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Rapid and Reliable Identification of *Staphylococcus aureus* by a Latex Agglutination Test

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A latex slide agglutination test detecting clumping factor and protein A simultaneously is recommended for rapid and reliable routine identification of *Staphyloccus aureus*. Strains (836) of staphylococci isolated from clinical specimens were examined, all *S. aureus* strains identified by conventional methods were correctly differentiated by the latex test, and no false-positive results occurred with other staphylococci. The reagent is easy to prepare since plasma is the coating material.

Rapid and reliable differentiation of *Staphylococcus aureus* from other strains of staphylococci is important in the clinical bacteriological laboratory not least because of the recent suggestion that coagulase-negative staphylococci may be pathogenic. The coagulase tube test was previously accepted for identification of *S. aureus*. However, false results due to nonspecific reactions, variability in plasma samples, and difficulties in evaluating the test (10, 12, 14, 16) have led to criticism of the method. This is the reason why several authors have sought for additional and more reliable procedures. Among such procedures are the heat-stable deoxyribonuclease (DNase) reaction (1, 10, 12), the hyaluronidase decapsulation test (3a, 15) and the protein A hemagglutination test (3a, 7, 9). Simultaneous detection of coagulase and heat-stable DNase (thermonuclease) by an agar method has also been recommended for improved *S. aureus* identification (2).

Slide tests for clumping factor or protein A hemagglutination give rapid results but are not very reliable (3a, 4, 5). Since latex agglutination is a very popular, sensitive, and rapid method currently employed routinely in serological laboratories, we have applied this method to *S. aureus* diagnosis. The present study describes a method for accurate *S. aureus* identification by using simultaneous detection of clumping factor and protein A by a latex agglutination test.

MATERIALS AND METHODS

Bacterial strains. All strains of staphylococci were isolated from human specimens sent to our laboratory for bacteriological examination. Reference strains were *S. aureus* ATCC 9342, NCTC 2530 (Cowan I), and A 676 (group IV, phage type 88, A. Forsgren), known to produce mainly extracellular protein A (8). Eighteen strains of well-characterized yeasts (two *Candida albicans*, two *Candida guilliermondii*, two *Candida krusei*, two *Candida parapsilosus*, two *Candida pseudotropicalis*, three *Candida tropicalis*, two *Saccharomyces cerevisiae*, and three *Torulopsis glabrata* strains) were tested with the latex reagent, as we accidentally observed nonspecific agglutination with a strain of yeast.

Characterization of strains. The 368 strains were identified by the following criteria: mannitol utilization (mannitol salt agar; Oxoid Ltd.), clumping factor, production of coagulase (tube test with human and rabbit plasma according to Fisk [6]), DNase (agar plate; Difco Laboratories), hyaluronidase (decapsulation test with *Streptococcus equi* [11]) and protein A (hemagglutination tube test [3a]).

An additional 495 strains were examined for DNase and hyaluronidase and, in case of different results, checked for coagulase and biochemical reactions (API staph system [3]; Analytab Products, Inc., Plainview, N.Y.).

Preparation of latex reagent. Latex suspension (Difco) was diluted 1:8 with glycine-saline buffer (pH 8.0). Equal volumes of the diluted latex suspension and human plasma (ethylenediaminetetraacetate treated, diluted 1:1,000 with glycine-saline buffer) were incubated for 30 min at 56°C in a shaking bath. The coated particles were washed two times with saline and suspended in phosphate-buffered saline (pH 7.4) containing 0.02% sodium azide and 0.05% human plasma.

Slide agglutination test. One to two colonies were mixed homogeneously with one drop of saline and one drop of the latex reagent on a glass slide. After the slide was rocked backward and forward a few times, the result was read.

RESULTS

The results of the first series of differentiation are shown in Table 1. Correct *S. aureus* diagnosis was indicated if three of the four reactions for coagulase, DNase, hyaluronidase, and protein A were positive. In one case the coagulase reaction was only in agreement with the other tests when pig plasma was used. No difference was seen between the results of the latex agglu-

TABLE 1. *Correlation between coagulase test and the other methods used for S. aureus identification in the first series*

Test	Coagulase[a] positive (n = 218)	Coagulase[a] negative (n = 150)
Mannitol		
Positive	211	23
Negative	7	127
Clumping factor[a]		
Positive	208	18
Negative	3	132
DNase		
Positive	218	4
Negative	0	146
Protein A		
Positive	216	1[b]
Negative	2	149
Hyaluronidase		
Positive	217	1[b]
Negative	1	149
Latex		
Positive	218	1[b]
Negative	0	149

[a] Human plasma.
[b] Positive with pig plasma.

TABLE 2. *Correlation between the two reference methods used in the second series*

Test (n = 495)	Hyaluronidase positive	Hyaluronidase negative
DNase		
Positive	313	10
Negative	2	170

tination test and the coagulase reaction. The data listed in Table 1 show furthermore that the combined testing of hyaluronidase and DNase used in the second series allows a reliable *S. aureus* identification. Different results occurred in about 2% of the cases (Table 2), and the decision was made according to the results of coagulase test and other biochemical reactions. The final results of all strains of the second series were in total agreement with the result of the latex reaction.

Of 18 strains of yeasts tested for nonspecific reactions, 14 caused agglutination with the latex test, but 5 strains reacted also in saline.

DISCUSSION

The good reliability of the reagent depended on the simultaneous detection of clumping factor and protein A, since clumping factor-negative and protein A-positive strains of *S. aureus* reacted as well as protein A-negative and clumping factor-positive species. Thus, the method is more accurate than the hemagglutination slide test for detecting protein A alone, which was correlated with the correct *S. aureus* diagnosis in 82.5% (4) and after subculture on an optimizing medium in 95% (5) of the strains tested.

The main problem was to prevent spontaneous agglutination of the coated latex particles. Addition of albumin alone (13) was not successful, but addition of a small amount of plasma to the final suspension led to longtime stability.

Since all coagulase-positive strains react with the latex agglutination test, the species *S. aureus*, *Staphylococcus intermedius* and *Staphylococcus hyicus* cannot be distinguished from each other. But the last two species are probably not often isolated from human specimens.

Staphylococci grown on culture media containing high salt concentrations are not suitable for the agglutination test since protein production is inhibited. Rough strains and yeasts may cause nonspecific reactions; rough strains could be recognized during the first step of the slide test, whereas yeasts should not cause any severe diagnostic problems because of their characteristic morphology.

Human plasma is a convenient and low-priced coating material as it contains the two substrates fibrinogen and immunoglobulin G needed for reaction. No further purification seems to be necessary. Thus, the latex slide agglutination test is a rapid and reliable method of low cost which can be recommended for identification of *S. aureus* in the bacteriological routine laboratory.

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