

## International Multicenter Evaluation of Latex Agglutination Tests for Identification of *Staphylococcus aureus*

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**A newly marketed rapid agglutination kit for the identification of *Staphylococcus aureus*, Slidex Staph Plus (bioMérieux), was compared to Staphaurex Plus (Murex Diagnostics) and Pastorex Staph-Plus (Sanofi Diagnostics Pasteur). The study took place in three clinical microbiology laboratories in three different European countries. A total of 892 staphylococcal isolates, including 278 methicillin-sensitive *S. aureus* (MSSA) isolates, 171 methicillin-resistant *S. aureus* (MRSA) isolates, and 443 coagulase-negative staphylococcal isolates, were analyzed. The sensitivities (MSSA/MRSA) and specificities, respectively, were 98.2% (98.9%/97.1%) and 98.9% for Slidex Staph Plus, 98.2% (98.2%/98.2%) and 96.2% for Staphaurex Plus, and 98.7% (98.6%/98.8%) and 95.7% for Pastorex Staph Plus. The specificity of the Slidex Staph Plus kit was statistically significantly higher than the specificities of Staphaurex Plus and Pastorex Staph-Plus. The Slidex Staph Plus is a very reliable test for the identification of *S. aureus*.**

*Staphylococcus aureus* is one of the most frequently isolated pathogens in clinical specimens (8). In fact, *S. aureus* is currently the most common cause of infections in hospitalized patients (1). Misidentification of *S. aureus* as coagulase-negative staphylococcus (CoNS) can result in a costly search for other pathogens or unwarranted broad-spectrum empiric antimicrobial coverage (11). Although the tube coagulase test for the detection of free coagulase is generally considered the standard test for differentiating *S. aureus* from CoNS, the time-consuming nature of the test (incubation for 4 to 24 h is required) often forces clinical microbiology laboratories to use more rapid alternatives. The slide coagulase test, which detects bound coagulase (clumping factor) is rapid (<1 min), but 10 to 15% of *S. aureus* strains may yield a false-negative result (7). Through the years many manufacturers have developed commercial agglutination kits for the rapid identification of *S. aureus*. The earliest of these tests consisted of erythrocytes sensitized with fibrinogen for the detection of clumping factor. Thereafter, a second generation of products was marketed, which employed coated latex particles and/or sensitized sheep erythrocytes to identify *S. aureus* by the simultaneous detection of clumping factor and protein A (12). Initially, these tests were reported to be very accurate, but later reports documented false-negative results among methicillin-resistant *S. aureus* (MRSA) strains (4, 8, 9, 13, 14). It was hypothesized that these false-negative MRSA strains do not expose clumping factor or protein A on their surface, which might be explained by the presence of a large amount of capsular polysaccharides masking other cell wall structures. The observation that capsular polysaccharide serotype 5 predominated among

MRSA isolates which were not identified by rapid agglutination methods offered a target for improvement of the available tests (4). Third-generation tests were developed, which incorporated antibodies against capsular polysaccharides or antibodies against group-specific antigens on the *S. aureus* cell surface (4, 5, 12).

In this international multicenter study, a newly marketed rapid latex agglutination test, Slidex Staph Plus (bioMérieux, Marcy-l'Étoile, France), was compared to two other latex agglutination tests of the same generation, Staphaurex Plus (Murex Diagnostics Ltd., Dartford, England) and Pastorex Staph-Plus (Sanofi Diagnostics Pasteur, SA, Marnes-La-Coquette, France). All three tests detect clumping factor and staphylococcal protein A, in addition, Slidex Staph Plus and Staphaurex Plus detect group-specific antigens on the *S. aureus* cell surface, and Pastorex Staph-Plus detects capsular polysaccharides.

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### MATERIALS AND METHODS

The study was conducted at three clinical microbiology laboratories in three European countries: Department of Medical Microbiology, University of Zurich, (Zurich, Switzerland); Hospital Edouard Herriot (Lyon, France); and St. Elisabeth Hospital (Tilburg, The Netherlands). During a 4-month period, from June until September 1999, staphylococcal isolates that were recovered from clinical specimens submitted to the laboratories were tested. Specimens were handled according to the laboratories' routine procedures. Isolates were included in the evaluation based upon colony and Gram stain morphologies and a positive catalase reaction. A single colony of the isolate on the primary plate was subcultured onto Columbia agar plus 5% sheep blood (bioMérieux) to obtain a pure culture. According to the study protocol, each center had to test approximately 300 isolates, composed of 150 *S. aureus* isolates (of which at least 50 had to be methicillin resistant) and 150 CoNS isolates. Only one isolate was tested per patient. If a center did not succeed in recovering these numbers during the study period, strains that belonged to the laboratory's own collection (stored at –70°C) were additionally tested. Frozen isolates were subcultured twice before testing.

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**Bacterial isolates.** A total of 905 staphylococcal isolates, including 449 *S. aureus* isolates (278 methicillin susceptible and 171 methicillin resistant) and 456 CoNS isolates, were tested. Ninety (52.6%) of the MRSA isolates were fresh clinical isolates. Of the remaining 81 isolates, 39 isolates were from the collection of the Division of Infectious Diseases and Hospital Epidemiology, Department of Medicine, University Hospital Zurich, Zurich, Switzerland, and 42 isolates were from the strain collection of the National Institute of Public Health and Environmental Protection (RIVM), Bilthoven, The Netherlands (16).

***S. aureus* identification tests.** Initial identification was based upon a tube coagulase test for the detection of free coagulase and three rapid slide latex agglutination tests. All tests were performed on the same day by the same person, using the pure subculture of the primary isolate. The result of the tube coagulase test was not available to the investigator at the time the latex agglutination tests were performed.

(i) **Tube coagulase test.** The tube coagulase test for the detection of free coagulase was performed using rabbit plasma (bioMérieux). A large colony was suspended into 0.5 ml of rabbit plasma, mixed, and incubated at 35°C. The tubes were inspected for clot formation after 4 and 24 h.

(ii) **Rapid slide latex agglutination tests.** Three rapid latex agglutination tests were performed: Slidex Staph Plus (bioMérieux), Staphaurex Plus (Murex Diagnostics Ltd.), and Pastorex Staph-Plus (Sanofi Diagnostics Pasteur, SA). All three tests are based on the same principle, i.e., latex particles sensitized with human fibrinogen and monoclonal antibodies for the simultaneous detection of clumping factor, staphylococcal protein A, and group-specific antigens on the *S. aureus* cell surface (Slidex Staph Plus and Staphaurex Plus) or capsular polysaccharides (Pastorex Staph-Plus). Tests were performed according to the manufacturers' instructions. All tests contained a negative control latex reagent to check for nonspecific agglutination. A test was considered positive if there was visible agglutination of the latex particles and clearing of the background in the test reagent and no agglutination in the control reagent. If autoagglutination, i.e., agglutination in both the test and the control reagent, occurred, the result was considered uninterpretable.

**Interpretation of test results and additional testing.** If the tube coagulase test and all latex agglutination tests were positive, the isolate was considered to be *S. aureus*. If all tests were negative, the isolate was considered to be a CoNS and further identification to the species level was determined with the ID32 Staph (bioMérieux). If the results of the tube coagulase test and the latex agglutination tests were discordant, these tests were repeated from a new subculture and an ID32 Staph test and an AccuProbe culture identification test (Gen-Probe, San Diego, Calif.) were performed. The result of the AccuProbe was considered to be the "gold standard."

(i) **ID32 Staph.** The ID32 Staph was performed on isolates with discordant test results and on all CoNS isolates. The ID32 Staph strip consists of 32 cupules, 26 of which are used as test cupules and contain dehydrated test substrates. The strips were inoculated according to the manufacturer's instructions, and results were read after 24 h of incubation at 35°C. Identification was done using the identification software. The species identification was accepted if the species identification probability was >80%.

(ii) **AccuProbe.** The AccuProbe culture identification test for *S. aureus* detects specific rRNA sequences that are unique for *S. aureus*. The test was performed according to the manufacturers' instructions. The result of the AccuProbe test was considered the gold standard for the identification of *S. aureus*.

**Methicillin susceptibility testing.** In addition to the laboratories' routine susceptibility testing procedures, all *S. aureus* isolates were inoculated onto Mueller-Hinton agar supplemented with 6 µg of oxacillin per ml and 4% NaCl (MRSA screen agar; bioMérieux) as recommended by the National Committee for Clinical Laboratory Standards (10). Plates were incubated at 35°C for 24 h. If any growth was detected, the isolate was considered to be oxacillin resistant. As control, on each plate an oxacillin-susceptible strain (*S. aureus* ATCC 25923) and an oxacillin-resistant strain (*S. aureus* ATCC 33593) were also tested. If there was a discrepancy between the routine susceptibility testing and the oxacillin agar screening test, PCR detection of the *mecA* gene or detection of PBP 2a with a latex agglutination test (MRSA Screen test; Denka Seiken Co., Ltd.) was performed to confirm the test result.

**Calculation of sensitivity and specificity.** For the calculation of test sensitivity and specificity, if an isolate was tested twice, the second test result was used. Isolates for which one of the latex agglutination tests gave an uninterpretable result were excluded from analysis.

**Statistical analysis.** The statistical significance of differences in sensitivity and specificity was determined by the McNemar test.

TABLE 1. Comparison of tests for the identification of *S. aureus*<sup>a</sup>

Test and center <sup>b</sup>	Sensitivity (%)			Specificity <sup>c</sup>
	MSSA	MRSA	All isolates	
Slidex Staph Plus	98.9	97.1	98.2	98.9
Zurich	98.0	95.9	97.3	99.3
Lyon	98.7	97.3	98.0	98.7
Tilburg	100	98.0	99.3	98.7
Staphaurex Plus	98.2	98.2	98.2	96.2
Zurich	98.0	100	98.7	95.1
Lyon	96.1	97.3	96.7	96.0
Tilburg	100	98.0	99.3	97.4
Pastorex Staph-Plus	98.6	98.8	98.7	95.7
Zurich	98.0	100	98.7	95.8
Lyon	98.7	97.3	98.0	95.3
Tilburg	99.0	100	99.3	96.0
Tube coagulase	99.3	100	99.6	99.5
Zurich	99.0	100	99.3	99.3
Lyon	100	100	100	100
Tilburg	99.0	100	99.3	99.3

<sup>a</sup> Values were determined with 892 staphylococcal isolates, composed of 449 *S. aureus* (171 methicillin resistant) and 443 CoNS isolates.

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<sup>c</sup> The specificity of Slidex Staph Plus is statistically significantly higher than those of Staphaurex Plus and Pastorex Staph-Plus ( $P < 0.05$ ).

## RESULTS

During the study period a total of 905 staphylococcal isolates were tested. Thirteen CoNS isolates (1.4%) gave uninterpretable results due to autoagglutination with at least one of the latex agglutination tests and were excluded from analysis. Of the 892 isolates analyzed, 449 isolates were identified as *S. aureus*, including 278 methicillin-sensitive *S. aureus* (MSSA) and 171 MRSA isolates, and 443 isolates were identified as CoNS. The majority of the CoNS isolates were *S. epidermidis* ( $n = 271$ ; 61%). The other CoNS included 46 *S. haemolyticus* isolates, 24 *S. hominis* isolates, 18 *S. lugdunensis* isolates, 17 *S. warneri* isolates, 11 *S. capitis* isolates, 9 *S. simulans* isolates, 8 *S. schleiferi* isolates, 5 *S. caprae* isolates, 3 *S. saprophyticus* isolates, 1 *S. cohnii* isolate, and 30 CoNS isolates which could not be identified by ID32 Staph.

The three rapid agglutination tests had comparable sensitivities for the identification of *S. aureus*. There was not much difference in the observed sensitivities and specificities of the tests among the centers (Table 1). The specificity of the Slidex Staph Plus test (98.9%) was significantly higher statistically than the specificities of the Staphaurex Plus (96.2%) and the Pastorex Staph Plus (95.7%) ( $P < 0.05$ ). False-positive results were observed mainly for *S. lugdunensis*, *S. schleiferi*, and *S. haemolyticus* (Table 2). In total, 29 CoNS isolates gave false-positive results by at least one of the latex agglutination tests. Four isolates (two *S. schleiferi*, one *S. lugdunensis*, and one *S. haemolyticus*) were positive with all three tests, three isolates (two *S. lugdunensis* and one *S. haemolyticus*), were positive with Staphaurex Plus and Pastorex Staph-Plus, and one *S. haemolyticus* isolate was positive with Pastorex Staph-Plus and Slidex Staph Plus. Two non-*S. aureus* isolates were tube coagulase

TABLE 2. Incidence of false-positive results among CoNS

Staphylococcal species	No. tested	No. (%) with false-positive results			
		Slidex Staph Plus	Staphaurex Plus	Pastorex Staph-Plus	Tube coagulase test
<i>S. epidermidis</i>	271	0	0	4 (1.5)	1 (0.4)
<i>S. haemolyticus</i>	46	2 (4.3)	3 (6.5)	3 (6.5)	0
<i>S. hominis</i>	24	0	0	1 (0.4)	0
<i>S. lugdunensis</i>	18	1 (5.6)	12 (66.7) <sup>b</sup>	3 (16.7)	0
<i>S. warneri</i>	17	0	0	0	0
<i>S. capitis</i>	11	0	0	1 (9.1)	0
<i>S. simulans</i>	9	0	0	0	0
<i>S. schleiferi</i>	8	2 (25.0)	2 (25.0)	6 (75.0)	1 (12.5)
<i>S. caprae</i>	5	0	0	0	0
<i>S. saprophyticus</i>	3	0	0	0	0
<i>S. cohnii</i>	1	0	0	0	0
CoNS <sup>a</sup>	30	0	0	1 (3.3)	0
Total	443	5 (1.1)	17 (3.8)	19 (4.3)	2 (0.5)

<sup>a</sup> No valid identification by ID32 Staph. Two isolates were identified as *S. aureus* by ID32Staph, but all other tests, including Accuprobe, were negative.

<sup>b</sup> Staphaurex Plus had significantly more false-positive results for *S. lugdunensis* than Slidex Staph Plus or Pastorex Staph-Plus ( $P < 0.05$ ).

positive; one was an *S. epidermidis* isolate which was negative in all latex agglutination tests, and the other was an *S. schleiferi* isolate which was also positive with Pastorex Staph-Plus.

The Staphaurex Plus had significantly more false-positive results among *S. lugdunensis* isolates than the other two tests ( $P < 0.05$ ) (Table 2).

## DISCUSSION

Due to stability problems, an earlier version of the Slidex Staph Plus kit was never made available commercially, although there were published reports that the test itself was very reliable for the identification of *S. aureus* (6, 12, 15). Using the same monoclonal antibodies and fibrinogen, a new manufacturing procedure has been developed, which proved to result in a stable product (J. Latour, bioMérieux, personal communication). Since the critical raw materials of the product were not changed, the test is now marketed under the same name (i.e., Slidex Staph Plus) as the earlier product. This study confirms that this optimized, newly marketed Slidex Staph Plus kit is a very reliable test for the identification of *S. aureus*.

The sensitivity of the Slidex Staph Plus is comparable to those of the other rapid agglutination kits tested. The specificity of the Slidex Staph Plus is significantly higher than those of Pastorex Staph-Plus and Staphaurex Plus and is not significantly different from that of the tube coagulase test. Interpretation of the test results was very easy and clear-cut. This in contrast to the results of the Pastorex Staph-Plus test, for which some interpretation problems were experienced at the center in Tilburg, The Netherlands. For 14 of the 151 CoNS isolates tested in this center (9.3%), the test showed equivocal, weak-positive agglutination without clearing of the background. For analysis purposes these test results were called negative, but we feel that in inexperienced hands these could easily be mistaken for positive. This would of course influence the specificity of the Pastorex Staph-Plus in a negative way.

The present study tried to reflect as closely as possible the normal routine practice of a clinical microbiology laboratory. Therefore, the evaluation was performed mainly with fresh isolates, which were tested in batches and not all at once on the

same day. This batchwise testing was also performed to see if the Slidex Staph Plus reagent was stable under routine laboratory usage conditions and could resist repeated suboptimal storage conditions, such as leaving the kit on a bench for a while before putting it back in the refrigerator on consecutive days, which is often the way a test is handled in routine practice. During the trial no evidence was found which indicated that there was a problem with reagent stability under these conditions.

If test results for the three latex agglutination tests or the tube coagulase test were discrepant, the tests were repeated the next day using a fresh subculture. In routine practice many laboratories perform a second test, like the tube coagulase test or a DNase test, to confirm the result of a rapid latex agglutination test. The results of these confirmation tests are often not available until the next day. If a discrepancy is noted in the test results, the latex agglutination test may be repeated before other tests, such as the Accuprobe culture confirmation test, are performed for definitive identification. Furthermore, if a technician finds the colony morphology inconsistent with the results of a latex agglutination test, the test is often repeated to confirm that the right colony was picked and that there wasn't a mix-up between the control and the test latex reagent. In these cases the result of the repeated test is often noted as the correct result; therefore, we also used the second test result for analysis purposes if a test was repeated.

Since geographical differences can correlate with antigenic variation of capsular polysaccharides and surface glycopolysaccharides of *S. aureus* and can therefore affect the outcome of an evaluation of an identification test for *S. aureus*, the study took place in three different centers in three European countries (15).

Since MRSA and MSSA have demonstrated different sensitivities with earlier generations of rapid agglutination kits, the aim was that at least one-third of the *S. aureus* isolates included in the evaluation would be methicillin resistant. Two of the three centers, however, were located in countries (Switzerland and The Netherlands) where the prevalence of MRSA is still low (3). Centers were therefore allowed to add MRSA isolates

from frozen collections. Eventually, 90 (52.6%) of the 171 MRSA isolates were fresh clinical isolates. As expected, the centers in Switzerland and The Netherlands needed additional frozen isolates. These frozen isolates had to be isolated in recent years. In Zurich, 39 MRSA isolates from the collection of the Division of Infectious Diseases and Hospital Epidemiology, Department of Medicine, University Hospital Zurich, Zurich, Switzerland, were additionally tested. In Tilburg, 42 MRSA isolates from the strain collection of RIVM, Bilthoven, The Netherlands, were tested. From the RIVM collection isolates with different phage types were selected. Most of the MRSA strains in this collection are recovered from patients who have been hospitalized in other countries, so this collection is considered to reflect MRSA strains from throughout the world, mostly Europe (16).

Most of the CoNS with false-positive test results in this evaluation were *S. lugdunensis*, *S. schleiferi*, or *S. haemolyticus* isolates (Table 2). *S. lugdunensis* and *S. schleiferi* produce clumping factor and can therefore give positive results in latex agglutination tests that detect clumping factor (7). *S. haemolyticus* has been reported in other studies to give false positive results. This is probably due to the production of type 8 capsular polysaccharide, which is detected by some latex agglutination tests (2, 5, 17).

In this study the Staphaurex Plus had significantly more positive results among *S. lugdunensis* isolates than the other two tests ( $P < 0.05$ ) (Table 2). An earlier study, performed at the laboratory in Zurich, showed that the Staphaurex Plus, combined with a negative result in the tube coagulase test, could in fact be used to detect clumping factor-positive *S. lugdunensis* (18).

The Slidex Staph Plus test seems to have overcome the problem of false-positive results for CoNS species, resulting in very high specificity, which in fact was not statistically different from that of the tube coagulase test.

The emergence of methicillin resistance in *S. aureus* has forced clinical microbiology laboratories to institute accurate and rapid detection methods. Recently, a reliable rapid slide latex agglutination test for detection of methicillin resistance in *S. aureus*, the MRSA Screen test (Denka Seiken Co., Ltd.) has been introduced. Since this test is based on the detection of PBP 2a, which is also the mechanism for methicillin resistance in CoNS, simultaneous reliable and rapid identification of the tested strain is necessary for optimal use of this rapid test (16). The Slidex Staph Plus is a valuable test for the rapid identification of *S. aureus*.

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