Detection of *Staphylococcus aureus* by a Sensitive Immuno-PCR Assay, Su-Hua Huang¹ and Tsung-Chain Chang² (¹ Division of Biological Science and Technology, MORE TOMY CO., LTD., Hsin-Chu City, Taiwan; ² Department of Medical Technology, Medical College, National Cheng Kung University, Tainan, Taiwan; address correspondence to this author at: Division of Biological Science and Technology, MORE TOMY CO., LTD., No. 194, Zing-fu St., Hsin-Chu City 300, Taiwan; fax 866-3-5339588, e-mail moretek.hcwei@msa.hinet.net)

*Staphylococcus aureus* causes a wide variety of diseases in humans, the clinical courses of which range from boils and furuncles to more serious diseases such as septicemia and pneumonia (1). Although a significant cause of community-acquired infection, most life-threatening cases of *S. aureus* disease are hospital-acquired and are associated, in many cases, with indwelling vascular devices or catheters (2). The standard method of diagnosing *S. aureus* is to culture an isolate from a blood agar plate and then use a latex test to identify *S. aureus*.

A specific product of *S. aureus* is protein A (3). Protein A is a cell-wall constituent of *S. aureus* and is mainly covalently linked to the peptidoglycan structure (4); however, ~8–30% of the protein is secreted into the medium during the exponential growth phase (5). This property has been used to develop a latex agglutination test and an ELISA to identify and detect *S. aureus*. We describe here a sensitive immuno-PCR assay to detect *S. aureus* protein A. After optimization of the reaction conditions and the use of flexible plates with 96 V-bottomed wells, which were compatible with both the ELISA washer and the thermal cycler for PCR, we automated both detection methods.

Anti-protein A antisera were created by immunizing rabbits with protein A purified from preparative polyacrylamide gels. The IgG fraction of the antisera was purified by DEAE ion-exchange chromatography (6). The protocol for biotinylating the antibody is described elsewhere (7). The avidin-biotinylated ADNA complex was prepared fresh before use by mixing avidin and the biotinylated ADNA at a molar ratio of 1:4. Avidin (2 g/L) was first diluted with bovine serum albumin (BSA) diluent to 2 µg/L, and then 10 µL was mixed with 2 µL of the biotinylated ADNA (120 mg/L). The mixture was incubated at room temperature for 10 min and then further diluted with BSA diluent before use.

The protocol for immuno-PCR was a modified version of the modified protocol of Chang and Huang (8) (Fig. 1A). Briefly, microtiter plates were coated with 50 µL/well of anti-protein A IgG (10 mg/L in phosphate-buffered saline) for 1 h at 37 °C, washed five times, and blocked with 75 µL of the BSA diluent for 1 h at 37 °C. The plates were washed five times, and 50 µL of 10-fold serial diluted antigen (protein A; 10⁻²–10⁻¹⁸ g/mL) was added to each well. After incubation for 1 h at 37 °C, the plates were washed five times, and 50 µL of the biotinylated anti-protein A (1 µg/L) was added to each well. The plates were incubated at 37 °C for 1 h and then washed 12 times; 50 µL of the avidin-biotinylated ADNA complex (1:10 000 dilution) was then added to each well. The plates were again incubated at 37 °C for 1 h and washed 12 times before the PCR step. The negative control was performed in the same way, except that the BSA diluent was substituted for the antigen solution. The buffer used throughout for plate washing was phosphate-buffered saline containing 0.5 mL/L Tween 20, whereas the BSA diluent was used for the dilution of antigen, biotinylated antibody, avidin, and the avidin-biotinylated ADNA complex.

PCR was performed under the following conditions: 50 µL of the isolate that underwent immuno-PCR contained 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂, 0.01 g/L gelatin, 0.2 mM deoxynucleotide triphosphates, 1.0 µM primers (5'-GATGAGTTCTGTGGTGCAACAATCGG-3' and 5'-GGTTCAGAAATCAGCCACAGGCCG-3'), and 2.5 µL of Taq DNA polymerase. PCR was run in a 96-well thermal cycler (OmniGen; Hybaid Limited), which was compatible with the microtiter plate, using the following temperature profile: initial denaturation step at 95 °C for 2 min; 30 cycles of denaturation (95 °C for 1 min), annealing (55 °C for 1 min), and extension (72 °C for 1 min); and final extension (72 °C for 5 min). The two primers flank a segment of the ADNA gene and should generate a 500-bp product on PCR amplification. PCR for the positive control was performed in the same way as for the negative

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**Fig. 1.** Schematic representation of immuno-PCR assay (A), and detection of protein A from *S. aureus* by immuno-PCR (B).

PCR products were obtained from 50 µL of 10-fold serial dilutions of antigen at concentrations from 1 × 10⁻⁹ g/mL (lane 1) to 1 × 10⁻¹⁸ g/mL (lane 10). Lane 11, negative control; lane 12, positive control; lane M, 100-bp ladder of DNA molecular weight markers.
control except that 0.5 ng (1 ng/μL) λDNA was added to the PCR reaction mixture before the PCR step. The PCR product was analyzed by 2.0% agarose gel electrophoresis, and after staining with ethidium bromide, the gels were viewed with a digital imaging system (IS-1000; Alpha Innotech Corporation).

A specific 500-bp product was observed in the lanes that contained antigen at concentrations >10^{-17} g/mL (Fig. 1B, lanes 1–10). The antigen was detected at 10^{-17} g/mL (Fig. 1B, lane 9), or 144 antigen molecules/mL (7 molecules/50 μL), assuming a molecular weight of 42 000 for the antigen. However, the 500-bp band was not visible in lanes 10 (antigen concentration, 10^{-18} g/mL) and 11 (negative control; Fig. 1B). This indicates that the biotinylated λDNA was specifically bound to the biotinylated anti-protein A through the bridging of avidin.

The patient specimens were all analyzed by the conventional method, ELISA, and immuno-PCR. Swab specimens from the wounds of patients were collected from Veterans General Hospital (Taipei, Taiwan). Of nine patient specimens analyzed, six were positive for S. aureus, and three were negative.

Several immuno-PCR methods have been developed for the detection of a variety of antigens and antibodies. Compared with ELISA, an enhancement in detection sensitivity ranging from 100-fold (9) to 10^5-fold (10) was obtained by immuno-PCR, and some claim that the methodology has the potential to detect very low concentrations of antigens such as cancer markers (11), cytokines, and neuroactive peptides (12).

In conclusion, we have described a method for the rapid detection of S. aureus by immuno-PCR using anti-protein A antibodies. Although immuno-PCR is commonly used for the detection of Escherichia coli (8) and other bacteria (13,14), we demonstrate that a modified immuno-PCR method can also detect S. aureus in patient specimens.

References

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Elimination of the Interference from Aminoglycoside Antibiotics in the Pyrogallol Red-Molybdate Protein Dye-Binding Assay, Thomas Marshall* and Katherine M. Williams (Analytical Biochemistry Group, Sunderland Pharmacy School, The University of Sunderland, Sunderland SR1 3RG, UK; *author for correspondence: fax 44-191-515-3405, e-mail tom.marshall@sunderland.ac.uk)

The Pyrogallol Red-molybdate (PRM) protein dye-binding assay is used for urinary protein determination (1–3). The assay is sensitive to interference from aminoglycoside antibiotics, and the extent of interference varies with different PRM reagents (1, 2, 4–7). Thus, the Dade Behring PRM and the Sigma PRM assays are sensitive to aminoglycoside interference, whereas the Cobas Fara and Roche Integra 700 PRM assays are not (4, 5). Furthermore, the PRM reagents used by Fujita et al. (1) and Orsonneau et al. (3) are more sensitive to interference than the reagent used by Watanabe et al. (2). We have previously shown that the addition of sodium dodecyl sulfate (SDS) to the PRM reagent increases the interference, whereas the addition of sodium oxalate reduces the interference (6, 7). In the present study, we indicate that the extent of interference also depends on the concentration of dye in the reagent and demonstrate that a decrease in the dye concentration combined with an increase in the sodium oxalate concentration eliminates the interference.

The aminoglycosides (Sigma-Aldrich) were dissolved in water at a concentration of 10 g/L. The neomycin and gentamicin were further diluted to 1 g/L. Pierce Predicted Protein Assay Standard (1 g/L bovine serum albumin) was purchased from Perbio Science UK Ltd. Urine control (cat. no. AU2353) was purchased from Randox Laboratories Ltd. and reconstituted in deionized water or aqueous aminoglycoside (final concentration, 0.2 or 1.0 g/L). The Watanabe dye reagent was freshly prepared by dissolving 5.9 g of succinic acid and 0.5 g of sodium benzoate in 900 mL of water and mixing with 40 mL of dye solution [60 mg of Pyrogallol Red (cat. no. P8759; Sigma-Aldrich Co. Ltd.) in 100 mL methanol]. We then added 4 mL of 2.4 g/L disodium molybdate and 4 mL of 35 g/L sodium oxalate, adjusted the mixture to pH 2.5 with 0.5 mol/L hydrochloric acid, and brought the final volume to 1 L with deionized water (2). We modified the