.05$, McNemar test).

dant results could be reproduced. Of the original 538 isolates, 30 (5.6%) were not included in the final analysis because the discordant test result was not reproducible. Since each isolate was evaluated with five tests, this represents a technical laboratory error rate of 1.1%. The overall rate of repeated inconsistent results was 5.9% (32 of 538), which corresponds with the false-negative and false-positive test results reported for the sensitivity and specificity determinations for the 508 isolates included in this study.

The four rapid agglutination test kits had comparable sensitivities (97.4 to 100%) for either MRSA (n = 154) or MSSA (n = 150) isolates (data not shown). The combined sensitivity of the four tests ranged from 98.7 to 99.0%, and the tube coagulase test had a sensitivity of 98.7%. The Slidex Staph Plus test demonstrated greater specificity than the Slidex Staph test against the 204 coagulase-negative staphylococcal isolates tested (Table 1). The majority (72%) of the coagulase-negative staphylococcal isolates were *S. epidermidis* (n = 147), with the remaining isolates including *Staphylococcus haemolyticus* (n = 13), *Staphylococcus hominis* (n = 10), *Staphylococcus warneri* (n = 7), *Staphylococcus capitis* (n = 6), *Staphylococcus simulans* (n = 4), *S. lugdunensis* (n = 4), *Staphylococcus equorum* (n = 1), and other non-*S. aureus* species (n = 12). Three of the tests had comparable specificities for 204 coagulase-negative staphylococcal isolates, i.e., 97.1% (Staphaurex), 97.5% (Staphyloslide), and 98.0% (Slidex Staph Plus) (Table 1); a significant improvement was achieved in the specificity of the Slidex Staph Plus test over the Slidex Staph test (93.1%) ($P <$ 0.05, McNemar test) (Table 1). The tube coagulase test demonstrated a specificity of 100%.

The false-positive test results for the four kits are listed in Table 2 and were distributed among 18 coagulase-negative isolates, with only 1 isolate positive by all four tests and 2 isolates positive by three of the four agglutination tests. These 18 isolates were identified as *S. epidermidis* (n = 12), *S. haemolyticus* (n = 1), *S. lugdunensis* (n = 2), and unidentified coagulase-negative staphylococci (n = 3). Of these 18 isolates, 50% (9 of 18) were methicillin resistant. One isolate was positive by all four agglutination tests, was negative by the tube coagulase test at 4 and 24 h, and was unsatisfactorily identified by either the API STAPH or the ID32 STAPH kit. This isolate was later identified as *S. aureus* by using the *S. aureus*-specific AccuProbe DNA probe hybridization kit.

TABLE 2. Incidence of false-positive results among coagulase-negative staphylococcal species

Staphylococcal species	No. tested	No. with false-positive result			
		Slidex Staph Plus	Slidex Staph	Staphaurex	Staphyloslide
S. epidermidis	147	2	11	1	3
S. capitis	6	0	0	0	0
S. equorum	1	0	0	0	0
S. haemolyticus	13	0	0	1	0
S. hominis	10	0	0	0	0
S. lugdunensis	4	1	1	2	2
S. simulans	4	0	0	0	0
S. warneri	7	0	0	0	0
Coagulase-negative staphylococci	12	1	2	2	0
Total	204	4	14	6	5

Of the 380 fresh clinical isolates from the Boston VA Medical Center, a total of 5 (1.3%) were eventually determined to be coagulase-negative *S. aureus*. All five isolates positively agglutinated in each of the four rapid agglutination kits, were reproducibly determined to be coagulase negative at 4 and 24 h by the tube coagulase method, and were methicillin sensitive. Of the five isolates, four were identified as *S. aureus* by the API STAPH and ID32 STAPH kits, with the fifth not satisfactorily identified by either kit. This isolate was confirmed to be *S. aureus* only by DNA probing.

Staphylococcal species are becoming increasingly important in the clinical microbiology laboratory and in the hospital setting due to the continued increase in methicillin resistance and the emergence and recognition of serious infections with coagulase-negative staphylococci in hospitalized patients exposed to invasive procedures, central lines, and intensive care units (2). The rapid differentiation of staphylococcal isolates has been complicated by an inability to detect MRSA deficient in clumping factor (14) and protein A (22, 25) that has also been reported with some rapid agglutination tests (13, 14, 26, 30). However, we found in this study that there was no significant difference in the sensitivities of the four test kits (Slidex Staph Plus, Slidex Staph, Staphaurex, and Staphyloslide) examined for MRSA and MSSA. Modification of second-generation agglutination tests to increase sensitivity by addition of antibodies directed against *S. aureus* surface antigens has resulted in the detection of organisms that might otherwise go undetected, leading to false-negative results. Several *S. aureus*-specific surface antigens have been targeted, including serotype 5 and 8 capsular polysaccharides (8) and a surface glycopolysaccharide called antigen 18 (7). It is important to note that geographical differences can correlate with antigenic variation and may affect an individual test's specificity (7).

In accordance with another report (31), we confirmed that the Slidex Staph kit was equally specific as but less sensitive than several other commercially produced agglutination tests. In this study we have compared the first-generation Slidex Staph kit with the modified Slidex Staph Plus kit to demonstrate that the specificity of the new test has been significantly increased and is now comparable to those of several other rapid agglutination tests on the market. Recently, the performance of the Slidex Staph Plus kit was also tested in a French study by Personne et al. (23) in which it exhibited an increase in both sensitivity and specificity compared to the Slidex Staph

kit. It is important to note that in our study, in which we examined U.S. isolates, we did not find a sensitivity of 100% for the Slidex Staph Plus kit, as was found in the French study (23). Additionally, we found no difference in the sensitivity of detecting MRSA ($n = 154$) with any of the four kits tested in our study, in contrast to the range of sensitivities (93.0 to 100%) found for MRSA in the French study (23) using the Slidex Staph and Slidex Staph Plus kits.

It is also of interest that the use of the tube coagulase test as the sole clinical laboratory test for identification of *S. aureus* would not have correctly identified the five confirmed clinical isolates of coagulase-negative *S. aureus* found in this study. These isolates did not express sufficient free coagulase activity to be detected by the tube coagulase method. Further molecular characterization of the nature of the free-coagulase deficiency of these isolates was not performed for this study. Since all five of these *S. aureus* isolates were positively identified with each of the four rapid agglutination tests, verification of suspected staphylococci negative by the tube coagulase test with a rapid agglutination test may prove useful.

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