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Simple and Reliable Enzyme-Linked Immunosorbent Assay with Monoclonal Antibodies for Detection of *Escherichia coli* Heat-Stable Enterotoxins

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We have developed a sensitive and specific competitive enzyme-linked immunosorbent assay for *Escherichia coli* heat-stable enterotoxins consisting of methanol-soluble, suckling mouse active peptides with similar core sequences (ST_a) by using monoclonal antibodies prepared against ST_a purified from a human isolate. The assay can detect 3 to 20 pg of purified ST_a, depending on the monoclonal antibody used in the assay. The assay is rapid, requiring ca. 1 h to complete. With this competitive enzyme-linked immunosorbent assay, we measured ST_a production by enterotoxigenic *E. coli* directly in Casamino Acid-yeast extract culture supernatants. The assay was suitable for measuring ST_a in culture supernatants from human, bovine, and porcine *E. coli* isolates. No cross-reactivity was observed with heat-labile enterotoxin, cholera toxin, or heat-stable enterotoxin ST_b, which is a methanol-insoluble peptide(s) active in the ligated pig jejunal loop test. A 100% correlation of toxin production was found by comparing the enzyme-linked immunosorbent assay with the previously established radioimmunoassay for ST_a and with suckling mouse activity.

Enterotoxigenic *Escherichia coli* is a pathogen that plays a major role in the diarrheal diseases of humans and domesticated animals (14, 17). Two distinct toxins have been implicated in the diarrheal process, a heat-labile enterotoxin (LT) (4, 19) and two families of heat-stable enterotoxins (ST). ST_as consist of methanol-soluble, suckling mouse active peptides with similar core sequences (2, 3, 7, 16). ST_b is a methanol-insoluble peptide(s) active in the ligated pig jejunal loop test but is less well characterized than the ST_as (2, 9).

ST_as have been purified, synthesized, and sequenced from human, bovine, and porcine isolates (1, 3, 10, 12, 13, 16, 18). The amino acid compositions of ST_as produced by additional strains have been determined as well (15). The toxins sequenced thus far consist of 18- or 19-amino acid peptides which share common core sequences and immunological domains. Twelve amino acids appear to be conserved with respect to their position in the toxins; six of these are cysteine. All of these are involved in disulfide bridges (13, 20). This high degree of apparent homology is likely responsible for the success in measuring ST_as produced in liquid culture by various ST_a-producing strains with a goat antibody and a radioimmunoassay (RIA) developed by Giannella et al. (8).

To further study structural relationships between these toxins and to develop tools for the rapid identification of ST_a-producing organisms, monoclonal antibodies were prepared against an ST_a which was previously purified and sequenced by one of us (3, 20; H. A. Brandwein, M. Deutsch, M. Thompson, and R. A. Giannella, submitted for publication). A family of five stable antibodies was obtained, which we have incorporated into a rapid, competitive enzyme-linked immunoassay (ELISA). The production and properties of the five monoclonal antibodies are described in a separate publication (Brandwein, Deutsch, Thompson, and Giannella, submitted).

The present work describes the ELISA developed for ST_a and presents data on the measurement of ST_a production in liquid culture by *E. coli*.

MATERIALS AND METHODS

Strains. Forty-two strains of *E. coli* encompassing ST_a, ST_b, and LT phenotypes and nontoxigenic isolates of human, porcine, and bovine origin were used in this study. Isolates included those which have been previously assayed and characterized for toxin production in the suckling mouse model and by RIA (8). *E. coli* strains were grown in Casamino Acid-yeast extract medium (CA-YE) in roller tubes as previously described (7, 8). Culture supernatants were coded and frozen at −20°C before the ELISA or RIA.

Preparation of monoclonal antibodies. Detailed procedures for the preparation of monoclonal antibodies to ST_a purified from strain 18D are presented elsewhere (Brandwein, Deutsch, Thompson, and Giannella, submitted). Briefly, BALB/c mice were immunized and boosted with ST_a-bovine serum albumin (BSA) conjugate. Coupling of ST_a to a protein carrier before immunization of animals is a procedure which was previously used to raise high-titer polyclonal antibodies in goats and rabbits (8, 11). ST_a-immune spleen cells were fused with myeloma cells and grown in selective medium, and positive hybridomas were recognized by using an ELISA protocol. Five stable subclones were used for the preparation of ascites tumors. One of the five purified monoclonal antibodies, 20C1B8, was used to optimize the ELISA procedure in this work.

Reagents. Purified ST_a produced by a human isolate (strain 18D, O42:K86:H37) was used as immunogen and as an ST_a standard. Toxin was purified as previously described (20). ST_a was conjugated to RIA grade BSA (Sigma Chemical Co., St. Louis, Mo.) exactly as described previously for coupling to bovine immunoglobulin G (IgG) (8) with 1-ethyl-

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3-(3-dimethylaminopropyl)carbodiimide hydrochloric acid (Bio-Rad Laboratories, Richmond, Calif.). The documented coupling ratios of ST_a molecules to BSA molecules was 5:1. The ST_a-BSA conjugate was used as the solid-phase antigen in the competitive ELISA. Conjugated ST_a-BSA was lyophilized and stored at −20°C. Conjugate prepared and stored in this manner was stable for at least 2 months, losing none of its ability to bind to plates or to bind antibody.

The following reagents were prepared for the ELISA procedure. A phosphate-buffered saline stock solution (PBS) (20 mM sodium phosphate [pH 7.2], 0.15 M NaCl) was used in all solutions in the assay except in substrate buffer. BSA-PBS was prepared at 0.1% (for antibody, sample, and conjugate dilutions), at 1% (for blocking), and at 0.05% (for wash solution). Wash solutions were also prepared in 20 mM Tween 20 (PBS-Tween). A sample of 20 mM carbonate-bicarbonate buffer (pH 9.6) (coating buffer) was prepared fresh before coating plates with ST_a-BSA. Antibodies were stored at −80°C in 0.1% BSA-PBS. Working stock dilutions of 10 μg of monoclonal antibody per ml of BSA-PBS were stored at −20 and 4°C for up to 1 month with no loss in binding characteristics. Horseradish peroxidase-labeled affinity-purified goat anti-mouse IgG (HRP-IgG) (Kirkegaard and Perry, Gaithersburg, Md.) was stored at 1 mg per ml in 0.1% BSA-PBS at −80°C. HRP-IgG was diluted to working concentrations just before its use in the ELISA. Substrate buffer (0.1 M sodium citrate, pH 4.0) was stored at 4°C. The chromogenic substrate 2,2′-azino-di-(3-ethylbenzthiazoline sulfonic acid) (Sigma) was diluted in water, aliquoted, and stored at −80°C. Substrate was added to buffer just before its use in the assay.

RIA of *E. coli* culture supernatants. Each coded culture supernatant tested in the ELISA was also tested for ST_a by RIA. A detailed description of the RIA was previously published (8). Antibodies to ST_a were obtained by immunizing goats with ST_a conjugated to bovine immunoglobulin. The RIA and ELISA determinations were performed at least twice for each of the supernatants tested. Reported values of ST_a are the means of two different assays performed on separate days.

Standard curves for the RIA and ELISA were obtained with an identical lot of pure ST_a; the concentration was previously determined by weight and by amino acid composition analysis (20).

ELISA procedure. (i) Standard assay. The competitive ELISA was developed based on principles outlined by Engvall and Perlmann (6). The ST_a-BSA conjugate was diluted to 5 μg of total protein per ml in coating buffer; 50 μl of diluted ST conjugate was added to wells of Immulon II microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.), and plates were incubated for at least 18 h at 4°C. Control wells were inoculated with coating buffer to detect nonspecific antibody adherence to the plastic wells. Plastic-wrapped, sealed plates were stable at 4°C for at least 60 days. Alternatively, ST_a-BSA conjugate was removed after 18 h, and 0.05 to 1% BSA in PBS was incubated in the wells for 1 h at 25°C to block remaining protein-binding sites, and then the plates were stored in blocking buffer at 4°C until use.

Before use in an assay, BSA-blocked plates were washed three times with 0.05% BSA-PBS. Routinely each plate was incubated with samples consisting of a standard curve with pure ST_a in addition to various dilutions of unknown sample. One well routinely received no HRP-IgG; color development in this well was subtracted from all others when plates were read. Antibodies and samples (either culture supernatant or purified ST_a) were diluted in 0.1% BSA-PBS before being mixed in glass tubes (12 by 75 mm). A 50-μl portion of the appropriate dilutions was added directly to wells, and incubation proceeded at room temperature for 2 h or at 4°C overnight, depending on the number of samples to be assayed (see Fig. 1). For screening large numbers of culture supernatant, incubation proceeded at 4°C overnight. Small numbers of samples or sample dilutions were incubated at room temperature for 2 h. No difference was observed in values obtained with the standard assay incubation of 2 h at room temperature or overnight at 4°C.

After the appropriate incubation time, the plates were rinsed five times with wash buffer to remove unbound antibody and free toxin. A 50-μl portion of HRP-IgG diluted 1/1,000 in 0.1% BSA-PBS was added to each well. After 1 h at room temperature, the plates were washed again, and freshly diluted chromogenic substrate 2,2′-azino-di-(3-ethylbenzthiazoline sulfonic acid) was added in 0.1 M citrate (pH 4.0) with 0.03% H_2O_2. Plates were scored visually, and the absorbance that developed was measured on an Artek automatic plate reader at 405 nm.

Each dilution of antibody and purified toxin or culture supernatant was assayed in triplicate, two in ST_a-BSA-coated wells and one in an uncoated well. The absorbance of the control well without antigen was subtracted from the average absorbance of the two coated wells to obtain the amount of specific antibody that bound to the ST_a-BSA wells. At least three dilutions of culture supernatant were assayed during each experiment. Experiments were repeated at least three times. Standard curves were plotted on semilog graph paper, and values for ST_a were calculated graphically. These values were correlated with those measured by RIA. Strains were considered toxin positive when the minimal dilution of culture supernatant tested (5 μl) yielded an absorbance ≤65% of that measured in the absence of competing toxin. This criterion was chosen with the first antibody tested, based upon assay conditions which yielded a measured concentration of 20 ng of pure ST_a per ml, the minimum detection limit in RIA. Depending on the monoclonal antibody tested, this corresponded to a concentration of 20 to 40 ng of ST_a per ml in culture supernatant. To determine whether or not toxin was present in culture supernatant, it was sufficient to assay one dilution and inspect the plate visually.

(ii) Rapid assay. The standard assay was modified to yield a rapid assay differing primarily in reagent concentrations and incubation times. Generally the rapid assay was performed on one plate at a time to minimize the time factor in loading wells with antibody, conjugate, and substrate. The exact conditions of the rapid assay are given in Fig. 1.

Antibody was mixed with standard ST_a, buffer, or culture supernatant just before assay as described above. A 50-μl portion of each antigen-antibody dilution was added to each well, and the plate was incubated for 20 min at room temperature. The plate was then washed, and 50 μl of HRP-goat anti-mouse IgG was added (1:250 dilution of stock) to the wells. After an incubation of 15 min at room temperature, unbound conjugate was discarded, the plate was washed, and 100 μl of 2,2′-azino-di-(3-ethylbenzthiazoline sulfonic acid) substrate was added to each well as previously described. Color development was arrested at 20 min with 25 μl of 0.05 M NaN_3. As in the standard assay, a reduction in absorbance to 65%, or less than that measured in the absence of competing toxin with 5 μl of culture supernatant, was considered a positive response for ST_a in screening isolates.

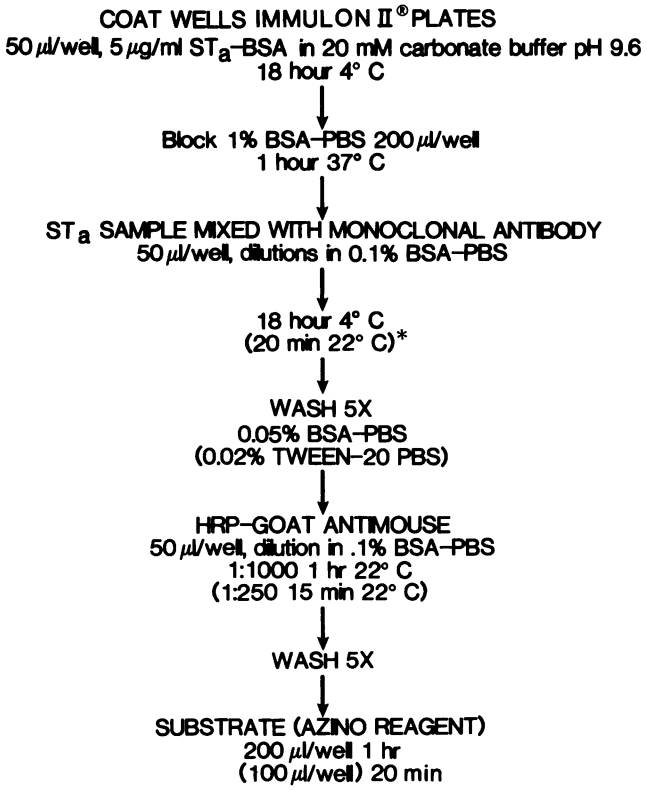

FIG. 1. Schematic flow diagram of competitive ST_a ELISA. Optimal conditions are shown for the overnight assay used for screening larger numbers of isolates (referred to in text as "standard assay"). *, Rapid assay conditions in parentheses.

RESULTS

Our approach to develop a sensitive nonisotopic competition assay was to first coat individual microtiter plate wells with ST_a conjugated to BSA. It had been our experience that native, unmodified ST_a did bind to plastic, but that the bound toxin lost most of its ability to bind antibodies. Covalent coupling of ST_a to protein carriers not only provided consistent coating of wells but also allowed the ST_a to retain its ability to bind antibody. Thus, sufficient monoclonal antibody was able to bind to the immobilized toxin conjugate to allow development of a competition assay. Checkerboard titration procedures were used to find appropriate reagent dilutions in the ELISA. Figure 1 shows a schematic diagram of the optimized assay.

ST_a-BSA conjugate was incubated in microtiter wells at concentrations of total protein of 0.1 to 20 µg per ml to optimize assay sensitivity and color development. An optimal coating concentration of 1 to 5 µg of ST_a-BSA per ml yielded the best compromise of maximal color development and sensitivity. At 1 µg per ml, color development was sufficient for plates to be read in an automatic plate reader but insufficient for visual scoring. At 3 to 5 µg per ml, plates were easily scored visually and retained sufficient sensitivity to correlate well with the RIA. Plates left in coating solution (ST_a-BSA in carbonate-bicarbonate buffer) gradually lost sensitivity over a period of months. Plates stored for several months at 4°C continued to function well in the ELISA, and ST_a-BSA removed from plates could be reused at least two additional times.

After coating plates, a blocking step that used PBS with BSA was found necessary for maximal assay sensitivity. No difference was found in reproducibility of the assay when plates were stored in PBS-BSA after coating or when plates were blocked just before use. Plates that were coated, blocked, and stored in PBS-BSA were also stable for at least several months. Adequate blocking was found with dilutions of BSA ranging from 0.05% to 1% in assay buffer. Blocking was performed at 37°C.

For convenience, three forms of the ELISA were developed, differentiated primarily by the incubation conditions of the antibody with samples. The standard ELISA used an antibody-antigen incubation time of 18 h at 4°C or 2 h at room temperature. The rapid ELISA used an incubation period of 20 min at room temperature.

The optimal concentration of each monoclonal antibody tested was determined under a variety of incubation conditions, including temperature, buffer, ionic strength, and pH. Binding was similar for a given concentration of antibody after 2 h of incubation at room temperature or after overnight at 4°C. Elevation of the incubation temperature increased binding of antibody to the immobilized ST_a, as reflected in increased absorbance, but the assay was reduced in sensitivity, as determined by competition with ST_a. Binding was reduced in low-ionic-strength buffers but was nearly identical in pH 7.2 PBS and in the pH 6.2 sodium acetate buffer used in the RIA (8). Furthermore, competition curves with pure ST_a were virtually identical in these buffers (data not shown).

The composition of the wash buffer consisted of dilute BSA (0.05%)-PBS or Tween 20 (0.02%) in PBS. Most of the isolate screening was done with the BSA buffer; however, PBS-Tween was found to be cheaper and worked equally well.

HRP-goat anti-mouse IgG antibody was incubated under various conditions to yield optimal color development. The enzyme conjugate gave similar color development at dilutions of 1:1,000 in BSA-PBS for 1 h or with 1:250 for 15 min. The rapid assay incorporated the latter dilution to increase the speed of the assay. All tests performed with overnight incubation (when six to eight plates were assayed) used an HRP-anti-IgG incubation time of 1 h, and the enzyme conjugate was used at 1:1,000 to reduce expense. Incubation of plates with HRP-anti-IgG at 37°C did not markedly increase color development but occasionally increased background color development.

Substrate incubation conditions were optimized for pH and H_2O_2 concentration. Best color development in a reasonable time (15 to 30 min) was found with citrate buffer at pH 4.0. Lower pH increased the rapidity of color development, which interfered with well-to-well consistency. The optimal concentration of H_2O_2 was 0.03%; above and below this concentration the color development was reduced.

To investigate the effect of enzyme-substrate incubation time on the linearity of response, antibody was incubated on plates with and without 100 pg of ST_a per well. Substrate color development was measured from 15 to 65 min. Although the maximum color development doubled during this time, the ratio of color development of antibody plus added ST compared with antibody alone was not significantly altered. Thus time of incubation with substrate was not critical for accurate evaluation of the competitive assay. Furthermore, normalized standard curves were found to be highly reproducible over a twofold variation in range of observed maximal absorbance. Thus although maximal absorbance varied from plate to plate, the percent displacement by pure toxin was in excellent agreement on a day-to-

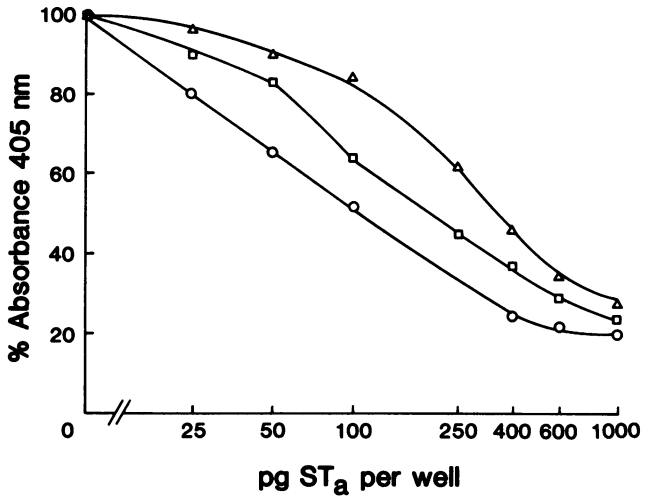

FIG. 2. Effect of concentration of monoclonal antibody 20B3 on standard ELISA competion curves. Symbols: △, 1:40,000 (maximum absorbance at 405 nm, 1.8); □, 1:80,000 (maximum absorbance, 1.0); ○, 1:200,000 (maximum absorbance, 0.36). Antibody dilutions from 1 mg per ml of stock incubated with and without pure ST_a in wells for 2 h at 22°C (standard assay conditions).

day basis. Plates could be scored visually with accuracy over a time period of at least 30 min. However, to aid in standardization of the assay, we have included the use of a 5× stop solution for the substrate, consisting of sodium azide and yielding a final concentration of 0.01 M.

An example of competitive inhibition curves with pure ST_a and one of the monoclonal antibodies in the standard assay is shown in Fig. 2. Antibody bound to the immobilized ST_a is signified by color development. Antibody binding to the immobilized ST_a, and subsequent color development, was decreased as the concentration of pure ST_a was increased. Various concentrations of antibody yielded competition standard curves with different sensitivities. At the highest concentration of antibody 20B3 tested, ca. 250 pg of ST_a was required to inhibit the binding of antibody to the solid phase by 35%; at the lowest concentration of antibody shown, ca. 50 pg of ST_a was capable of inhibiting binding to the same level. No preincubation was required of ST_a and monoclonal antibody. Incubation of antibody and toxin dilutions overnight at 4°C or at room temperature for 1 h before plating yielded competition curves identical to those observed when dilutions were immediately plated. The assay was sensitive and could detect as little as 3 to 10 pg of ST_a under optimal conditions (1 μg/ml of ST_a-BSA coating solution). For the purpose of this paper, however, concentrations of coating conjugate and antibody were chosen to yield competition curves easily read by eye. The increased antibody and ST_a-BSA conjugate concentration required to meet this criterion decreased sensitivity of the screening assay to 20 to 40 pg per well. The ST_a competition curves obtained with the 2-h antibody incubation assays and the rapid (total 1 h) ELISA with monoclonal antibody 20C1 are compared in Fig. 3. These competition curves showed nearly identical sensitivity for ST_a. Antibody dilutions were chosen which yielded equivalent antibody binding in the absence of competing toxin. The approximate useful range for these curves is represented by the bar in Fig. 3. Dilutions of culture supernatant yielding absorbance outside of this range were discarded.

No cross-reactivity was observed in this assay with pure *E. coli* LT (obtained from R. Finklestein, University of Missouri College of Medicine), cholera toxin (Schwarz/Mann, Orangeburg, N.Y.), or staphlococcal enterotoxin B (W. Beisel, Fort Dietrick, Frederick, Md.).

Sample buffers were tested to determine the sensitivity of the assay to extraneous matter. None of the conventional media tested, including tryptic soy broth, CA-YE, or defined media (8), had any effect on standard competition curves when tested at dilutions customarily used to assay culture supernatants.

Reproducibility of the ELISA was checked by using dilutions of pure ST_a and several culture supernatants. Intraplate and interplate variation was measured on the same day and on three different days. Same-day interplate assays were found to have a coefficient of variation of 10%. Intraplate variation was smaller, yielding a coefficient of variation of ca. 5%.

Forty-two *E. coli* isolates of known toxin phenotype were tested concurrently in the screening ELISA and the RIA. These strains included human, porcine, and bovine isolates. Overnight plate incubation of the culture filtrate and monoclonal antibody dilutions was required because of the large number of samples and dilutions tested. Twelve culture supernatants from toxin-producing strains were additionally tested in the rapid assay (20-min incubation of antibody plus culture supernatant) to verify the validity of the rapid assay.

Initial screening of all culture supernatants was done successfully at three dilutions equivalent to 5, 1, and 0.2 μl of supernatant per well. Visual scoring of plates with these dilutions and confirmatory measurement with the Artek plate reader demonstrated that plates could be visually interpreted for ST_a production. We quantitated toxin production by averaging the values obtained from dilutions of culture supernatant yielding an absorbance that fell within the linear range of the standard curve obtained on each plate.

Of the 42 strains tested, 20 were previously found to produce ST_a in liquid culture by the suckling mouse assay (8). These 20 strains were also found to be positive in both the ELISA, with each of the four monoclonal antibodies,

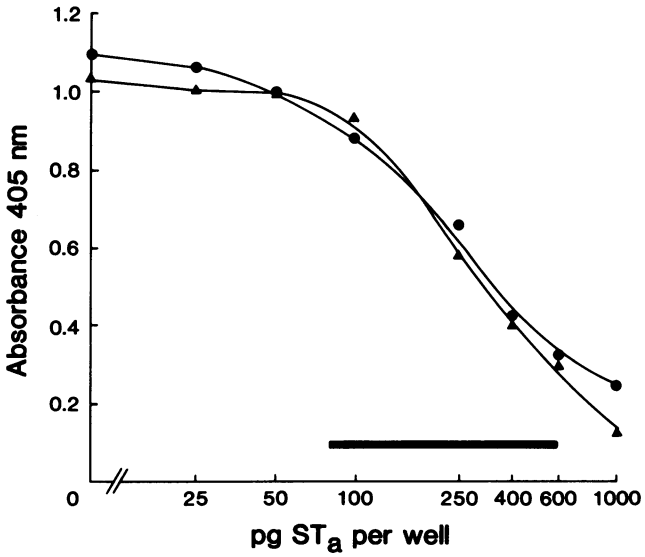

FIG. 3. Comparison of standard ELISA versus rapid ELISA competition curves with monoclonal antibody 20C1. Symbols: ▲, rapid assay, 1:40,000 dilution of antibody; ●, standard assay, 1:120,000 antibody dilution.

TABLE 1. Correlation of ST_a production by *E. coli*: RIA versus ELISA

Toxin	Phenotype	No. of strains	ST_a detected			
			RIA		ELISA	
			+	−	+	−
ST_a^+	LT^-	18	18		18	
ST_a^+	LT^+	2	2		2	
ST_a^-	LT^+	13		13		13
ST_a^-	LT^-	6		6		6
ST_a^-	LT^- ST_b^+	3		3		3

and the RIA (Table 1). No LT^+, ST_a^-, or ST_b^+ strains were detected as ST_a^+. Thus there was complete agreement between the ELISA, the RIA, and previously obtained suckling mouse assay data.

Figure 4 shows the correlation between the ST_a quantitated by the ELISA with monoclonal antibodies 20B3 and 20F5 and that quantitated by the RIA. Linear regression analysis of these data showed regression coefficients of 0.71 and 0.94, respectively. Concentrations of ST_a in culture supernatants measured by the ELISA were generally approximately double those measured by the RIA.

An occasional strain produced toxin which yielded concentrations in the ELISA very different from the best-fit regression line. Furthermore some of these isolates (e.g., 431 or NP32) yielded very different measured concentrations, depending on the particular monoclonal antibody used. However, none of these isolates were found to be ST_a^- when any one of the monoclonal antibodies was used in the ELISA. Thus, although the measured concentration of ST_a obtained by ELISA could depend upon the monoclonal antibody used in the assay, any one of these antibodies was suitable for screening isolates for toxin production.

Six culture supernatants from isolates producing low, medium, and high ST_a concentrations were tested in both the overnight ELISA and in the rapid, 1-h assay for ST_a production. Table 2 compares the measured values for ST_a obtained in both assays with monoclonal antibody 20C1. Comparable values were obtained between the two assays for each of these culture supernatants.

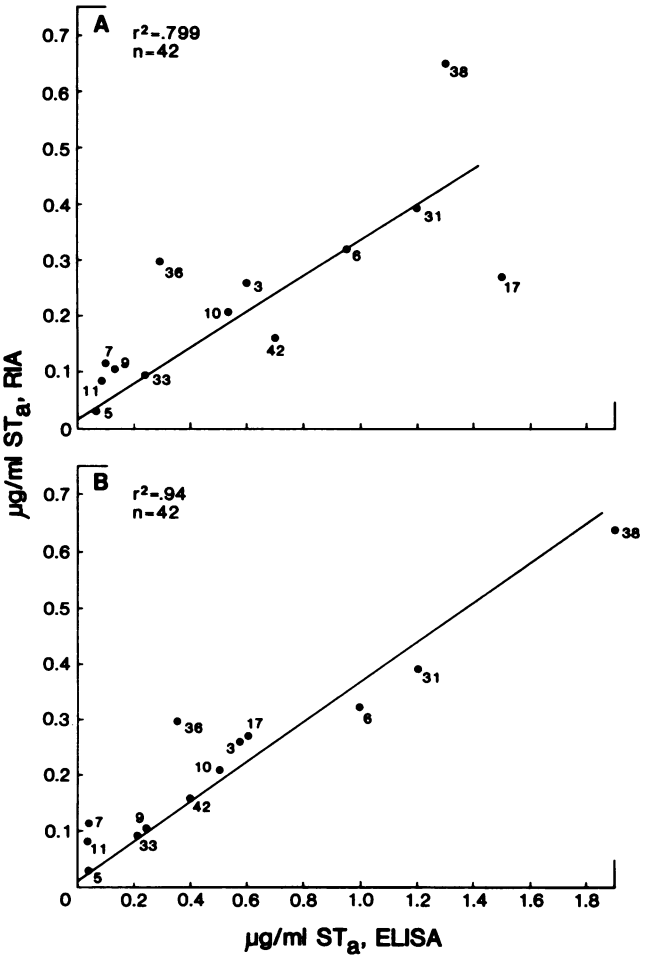

FIG. 4. Correlation of measured ST_a in culture broth by RIA and ELISA. Values are mean determinations from three dilutions of culture broth in the linear range of the standard curves. ELISA values are presented with monoclonal antibodies 20B3 (A) and 20F5 (B). Origins of isolates listed below are signified by B (bovine), P (porcine), and H (human). Representative isolates are 3, B41(B); 5, 483(B); 6, C59C4(H); 7, F10-2(H); 9, 987(P); 10, 1362(P); 11, P127-4(H); 17, 431(P); 31, 18D(H); 33, 490(B); 36, B44(B); 38, NP32(H); 42, 505(B). Data not shown for low or nontoxin producers.

DISCUSSION

Recent amino acid sequence work in a number of laboratories has demonstrated that ST_a, elaborated by toxigenic *E. coli* of human, porcine, and bovine origin, comprises a family of closely related peptides (1, 3, 10, 12, 13, 16, 18). These toxins all have a high degree of homology in primary structure. Currently, two classes of these toxins can be differentiated by primary sequence analysis. Chan and Giannella originally purified and sequenced a toxin produced by a human isolate which consisted of an 18-amino acid peptide containing six cysteines (3). Subsequently other 18-amino acid ST_as have been purified and sequenced from human, porcine, and bovine isolates, which share large regions of homology with this peptide. Recently, a second class of ST_a has been described consisting of 19-amino acid peptides which contain two serines, residues which are not found in the 18-amino acid toxins (1, 15). The 19-amino acid ST_a also shares a large region of homology with the 18-amino acid ST_as. Maximum homology occurs within the carboxy-terminal 14 amino acids in these toxins. That both classes of toxins share large regions of homology suggests that these toxins likely share immunodominant regions (16). This has been demonstrated by the fact that polyclonal antisera raised against the 18-amino acid ST_a recognize both classes of ST_a in the RIA (5, 8).

TABLE 2. Comparison of rapid versus standard overnight assay for ST_a[a] in quantitating ST_a in culture supernatant fluids

Strain	ST_a in ng/ml (± SD) by:	
	Rapid assay	Overnight assay
C59C4	1,207 ± 187	1,374 ± 368
1362	443 ± 83	483 ± 177
431	777 ± 116	1,098 ± 223
18D	1,021 ± 285	1,014 ± 339
987	175 ± 35	168 ± 30
B41	450 ± 20	545 ± 95

[a] Values reported were obtained with monoclonal antibody 20C1. Each value represents the average of three dilutions assayed on six different occasions.

We have presented data in this report by using monoclonal antibodies as probes for toxin structure which show that a common epitope(s) exists on both classes of ST_as. The *E. coli* strains tested in this report encompass human, porcine, and bovine isolates. Of the 20 ST_a^+ isolates tested, only three of the strains tested in the ELISA have toxins for which sequence data exist that are representative of each class of ST_a (3, 4, 18). It seems likely, however, that one or more of the other strains tested produces toxin(s) with sequences also differing from those previously reported for the toxin used to raise monoclonal antibodies. Thus the existence of common epitopes observed with the monoclonal antibodies is consistent with the previously inferred conservation of tertiary structure within the carboxy-terminal 14 amino acids of these toxins.

The majority of the ST_a^+ culture supernatants tested in the ELISA yielded consistently higher concentrations of toxin (two- to threefold) than those measured in the RIA. Several factors may contribute to this observation. We have found that monoclonal antibodies can only bind about half as much [^{125}I]ST_a as can polyclonal antibodies (8; Brandwein, Deutsch, Thompson, and Giannella, submitted). Since [^{125}I]ST_a retains >95% of its biological activity, it seems that upon iodination there is modification of the epitopes responsible for monoclonal antibody binding. This modified toxin may have altered binding characteristics compared with native toxins in the RIA. Likewise the ELISA relies on a solid-phase antigen, ST_a conjugated to BSA, and binding affinities of monoclonal antibody to the immobilized ST_a may differ from those for freely soluble ST_a.

The complete concordance of the RIA and ELISA in determining whether or not a strain produces ST_a demonstrates the usefulness of the ELISA for ST_a detection. The assay is simple, rapid, reproducible, nonisotopic, and does not require sophisticated instrumentation. Although the conditions of incubation described have been optimized for rapid screening of bacterial culture supernatants, they are by no means absolute, as it is possible to run the assay with wash reagents and substrate in a commercial EIA kit (Kierkegaard and Perry) with only a small loss in sensitivity (unpublished data). With appropriate antibody dilutions, it is possible to visually interpret the rapid assay plates when isolates producing 20 ng more of ST_a per ml of culture supernatant are tested. Thus, the ST_a ELISA shows promise for epidemiological screening and for rapid identification of stable-toxin-producing organisms.

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