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Comparison of Five Agglutination Tests for Identification of *Staphylococcus aureus*

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Various commercially produced agglutination kits are widely used for the identification of *Staphylococcus aureus*. These kits detect the presence of protein A and/or clumping factor on *S. aureus*. The literature has shown that methicillin-resistant *S. aureus* (MRSA) isolates which are deficient in both clumping factor and protein A may be misidentified. Two products, Slidex and Staphaurex Plus, utilize specific anti-*S. aureus* antibodies, potentially giving them greater sensitivity compared to products without these antibodies. We report a prospective study designed to compare the performance characteristics of Fastaph, Slidex, Staphaurex, Staphaurex Plus, Staphyloslide, and the tube coagulase test for the identification of staphylococcal isolates. All discrepant isolates were tested with the Gen-Probe AccuProbe *S. aureus* test and were identified to the species level with conventional reference biochemicals. A total of 1,193 isolates were tested, including 33 MRSA and 423 methicillin-sensitive *S. aureus* isolates. The sensitivities and specificities of the tests, respectively, were as follows: Fastaph, 99.1 and 98.9%; Slidex, 99.6 and 96.4%; Staphaurex, 98.9 and 99.9%; Staphaurex Plus, 99.6 and 93.9%; Staphyloslide, 99.1 and 98.9%; and tube coagulase, 99.3 and 100%. Sensitivity was excellent for all of the products tested. The specificities of Fastaph, Staphaurex, and Staphyloslide were excellent, while Staphaurex Plus and Slidex demonstrated less optimal results.

Several commercially produced kits are available to aid clinical laboratories in the rapid identification of *Staphylococcus aureus*, providing alternatives to the classic slide and tube coagulase tests. The slide and tube coagulase tests detect bound and free clumping factor, respectively, but 10 to 15% of *S. aureus* strains may yield a negative result with the slide test (6), and 2 to 5% may do so with the tube test (11).

Although the tube coagulase test using rabbit plasma is still considered the definitive method, many laboratories now use test kits that employ either latex particles or sheep erythrocytes coated with fibrinogen to detect the presence of clumping factor. In order to improve the sensitivity and specificity of these kits, many manufacturers have also coated the latex particles with immunoglobulin G to allow detection of protein A (11, 15), a cell surface protein present on approximately 90% of *S. aureus* strains that has an affinity for the Fc portion of immunoglobulin G (1).

A reported weakness of some of the commercial kits is their inability to detect methicillin-resistant *S. aureus* (MRSA) (7, 8, 13, 14). Certain strains of MRSA have been misidentified as coagulase-negative staphylococci, with false-negative rates as high as 25% (15). In an attempt to improve test accuracy, some manufacturers have attached antibodies against staphylococcal capsular types 5 and 8 and other antigens to the latex particles in their kits. Staphylococcal capsular types 5 and 8 account for 70 to 80% of the capsular types of clinical isolates of *S. aureus* (3), with capsular type 5 predominant among MRSA strains (4).

This study was designed to compare the performance characteristics of two of these newer kits containing anti-staphylococcal capsule antibodies with several of the widely used commercial kits and the classic tube coagulase method for the identification of freshly isolated strains of staphylococci.

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MATERIALS AND METHODS

A total of 1,193 isolates of *Staphylococcus* species were cultured from consecutive, fresh clinical specimens submitted to the microbiology laboratory of the Geisinger Medical Center for routine bacteriologic culture. All isolates were cultured on 5% sheep blood agar (Remel, Lenexa, Kans.) and were chosen on the basis of their colonial and Gram stain morphologies and a positive catalase reaction. A tube coagulase test was performed on each isolate with rabbit plasma, using the direct tube method as stated in the package insert (BBL Microbiology Systems, Cockeysville, Md.), with results read at 4 and 24 h.

Five commercial agglutination kits were evaluated in this study. Two, Slidex Staph-Kit (bioMerieux Vitek, Inc., Hazelwood, Mo.) and Staphaurex Plus (Murex Diagnostics Inc., Norcross, Ga.), are manufactured with antibodies specific for anti-staphylococcal capsule antigens. The Slidex Staph-Kit is a combined latex-hemagglutination product, while the Staphaurex Plus is a latex agglutination kit.

Three products that do not have specific anti-staphylococcal capsule antibodies were evaluated. Two of the three, Fastaph (Carr-Scarborough Microbiologicals, Inc., Stone Mountain, Ga.) and Staphaurex (Murex Diagnostics Inc.), are latex agglutination products, while the third product, Staphyloslide (BBL Microbiology Systems), utilizes a hemagglutination method.

All of the commercial products were tested by following instructions in the manufacturers' package inserts. Appropriate quality control strains were included in the evaluation of each product. Three of the kits (Slidex, Staphaurex Plus, and Staphyloslide) include an internal control that is run concurrently with each isolate tested; if the control reacts with the test organism, the test is considered noninterpretable.

All isolates were tested for their susceptibility to oxacillin either by an MIC determination with the Vitek GPS-SC card (bioMerieux Vitek, Inc.) or by the disk diffusion method performed according to the National Committee for Clinical Laboratory Standards protocol (10).

All tube coagulase tests as well as the five commercial agglutination tests were performed by the same individual on all isolates. Isolates yielding discrepant results among the six methods were initially retested by that same individual. Those isolates with repeat discrepant results were retested by all six methods by a second individual, blinded to the previous results. All isolates with discrepant test results from both individuals were further tested by a DNA probe method using the AccuProbe *Staphylococcus aureus* Culture Identification Test kit (Gen-

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TABLE 1. Performance characteristics of six tests for identification of *S. aureus*

Test method	No. of isolates[a] with interpretable results	No. of results				Sensitivity (%)	Specificity (%)	Specificity 95% CI[b]	Predictive value of positive result (%)	Predictive value of negative result (%)
		True positive	False negative	True negative	False positive					
Fastaph	1,193	452	4	729	8	99.1	98.9	98.2–99.6	98.3	99.5
Slidex	1,172	450	2	694	26	99.6	96.4	95.0–97.8	94.5	99.7
Staphaurex	1,193	451	5	736	1	98.9	99.9	99.7–99.9	99.8	99.3
Staphaurex Plus	1,190	454	2	689	45	99.6	93.9	92.9–95.6	91.0	99.7
Staphyloslide	1,177	449	4	719	5	99.1	99.3	98.7–99.9	98.9	99.4
Tube coagulase	1,193	453	3	737	0	99.3	100		100	99.6

[a] In all, 1,193 isolates were tested.
[b] CI, confidence interval.

Probe Inc., San Diego, Calif.). Discrepant organisms that were not identified as *S. aureus* by the DNA probe method were sent to the Diagnostic Microbiology Laboratory at the Centers for Disease Control and Prevention (CDC) for full species determination using a full battery of standard biochemical tests (12).

Large sample confidence intervals were calculated by standard methods (2).

RESULTS

A total of 1,193 staphylococcal isolates were tested, with 1,119 isolates yielding the same results by all test methods (448 *S. aureus* isolates and 671 isolates of other staphylococcal species) and 74 isolates yielding discrepant results. Biochemical and DNA probe testing of the 74 discrepant isolates determined that 8 were *S. aureus* and 66 were other staphylococcal species.

Table 1 summarizes the performance characteristics of the six test methods. Including the 74 discrepant isolates, there were a total of 456 *S. aureus* isolates and 737 isolates of other staphylococcal species. A total of 21, 3, and 16 test results were noninterpretable for Slidex, Staphaurex Plus, and Staphyloslide, respectively. For purposes of statistical analysis, we included only interpretable test results. Thus, as is shown in Table 1, there are differences in the number of total positive and total negative results for Slidex, Staphaurex Plus, and Staphyloslide compared with results for the other test methods. There were no significant differences between the sensitivities of the six methods. On the other hand, the specificities of Slidex and Staphaurex Plus were significantly lower than those of the other test methods.

Table 2 summarizes the test results by species for the 74 isolates with discrepant results. There were eight isolates of *S. aureus* with discrepant test results. Of the 66 non-*S. aureus* isolates of staphylococci with discrepant results, 65 were identified as belonging to one of eight different species and 1 was unidentified.

Three of the discrepant isolates identified as *S. aureus* produced negative tube coagulase test results both when initially tested and upon a single repeat test. Following subculture in the laboratory, all three isolates produced positive tube coagulase test results when tested in the CDC and Geisinger Medical Laboratories. For purposes of analysis, these three isolates were classified as giving negative tube coagulase results.

A total of 456 isolates were identified as *S. aureus*, 33 of which were methicillin resistant (7.2%). There were no significant differences in the sensitivities between the various test methods for identification of MRSA (Table 3). All kits demonstrated greater than 99.0% sensitivity for identification of methicillin-sensitive strains of *S. aureus.*

DISCUSSION

Commercially produced kits for the identification of *S. aureus* are widely used in the clinical microbiology laboratory. Although the tube coagulase test is generally accepted as the "gold standard" for identification of *S. aureus*, the 4- to 24-h time required for a final negative test result makes the rapid agglutination tests attractive alternatives.

In this study we compared the performance characteristics of five commercially available kits used for the identification of *S. aureus*. We were particularly interested in the Slidex and Staphaurex Plus kits because they have antibodies specific for capsular types 5 and 8 of *S. aureus*. The anticapsular antibodies of the Slidex kit are monoclonal, while those of the Staphaurex Plus kit are polyclonal. The use of anticapsular antibodies should, theoretically, improve detection of *S. aureus* isolates, particularly MRSA strains deficient in clumping factor and protein A.

There were no significant differences in the sensitivities of the five agglutination methods and the tube coagulase test when results for MRSA and methicillin-sensitive *S. aureus* isolates were combined or examined separately. Overall sensitivities ranged from 98.9 to 99.6%. Sensitivities for MRSA strains ranged from 93.9 to 97.0%.

On the other hand, the specificities of Slidex (96.4%) and Staphaurex Plus (93.9%) were significantly lower than those of the other three agglutination methods. The specificities of the other three kits were all ≥98.9%. Any benefit from increased sensitivity afforded by the anticapsular antibodies present on the Slidex and Staphaurex Plus kits was more than offset by the increase in false-positive test results. We recommend that laboratories using Slidex and Staphaurex Plus kits confirm all positive test results with a tube coagulase test.

While the presence of clumping factor is a defining characteristic of *S. aureus*, other staphylococcal species can also be positive for clumping factor, including *S. lugdunensis*, *S. schleiferi* subsp. *schleiferi*, and *S. intermedius* (6). Ten isolates of *S. lugdunensis* from this study did have false-positive results, but, interestingly, the five agglutination methods varied in the frequency with which this occurred. Slidex, Staphaurex Plus, and Staphyloslide gave 5, 10, and 1 false-positive reactions, respectively, for isolates of *S. lugdunensis*, while there were no false-positive results with Fastaph and Staphaurex for these same 10 isolates. No isolates of *S. schleiferi* subsp. *schleiferi* or *S. intermedius* associated with discrepant results were encountered.

Some isolates of *S. saprophyticus* and *S. sciuri* have also been associated with false-positive agglutination reactions (6). Seven isolates of *S. saprophyticus* from this study yielded five false-positive and 13 noninterpretable test results, including the only

TABLE 2. Analysis of 74 isolates producing discrepant test results

Species	No. of isolates	No. oxacillin sensitive/oxacillin resistant	Result[a] of:					
			Fastaph	Slidex	Staphaurex	Staphaurex Plus	Staphyloslide	Tube coagulase
S. aureus	1	0/1	+	NI	0	0	0	0
	2	1/1	0	0	0	+	0	0
	1	1/0	0	+	0	0	+	+
	3	3/0	+	NI	+	+	NI	+
	1	1/0	0	+	0	+	0	+
S. haemolyticus	2	2/0	0	0	0	+	0	0
	1	1/0	0	+	0	+	0	0
S. hominis	1	1/0	+	0	0	+	0	0
S. lugdunensis	5	5/0	0	0	0	+	0	0
	2	2/0	0	+	0	+	0	0
	3	3/0	0	+	0	+	+	0
S. pasteuri	1	1/0	0	NI	0	0	NI	0
S. saprophyticus	1	1/0	0	NI	0	0	NI	0
	2	2/0	0	NI	0	NI	NI	0
	1	1/0	+	NI	0	NI	NI	0
	1	0/1	0	0	0	+	0	0
	1	0/1	0	NI	0	+	NI	0
	1	1/0	0	+	0	+	0	0
S. simulans	1	1/0	+	+	0	0	0	0
	1	1/0	0	NI	0	0	NI	0
S. xylosus	1	1/0	0	+	0	0	+	0
S. epidermidis	3	2/1	0	NI	0	0	0	0
	1	0/1	0	NI	0	0	NI	0
	1	0/1	0	NI	0	0	+	0
	3	2/1	0	+	0	0	0	0
	1	1/0	+	+	0	0	0	0
	2	0/2	0	+	0	0	NI	0
	1	0/1	+	+	0	0	NI	0
	13	8/5	0	0	0	+	0	0
	1	0/1	+	0	0	+	0	0
	3	0/3	0	NI	0	+	0	0
	1	0/1	0	NI	0	+	NI	0
	7	3/4	0	+	0	+	0	0
	1	1/0	+	+	0	+	0	0
	1	1/0	0	+	+	+	0	0
S. auricularis	1	1/0	0	+	0	+	0	0
Staphylococcus species unknown	1	1/0	+	NI	0	0	NI	0
Subtotal no.								
Noninterpretable			NA	21	NA	3	16	NA
False positive			8	26	1	45	5	0
False negative			4	2	5	2	4	3
Total no. of discrepant results	74	49/25	12	49	6	50	25	3

[a] NI, not interpretable; 0, negative; +, positive; NA, not applicable.

3 noninterpretable test results from the entire study produced by the Staphaurex Plus test. These results indicate that at least some of the false-positive reactions observed with *S. saprophyticus* are probably due to nonspecific agglutination.

Pastorex Staph-Plus (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France) is another new kit for identifying *S. aureus* isolates. This product has monoclonal antibodies specific for staphylococcal capsular polysaccharide antigens types 5 and 8. In an evaluation of Pastorex Staph-Plus, Fournier et al. reported a specificity of 96.1% (5). Among the isolates with false-positive results were two isolates of *S. haemolyticus* and one of *S. hominis* which reacted with the monoclonal antibody to the type 8 capsular polysaccharide antigen. In addition, the Pastorex Staph-Plus kit yielded false-positive results for 1 of 89 *S. epidermidis* and 1 of 4 *S. lugdunensis* isolates tested.

Luijendijk et al. recently reported 100% sensitivity for both the Staphaurex Plus and Pastorex StaphPlus kits for the identification of 271 isolates of *S. aureus*, including 59 MRSA isolates (9). However, their study included only 30 non-*S. aureus* isolates, precluding an adequate assessment of test specificity.

In our study, the largest number of false-positive reactions for any staphylococcal species tested for the Fastaph, Slidex, and Staphaurex Plus kits was from isolates of *S. epidermidis*, with 4, 16, and 27 isolates, respectively, having false-positive results. Since only the Staphaurex Plus and Slidex kits among the products evaluated in this study utilize anticapsular antibodies, it would be worthwhile to know if the false-positive reactions are due, at least in part, to the presence of type 5 or 8 capsular antigens on the discrepant isolates. False-positive reactions could also be due to other antigens on these isolates that cross-reacted with the particular antibodies utilized in these assays.

Three isolates, all confirmed as *S. aureus*, had negative tube

TABLE 3. Comparative sensitivities of methods for identification of MRSA isolates[a]

Test method	No. of MRSA isolates		Sensitivity (%)
	True positive	False negative	
Fastaph	32	1	97.0
Slidex[b]	31	1	96.9
Staphaurex	31	2	93.9
Staphaurex Plus	32	1	97.0
Staphyloslide	31	2	93.9
Tube coagulase	31	2	93.9

[a] 33 total isolates.
[b] 32 interpretable results; 1 isolate noninterpretable.

coagulase results at 4 and 24 h of incubation. Repeat tube coagulase testing, performed to rule out any technical error in test performance, was negative for the three isolates. After repeated subcultures, all three of these isolates had positive tube coagulase test results in both the CDC and Geisinger Medical Laboratories. For the purpose of data analysis, we accepted the original negative results as valid. We believe that, for whatever reason, these isolates did not initially produce detectable free coagulase but that repeat subculture in the laboratory resulted in sufficient coagulase production for positive test results. We must bear in mind that in the day-to-day routine in clinical laboratories, subculturing and retesting of isolates are not the standard practice. Although generally considered the definitive test for identification of *S. aureus*, the tube coagulase test can nonetheless yield false-negative results. Indeed, Luijendijk et al. recently reported 3 isolates among 59 MRSA isolates tested gave negative tube coagulase test results (9).

Although the rapid agglutination tests proved to be very accurate in identifying staphylococcal isolates tested in this study, none of the tests correctly identified all isolates. What should be the standard test for identification of *S. aureus* in clinical laboratories? At Geisinger Medical Laboratories, we confirm all negative latex agglutination results with a tube coagulase test on isolates from sterile tissues and body fluids as well as any other suspicious isolates, as judged by the bench technologists. Positive latex agglutination test results are confirmed by a tube coagulase test for first-time MRSA isolates from a particular patient. To avoid the possibility of reporting isolates of *S. saprophyticus* as *S. aureus*, agglutination-positive results from female urine specimens are not reported as *S. aureus* until the novobiocin susceptibility test results are known.

The AccuProbe *S. aureus* Culture Identification Test kit correctly identified all 74 isolates with discrepant test results. Although these 74 isolates were only a small portion of the 1,193 total isolates included in this study, they were the isolates that proved most challenging to the six methods evaluated in this study. While we do not advocate testing all isolates with the AccuProbe kit, our results indicate that this kit may be very useful for identification of problem isolates. We also suggest that the AccuProbe kit be utilized in the evaluation of new products for the identification of *S. aureus*, particularly those isolates that have discrepant test results with other test methods or commercial kits.

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REFERENCES

1. **bioMerieux Vitek, Inc.** 1992. Package insert 10/92. Slidex Staph-Kit, product 73113. bioMerieux Vitek, Inc., Hazelwood, Mo.
2. **Fisher, L. D., and G. van Belle.** 1993. Biostatistics: a methodology for the health sciences. John Wiley & Sons, Inc., New York, N.Y.
3. **Fournier, J. M., A. Boutonnier, and A. Bouvet.** 1989. *Staphylococcus aureus* strains which are not identified by rapid agglutination methods are of capsular serotype 5. J. Clin. Microbiol. **27:**1372–1374.
4. **Fournier, J. M., A. Bouvet, A. Boutonnier, A. Audurier, F. Goldstein, J. Pierre, A. Bure, L. Lebrun, and H. K. Hochkeppel.** 1987. Predominance of capsular polysaccharide type 5 among oxacillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **25:**1932–1933.
5. **Fournier, J. M., A. Bouvet, D. Mathieu, F. Nato, A. Boutonnier, R. Gerbal, P. Brunengo, C. Saulnier, N. Sagot, B. Slizewicz, and J. C. Mazie.** 1993. New latex reagent using monoclonal antibodies to capsular polysaccharide for reliable identification of both oxacillin-susceptible and oxacillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **31:**1342–1344.
6. **Kloos, W. E., and T. L. Bannerman.** 1995. *Staphylococcus* and *Micrococcus*, p. 282–298. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. ASM Press, Washington, D.C.
7. **Lairscey, R., and G. Buck.** 1987. Performance of four slide agglutination methods for identification of *Staphylococcus aureus* when testing methicillin-resistant staphylococci. J. Clin. Microbiol. **25:**181–182.
8. **Lally, R., and B. Woolfrey.** 1984. Clumping factor defective methicillin-resistant staphylococcus. Eur. J. Clin. Microbiol. **3:**151–152.
9. **Luijendijk, A., A. van Belkum, H. Verbrugh, and J. Kluytmans.** 1996. Comparison of five tests for identification of *Staphylococcus aureus* from clinical samples. J. Clin. Microbiol. **34:**2267–2269.
10. **National Committee for Clinical Laboratory Standards.** 1994. Performance standards for antimicrobial susceptibility tests, 5th ed. (M2-A5). National Committee for Clinical Laboratory Standards, Villanova, Pa.
11. **Piper, J., T. Hadfield, F. McCleskey, M. Evans, S. Friedstrom, P. Lauderdale, and R. Winn.** 1988. Efficacies of rapid agglutination tests for identification of methicillin-resistant staphylococcal strains as *Staphylococcus aureus*. J. Clin. Microbiol. **26:**1907–1909.
12. **Rhoden, D. L., G. A. Hancock, and J. M. Miller.** 1993. Numerical approach to reference identification of *Staphylococcus*, *Stomatococcus*, and *Micrococcus* spp. J. Clin. Microbiol. **31:**490–493.
13. **Ruane, P. J., M. A. Morgan, D. M. Citron, and M. E. Mulligan.** 1986. Failure of rapid agglutination methods to detect oxacillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **24:**490–492.
14. **Wanger, A. R., S. L. Morris, C. Ericsson, K. V. Singh, and M. T. LaRocco.** 1992. Latex agglutination-negative methicillin-resistant *Staphylococcus aureus* recovered from neonates: epidemiological features and comparison of typing methods. J. Clin. Microbiol. **30:**2583–2588.
15. **Weers-Pothoff, G., C. E. M. Moolhuijzen, and G. P. A. Bongaerts.** 1987. Comparison of seven coagulase tests for identification of *Staphylococcus aureus*. Eur. J. Clin. Microbiol. **6:**589–591.