The Newly Developed Monoclonal Antibody SA7D6 Exhibits Potential for Detection of *Staphylococcus aureus*

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Abstract Lack of a monoclonal antibody (MAb) with a high specificity and sensitivity against *Staphylococcus aureus* hampers easy and fast detection of the pathogen. Cell wall proteins of *S. aureus* were used for immunization and spleenocytes were fused with SP2/0-Ag14 cells. After cloning, SA7D6 hybridoma cells were selected for characterization due to high specificity to *S. aureus* surface proteins. SA7D6 reacted with all *S. aureus* reference strains and 100% of food and human isolates (50/50), but not with other species. Western Blotting revealed that SA7D6 reacted with a specific 75 kDa antigen but not with protein extracts of other bacterial species. The sensitivity of SA7D6 in ELISA was 10^2 and 10^3 CFU/mL in pure culture and sterile milk, respectively, which was 100× more sensitive than commercially available MAb. The newly developed SA7D6 was specific and has potential for detection of *S. aureus*.

Keywords: *S. aureus*, pathogenic bacteria, monoclonal antibody, enzyme-linked immunosorbent assay, Western Blot

Introduction

Staphylococcus aureus is a Gram-positive bacterium that is a common cause of skin infections, respiratory disease, and food poisoning (1). Particularly in food poisoning, it is ranked in the top third among all reported food poisoning cases (2). In the United States, hospital expenses, loss of productive time, and post-occurrence sanitation account for

costs of 1.5 billion \$ per annum (3). More than 20 staphylococcal enterotoxins have been described to date and half of these toxins are known hazards for consumers (4). These enterotoxins are pepsin resistant and heat stable and, therefore, are not inactivated by simple exposure to heat (1,5,6). A level as low as 10^4 CFU/g is required for an outbreak of *S. aureus* (7). Thus, early detection and prevention of *S. aureus* contamination in foods is pivotal. Conventional culture methods for *S. aureus* detection are complicated, laborious, and time-consuming (8). PCR-based detection systems are relatively fast and sensitive, theoretically being able to detect a level of 1 CFU/g. However, PCR systems require special equipment and well-trained personnel for accurate results.

Antibody-based detection systems can be fast and easy-to-use alternatives. A monoclonal antibody (MAb) with appropriate selectivity and specificity can be effectively used in detection protocols for specific bacteria (9–11), while the majority of *S. aureus* MAb systems (MAbs) were designed to detect enterotoxins, which neutralize the pathogen in a clinical context (12–16). A few MAbs were reported to detect *S. aureus* whole cells. However, these systems exhibited relatively low specificity and low sensitivity values to *S. aureus*. Wiriyaichaiyorn *et al.* (17) reported development of an *S. aureus* specific antibody with a sensitivity of 10^6 CFU/mL using a lateral flow technique. Yamada *et al.* (18) also developed chicken IgY for use against the PBP2a protein and showed that the sensitivity was greater than 1×10^6 CFU/well using ELISA. Yazdankhah *et al.* (19) showed that the 3 clones 2F3, 5G6, and 5G3 cross-reacted with other *Staphylococcus* species with a detection sensitivity of $>10^5$ CFU/mL using ELISA. In this study, *S. aureus*-specific MAb SA7D6 was developed using cell wall associated proteins for immunization. Characterization of SA7D6 showed no reaction with non-*S. aureus* bacterial species and a high specificity to *S.*

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aureus and a broad reaction spectrum with a number of *S. aureus* isolates. SA7D6 detected *S. aureus* at a level of 10^2 CFU/mL in a pure culture using ELISA, which will lead to development of different detection systems for *S. aureus*.

Materials and Methods

Bacterial strains and culture conditions The *Staphylococcus* strains used in this study included the 8 *S. aureus* reference strains ATCC 25923, ATCC 12600, KACC 10768, ATCC14458, ATCC 9144, ATCC 27664, ATCC 29213, and ATCC 6538, 50 *S. aureus* food and human isolates, *S. simulans* ATCC 11631, *S. xylosus* ATCC 29971, and *S. leutus* ATCC 49574. In addition, the Gram-positive bacteria *B. cereus* ATCC 14579, *B. licheniformis* JCM 2505, *B. subtilis* KACC 12680, *L. monocytogenes* EGDe, and *L. monocytogenes* ScottA and the Gram-negative bacteria *E. coli* O157:H7 ATCC 35150, *E. coli* O157:H7 ATCC 43890, *E. coli* O157:H7 ATCC 43894, *E. coli* O157:H7 ATCC 43895, *E. coli* O169, *E. coli* O112ac, *Salmonella* Typhimurium ATCC 14028, and *Cronobacter sakazakii* ATCC 29544 were also used (Strains from the Department of Food Science and Technology, Chonbuk National University, Jeonju, Korea). All bacteria were grown on Luria-Bertani (Difco Laboratories Inc., Franklin Lakes, NJ, USA) medium at 37°C with shaking (Wise Cube WIS-20; DAIHAN Scientific Co., Ltd., Seoul, Korea) at 160 rpm for 12 h, unless otherwise indicated.

Protein isolation from Gram-positive bacteria Bacteria were grown on trypticase soy broth (TSB) (DAIHAN Scientific Co., Ltd.) for 16 h at 37°C with shaking (Wise Cube; DAIHAN Scientific Co., Ltd.) at 160 rpm. Extraction of cell wall associated proteins was carried out according to He and Buck (20) with minor modification. Briefly, cells from 1 L of culture were harvested using centrifugation (CR22GIII; Hitachi, Tokyo, Japan) at $4,400\times g$ for 10 min and washed with an NaCl solution (0.16 M). The wet weight of the cell pellet was determined by weight measurement (PC3002; Mettler Toledo, Greifensee, Switzerland) and for each g of bacteria, 1 mL of lysis buffer (0.05 M potassium phosphate (P5629; Sigma-Aldrich, St. Louis, MO, USA), 0.022% [v/v] β -mercaptoethanol (M3148; Sigma-Aldrich), pH 6.5) was added, followed by addition of lysozyme (Bioshop, Burlington, Canada) to a final concentration of 2.4 mg/mL. Cells were incubated (SSI-203; Shin Saeng Scientific Co., Ltd., Seoul, Korea) at 37°C for 2 h, then disrupted 6 times for 1.5 min each time using a bead beater (Biospec, Bartlesville, OK, USA). The lysate was centrifuged (CR22GIII; Hitachi) at $600\times g$ for 10 min to remove cell debris. To obtain the cell wall fraction, centrifugations (CR22GIII; Hitachi) was repeated 5 times at $22,000\times g$ for

40 min. All pellets were re-suspended and pooled in lysis buffer and the pooled pellet was subjected to a second round of lysis, as described above. The sample was centrifuged (CR22GIII; Hitachi) at $22,000\times g$ for 20 min at 4°C, then the pellet was re-suspended in PBS (137 mM NaCl (S7653; Sigma-Aldrich), 2.7 mM KCl (P9333; Sigma-Aldrich), 10 mM Na_2HPO_4 (S8282; Sigma-Aldrich), 2 mM KH_2PO_4 (P5655; Sigma-Aldrich)) and a protease inhibitor (P8465; Sigma-Aldrich) was added according to the manufacturer's instructions (1 mL of inhibitor/4 g of cells). Proteins were resolved in polyacrylamide gel electrophoresis and stained with Coomassie blue.

Protein isolation from Gram-negative bacteria Membrane protein preparation from Gram-negative bacteria was performed according to Thein *et al.* (21) with minor modification. Bacteria were grown on TSB (BBL) for 16 h at 37°C with shaking (Wise Cube WIS-20; DAIHAN Scientific Co., Ltd.) at 160 rpm. Cells from 1 L of culture were harvested by centrifugation (CR22GIII; Hitachi) at $4,400\times g$ for 10 min at 4°C. The cell pellet was re-suspended in 500 μL of resuspension buffer (0.2 M Tris-HCl (T5941; Sigma-Aldrich) pH 8.0, 1 M sucrose (S5016; Sigma-Aldrich), 1 mM EDTA (E3889; Sigma-Aldrich)), then 100 μL of a lysozyme solution (Bioshop) (5 mg/mL in distilled water) (dH_2O) was added, vortexed and incubated for 5 min at room temperature (RT). Two mL of dH_2O was added, followed by incubation for 20 min at RT until spheroblast formation was observed under a microscope. Then, 3 mL of lysis buffer (50 mM Tris-HCl (T5941; Sigma-Aldrich) pH 8, 0.2% [w/v] Triton X-100 (USB, Cleveland, OH, USA), 10 mM MgCl_2 (M2670; Sigma-Aldrich), and 50 μL of DNase I (AppliChem GmbH, Darmstadt, Germany) (1 mg/mL in dH_2O) was added and mixed and kept on a rocker (Labogen, R100) at 25 rpm until the suspension became clear. The mixture was ultra-centrifuged (LE-80K; Beckman, Brea, CA, USA) at $85,000\times g$ for 30 min at 4°C. The pellet containing the outer membrane fraction was washed using 750 μL of washing buffer (50 mM Tris-HCl (T5941; Sigma-Aldrich) pH 8.0, 2% [w/v] Triton X-100 (USB), 10 mM MgCl_2 (M2670; Sigma-Aldrich)), centrifuged at $85,000\times g$ for 20 min at 4°C, washed in 500 μL of dH_2O 3 times, then resuspended in PBS (137 mM NaCl (S7653; Sigma-Aldrich), 2.7 mM KCl (P9333; Sigma-Aldrich), 10 mM Na_2HPO_4 (S8282; Sigma-Aldrich), 2 mM KH_2PO_4 (P5655; Sigma-Aldrich)). A protease inhibitor (P8465; Sigma-Aldrich) was added and resulting aliquots were stored at -20°C . The protein concentration was determined following the Bradford assay method according to manufacturer's instruction (B6916; Sigma-Aldrich).

Immunization The cell wall fraction of *S. aureus* ATCC 25923 was used to immunize BALB/c mice, 5 mice per

treatment (DBL, Eumseong, Korea). The total cell wall fraction (100 µg per animal) was mixed with an equal volume of Complete Freund's adjuvant (F5881; Sigma-Aldrich) for primary intraperitoneal (i.p) injection at lower left quadrant of abdomen. Every 2 weeks, boosting was performed as above except with use of Incomplete CFA (F5506; Sigma-Aldrich). Blood were withdrawn using tail vein and antibody titers were checked in blood serum in ELISA. When the serum antibody titer reached 1:10,000 (using ELISA, see below), animals were euthanized by carbon dioxide asphyxiation. Spleens were aseptically removed and spleenocytes were collected for fusion (22). All institutional and national guidelines for the care and use of laboratory animals were followed.

Fusion and cloning Fusion and cloning were performed according to the procedure of Galfre (23), with minor modification. Briefly, spleenocytes from immunized mice were fused with Sp2/0-Ag14 cells (KCLB, Seoul, Korea) using 50% (w/v) polyethylene glycol (PEG 1450 mw HybriMax; Sigma-Aldrich). Fused cells were grown in a selective medium containing hypoxanthine, aminopterin, and thymidine (H0262; Sigma-Aldrich) in Dulbecco's Modified Eagle's Medium (DMEM; Lonza, Walkersville, MD, USA) with 20% fetal bovine serum (FBS; Serana WA Pty Ltd., Bunbury, Australia), 10 mM HEPES (H4034; Sigma-Aldrich), and 1 mM sodium pyruvate (P5280; Sigma-Aldrich) in 96 well tissue culture plates (Falcon One, Inc., Franklin Lakes, NJ, USA) (Supernatants from hybridoma-growing wells were screened for reaction with the cell wall fraction of *S. aureus* in an indirect enzyme-linked immunosorbent assay (ELISA). Hybridoma of interest were cloned twice based on limiting dilution to 0.5 and 0.1 cell per well in DMEM containing 20% FBS, 10 mM HEPES, and 1 mM sodium pyruvate and were confirmed for antibody production.

Commercial *S. aureus* monoclonal antibodies Commercially available MAb (Product number SC 80945; Santa Cruz Biotechnology, Santa Cruz, CA, USA) reactive with *S. aureus* was used to assess sensitivity in ELISA.

ELISA ELISA plates were coated with proteins (100 ng/well) or bacterial cells in a coating buffer (0.05 M NaHCO₃ (S5761; Sigma-Aldrich)/Na₂CO₃ (S7795; Sigma-Aldrich), 9.5 pH) overnight at 4°C. For preparation of bacteria, fresh medium was added with overnight culture of bacteria (2% inoculum) and incubated as above. When the optical density at 600 nm (OD₆₀₀) reached 0.6, (Libra S22; Biochrom, Cambridge, UK) bacteria were collected by centrifugation in microcentrifuge (Vision, VS-15000N) at 15000×g for 5 min and washed 2 times with PBS (137 mM NaCl (S7653; Sigma-Aldrich), 2.7 mM KCl (P9333;

Sigma-Aldrich), 10 mM Na₂HPO₄ (S8282; Sigma-Aldrich), 2 mM KH₂PO₄ (P5655; Sigma-Aldrich)), then resuspended in a coating buffer. Wells were washed twice with a washing buffer (0.01 M PBS-T, pH7.2). Blocking was performed using a blocking buffer (0.10 M PBS with 3% BSA (Affymetrix Inc., Santa Clara, CA, USA), pH 7.5) for 2 h. Wells were washed 3× with 200 µL of washing buffer and the culture supernatant (primary antibody) at a 1:100 dilution for hybridoma screening (or 1 µg/mL of purified MAb) was added, followed by 2 h of incubation at RT. After washing as above, horseradish peroxidase-conjugated goat-anti mouse IgG (1:10000 in PBS-T) (Life Technologies Inc., Carlsbad, CA, USA) was added, followed by incubation for 2 h at room temperature. Wells were washed as above and a 3,3',5,5' tetramethylbenzidine (TMB; Sigma-Aldrich) substrate was added, followed by 15–45 min of incubation at room temperature. The reaction was stopped using addition of 2 N HCl and OD values were measured at 450 nm using a microplatereader (BioTeck, Winooski, VT, USA).

Detection of *S. aureus* in milk *S. aureus* cells prepared as above using ELISA were added to sterile milk (Seoul Milk) to obtain 10⁶, 10⁵, 10⁴, 10³, and 10² CFU/mL, which was used to coat ELISA plates either directly or after centrifugation (Vision, VS-15000N) at 10,000×g for 10 min at 4°C, followed by re-suspension in a coating buffer. ELISA was then performed as described above.

Ascitic MAb production Ten BALB/c mice were primed using i.p. injection of 2,6,10,14-tetramethyl pentadecane (Pristane; Sigma-Aldrich) (0.5 mL each). After 10 days, mice were injected with 2×10⁶ hybridoma cells and ascetic fluid was collected 20 days post-injection. First, abdominal area was sterilized using 70% ethanol. Lower abdominal quadrant were pierced using sterile 18G needle and dripping fluid was collected in to a 15 mL sterile falcon tube. Cell debris was removed by centrifugation (CR22GIII, Hitachi) at 1500×g for 10 min and antibody containing ascetic fluid was membrane filtered and made in to aliquots and stored in −20°C.

Purification of MAb MAb from ascetic fluid was purified using a HiTrap protein G column (GE Healthcare Bio-Sciences, Little Chalfont, UK) according to the manufacturer's instructions. Briefly, ascetic fluid was centrifuged (CR22GIII; Hitachi) at 10,000×g for 20 min, and a binding buffer (20 mM sodium phosphate, pH 7) was added to the supernatant at a 3:1 ratio before the solution was applied to the column, which was washed 5–10× using a binding buffer, followed by elution of the antibody with an elution buffer (0.1 M glycine-HCl (G8898; Sigma-Aldrich) pH 2.7). Fractions containing the antibody were dialyzed

against a 20 mM phosphate buffer (pH 7.0). The antibody concentration was determined following the bicinchoninic acid (BCA) method, according to manufacturer's instruction using a Pierce BCA assay kit (Thermo Scientific, Rockford, IL, USA).

Immunoglobulin isotyping Immunoglobulin isotyping was performed using a mouse immunoglobulin isotyping kit (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions.

Immunoblotting Purified cell wall associated proteins were separated using SDS-PAGE. Resolved proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane (Roche, Indianapolis, IN, USA) using a semi-dry transfer apparatus (ATTO-AE6687 ATTO Co., Tokyo, Japan) (300 mA, 15 V for 20 min). Blocking was performed using 5% skim milk (70166; Sigma-Aldrich) for 2 h in TBS-T (10 mM Tris (T1503; Sigma-Aldrich), 125 mM NaCl (S7653; Sigma-Aldrich), 0.01% Tween (P1379; Sigma-Aldrich) at RT, followed by washing with 0.01% TBS-T. MAb was added, followed by incubation for 2 h at room temperature, then by washing 3× with TBS-T. Alkaline phosphatase-conjugated goat-anti mouse IgG (Life Technologies) was added at a 1:20,000 dilution, followed by incubation for 2 h at RT, then by washing 4× for 40 min with TBS-T. A BCIP/NBT substrate system (B5655; Sigma-Aldrich) was used for visualization.

Statistical analysis All experiments were carried out in triplicate. Comparisons of mean values were conducted using Duncan's multiple range test at $p < 0.05$ with SAS software (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Mouse MAb SA7D6 was developed using the cell wall fraction of *S. aureus* ATCC 25923 Cell wall associated proteins were purified from *S. aureus* ATCC 25923 and resolved in SDS PAGE (Fig. 1A), which were used for immunization, followed by fusion and cloning. MAb SA7D6 reacted with cell wall associated proteins of *S. aureus* (OD450 of 2.331) but not with surface proteins of gram negative and gram positive non-*S. aureus* species (OD450 of <0.057) in ELISA (Fig. 1B). In addition, isotyping of SA7D6 revealed the presence of IgG2b and lambda for heavy and light chains, respectively (data not shown).

SA7D6 exhibited reactivity specific for *S. aureus* The specificity of SA7D6 was further confirmed using ELISA with reference strains of Gram-negative (*S. Typhimurium*, *C. sakazakii*, *E. coli* O157:H7, *E. coli* O169, and *E. coli* O112ac) (Fig. 2A), and Gram-positive (*L. monocytogenes* EGDe, *L. monocytogenes* ScottA, *B. licheniformis* JCM 2505, *B. subtilis* KACC 1268, and *B. cereus* ATCC 14579) bacteria (Fig. 2B). SA7D6 reacted only with *S. aureus* strains that exhibited OD450 values of 1.717 to 4.47, but not with tested non-*Staphylococcus* species (OD450 <0.076). In addition, SA7D6 did not react with the non-*S. aureus* *Staphylococcus* species *S. stimulans*, *S. xylosus*, and *S. leutus* (Fig. 2B).

SA7D6 specifically reacted with a single antigen with an approximate size of 75 kDa found on *S. aureus* using Western Blot analysis. No cross reaction was observed with antigens of the other test species *B. cereus*, *L. monocytogenes*, *E. coli* O157, *E. coli* O169, *E. coli* O112ac, or *C. sakazakii*

Fig. 1 (A) SDS PAGE of cell wall associated proteins isolated from *S. aureus* ATCC 25923, stained using Coomassie Blue. Lane 1, marker; L2, L3, and L4 for 3, 2, and 1 µL of isolated proteins, respectively; L5, L6, and L7 for 1, 2 and 3 µg of BSA, respectively. (B) The specificity of SA7D6 toward purified surface proteins of *S. aureus* ATCC 25923 and other Gram-positive and negative strains in ELISA.

Fig. 2. Reactivity of SA7D6 using Gram-positive and Gram-negative reference strains evaluated using ELISA (A and B) and Western Blotting using whole cell extracts (C). L1, marker; L2, Coomassie Blue stained proteins of *S. aureus* ATCC 25923; L3, *B. cereus* ATCC 14579; L4, *L. monocytogenes* EDGe; L5, *S. aureus* ATCC 25923; L6, *S. Typhimurium* ATCC 14028; L7, *E. coli* O157:H7 ATCC 35150; L8, *E. coli* O157:H7 ATCC 43890; L9, *E. coli* O157:H7 ATCC 43894; L10, *E. coli* O157:H7 ATCC 43895; L11, *E. coli* O169; L12, *E. coli* O112ac; L13, *C. sakazakii* ATCC29544.

(Fig. 2C). The specificity of SA7D6 to *S. aureus* was confirmed based on no cross reaction with any tested non-*S. aureus* bacterial species. The exact identity of the target was not identified, but the antigen must be exposed on the surface and be specific to *S. aureus*.

Many MAbs have been developed for *S. aureus* using recombinant, synthetic proteins (12) or attenuated whole bacterial cells (24). In this study, purified cell wall proteins instead of whole cells were used in order to avoid suppression of the immune response by the polysaccharide capsule that is usually present in pathogenic strains of *S. aureus* (25,26) to provide a variety of protein antigens for mouse immune system development using different lymphocytes for better selection of clones.

SA7D6 reacted to all *S. aureus* environmental isolates

The reactivity of SA7D6 was tested using *S. aureus* isolates (25 food and 25 human isolates) with ELISA (Fig. 3). SA7D6 was reactive with all 50 isolates, but absorbance values varied among isolates ranging from 0.564 to 4.7. Forty-eight isolates (96%) showed OD450 values greater than 1.5 and the 2 isolates 93 and 96 had values of 0.777 and 0.564 respectively, which were significantly ($p < 0.05$) higher than for the negative control *E. coli* (OD450=0.156) (Fig. 3A and B). Thus, SA7D6 exhibited broad spectrum reactivity among *S. aureus* isolates.

Different reactivity values of *S. aureus* isolates with SA7D6 could have been due to differences in antigen expression among isolates (27). On the other hand, the presence of a polysaccharide capsule that inhibited the antigen-antibody interaction was also a possibility (28). The microstructure of the cell envelope could also vary between isolates (25,26). Nevertheless, SA7D6 was able to overcome hindrances for detection of all *S. aureus* isolates tested, which is essential for detection of *S. aureus* in the environment.

SA7D6 detected a level of 10^2 CFU/mL *S. aureus* using ELISA

The sensitivity of SA7D6 ($1 \mu\text{g/mL}$) for detection of *S. aureus* was evaluated using ELISA. When 10^6 , 10^5 , 10^4 , 10^3 , and 10^2 CFU/mL of *S. aureus* pure cultures were coated on ELISA plates, OD450 values after 15 min of incubation were 2.24, 1.69, 0.54, 0.136, and 0.111, respectively, which were significantly ($p < 0.05$) higher than the values of 0.049, 0.034, 0.017, 0.03, and 0.03 for *C. sakazakii* used as a negative control (Fig. 4A). No significant ($p > 0.05$) difference was observed with an extended incubation time up to 45 min for *S. aureus* against the control *C. sakazakii* (Fig. 4B).

When a commercially available MAb for *S. aureus* was used under the same experimental conditions, ELISA values were 2.26, 1.41, 0.109, 0.056, and 0.058 for 10^6 ,

Fig. 3. ELISA reactivity of SA7D6 with food (A) and human isolates (B) of *S. aureus* whole cells.

10^5 , 10^4 , 10^3 , and 10^2 CFU/mL of *S. aureus*, respectively (Fig. 4A), confirming a detection limit of 10^4 CFU/mL. An extended incubation time for color development did not

result in a significant ($p > 0.05$) difference between the original and extended times (Fig. 4B). Thus, SA7D6 will be an appealing choice among MAbs for *S. aureus* detection.

SA7D6 detected a level of 10^3 CFU/mL of *S. aureus* in sterile milk using ELISA The sensitivity of SA7D6 was evaluated using ELISA with sterile milk. When artificially contaminated milk was directly coated on to ELISA plates, OD450 values for 10^6 , 10^5 , 10^4 , and 10^3 CFU/mL of *S. aureus* were 1.06, 0.95, 0.23, and 0.13, respectively. Control (*C. sakazakii*) values were 0.03, 0.01, 0.04, and 0.03, respectively. A significant ($p < 0.05$) difference was observed between *S. aureus* and *C. sakazakii* (negative control) for all contamination levels, except at 10^2 CFU/mL (Fig. 5A). Similar results were found when a coating buffer was used after collection of *S. aureus* by centrifugation. Prolonged incubation with the substrate did not increase the sensitivity. In contrast, a commercial antibody detected levels of 10^6 and 10^5 CFU/mL of *S. aureus* in a 15 min incubation after addition of substrates (Fig. 5A and B) when coating was done using milk and a coating buffer, respectively. With prolonged incubation, the detection limit was reduced to 10^4 CFU/mL, regardless of the coating conditions. Lower sensitivity values with milk samples than under pure culture conditions (Fig. 4) were probably due to the presence of fat and other nutrients present in milk that imposed a covering effect over cell surface receptors.

Fast and sensitive detection techniques are dependent upon use of a good capturing molecule that interacts with the specific antigen of interest. These techniques include lateral flow immunoassay, magnetic bead separation, and use of biosensors (11,29). For example, MAb against *B. anthracis* detected a level of 10^2 spores/mg in milk using a lateral flow immune assay (28) and *C. mytjensii* detected a level of 2.0×10^4 CFU/mL using an ELISA-based detection

Fig. 4. Sensitivity testing for SA7D6 using indirect ELISA and comparison with commercial MAb. (A) OD450 values after 15 min incubation with the TMB substrate. (B) OD450 values after 45 min incubation with the TMB substrate.

Fig. 5. Sensitivity testing for SA7D6 using sterile milk with indirect ELISA and comparison with commercial MAb. (A) ELISA plates were coated using bacteria suspended in milk and OD450 values were measured after 15 min of incubation with the TMB substrate. (B) ELISA plates were coated using bacteria re-suspended in a coating buffer after centrifugation, and OD450 values were measured after 15 min of incubation with the TMB substrate.

method (10). On the other hand, lack of good MAb with high specificity and sensitivity values against *S. aureus* causes difficulty for easy and fast detection of the pathogen (30). For example, a MAb coated latex and capillary electrophoresis method is available for *S. aureus* with a sensitivity of 9×10^5 CFU/mL (11). The levels of specificity and sensitivity demonstrated by SA7D6 in this study provided advantage over other available MAbs for detection of *S. aureus* in food and environmental samples.

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Disclosure The authors declare no conflict of interest.

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