Rapid Identification of Staphylococcus aureus by Using Fluorescent Staphylocoagulase Assays

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Two rapid (1-h) assays for the detection of Staphylococcus aureus staphylocoagulase were developed by using the fluorogenic thrombin substrates N-t-boc-Val-Pro-Arg-7-amido-4-methylcoumarin (VPA) and N-t-boc-β-benzyl-Asp-Pro-Arg-7-amido-4-methylcoumarin (BB). The assays were compared to the tube coagulase test and latex agglutination (LA) (Sanofi Diagnostics Pasteur, Guildford, Surrey, United Kingdom) by using 406 clinical isolates of staphylococci, and they produced positive and negative predictive values of 99.2 and 99.1% for LA, 98.9 and 92.7% for VPA, and 98.9 and 99.1% for BB. Fluorescent assays used colonies from solid media, thereby eliminating the need for broth cultures, and were performed in microtiter trays, thus making them suitable for large-scale screening.

Staphylococcus aureus is one of the most frequently encountered pathogens in clinical samples (7, 10), while methicillin-resistant S. aureus (MRSA) is an important nosocomial pathogen (9, 15). A rapid, reliable test to identify these organisms is of considerable relevance (10, 15). The detection of staphylocoagulase by the tube coagulase test (1, 4, 16) is the “gold standard” for identification of S. aureus by the tube coagulase test (1, 4, 16) and the Pastorex Staph Plus test (Sanofi Diagnostics Pasteur, Guildford, United Kingdom), which detects clumping factor, staphyloccocal protein A, and capsular polysaccharides was used according to the manufacturer’s instructions. The Pastorex Staph Plus test (Sanofi Diagnostics Pasteur, Guildford, United Kingdom), which detects clumping factor, staphyloccocal protein A, and capsular polysaccharides was used according to the manufacturer’s instructions. Fluorescent staphylocoagulase assays. Isolates were subcultured onto Columbia blood agar overnight before testing. Tests were performed in round-bottomed microtiter trays. A 25-μl volume of BHI broth containing 0.2% bovine albumin/fraction V (Sigma Ltd., Poole, United Kingdom) and 25 μl of prothrombin (factor V) diluted at 1 mg/ml in distilled water (Sigma) were pipetted into each well. Four distinct colonies or a 2-mm streak through confluent growth of the organism tested was emulsified in the BHI-prothrombin solution to a turbidity greater than a no. 10 MacFarland standard. The plates were incubated at 37°C for 40 min before 100 μl of N-t-boc-Val-Pro-Arg-7-amido-4-methylcoumarin (VPA) (Sigma) (0.5 mg/ml in 0.05 M Tris, pH 8.0) was added, the solution was mixed, and the plates were incubated for a further 20 min. The degree of fluorescence was determined by using a Labtech Biolite F1 fluorescent microtiter plate reader (Labtech Ltd., Litchfield, United Kingdom) with filters set at 365 and 440 nm and sensitivity at 28. Readings above 8,000 were taken as positive. Positive, negative, and reagent controls were included with each batch, with S. aureus NCTC 6571 as the positive control and S. epidermidis NCTC 11047 as the negative control. The same technique was repeated with N-t-boc-β-benzyl-Asp-Pro-Arg-7-amido-4-methylcoumarin (BB) (Sigma) (0.5 mg/ml in 0.05 M Tris, pH 8.0).

Of the 406 clinical isolates tested, 297 were positive by the tube coagulase test and were identified as S. aureus. Of these, 295 (99.3%) were LA positive while 294 (98.9%) were positive by the two fluorescent assays (Table 1). Two strains identified as S. aureus by the API STAPH system (Biomerieux, Basingstoke, United Kingdom) and by the tube coagulase test were reproducibly LA negative. Forty MRSA strains were positive with all three assays.

Two S. aureus strains gave negative results with both VPA and BB fluorescent assays. One S. aureus strain was VPA negative and BB positive, and one strain was VPA positive and BB negative (Table 1). Of the 109 coagulase-negative strains, one
was repeatedly LA positive and was identified by the API STAPH system as *S. epidermidis*. Eight strains (7%) were VPA positive, while one strain (0.9%) (different from the previous eight) was BB positive (Table 2).

The positive and negative predictive values of the Pastorex LA test, the VPA fluorescent assay, and the BB fluorescent assay were 99.3 and 99.1%, 98.9 and 92.7%, and 98.9 and 99.1%, respectively.

Tube coagulase tests are widely used to detect staphylocoagulase production for identification of *S. aureus* (1, 3, 4, 7, 16). Problems have been encountered with this test due to variations in the type of plasma used, incubation time, and degree of clotting achieved, and definitive results may be unavailable until up to 24 h after initial isolation (3, 12, 13, 16).

Direct detection of the staphylocoagulase—prothrombin complex could offer a rapid, reliable assay for identification of *S. aureus* (4), and specific and sensitive chromogenic assays for staphylocoagulase have been described (3, 4). The more sensitive fluorescent substate VPA has been used to study staphylocoagulase kinetics and proved to be rapid, accurate, and simple to use (5, 8). Previous chromogenic and fluorogenic assays have been based on detection of staphylocoagulase in broth culture fluids or in semipurified form. These assays are not suitable for use in routine laboratories, where most organisms are grown on solid media. Staphylocoagulase is present and exposed on the cell surface (2) and is released into culture fluids through proteolysis (5). False-positive reactions observed with culture fluid staphylocoagulase assays may be due to direct activation of prothrombin by trypsin or papain-like proteases (4). Proteolytic release of staphylocoagulase may result in degradation of its COOH terminus, producing staphylo-like proteases (4). Proteolytic release of staphylocoagulase could offer a rapid, reliable assay for identification of *S. aureus colonies* grown on solid media. The BB assay is as sensitive as the VPA assay but is more specific. Our results suggest that VPA or BB assays are valid alternatives to LA or the tube coagulase test for the identification of *S. aureus* or could provide rapid and reliable confirmation of LA tests in the laboratory. Fluorogenic assays are well suited to large-scale screening and can provide quantitative values with standardized inocula.

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### REFERENCES


