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Quantitative Determination of Staphylococcal Enterotoxin A by an Enzyme-Linked Immunosorbent Assay Using a Combination of Polyclonal and Monoclonal Antibodies and Biotin-Streptavidin Interaction

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A sandwich enzyme-linked immunosorbent assay to detect staphylococcal enterotoxin A (SEA) was developed by using monoclonal antibodies (MAb) to SEA as primary capture antibodies. The antigen was detected with purified rabbit anti-SEA antibody as the secondary antibody. The secondary antibody was identified by direct conjugation with biotin or via biotinylated sheep $F(ab')_2$ fragments to rabbit antibody. The biotin was then reacted with avidin-alkaline phosphatase (AP) conjugate, avidin-biotin-AP conjugated complex, or streptavidin-AP conjugate. The enzyme was identified by using *p*-nitrophenylphosphate. The incorporation of the avidin-biotin-AP conjugated complex or streptavidin-AP conjugate augmented the sensitivity 32-fold over that of the enzyme-linked immunosorbent assay without these reagents. Controls were run by substitution of the anti-SEA MAb with unrelated MAb of the same isotype. Sample values were considered positive when the A_{405} exceeded those of the negative controls by 3 standard deviations (>99% confidence interval). The toxin could be quantitated with purified SEA standards through linear regression analysis with lower detection limits of 4 ng/ml ($r = 0.99$) and 0.25 ng/ml ($r \geq 0.98$). Concentrations of protein A up to 10 μg/ml did not cause interference. Analyses of crude growth extracts of SEA-secreting strains of *Staphylococcus aureus* were reproducible and were expressed in terms of 95% confidence intervals. Lack of cross-reactivity was seen with extracts of other toxigenic and nontoxigenic strains of *S. aureus*. The assay can be completed in one working day, provided that MAb-coated plates are available.

Several reports have described the use of enzyme-linked immunosorbent assays (ELISA) for the detection of staphylococcal enterotoxins A, B, C, and D (SEA, SEB, SEC, and SED [2, 9, 10, 16, 19, 24]). However, most of these immunoassays have frequently been hampered by false-positive results elicited by extraneous protein contaminants, including protein A, associated with a large percentage of *Staphylococcus aureus* strains. Attempts to eliminate this lack of specificity often involved tedious, time-consuming processing of either the constitutive reagents (19) or the test samples analyzed for the presence of toxin (2, 8).

In this communication we describe the development of an ELISA for the detection of SEA, the serotype most commonly implicated in staphylococcal food poisoning (3). The format is a version of the double-antibody sandwich ELISA. A primary monoclonal antibody (MAb) in concert with a secondary polyclonal antibody that was directly or indirectly biotinylated were used for detection of the antigen. Three different combinations of biotin with avidin or streptavidin conjugated to alkaline phosphatase (AP) were screened as detection probe (Fig. 1).

The sensitivity of the assay was increased through enzyme amplification by the incorporation of the biotin-avidin or streptavidin system. This system was previously used in immunocytochemical staining (1) and immunofluorescence (17) and is a common feature of solid-phase immunoassays (14).

The system was patterned on a quantitative basis, with the use of purified SEA standards to estimate levels of SEA in test samples. High correlations (>0.98) over a linear range of 0.25 to 250 ng of purified toxin per ml were obtained.

MATERIALS AND METHODS

Antibodies. Hybridomas were produced by fusing spleen cells from BALB/c mice hyperimmunized with purified SEA and murine myeloma cells (6). Clones testing positive for MAb reacting with SEA were cultured in growth medium consisting of Iscove modified, Dulbecco modified Eagle medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 15% fetal calf serum, 0.002 mM ethanolamine, 12 mM hypoxanthine, 8 mM thymidineglycine, 100 mM sodium pyruvate, 0.5% amphotericin B, and 0.1% gentamicin sulfate. The supernatants from culture growth medium were dialyzed against 20 mM sodium phosphate-buffered saline (PBS; 20 mM sodium phosphate, 140 mM sodium chloride [pH 7.3]) for 24 h at 4°C and stored at −20°C. The MAb had an immunoglobulin G1 IgG1 heavy-chain isotype and κ light chains (4).

The MAbs were also partially purified by being precipitated three times with 40% ammonium sulfate solution and dialyzed against PBS.

Purification of rabbit antibodies. Partially purified rabbit antiserum to SEA (precipitated once with ammonium sulfate and dialyzed against PBS) was kindly provided by Jeffrey Parsonnet, Channing Laboratory, Boston, Mass. The antiserum was subjected to affinity chromatography over a protein A-Sepharose CL-4B column (Sigma Chemical Co., St. Louis, Mo.) as described by Goding (11).

Biotinylation of rabbit anti-SEA antibodies. The rabbit anti-SEA antibodies were biotinylated by the method described by Goding (13). The biotinylated antibody conjugates were stored either at 4°C or in an equal volume of glycerol at −20°C.

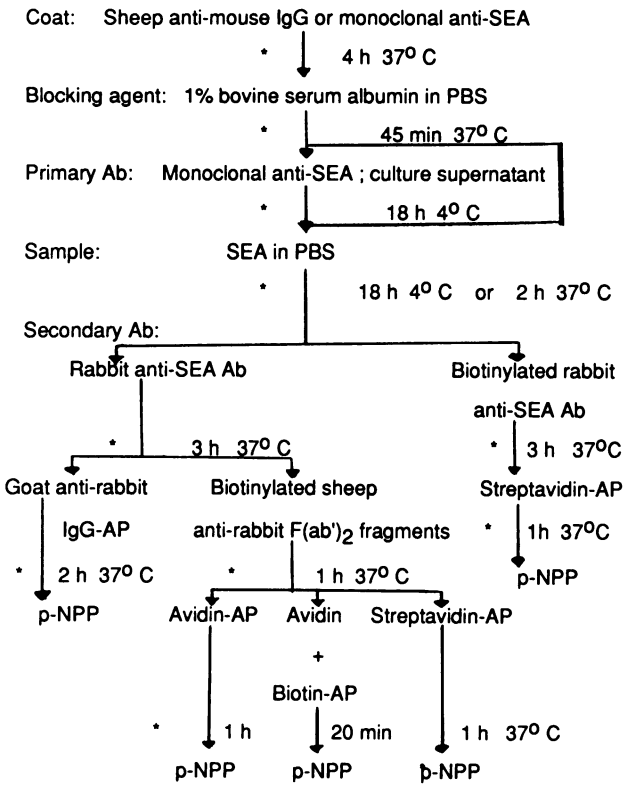

FIG. 1. Schematic of the variations of the ELISA for detection of SEA. Each procedure was optimized, and the conditions for every step in the assay are shown. Abbreviation: p-NPP, *p*-Nitrophenylphosphate. Symbol: *, three washes with PBS until the blocking stage, followed by washing with PBS–0.05% Tween 20.

Affinity-purified sheep anti-mouse IgG (heavy and light chain specific with no reaction to human IgG) and AP-conjugated goat anti-rabbit IgG were obtained from Cooper Biomedical, Inc., West Chester, Pa.

Biotin-avidin reagents. Biotinylated sheep $F(ab')_2$ fragments directed to rabbit IgG were obtained from Sigma. Unconjugated avidin and biotin-AP and avidin-AP conjugates were obtained from Cooper Biomedical. Streptavidin-AP conjugate was obtained from Zymed Laboratories, Inc., South San Francisco, Calif.

Antigens. All optimization was done with standards of purified SEA (Toxin Technology, Inc., Madison, Wis.). Two- or fourfold dilutions of SEA at concentrations ranging from 0.25 to 250 ng/ml were used. Clinical isolates of *S. aureus* strains (including secretors and nonsecretors of SEA and SEB [provided by Jeffrey Parsonnet]) were cultivated by the membrane-over-agar method (22). Crude culture extracts were obtained by harvesting the culture growth with PBS and pelletting the bacteria by centrifugation. The decanted supernatants were aliquoted, stored at −20°C, and tested for the presence of toxin.

ELISA. Flat-bottomed, 96-well polystyrene microdilution plates (Immunolon II; Dynatech Laboratories, Inc., Alexandria, Va.) were coated with sheep anti-mouse IgG at 2.5 μg/ml or partially purified MAb (1:50 dilution) in 50 mM sodium carbonate-bicarbonate buffer (pH 9.5) for 4 h at 37°C. After three washes with 10 mM PBS containing 0.01% sodium azide (pH 7.4), the wells were blocked with 1% bovine serum albumin in PBS for 45 min at 37°C. The wells were washed three times with PBS containing 0.05% Tween 20. The plates coated with sheep IgG were incubated overnight with MAb culture supernatant (1:50 dilution in PBS-Tween 20) at 4°C and then washed as described above. At this stage, the plates could be stored for at least 3 months at 4°C.

Dilutions of test samples (SEA) in PBS-Tween 20 were added in duplicate to the wells. The plates were incubated either for 2 h at 37°C or overnight at 4°C. The polyclonal secondary rabbit anti-SEA antibodies (unconjugated or conjugated to biotin) were added at a 1:200 dilution to the washed plates, which were then maintained at 37°C for 3 h.

Detection of rabbit anti-SEA antibodies. The unconjugated antibodies were probed by exposure to a 1:1,000 dilution of conventional goat anti-mouse-AP conjugate in PBS-Tween 20 for 2 h at 37°C or a 1:1,000 dilution of sheep $F(ab')_2$ fragments (directed to rabbit IgG) conjugated to biotin for 1 h at 37°C. The biotin was then reacted with avidin by one of three processes: (i) avidin-AP conjugate (1:1,000 dilution) for 1 h at 37°C; (ii) avidin-biotin-AP complex (ABAC) for 20 min at 37°C (the ABAC was formulated by preincubating equal volumes of avidin [1:800] and biotin-AP conjugate [1:1,000] for 20 min at 37°C before addition to the wells); or (iii) streptavidin-AP conjugate (1:1,000) for 1 h at 37°C.

The biotinylated rabbit anti-SEA antibodies were reacted with a 1:1,000 dilution of streptavidin-AP for 1 h at 37°C.

The wells were washed, and enzymatic activity was determined by reacting the contents with 1 mg of *p*-nitrophenylphosphate per ml (Sigma) (100 mM sodium carbonate-bicarbonate buffer with 1 mM $MgCl_2 \cdot 6H_2O$ [pH 9.5]). The enzyme-substrate reactivity was quenched with 50 μl of 3 M NaOH per well. The optical density at 405 nm (OD_{405}) was read with a Microelisa reader (no. EL307; Biotek, Burlington, Vt.).

Negative controls were run simultaneously by substitution of the SEA-directed MAb with an equivalent concentration of a different MAb with the same heavy- and light-chain isotypes (in this instance, anti-toxic shock syndrome toxin type 1 [TSST-1] MAbs with no reactivity to purified SEA).

The standard volume of reagents added per well, unless specified otherwise, was 200 μl.

Statistical analyses. The mean OD value for replicates of all samples was calculated, and standard curves were generated by linear regression analysis (21). The OD_{405} was transformed into logit units by the following formula: logit = log OD (test)/[OD (max) − OD (test)], where OD (test) is the OD of test samples and OD (max) is the maximum OD for infinite concentration of test samples (2.0). The concentration of purified SEA (in nanograms per milliliter) was represented in logarithm units.

A test sample was regarded as positive and the SEA levels were quantitated when the OD was greater than the mean OD plus 3 standard deviations (SD) of the negative controls. The log SEA concentration was converted to the antilog, adjusted for sample dilution, and expressed in nanograms per milliliter. The reproducibility of *n* determinations was expressed in terms of 95% confidence intervals by the following equation: 95% confidence interval = mean ± $1.96(SD/\sqrt{n})$.

RESULTS

Standard curves. All variations of this ELISA were conducted with two- or fourfold sequential dilutions of purified SEA standards. Linearity was observed in the range of 4 to 250 ng/ml by all assays. The correlation coefficient over the range of linearity was 0.97 to 0.99. The sensitivity (detection

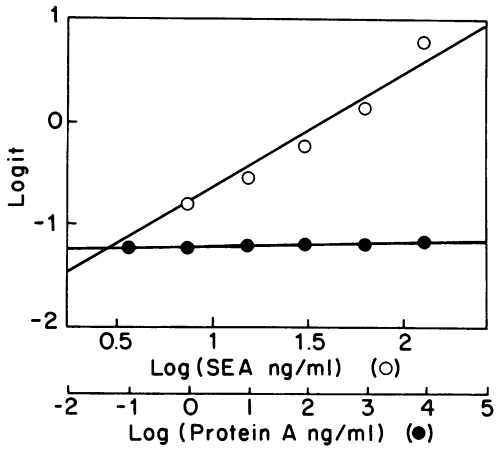

FIG. 2. Detection of SEA and protein A tested simultaneously by the conventional ELISA. Purified SEA standards in twofold dilutions were in the range of 8 to 126 ng/ml (log SEA concentrations, 0.9 to 2.1; $r = 0.98$). Tenfold dilutions of protein A were in the range of 0.1 to 10,000 ng/ml (log protein A concentrations, −1.0 to 4). Each datum point is the mean for duplicate wells. OD_{405} (y ordinate) were converted to logit units. The protein concentrations (x abscissa) were expressed in logarithm units.

limit) was found to differ with concentrations below 4 ng/ml with the different formats. The lowest concentration of toxin tested was 0.25 ng/ml. ODs which were greater than the mean plus 3 SDs of the control wells with anti-toxic shock syndrome toxin type 1 MAbs were considered positive.

ELISA format. The initial coating of the wells of the microdilution plate with sheep anti-mouse antibody was performed to enhance sensitivity. Checkerboard titrations with 5, 2.5, and 1.25 μg of the sheep antibody per ml indicated that 2.5 μg/ml was optimal, with low background reactivity and binding of sufficient MAb to detect SEA.

Initial experiments were performed to standardize levels of primary antibody (MAb) and secondary antibody (polyclonal rabbit anti-SEA) for the detection of SEA with conventional goat anti-rabbit AP conjugate used for identification of the secondary antibody. It was determined that a 1:50 dilution of MAb culture supernatant and a 1:200 dilution of purified rabbit anti-SEA antibody were optimal. A determination limit of 8 ng of SEA per ml (log SEA concentration, 0.9) was recorded (Fig. 2).

Interference with protein A. Tenfold dilutions of purified protein A (Pharmacia, Inc., Piscataway, N.J.) ranging from 0.1 to 10,000 ng/ml (log protein A concentrations, −1.0 to 4.0) were tested, and no reactivity was observed (Fig. 2).

Biotin-avidin interaction. The relative sensitivities were enhanced by the amplification of AP through biotin-avidin or biotin-streptavidin interaction in indirect immunoassays with unlabeled rabbit anti-SEA antibody. Biotinylated sheep $F(ab')_2$ fragments directed to rabbit antibody were interacted with avidin-AP, ABAC, and streptavidin-AP. The detection limit obtained with a 1:1,000 dilution of avidin-AP was 4 ng/ml (log SEA concentration, 0.6 [Fig. 3A]). Lower avidin-AP concentrations revealed a further decline in sensitivity.

Calibration curves with purified SEA standards in ELISAs incorporating ABAC (Fig. 3B) and streptavidin-AP (Fig. 3C) were similar. The detection limit in both instances was 0.25 ng/ml (log SEA concentration, −0.6) and therefore was approximately 16 times higher than with avidin-AP and 32 times higher than in the conventional ELISA. Linearity was observed in the range of 0.25 to 250 ng/ml through regression

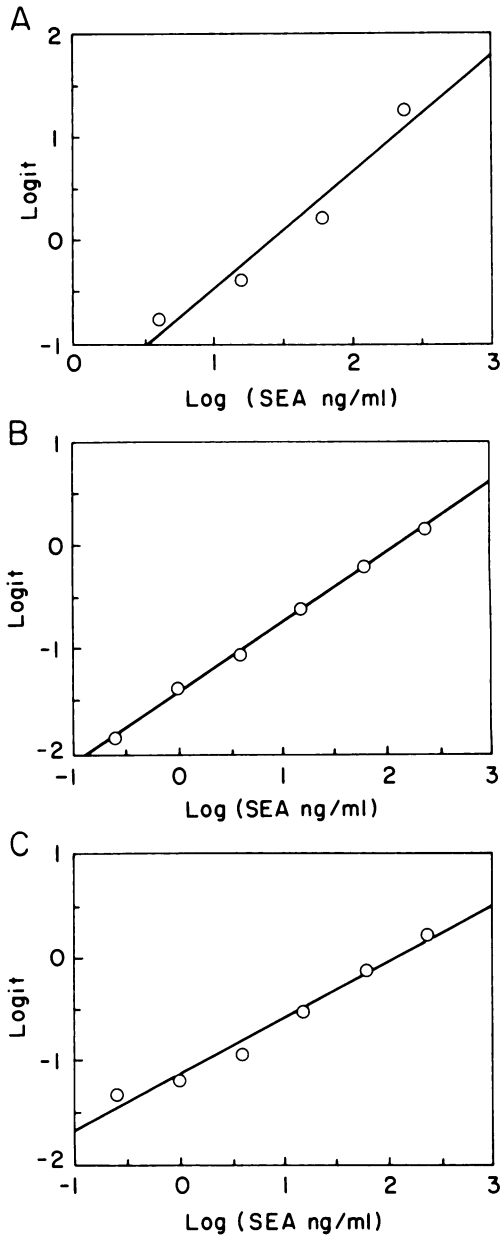

FIG. 3. Comparison of ELISAs for the detection of SEA by using three versions of the biotin-avidin or biotin-streptavidin interaction for enzyme amplification. Variations were with the probes used for the detection of the secondary antibody as described in the text. Graphs show standard curves of purified SEA ranging from 4 to 250 ng/ml (log SEA concentrations, 0.6 to 2.4 [A]) or 0.25 to 250 ng/ml (log SEA concentrations, −0.6 to 2.4 [B and C]). (A) Biotinylated sheep anti-rabbit $F(ab')_2$ fragments followed by avidin-AP conjugate. Each datum point is the mean of three replicates (duplicate wells with each trial) ($r = 0.97$). (B) Biotinylated sheep anti-rabbit $F(ab')_2$ fragments followed by ABAC. Each datum point is the mean of three replicates (duplicate wells with each trial) ($r = 1.00$). (C) Biotinylated sheep anti-rabbit $F(ab')_2$ fragments followed by streptavidin-AP conjugate. Each datum point is the mean of six replicates (duplicate wells with each trial) ($r = 0.99$).

analysis of the logit OD versus the log SEA concentration ($r = 1.00$ for ABAC and 0.99 for streptavidin-AP).

The enhanced detectability of the ABAC as opposed to the avidin-AP conjugate has been reported earlier (14, 25). The

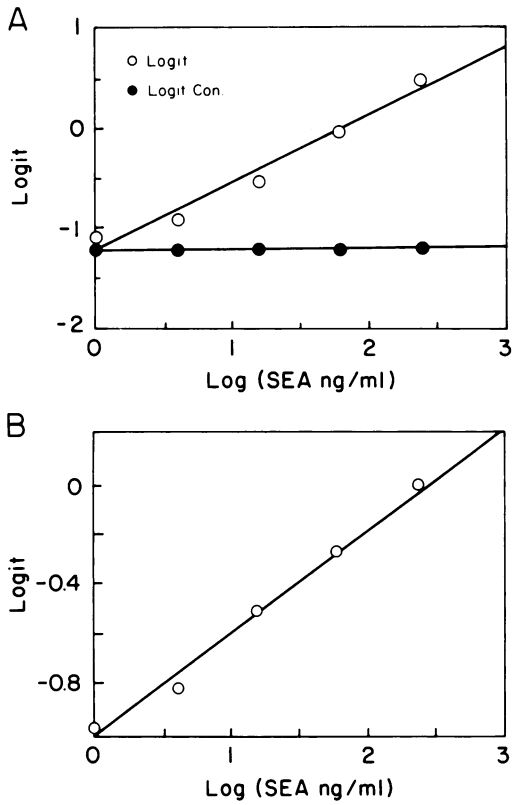

FIG. 4. Detection of SEA by an ELISA using biotinylated rabbit anti-SEA followed by streptavidin-AP conjugate. Standards of purified SEA ranging from 1 to 250 ng/ml (log SEA concentrations, 0 to 2.4) were tested. Controls were run simultaneously, with the substitution of anti-SEA MAb with an unrelated MAb of the same isotype. (A) The primary antibody (MAb) was indirectly bound to the plate with sheep anti-mouse antibody. (B) The primary antibody (MAb) was directly bound to the plate.

working dilutions of avidin and biotin-AP were determined by checkerboard titration. The sensitivity of the assay was dependent on the ratio of avidin and biotin-AP used in the complex. The optimal complex was determined to involve dilutions of 1:800 and 1:1,000 with avidin and biotin-AP, respectively.

The sensitivities of the immunoassays using MAbs which were bound indirectly (via sheep anti-mouse antibody) (Fig. 4A) and directly (Fig. 4B) to the microdilution plate followed by SEA and biotinylated rabbit anti-SEA antibodies were similar. Regression analysis with purified SEA standards was linear at concentrations of 4 to 250 ng/ml, with a correlation coefficient of 0.99 (Fig. 4). The detection limit was 1 ng/ml (r = 0.95 [Fig. 4A] and 0.98 [Fig. 4B] over a range of 1 to 250 ng/ml).

The fourfold increase in sensitivity of the assay in which indirectly biotinylated rabbit anti-SEA antibody was used as opposed to directly biotinylated rabbit anti-SEA (0.25 versus 1 ng/ml) could be attributed to the additional immunoreactive layer of biotinylated sheep anti-rabbit $F(ab')_2$ fragments. The enhancement in detection limits was accompanied by an increase in background reactivity. This effect was not negated with a variety of blocking agents such as 1% fetal calf serum and 5% skim milk. Changes in the ionic strength of PBS (0.5 M versus 0.15 M NaCl) and enhancement in pH (8.05 versus 7.4) were also ineffective. It is possible that the polyclonal $F(ab')_2$ fragments showed low-level cross-reactivity with sheep antibody or MAb or both. In these instances, the background ODs were subtracted from all test sample ODs. The slight decline in detection limit with the assay in which biotinylated rabbit anti-SEA antibody was used was counteracted by a negation of nonspecific binding (evidenced by a decline in background reactivity) and a decrease in total ELISA time.

TABLE 1. ELISA quantitation of SEA in replicate crude culture growth extracts of *S. aureus* strains

S. aureus strain	Toxin secreted	Mean (range) amt of toxin (ng/ml)[a] in:	
		Expt 1[b]	Expt 2[c]
7	SEA, SEB	170[d]	176 (157–196)[e]
8	SEB	–	–
13	None	–	–
16	SEA	208 (182–234)	236 (186–276)
31	SEA, SEB	194 (163–225)	219 (205–234)
33	SEA, SEB	146 (106–186)	133 (115–151)

[a] Mean (95% confidence interval = mean ± 1.96 [SD/$n^{1/2}$]). –, Negative value.
[b] Culture growth; mean of five trials.
[c] Culture growth; mean of six trials.
[d] Single trial.
[e] Five trials.

The ratio of biotin to antibody in the preparation of biotinylated rabbit anti-SEA conjugates was varied, with a twofold increase in biotin levels to a constant level of antibody. Ratios of 0.06:1, 0.12:1, and 0.24:1 (vol/vol) showed similar calibration curves (data not shown). The stability of the conjugates was ascertained upon storage either in PBS-sodium azide at 4°C or with an equal volume of glycerol at −20°C.

Detection of SEA in crude culture extracts and reproducibility. Clinical isolates of *S. aureus* strains previously determined to be toxigenic or nontoxigenic for SEA were cultured by the membrane-over-agar method. The toxin concentration in each trial was calibrated only when the mean OD of duplicate test wells was within the linear portion of the standard curve. The correlation coefficient over this range was >0.98. Finally, the mean SEA concentration in all trials and the standard deviation was calculated. The toxin concentrations of five or six trials (different aliquots) for *S. aureus* 16, which secreted SEA, were in the range of 180 to 240 ng/ml. The reproducibility was evaluated further by repeating the entire experiment under similar growth conditions. Strain 16 yielded mean SEA concentrations of 208 and 236 ng/ml in each experiment (Table 1).

Specificity. Three strains which were both SEA and SEB secretors were also screened. Toxin concentrations in the range of 133 to 219 ng/ml were obtained (Table 1). To rule out the possibility of inadvertent detection of SEB in conjunction with SEA, we tested a strain exclusive for the production of SEB. No reactivity was observed, indicating that the toxin levels determined were not due to SEB. Immunoblotting followed by autoradiography of purified and crude SEB had previously revealed low-level cross-reactivity on exposure to the MAbs (5). This factor was perhaps obviated in the ELISA by the inclusion of rabbit anti-SEA antibody as the secondary antibody. A nontoxigenic control strain was consistently not reactive. This could also help ascertain the lack of interference with protein A at this level.

DISCUSSION

The procedure developed in this report is geared toward the accurate, quantitative determination of nanogram quan-

tities of SEA from *S. aureus* isolates, with minimal obstruction from extraneous protein contaminants, including protein A. The enhanced sensitivity of the biotin-streptavidin interactive system was exploited to augment the detection limit of the assay.

It has been reported that the antigen-binding capacity of the primary trapping MAb in immunoassays is depreciated considerably owing to alterations at the antigen-binding sites by the direct hydrophobic interaction of the MAb with the polystyrene solid phase (23). To avoid this problem, we initially used sheep anti-mouse IgG to capture the MAb. One drawback of inclusion of this coat was the nonspecific reactivity between the polyclonal sheep antibody and the other reagents used in the system. However, similar detection limits with ELISAs which used MAb directly bound to the plate and indirectly bound (through sheep anti-mouse antibody) imply that other variables may also be instrumental in determining the sensitivity of the assay and that the binding capacity of the MAbs in this instance was not deterred by direct interaction with the polystyrene surface.

The high affinity of the MAbs in the range of 2×10^{-8} to 5×10^{-8} M would enable minute quantities of SEA to be captured (4). Two measures undertaken to minimize interference due to protein A were (i) the use of sheep antibodies and murine antibodies of the IgG1 isotype (both of which show a low affinity to protein A) as the coating and capture antibodies respectively (7, 12), and (ii) the biotinylation of the secondary rabbit antibody with the obstruction of binding sites to protein A on the antibody molecule (16). Notermans et al. (20) have detected protein A levels above 100 ng/ml with sheep antibody as the primary antibody and rabbit anti-SEA conjugated to AP as the secondary antibody. However, it is unknown whether higher levels of protein A (>10 μg/ml) in regular culture supernatants of *S. aureus* growth would cause interference.

Direct conclusions on the enhanced sensitivity of streptavidin-AP as opposed to avidin-AP could not be drawn. It is suggested that there is a reduction in nonspecific binding with streptavidin which is attributed to the absence of glycosylation and a lower isoelectric point than that of avidin (15, 18).

A comparative study of ELISA systems for the detection of staphylococcal enterotoxins showed the preponderance of the double-antibody system over other versions (9). Initial attempts at a competitive ELISA with sheep antibody- and MAb-coated microdilution plates followed by the simultaneous application of SEA-AP conjugate and unlabeled purified SEA were futile. The MAbs revealed a preferential binding of the SEA conjugate as opposed to the unlabeled test samples. This probably reflects a conformational change in the functional epitope as a result of conjugation, with a subsequent increased binding affinity with the MAb. A sandwich ELISA with an alternation of primary and secondary antibodies, i.e., rabbit anti-SEA antibody as the capture antibody followed by the MAb to SEA, gave extremely low detectability levels. In this instance, the epitope detected by the MAb was perhaps occupied by the polyclonal rabbit antibody, obliterating any interaction with the MAb. Therefore, only a narrow range of strategies exsist when a combination of MAbs and polyclonal antibodies is used. A restraint encountered with the exclusive use of MAbs in a sandwich ELISA was a decline in sensitivity with a reciprocity of coating and probing MAb pairs (24). Therefore, the classification of MAbs into those that are effective as either coating or probing antibodies could be crucial.

The system may be practically used as a routine diagnostic assay. None of the reagents constitute health hazards, and they do not require standardized safety precautions with regard to either usage or disposal. The coated plates (up to MAb stage), as stated above, can be stored in the cold for prolonged periods. The subsequent steps, starting with the addition of test samples, would allow completion of the assay in an 8-h work day. An overnight incubation with the test samples would allow a reduction of ELISA time by 3 h. Adaptability to the detection of enterotoxins in food samples implicated in food-poisoning incidences appears feasible, since minute quantities of SEA could be identified in unprocessed culture extracts of *S. aureus* strains.

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LITERATURE CITED

1. **Bayer, E. A., M. Wilchek, and E. Skutelsky.** 1976. Affinity cytochemistry: the localization of lectin and antibody receptors on erythrocytes via the avidin-biotin complex. FEBS Lett. **68**:240–244.
2. **Berdal, B. P., O. Olsvik, and T. Omland.** 1981. A sandwich ELISA method for the detection of *Staphylococcus aureus* enterotoxins. Acta Pathol. Microbiol. Scand. Sect. B **89**:411–415.
3. **Bergdoll, M. S.** 1970. Enterotoxins, p. 265–326. *In* T. C. Montie, S. Kadis, and S. J. Ajl (ed.), Microbial toxins, vol. 3. Academic Press, Inc., New York.
4. **Edwin, C., S. R. Tatini, and S. K. Maheswaran.** 1986. Nature and reactivity of staphylococcal enterotoxin A monoclonal antibodies. Appl. Environ. Microbiol. **52**:1247–1252.
5. **Edwin, C., S. R. Tatini, and S. K. Maheswaran.** 1986. Specificity and cross-reactivity of staphylococcal enterotoxin A monoclonal antibodies with enterotoxins B, C_1, D, and E. Appl. Environ. Microbiol. **52**:1253–1257.
6. **Edwin, C., S. R. Tatini, R. S. Strobel, and S. K. Maheswaran.** 1984. Production of monoclonal antibodies to staphylococcal enterotoxin A. Appl. Environ. Microbiol. **48**:1171–1175.
7. **Ey, P. L., S. J. Prowse, and C. R. Jenkin.** 1978. Isolation of pure IgG1, lgG2a and IgG2b immunoglobulins from mouse serum using Protein A-Sepharose. Immunochemistry **15**:429–436.
8. **Fey, H., and G. Burkhard.** 1981. Measurement of staphylococcal protein A and detection of Protein A carrying staphylococcus strains by a competitive ELISA method. J. Immunol. Methods **47**:99–107.
9. **Fey, H., H. Pfister, and O. Ruegg.** 1984. Comparative evaluation of different enzyme-linked immunosorbent assay systems for the detection of staphylococcal enterotoxins A, B, C, and D. J. Clin. Microbiol. **19**:34–38.
10. **Freed, R. C., M. L. Evenson, R. F. Reiser, and M. S. Bergdoll.** 1982. Enzyme-linked immunosorbent assay for the detection of staphylococcal enterotoxins in foods. Appl. Environ. Microbiol. **44**:1349–1355.
11. **Goding, J. W.** 1976. Conjugation of antibodies with fluorochromes: modifications to the standard methods. J. Immunol. Methods **13**:215–226.
12. **Goding, J. W.** 1978. Use of staphylococcal protein A as an immunological reagent. J. Immunol. Methods **20**:241–253.
13. **Goding, J. W.** 1983. Conjugation of antibodies with biotin succinimide ester p. 230–233. *In* J. W. Goding (ed.), Monoclonal antibodies: principles and practice. Academic Press, Inc. (London), Ltd., London.
14. **Guesdon, J.-L., T. Ternynck, and S. Avrameas.** 1979. The use of avidin-biotin interaction in immunoenzymatic techniques. J. Histochem. Cytochem. **27**:1131–1139.
15. **Haeuptle, M.-T., M. L. Aubert, J. Djiane, and J.-P. Kraehenbuhl.** 1983. Binding sites for lactogenic and somatogenic hor-

mones from rabbit mammary gland and liver. J. Biol. Chem. **258**:305–314.

16. **Hahn, I. F., P. Pickenhahn, W. Lenz, and H. Brandis.** 1986. An avidin-biotin ELISA for the detection of staphylococcal enterotoxins A and B. J. Immunol. Methods **92**:25–29.
17. **Heitzmann, H., and F. M. Richards.** 1974. Use of the avidin-biotin complex for specific staining of biological membranes in electron microscopy. Proc. Natl. Acad. Sci. USA **71**:3537–3541.
18. **Hofmann, K., S. W. Wood, C. C. Brinton, J. A. Montibeller, and F. M. Finn.** 1980. Iminobiotin affinity columns and their application to retrieval of streptavidin. Proc. Natl. Acad. Sci. USA **77**:4666–4668.
19. **Koper, J. W., A. M. Hagenaars, and S. Notermans.** 1980. Prevention of cross-reactions in the enzyme-linked immunosorbent assay (ELISA) for the detection of *Staphylococcus aureus* enterotoxin type B in culture filterates and foods. J. Food Saf. **2**:35–45.
20. **Notermans, S., P. Timmermans, and J. Nagel.** 1982. Interaction of staphylococcal Protein A in enzyme-linked immunosorbent assays for detecting staphylococcal antigens. J. Immunol. Methods **55**:35–41.
21. **Ritchie, D. G., J. M. Nickerson, and G. M. Fuller.** 1981. Two simple programs for the analysis of data from enzyme-linked immunosorbent (ELISA) assays on a programmable desk-top calculator. Anal. Biochem. **110**:281–290.
22. **Robbins, R., S. Gould, and M. Bergdoll.** 1974. Detecting the enterotoxigenicity of *Staphylococcus aureus* strains. Appl. Microbiol. **28**:946–950.
23. **Suter, M., and J. E. Butler.** 1986. The immunochemistry of sandwich ELISAs. II. A novel system prevents the denaturation of capture antibodies. Immunol. Lett. **13**:313–316.
24. **Thompson, N. E., M. Razdan, G. Kuntsmann, J. M. Aschenbach, M. L. Evenson, and M. S. Bergdoll.** 1986. Detection of staphylococcal enterotoxins by enzyme-linked immunosorbent assays and radioimmunoassays: comparison of monoclonal and polyclonal antibody systems. Appl. Environ. Microbiol. **51**: 885–890.
25. **Yolken, R. H., F. J. Leister, L. S. Whitcomb, and M. Santosham.** 1983. Enzyme immunoassays for the detection of bacterial antigens utilizing biotin-labeled antibody and peroxidase biotin-avidin complex. J. Immunol. Methods **56**:319–327.