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New Latex Reagent Using Monoclonal Antibodies to Capsular Polysaccharide for Reliable Identification of Both Oxacillin-Susceptible and Oxacillin-Resistant *Staphylococcus aureus*

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A new latex agglutination test (Pastorex Staph-Plus, Sanofi Diagnostics Pasteur), consisting of a mixture of latex particles coated with fibrinogen and immunoglobulin G for the detection of clumping factor and protein A and latex particles sensitized with monoclonal antibodies directed to *Staphylococcus aureus* serotype 5 and 8 capsular polysaccharides, was compared with three commercially available rapid agglutination methods for the identification of 220 isolates of *S. aureus* (61 oxacillin resistant) and 128 isolates of coagulase-negative staphylococci. The sensitivity for identification of *S. aureus* was high with the Pastorex Staph-Plus test (98.6%) compared with those of the other tests, which ranged from 91.8 to 84.5%. Test sensitivities for the identification of oxacillin-resistant *S. aureus* were as follows: Pastorex Staph-Plus, 95.1%; Pastorex Staph, 73.8%; Staphyslide, 72.1%; and StaphAurex, 49.2%.

Agglutination procedures have been developed for the rapid differentiation of *Staphylococcus aureus* from coagulase-negative staphylococci. These methods use either latex particles coated with human sera for the simultaneous detection of protein A and clumping factor (6) or sheep erythrocytes sensitized with fibrinogen for the detection of clumping factor (7). Some clinical evaluations of the commercially available systems have noted that oxacillin-resistant *S. aureus* isolates may yield false-negative reactions with these kits (1, 3, 15, 20, 21).

Capsular polysaccharides (CPs) have been identified in clinical isolates of *S. aureus* (13), and two capsular serotypes, 5 and 8, account for about 70 to 80% of the serological types (2, 12, 18). It was found that all oxacillin-resistant isolates not identified by rapid agglutination methods were capsular serotype 5 (8). We have hypothesized that the cell wall structures recognized by these reagents might be masked by the CP. This hypothesis encouraged us to improve the currently available kits by adding latex particles sensitized with monoclonal antibodies which react with *S. aureus* CPs to the reagents. The performance of this new reagent, Pastorex Staph-Plus (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France), was compared with the performances of classical reagents.

A total of 348 fresh staphylococcal isolates were recovered from specimens submitted to the clinical microbiology laboratories of two hospitals (Hôtel-Dieu, Paris, and Paul Brousse, Villejuif, France). Staphylococci were identified as *S. aureus* or coagulase-negative staphylococci by colonial and microscopic morphology, catalase, free coagulase, and DNase. DNase was revealed by toluidine blue on an overnight culture on DNA medium (Sanofi Diagnostics Pasteur). The tube coagulase test, which is considered the reference method, was done by mixing 0.5 ml of an overnight broth culture, obtained by inoculation of a single colony into coagulase test broth (Sanofi Diagnostics Pasteur), with 0.5 ml of oxalated rabbit plasma (Sanofi Diagnostics Pasteur) in a sterile hemolysis tube. The tube was incubated at 37°C and was examined at 4 and 24 h. Clot formation at either reading was recorded as positive. Strains of CNS that did not coagulate plasma and failed to produce DNase were identified at the species level by using Api-Staph (BioMérieux, Charbonnières-les-Bains, France). Oxacillin resistance was detected with a 5-μg oxacillin disk (Sanofi Diagnostics Pasteur) by using the conventional diffusion method on Mueller-Hinton agar (Sanofi Diagnostics Pasteur) incubated for 24 h at 30°C.

Four rapid agglutination methods were tested: (i) Staphyslide (BioMérieux), which consists of two reagents, sheep erythrocytes sensitized with fibrinogen with 0.1% sodium azide preservative and nonsensitized sheep erythrocytes with 0.1% sodium azide preservative as a negative control; (ii) StaphAurex (Wellcome, Research Triangle Park, N.C.), which consists of latex particles coated with fibrinogen for the detection of clumping factor and with immunoglobulin G for the detection of protein A; (iii) Pastorex Staph (Sanofi Diagnostics Pasteur), which consists of latex particles sensitized with human plasma for the simultaneous detection of clumping factor and protein A; and (iv) a new reagent, Pastorex Staph-Plus (Sanofi Diagnostics Pasteur), which consists of two reagents. The latex test is a 2:1 mixture of latex particles coated with fibrinogen for the detection of clumping factor and with immunoglobulin G for the detection of protein A and latex particles sensitized with monoclonal antibodies directed to *S. aureus* serotype 5 and 8 CPs. The negative control consists of latex particles sensitized with bovine serum albumin.

Testing was done, as recommended by the manufacturers, on isolates grown on either Mueller-Hinton agar (Sanofi Diagnostics Pasteur), Columbia agar enriched or not en-

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TABLE 1. Comparison of tests for the identification of *S. aureus*[a]

Test	Identification of all *S. aureus* isolates (%)		Sensitivity for *S. aureus* (%)	
	Sensitivity	Specificity	Oxacillin susceptible	Oxacillin resistant
Pastorex Staph-Plus	98.6	96.1	100.0	95.1
Pastorex Staph	91.8	96.1	98.7	73.8
Staphyslide	91.4	96.9	98.7	72.1
StaphAurex	84.5	97.7	98.1	49.2

[a] Values were derived with 220 isolates of *S. aureus* (61 oxacillin resistant) and 128 isolates of coagulase-negative staphylococci.

riched with blood (BioMérieux), or the solid phase of a blood culture bottle (BCB System Roche, F. Hoffmann-La Roche and Co. Ltd., Diagnostica, Basel, Switzerland). Each of the strains was tested with each method on the same day by the same operator.

Capsular serotyping was done by the detection of type 5 and 8 CPs of bacteria grown on Columbia agar (Difco Laboratories) slants. The bacterial cells were suspended in 2 ml of phosphate-buffered saline (pH 7) and autoclaved at 121°C for 60 min. After centrifugation, the supernatants were retained and stored at −20°C. Serotype 5 and 8 CPs were detected in these supernatants by an enzyme-linked immunosorbent assay with type 5 or 8 CPs and the corresponding monoclonal antibodies (4).

Among 220 isolates of *S. aureus*, 159 were susceptible and 61 were resistant to oxacillin. Among 128 coagulase-negative staphylococci, 10 species were identified. There were 89 *S. epidermidis*, 15 *S. haemolyticus*, 6 *S. hominis*, 5 *S. capitis*, 4 *S. lugdunensis*, 2 *S. cohnii*, 2 *S. sciuri*, 2 *S. schleiferi*, 2 *S. xylosus*, and 1 *S. warneri* isolate. The results of the tests are shown in Table 1. The sensitivities of the four tests for the identification of *S. aureus* ranged from 84.5 to 98.6%. The specificities ranged from 96.1 to 97.7%. With 159 isolates of oxacillin-susceptible *S. aureus*, the sensitivities of the four tests ranged from 98.1 to 100%. With 61 isolates of oxacillin-resistant *S. aureus*, the sensitivities ranged from 49.2% (StaphAurex) to 95.1% (Pastorex Staph-Plus).

Among the 220 *S. aureus* isolates tested, 100 (45%) were capsular type 5, 99 (45%) were capsular type 8, and 21 (10%) were nontypeable. Nontypeable isolates are isolates which either do not elaborate detectable type 5 or 8 CP, elaborate CP belonging to other capsular types, or do not produce CP. Among the 159 oxacillin-susceptible isolates, 49 (31%) contained type 5 CP, 94 (59%) contained type 8 CP, and 16 (10%) were nontypeable with monoclonal antibodies specific for type 5 or type 8 CP. Among the 61 oxacillin-resistant isolates, 51 (84%) were type 5, 5 (8%) were type 8, and 5 (8%) were nontypeable. Among the 37 isolates of *S. aureus* not agglutinated with one or more of the tests, 35 isolates were resistant to oxacillin, 31 isolates were capsular type 5, 4 isolates were nontypeable, and none were capsular type 8.

Among 128 isolates of coagulase-negative staphylococci, 7 isolates (five *S. haemolyticus* and two *S. hominis*) produced type 8 CP. No isolate produced type 5 CP. Eleven isolates of coagulase-negative staphylococci were agglutinated with one or more of the tests (Table 2). These isolates belonged to six species: *S. epidermidis*, *S. lugdunensis*, *S. haemolyticus*, *S. hominis*, *S. cohnii*, and *S. warneri*. Among these, three isolates (two *S. haemolyticus* and one *S. hominis*) produced type 8 CP.

We compared Pastorex Staph and Pastorex Staph-Plus with two kits which are among the most commonly used in Europe, where more than 10 agglutination kits are presently on the market. Moreover, one of the kits (Staphyslide) is a hemagglutination method, and the other (StaphAurex) is a latex agglutination test. The sensitivities of the four tests in the detection of oxacillin-susceptible *S. aureus* isolates were comparable. In contrast, there was a variation in the sensitivity of the tests in the detection of oxacillin-resistant *S. aureus* isolates. The relatively high failure rate of the StaphAurex, Staphyslide, and Pastorex Staph tests to detect oxacillin-resistant *S. aureus* isolates is comparable with previous results (1, 3, 8, 15, 20, 21). Pastorex Staph-Plus correctly identified 95.1% of the oxacillin-resistant *S. aureus* isolates. This high sensitivity is particularly noteworthy because the specificity of this test is comparable with those of the other tests.

The capsular typing of the *S. aureus* isolates confirmed the predominance of capsular types 5 and 8 among clinical isolates of *S. aureus*, as previously shown in the United States (2) and Europe (4, 9, 12, 18). The published observation that capsular type 5 is predominant among oxacillin-resistant *S. aureus* isolates (9) was also confirmed in this study, because 84% of the oxacillin-resistant *S. aureus* isolates were capsular type 5. The fact that most isolates not recognized by these tests are capsular type 5 could have been attributed to the predominance of capsular type 5 among oxacillin-resistant *S. aureus* (9). However, in this study and in a previously published study, no type 8 isolate

TABLE 2. Coagulase-negative staphylococci agglutinated with one or more of the tests

Strain	Species	Presence of type 8 CP[a]	Agglutination with reagent			
			Pastorex Staph-Plus	Pastorex Staph	Staphyslide	StaphAurex
1	*S. epidermidis*	−	−	+	−	−
2		−	+	−	−	−
3		−	−	−	+	−
4	*S. lugdunensis*	−	−	+	+	+
5		−	+	+	−	+
6		−	−	+	−	−
7	*S. haemolyticus*	+	+	−	−	−
8		+	+	+	−	−
9	*S. hominis*	+	+	−	−	−
10	*S. cohnii*	−	−	−	+	+
11	*S. warneri*	−	−	−	+	−

[a] All isolates were negative with antibody specific for type 5 capsular polysaccharide.

was identified among the isolates not detected by the tests (8). Furthermore, the five oxacillin-resistant type 8 isolates of this study were agglutinated, and the two oxacillin-resistant type 8 isolates of the previous study were agglutinated (8). On the contrary, two oxacillin-susceptible type 5 isolates could not be detected by the tests. These observations confirm that the presence of type 5 CP is a typical feature of the *S. aureus* isolates not recognized by these agglutination tests.

We have hypothesized that the cell wall structures (clumping factor and protein A) recognized by these reagents are not exposed on the surface of the *S. aureus* isolates not agglutinated by these reagents (8). Such modification might be due to the presence of large amounts of CP masking other cell wall structures. Indeed, variation in the production of CP by *S. aureus*, depending on the culture conditions, has been shown (5, 12, 19). Such modification could also be due to a decrease in the amount of protein A in the *S. aureus* isolates unable to be agglutinated. Indeed, such phenomena have been described for oxacillin-resistant *S. aureus* (14, 16, 21). Our results are in agreement with the hypothesis that CP masks the cell wall structures recognized by these reagents. However, further experiments are necessary to explain why this feature is restricted to capsular type 5 isolates.

The presence of type 8 CP in three isolates of coagulase-negative staphylococci might explain the agglutination of these isolates with the reagent Pastorex Staph-Plus, which contains monoclonal antibodies to type 8 CP. Type 8 CP has been identified in coagulase-negative staphylococci isolated from cow and goat milk (17). Because *S. aureus* type 8 isolates were not missed in this study and in our previous study (8), it might be interesting to use only latex particles coated with anti-type 5 monoclonal antibodies in addition to the fibrinogen- and immunoglobulin G-coated particles.

A total of 11 isolates of coagulase-negative staphylococci were agglutinated with one or more of the tests, and the numbers of strains agglutinated with each test are comparable. The agglutination by one or more of these reagents of three of four isolates of *S. lugdunensis* might be due to the fact this species does produce a clumping factor (10). We tested a small number of isolates of coagulase-negative staphylococci belonging to species other than *S. epidermidis*, and we did not test *S. saprophyticus* isolates, for which false-positive results have been observed (3, 11). More extensive studies with a wider range of staphylococcal species might be useful.

In conclusion, the new reagent Pastorex Staph-Plus, which detects clumping factor, protein A, and CPs of *S. aureus*, appeared reliable for detection of oxacillin-susceptible *S. aureus* as well as for identification of oxacillin-resistant *S. aureus*.

REFERENCES

1. **Aldridge, K. E., C. Kogos, C. V. Sanders, and R. L. Marier.** 1984. Comparison of rapid identification assays for *Staphylococcus aureus*. J. Clin. Microbiol. **19**:703–704.
2. **Arbeit, R. D., W. W. Karakawa, W. F. Vann, and J. B. Robbins.** 1984. Predominance of two newly described capsular polysaccharide types among clinical isolates of *Staphylococcus aureus*. Diagn. Microbiol. Infect. Dis. **2**:85–91.
3. **Berke, A., and R. C. Tilton.** 1986. Evaluation of rapid coagulase methods for the identification of *Staphylococcus aureus*. J. Clin. Microbiol. **23**:916–919.
4. **Boutonnier, A., F. Nato, A. Bouvet, L. Lebrun, A. Audurier, J. C. Mazié, and J. M. Fournier.** 1989. Direct testing of blood cultures for detection of the serotype 5 and 8 capsular polysaccharides of *Staphylococcus aureus*. J. Clin. Microbiol. **27**:989–993.
5. **Dassy, B., W. T. Stringfellow, M. Lieb, and J. M. Fournier.** 1991. Production of type 5 capsular polysaccharide by *Staphylococcus aureus* grown in a semi-synthetic medium. J. Gen. Microbiol. **137**:1155–1162.
6. **Essers, L., and K. Radebold.** 1980. Rapid and reliable identification of *Staphylococcus aureus* by a latex agglutination test. J. Clin. Microbiol. **12**:641–643.
7. **Flandrois, J. P., and G. Carret.** 1981. Study of the staphylococcal affinity to fibrinogen by passive hemagglutination; a tool for the *Staphylococcus aureus* identification. Zentralbl. Bakteriol. Parasitenkd. Infectionskr. Hyg. Abt. 1 Orig. Reihe A **251**:171–176.
8. **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.
9. **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.
10. **Freney, J., Y. Brun, M. Bes, H. Meugnier, F. Grimont, P. A. D. Grimont, C. Nervi, and J. Fleurette.** 1988. *Staphylococcus lugdunensis* sp. nov. and *Staphylococcus schleiferi* sp. nov., two species from human clinical specimens. Int. J. Syst. Bacteriol. **38**:168–172.
11. **Gregson, D. B., D. E. Low, M. Skulnik, and A. E. Simor.** 1988. Problems with rapid agglutination methods for identification of *Staphylococcus aureus* when *Staphylococcus saprophyticus* is being tested. J. Clin. Microbiol. **26**:1398–1399.
12. **Hochkeppel, H. K., D. G. Braun, W. Vischer, A. Imm, S. Sutter, U. Staeubli, R. Guggenheim, E. L. Kaplan, A. Boutonnier, and J. M. Fournier.** 1987. Serotyping and electron microscopy studies of *Staphylococcus aureus* clinical isolates with monoclonal antibodies to capsular polysaccharide types 5 and 8. J. Clin. Microbiol. **25**:526–530.
13. **Karakawa, W. W., J. M. Fournier, W. F. Vann, R. Arbeit, R. S. Schneerson, and J. B. Robbins.** 1985. Method for the serological typing of the capsular polysaccharides of *Staphylococcus aureus*. J. Clin. Microbiol. **22**:445–447.
14. **Lindmark, R., J. Movitz, and J. Sjoquist.** 1977. Extracellular protein A from a methicillin resistant *Staphylococcus aureus*. Eur. J. Biochem. **74**:623–628.
15. **Mathieu, D., and V. Picard.** 1991. Comparative evaluation of five agglutination techniques and a new miniaturized system for rapid identification of methicillin-resistant strains of *Staphylococcus aureus*. Zentralbl. Bakteriol. **276**:46–53.
16. **Maxim, P. E., H. L. Mathews, and H. F. Mengoli.** 1976. Single-tube mixed agglutination test for the detection of staphylococcal protein A. J. Clin. Microbiol. **4**:418–422.
17. **Poutrel, B., C. Mendolia, L. Sutra, and J. M. Fournier.** 1990. Reactivity of coagulase-negative staphylococci isolated from cow and goat milk with monoclonal antibodies to *Staphylococcus aureus* capsular polysaccharide types 5 and 8. J. Clin. Microbiol. **28**:358–360.
18. **Sompolinsky, D., Z. Samra, W. W. Karakawa, W. F. Vann, R. Schneerson, and Z. Malik.** 1985. Encapsulation and capsular types in isolates of *Staphylococcus aureus* from different sources and relationship to phage types. J. Clin. Microbiol. **22**:828–834.
19. **Stringfellow, W. T., B. Dassy, M. Lieb, and J. M. Fournier.** 1991. *Staphylococcus aureus* growth and type 5 capsular polysaccharide production in synthetic media. Appl. Environ. Microbiol. **57**:618–621.
20. **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: epidemiologic features and comparison of typing methods. J. Clin. Microbiol. **30**:2583–2588.
21. **Winblad, S., and C. Ericson.** 1973. Sensitized sheep red cells as a reactant for *Staphylococcus aureus* protein A. Acta Pathol. Microbiol. Scand. Sect. B **81**:150–156.