APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1982, p. 877–883
0099-2240/82/040877-07$02.00/0
Vol. 43, No. 4

Enzyme Immunoassay for Detection of Salmonellae in Foods†

SCOTT A. MINNICH,[1]‡ PAUL A. HARTMAN,[1]* AND RICHARD C. HEIMSCH[2]

Department of Microbiology, Iowa State University, Ames, Iowa 50011,[1] and Department of Bacteriology and Biochemistry, University of Idaho, Moscow, Idaho 83843[2]

Received 13 October 1981/Accepted 28 December 1981

An enzyme immunoassay was developed to detect *Salmonella* in foods. Indirect test protocols were developed for use with microtitration plates or Gilford microcuvettes. Samples from enrichment cultures were mixed with H-specific immunoglobulin G and allowed to react; unbound antibody was removed by three 5-min centrifugation washes; goat anti-rabbit antibody conjugated to alkaline phosphatase was added and allowed to react; and unbound conjugate was removed by centrifugation washing as before. *Salmonella*-positive samples were indicated by the production of a chromogenic reaction product after the addition of alkaline phosphatase substrate. The color could be read visually or quantified by absorbance. Ninety-eight food samples were examined to compare the enzyme immunoassay with enrichment serology, immunofluorescence, and the Food and Drug Administration pure culture technique. The enzyme immunoassay was sensitive and specific, and it possessed advantages over methods currently in use. Furthermore, when the enzyme immunoassay was used to screen preenrichment media, the results indicated that it might be decidedly more sensitive than the conventional pure culture technique.

The detection of salmonellae in foods and foodstuff involves a series of enrichment steps because these pathogens, when present in foods, are generally found in low numbers and are often sublethally injured. Therefore, detection methodologies for salmonellae must be sensitive and allow for the resuscitation and growth initiation of injured cells. Two methods of *Salmonella* detection are currently recommended by the Food and Drug Administration (3): (i) a pure culture technique (PCT) involving preenrichment, selective enrichment, and selective plating; and (ii) immunofluorescence (IF) after selective enrichment. The PCT is an expensive and time-consuming process that requires 4 or 5 days for analysis (3). In contrast, IF is more rapid; it is sensitive, but many false-positives are obtained because polyvalent OH antisera are used and anti-O antibodies cross-react with a wide spectrum of related enteric bacteria (16, 17). Enrichment serology (ES; 13), a third method used by some laboratories, is more specific than IF, but ES is more cumbersome and has not met with widespread acceptance. The list of additional techniques for the detection of salmonellae is lengthy; however, the acceptance of a new procedure requires that it demonstrate decided advantages over existing methods. Sensitivity, technical expertise required, time until results are obtained, cost effectiveness, and amenability to automation are factors that should be considered.

Enzyme-labeled antibodies were used by Krysinski and Heimsch (5) to detect salmonellae on membrane filters. This technique was sensitive, permitted multisample analysis, and required a minimum of equipment. The major obstacle to perfecting the technique was the requirement for a polyvalent H-antiserum that was free of cross-reacting O antibodies. Minnich and Heimsch (S. A. Minnich, M.S. thesis, University of Idaho, Moscow, 1978) improved the Krysinski-Heimsch (5) procedure by purifying immunoglobulin G (IgG) from commercial antisera. IgM, which is the primary immunoglobulin class elicited by somatic antigens (6, 7), was removed. The use of purified IgG has been recommended by others for increased specificity of IF methods (15, 17).

The present study was undertaken to further improve an enzyme immunoassay (EIA) to detect salmonellae in foods. An indirect EIA that was sensitive and specific was developed; the EIA was compared with the PCT, IF, and ES methods.

MATERIALS AND METHODS

Stock cultures and media. Most of the bacterial cultures were obtained from collections maintained at the University of Idaho and Iowa State University.

† Journal paper no. J-10419 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, and Research Paper no. 81519 of the Idaho Agricultural Experiment Station, Moscow, Idaho.

‡ Present address: Department of Biological Sciences, Purdue University, West Lafayette, IN 47906.

Salmonella serotypes utilized in the pure culture studies were obtained from R. W. Ryder, Centers for Disease Control, Atlanta, Ga.

Media were commercial products (Difco Laboratories, Detroit, Mich.), except M-broth, which was prepared as described by Sperber and Deibel (13).

Antibody preparations. Spicer-Edwards polyvalent anti-H IgG (Difco) was purified by using staphylococcal protein A (SPA) affinity chromatography. A 40-mg portion of SPA (Sigma Chemical Co., St. Louis, Mo.) was coupled to Sepharose 4B (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) as described by March et al. (9) and modified by Swaminathan et al. (15). Routinely, 4 to 6 ml of pooled Spicer-Edwards antiserum was added to a column (0.5 by 10 cm) containing SPA-Sepharose 4B. The column was washed with phosphate-buffered saline (PBS; pH 7.4), and the protein content of the eluate was monitored by absorbance at 280 nm. When the absorbance readings dropped below 0.05, 0.1 M glycine-HCl buffer, pH 3.0, was added to the buffer reservoir and bound IgG was eluted. The IgG peak was pooled and dialyzed overnight against PBS to bring the pH back to neutrality. Purity of the antibody preparations was checked by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The purified IgG was stored at 4°C in PBS containing 0.2% NaN_3. Before use, the purified IgG was diluted in polyvinyl pyrrolidone-bovine serum albumin-PBS (2.0 g of polyvinyl pyrrolidone-40, 0.5 g of bovine serum albumin, and 0.6 ml of Formalin in 100 ml of PBS; pH 7.2). The appropriate dilution of purified IgG was determined by using the highest dilution that produced positive tube agglutination tests on three known *Salmonella* serotypes. Regeneration of the SPA-Sepharose 4B column was accomplished by flushing the column thoroughly with PBS, pH 7.2

Calf intestinal alkaline phosphatase (type VII; Sigma) was conjugated to goat anti-rabbit immunoglobulin (GIBCO Laboratories, Grand Island, N.Y.) by using the procedure described by Voller et al. (18, 19). A commercial conjugate became available during the course of this study, and one preparation (Sigma) was highly satisfactory; it possessed a higher titer and was more specific than conjugates that we prepared.

EIA. An EIA was developed by modifying the procedure of Polin and Kennett (12). A 0.1-ml amount of enrichment broth was transferred to 10 ml of M-broth and incubated at 35°C for 4 to 6 h; then 0.1 ml of the M-broth culture was added to either a Gilford cuvette (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) or a microtitration plate well (Micro-Elisa plate, Dynatech Laboratories, Inc., Alexandria, Va.). Unless specified otherwise, three wells were used per sample, two for replicate analyses and the third for a control. A 0.1-ml portion of purified Spicer-Edwards IgG that had been diluted in polyvinyl pyrrolidone-bovine serum albumin-PBS was added to two of the test sample wells. Diluent without IgG was added to the control well, which served to monitor washing efficiency and nonspecific binding of conjugate. The cuvettes or microtitration plates were incubated at room temperature for 1 h and then were rinsed three times with PBS-Tween 20 (0.5% Tween 20 in PBS, pH 7.2) to remove unbound IgG. Rinsing was accomplished by centrifugation at 3,000 rpm for 5 min in an International centrifuge equipped with a rotor designed for microtitration plates; plastic spacers were made to adapt the plate holders for the Gilford cuvette packs. A 0.1-ml amount of Sigma goat anti-rabbit–alkaline phosphatase conjugate diluted 1:1,000 was added to each well; the plates were incubated for 1 h at room temperature, and unbound conjugate was removed by washing, as before. Finally, each well received 0.2 ml of alkaline phosphatase substrate. The substrate was prepared on the day of use by adding 5 mg (one tablet of Sigma phosphatase substrate 104) to 5 ml of diethanolamine buffer (97 ml of diethanolamine, 0.2 g of NaN_3, and 100 mg of $MgCl_2 \cdot 6H_2O$ in 800 ml of distilled water; pH adjusted to pH 9.6 with 1 M HCl and volume made to 1 liter). After incubation at 35°C for 30 min, the production of a yellow reaction product indicated a positive sample. Although the color could be observed visually, in some instances to obtain quantitative results we also read absorbancies at 405 nm by using a Titertek EIA reader (Flow Laboratories, McLean, Va.) or a Gilford manual EIA reader (Gilford Instrument Laboratories, Inc.).

Detection of salmonellae in foods. For this study, a total of 98 naturally contaminated food samples were obtained from private and federal agency laboratories and local retail food markets. Four different detection procedures were compared. One procedure was the EIA described above. IF assays were conducted by using commercial polyvalent OH antisera (Clinical Sciences, Inc., Whippany, N.J.), as described by Thomason (16). ES was performed as described by Sperber and Deibel (13); pooled Spicer-Edwards serum diluted to give a final concentration of 1:500 was used. After postenrichment in M-broth, tube agglutinations were performed at 50°C, and the reactions were scored after 4 h. The conventional PCT was performed as described by Poelma and Silliker (11). Samples, 25 or 50 g, were preenriched in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) (dried yeast), reconstituted nonfat dry milk (chocolate candy), or lactose broth (all other samples). Tetrathionate and selenite-cystine broths were used for selective enrichment, and salmonella-shigella, brilliant green, and bismuth sulfite agars were used for selective plating. Suspect *Salmonella*-like colonies were transferred to tubes of lysine iron and triple sugar iron agars. Isolates positive for *Salmonella* in lysine iron and triple sugar iron agars were further characterized by using the Mini-Tek identification system for *Enterobacteriaceae* and were also retested by tube agglutination with Spicer-Edwards antiserum. Isolates conforming to the definition of the genus *Salmonella* were sent to the National Veterinary Service Laboratory, Ames, Iowa, for serotyping.

Sensitivity of the EIA procedure. Studies with *Salmonella anatum*, *S. schwarzengrund*, and *S. rubislaw* were conducted to determine the level of sensitivity of the EIA procedure. Serial decimal dilutions of overnight M-broth cultures were made in PBS. A viable cell count was made by plating appropriate dilutions in Trypticase soy agar (BBL). Concomitantly, 0.1-ml amounts of dilutions 10^{-1} through 10^{-7} were dispensed into microtitration plate wells and subjected to EIA analysis. A control, *Escherichia coli* B, was used to determine background levels of absorbance. Results of the completed tests were scored by visual observation.

A preliminary study also was conducted by using a fluorogenic substrate (20), 4-methyl umbelliferyl phos-

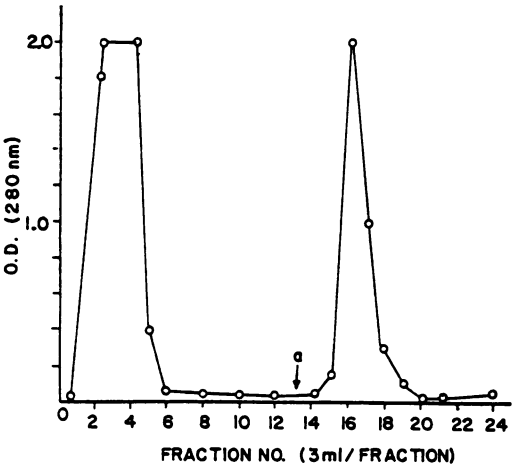

FIG. 1. Fractionation of Spicer-Edwards antiserum on an SPA-Sepharose 4B column. The first peak (fractions 1 to 6) is composed of serum proteins other than IgG that were eluted in PBS. Glycine-HCl buffer was added to the buffer reservoir at point "a." The second peak (fractions 14 to 20) contains purified IgG. O.D., Optical density.

phate (Sigma), at a concentration of 25 μg/ml in diethanolamine buffer. An overnight broth culture of *S. anatum* was diluted as described earlier, and viable counts and EIA analyses were performed as described above. After 30 min of incubation with substrate, the plates were held under a long-wave UV light to detect fluorescence of the reaction product, 4-methyl umbelliferone.

RESULTS

A typical elution profile of Spicer-Edwards polyvalent antiserum from SPA-Sepharose 4B is shown in Fig. 1. About 60 mg of purified IgG (fractions 15 through 18, Fig. 1) was obtained from 15 ml of antiserum.

A sodium dodecyl sulfate-polyacrylamide gel procedure was used to compare preparations of whole Spicer-Edwards serum, commercial goat anti-rabbit IgG, and Spicer-Edwards serum purified by SPA-Sepharose 4B chromatography. Whereas numerous bands were observed in unpurified Spicer-Edwards antiserum (A, Fig. 2), only two protein bands were present in the goat anti-rabbit IgG (B, Fig. 2) and purified Spicer-Edwards (C, Fig. 2). The two bands in the purified preparations (B and C, Fig. 2) represented the light and heavy chains of IgG. The purified Spicer-Edwards IgG agglutinated all *Salmonella* serotypes tested. No IgM was detectable by Ouchterlony double diffusion against anti-rabbit IgM. Titers of the purified IgG preparations ranged from 1:40 to 1:100, depending on the amount of material placed on the SPA-Sepharose 4B column.

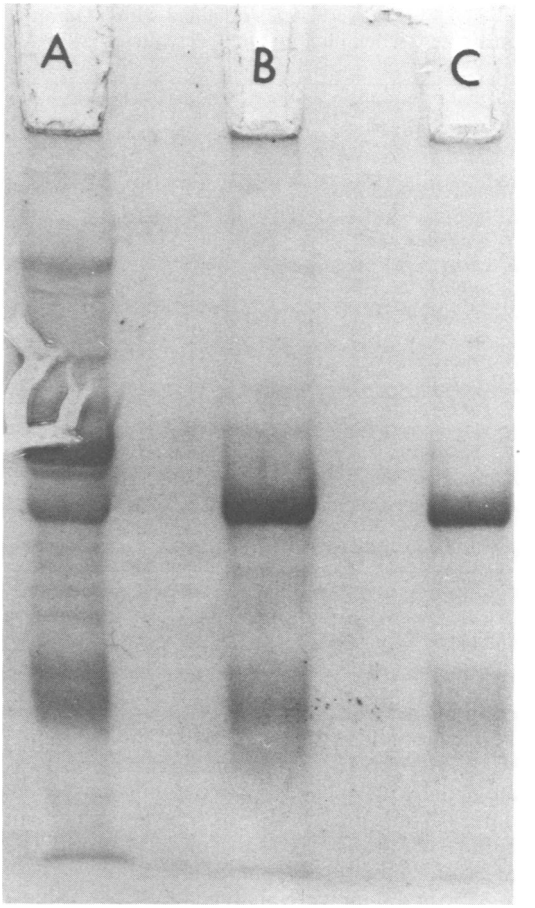

FIG. 2. Sodium dodecyl sulfate-polyacrylamide gels of: (A) unpurified Spicer-Edwards antiserum, (B) commercial goat anti-rabbit IgG, and (C) IgG purified from Spicer-Edwards antiserum by passage through a column containing SPA-Sepharose 4B.

Table 1 contains results obtained with pure cultures by using a Gilford EIA reader. Duplicate samples were run with each culture, and a control cuvette was used to monitor the efficiency of washing of unbound antibody and enzyme-antibody conjugate. Absorbancies of cuvettes that contained salmonellae all exceeded 0.9, and absorbancies of control cuvettes and control cultures were all <0.5. Table 2 contains the results of a similar study, except the analyses were conducted in microtitration plates and a Flow Titertek EIA reader was used. Absorbancies of wells containing salmonellae all exceeded 1.0, and absorbancies of control wells were all <0.1. Representative *Salmonella*-positive and -negative EIA results, using microcuvettes and microtitration plates, are shown in Fig. 3 and 4, respectively.

A comparison of the EIA procedure conduct-

TABLE 1. Absorbancies obtained with a Gilford instrument when pure cultures were analyzed by an EIA

Organism	Absorbance		
	Sample 1	Sample 2	Control[a]
Salmonella anatum	0.87	1.58	0.10
S. blockley	2+	2+	0.30
S. bredeney	2+	2+	0.42
S. cerro	2+	1.84	0.22
S. illinois	2+	2+	0.49
S. kaapstad	1.92	1.98	0.10
S. miami	2+	2+	0.21
S. paratyphi B	2+	2+	0.16
S. rubislaw	2+	2+	0.41
S. sandiego	2+	2+	0.09
S. senftenberg	2+	2+	0.28
S. zuerich	2+	2+	0.22
Citrobacter freundii	0.24	0.18	0.09
Enterobacter freundii X539A	0.25	0.20	0.03
Enterobacter freundii	0.15	0.24	0.15
Escherichia coli 2B14	0.34	0.34	0.18

[a] Control wells received no Spicer-Edwards IgG, but did receive goat anti-rabbit–alkaline phosphatase conjugate.

ed in microtitration plates with IF, ES, and the PCT was made by using 98 food samples. In this study, the preenrichment medium was also examined by EIA by using the following procedure: 0.1 ml of each 18- to 24-h preenrichment culture was transferred to 10 ml of M-broth. The M-broth subcultures were incubated for 4 to 6 h at 35°C and then were analyzed by EIA. Table 3 contains data from individual subsamples of naturally contaminated foods that resulted in positive identification of salmonellae by EIA. These foods all contained less than one viable *Salmonella* cell per gram. Thirteen subsamples were positive by all methods. The *Salmonella* serotypes from the chicken, yeast, and spice samples were *S. heidelberg*, *S. minnesota*, and *S. rubislaw*, respectively. Five other subsamples of these same foods were positive by one or more IF test, but were negative by all other methods; these probably were false-positive reactions. False-positive IF reactions were also obtained on subsamples of pork liver and chocolate candy, but no salmonellae were detected by any other method. Because of the tendency of IF to give false-positive reactions, we examined closely those instances when EIA yielded positive results in the absence of conclusive cultural evidence for the presence of salmonellae (samples 2, 4, 7, 9, 10, 11, and 19, Table 3).

Two criteria were used to score positive EIA samples. During the analysis of a large number of pure cultures and food samples in microtitration plates, we noted that control values varied, with respect to absorbance, from 0.06 to 0.38.

TABLE 2. Absorbancies obtained by using a Flow Titertek instrument when pure cultures were analyzed by an EIA

Organism	Absorbance	
	Sample	Control[a]
Salmonella anatum	1.01	0.03
S. rubislaw	1.27	0.03
S. senftenberg	1.34	0.07
S. zuerich	1.25	0.03
Citrobacter freundii	0.26	0.01
Escherichia coli B	0.16	0.02

[a] Control wells received no Spicer-Edwards IgG, but did receive goat anti-rabbit–alkaline phosphatase conjugate.

Hence, an absorbance above 0.40 was one criterion that was used to conclude that a sample was positive. With a few food samples, however, the difference between the control and replicate test wells was appreciable, although the samples gave absorbance values of <0.40. Therefore, a difference of 0.20 absorbance unit between control and sample wells was considered a borderline positive. If these interpretations were correct, then subsamples 2 and 4 of Table 3 contained *S. heidelberg* detected only by EIA and, in one instance, only by EIA of the preenrichment broth. Likewise, subsamples 7, 9, 10, 11, and 19 may have contained low levels of *S. minnesota* that did not survive selective enrichment. An alternative explanation is that these subsamples did not contain salmonellae and that false-positive results were obtained by EIA. These possible false-positive EIA results could have been eliminated if the sole criterion of a positive test was an absorbance reading of 0.40 or higher. But we did not choose to neglect these borderline cases because they may be true positive tests, and they represented 25% of the subsamples. Also, these borderline reactions were never observed when 100 or more subsamples of food that did not contain *Salmonella* were examined. In all likelihood, each laboratory will have to set its own parameters, based on the sensitivity of the instrument, properties of the reagents, and type of food involved.

Sensitivity thresholds of EIA. Results obtained by using three *Salmonella* serotypes for sensitivity threshold determinations indicated that approximately 10^6 *Salmonella* cells per ml (10^5 cells per microtitration plate well) gave a strongly positive reaction that was sufficient for visual discrimination. *S. rubislaw* gave a weakly positive reaction at the level of 10^5 cells per ml (10^4 cells per microtitration plate well). Thus, the limit of detection of salmonellae by EIA was as good as, if not better than, the lower limit of sensitivity of IF.

A single experiment was conducted by using a

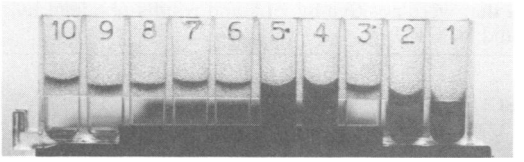

FIG. 3. Microcuvette showing representative *Salmonella*-positive and -negative samples. Samples 1 (wells 1 to 3) and 2 (wells 4 to 6) are positive tests as indicated by the chromogenic reaction product. Sample 3 (wells 7 to 9) is negative. Wells 3, 6, and 9 are control wells that received goat anti-rabbit–alkaline phosphatase conjugate but no Spicer-Edwards IgG.

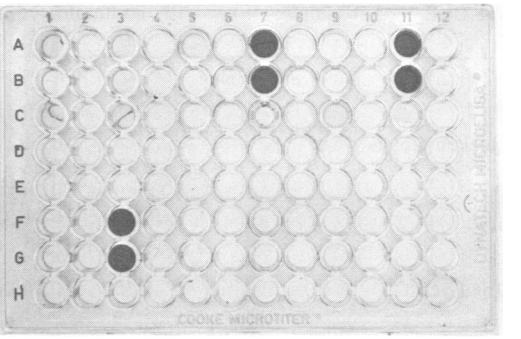

FIG. 4. Microtitration plate showing representative *Salmonella*-positive and -negative samples. Samples (three wells per sample) were added to vertical columns 1, 3, 5, 7, 9, and 11. Wells in rows C and H that received test material were used as controls. Wells in rows D and E were not used. Of the 12 samples tested here, 3 were strongly positive (vertical columns 3, 7, and 11).

fluorogenic substrate, 4-methyl umbelliferyl phosphate. When *S. anatum* was used as the test organism, 10^5 cells per ml were sufficient for a positive identification. Also, fluorogenic reactions were easier to read than the yellow reaction product of *p*-nitrophenyl phosphate, and no instrumentation was necessary to detect a positive reaction.

DISCUSSION

Enzyme-labeled antibodies are powerful assay tools (2, 8, 12, 19), and two publications have appeared on the detection of salmonellae with EIA (5, 14). Krysinski and Heimsch (5) advocated the use of goat anti-rabbit immunoglobulin-horseradish peroxidase conjugates in an indirect test to detect salmonellae fixed to membrane filters. Swaminathan and Ayres (14) developed a direct EIA for detecting salmonellae in foods; cell preparations were fixed to glass slides and were observed microscopically, as in IF. Although their method is excellent, the microscopy requirement is a deterrent to widespread adoption of the method.

Our original approach was to improve the method of Krysinski and Heimsch (5) and to use cellulose acetate membrane filters and peroxidase conjugates. First, we purified IgG from commercial polyvalent Spicer-Edwards antisera to obtain a reagent free of cross-reactive antibodies (Fig. 1 and 2). This approach was based on reports that O antigens stimulated almost entirely an IgM response in rabbits (6, 7) and flagellar antigens elicited primarily an IgG response (1, 10). We expected that the use of purified H-antibodies would solve what seemed to be a problem caused by cross-reactions between nonsalmonellae and antibodies to somatic antigens. Later, we discovered that most of the apparent nonspecificity encountered was caused by the expression of intracellular peroxidases or catalases or both in a variety of food-borne bacteria. This latter problem could not be overcome, even though several methods were used to inactivate the bacterial enzymes before EIA analysis. For example, fixed cells were treated with NaN_3, 30% H_2O_2 in absolute methanol, formaldehyde vapors, or by heating. None of these treatments was successful. Furthermore, cell mats frequently sloughed off the membranes during manipulation, which could lead to false-negative results.

Consequently, we used alkaline phosphatase conjugates. Since the products of alkaline phosphatases on various substrates are soluble, unlike the product of peroxidase, the products would not remain localized on membrane filters. Therefore, microtitration plate assays were developed. Fixation of cells to the polystyrene of microtitration plates likewise posed a problem. Although success was obtained in preventing the sloughing of cells in pure culture studies (S. A. Minnich, Ph.D. thesis, Iowa State University, Ames, 1980), inconsistencies were encountered when foods were examined. This problem was only partly solved by using a direct EIA procedure (4) to reduce the number of wash steps required. Consequently, we adapted the method of Polin and Kennett (12), which does not require cell fixation because unbound antibody and conjugated antibody were removed by wash steps that used low-speed, short-time centrifugation.

The results obtained in pure culture studies with the indirect EIA procedure indicated that the procedure was sensitive and specific. Either visual examination or absorbance determinations could be used to differentiate positive from negative samples (Tables 1 and 2).

Naturally contaminated foods that contained very low numbers of salmonellae were then analyzed to compare the modified EIA with the three most popular *Salmonella* screening proce-

TABLE 3. Subsamples of naturally contaminated foods that were positive by EIA and results obtained by using IF, ES, and a PCT

Food	Subsample no.	EIA (absorbance)			IF[a]		ES		PCT	
		Pre[b]	Tet[c]	SC[d]	Tet	SC	Tet	SC	Tet	SC
Chicken giblets	1	1.24	0.61	0.39	4+	3+	+	+	+	+
	2	0.82	0.16	0.13	−	−	−	−	−	−
	3	0.50	0.19	0.59	3+	3+	+	+	+	+
	4	0.59	0.51	0.23	−	2+	−	−	−	−
	5	0.45	0.14	0.41	−	4+	−	+	+	+
	6	0.46	0.35	0.39	4+	4+	+	+	+	+
Dried yeast	7	0.35	0.16	(−)[e]	−	−	−	−	−	−
	8	1.26	0.61	(+)	4+	4+	+	+	+	+
	9	0.26	0.04	(−)	2+	−	−	−	−	−
	10	0.33	0.06	(−)	−	−	−	−	−	−
	11	0.34	0.07	(−)	−	−	−	−	−	−
Spice condiment	12	0.77	0.83	0.56	4+	4+	+	+	+	+
	13	0.91	1.45	0.79	4+	4+	+	+	+	+
	14	0.90	0.90	1.02	4+	4+	+	+	+	+
	15	0.67	0.93	0.85	4+	4+	+	+	+	+
	16	0.73	1.05	1.26	4+	4+	+	+	+	+
	17	0.91	0.55	0.99	4+	4+	+	+	+	+
	18	0.54	0.62	0.83	4+	4+	+	+	+	+
	19	0.35	0.18	0.20	3+	−	±	−	−	−
	20	0.87	1.09	0.97	4+	4+	+	+	+	+

[a] IF results were scored: −, very weak; +, weak; 2+, doubtful positive; 3+, definitely positive; and 4+, very strong. Only 3+ and 4+ reactions were considered positive in this study.
[b] Absorbance readings were obtained by EIA after overnight preenrichment (Pre).
[c] Tet, Tetrathionate broth.
[d] SC, Selenite-cystine broth.
[e] Not determined quantitatively (+ or − by visual observation).

dures. The most striking discovery of this series of experiments was that the EIA procedure could detect the presence of salmonellae in preenrichment broths. All positive confirmed samples were initially identified as positive by EIA when preenrichment broths were subcultured for 4 to 6 h in M-broth before the EIA test (Table 3). Furthermore, in many instances higher absorbance values were obtained from the preenrichment broths than from subcultures of the selective enrichment broths. Also, some "doubtful positive" preenrichment broths were detected by EIA; these invariably were obtained from foods, subsamples of which had yielded salmonellae upon other occasions. Doubtful positive EIA tests were never obtained on subsamples of foods, other subsamples of which did not yield a *Salmonella* sp. by culture. These results suggest that selective enrichment may be too selective against many salmonellae and that preenrichment broths should be examined for the presence of salmonellae. The EIA test is sufficiently sensitive to do this. The fluorescent-antibody and possibly the ES tests also may possess sufficient sensitivity, but the fluorescent-antibody test would yield too many false-positive reactions and no one has studied the efficacy of ES tests when applied to preenrichment broths.

Both instruments used to measure product color (the Gilford and Flow Laboratories units) were satisfactory. Gilford microcuvettes hold 0.5 ml in deep wells; therefore, cells could be thoroughly resuspended by adding 0.3 ml of buffer and gently shaking the cuvettes. In contrast, the microtitration plates that we used had a well capacity of 0.25 ml. Occasionally, the use of wooden applicator sticks was required to resuspend the cell pellets.

We obtained consistent and reproducible EIA results when the M-broth was used in all postenrichment procedures. Similarly, good EIA results were obtained by another laboratory when M-broth was used (F. Delacroix, personal communication), but abnormalities arose when brain heart infusion or Trypticase soy broth was used for postenrichment. Problems may also arise with various brands of microtitration plates; therefore, we recommend standardization of the EIA procedure with pure cultures before food samples are tested.

The EIA procedure described here has several advantages over other *Salmonella* screening methods. Sensitivity of the EIA is equivalent to or better than the lower limit of IF of 10^5 salmonellae per ml (16). Tube agglutination, as performed in ES, requires an optimal concentration of about 10^7 salmonellae per ml (13), and

this may be a drawback when preenrichment broths are examined. Problems in IF analyses also are encountered when high concentrations of salmonellae are present (16) because decreased fluorescence of cells is obtained in the presence of high concentrations of antigen; this would not be a problem with EIA. Further advantages of the EIA are that the results can be quantitated and the reaction product is stable. In contrast, IF results are subjective, and the fluorescence fades rapidly when the slides are exposed to UV light. The use of purified IgG in EIA results in specific reactions, whereas polyvalent OH conjugates conventionally used in IF result in a false-positive rate of about 9% (16). Lastly, EIA is amenable to automation for rapid multisample analysis. Automated enzyme-linked immunosorbent assay systems are currently in use, and the adaptation of the *Salmonella* EIA test to present commercial equipment could be accomplished readily.

The present *Salmonella* EIA has two drawbacks. A centrifuge is needed, and we proposed an indirect procedure that required two centrifugation steps. A direct test was shown to be feasible (4), but its routine use in the laboratory is dependent upon reliable means of fixing cell sheets to microtitration plates. More potent anti-H antibody preparations also would be helpful. Lastly, the use of fluorogenic substrates, which was only briefly addressed in this study, shows promise of increased sensitivity. We are examining some of these factors in further detail.

ACKNOWLEDGMENTS

This study was primarily supported by the Iowa Agriculture and Home Economics Experiment Station under Project 2377 and, to a lesser extent, by the Idaho Agricultural Experiment Station under Project 708-R670.

LITERATURE CITED

1. **Ada, G. L., G. J. V. Nossal, J. Pye, and A. Abbot.** 1964. Preparation and properties of flagellar antigens from *Salmonella adelaide*. Aust. J. Exp. Biol. Med. Sci. **42**:267–282.
2. **Engvall, E., and A. J. Pesce (ed.).** 1978. Quantitative enzyme immunoassay. Scand. J. Immunol. **8**(Suppl. 7):1–129.
3. **Food and Drug Administration.** 1978. Bacteriological analytical manual for foods, 5th ed. Association of Official Analytical Chemists, Washington, D.C.
4. **Hartman, P. A., and S. A. Minnich.** 1981. Automation for rapid detection of salmonellae in foods. J. Food Prot. **44**:385–393.
5. **Krysinski, E. P., and R. C. Heimsch.** 1977. Use of enzyme-labeled antibodies to detect *Salmonella* in foods. Appl. Environ. Microbiol. **33**:947–954.
6. **Landy, M., R. P. Sanderson, and A. L. Jackson.** 1965. Humoral and cellular aspects of the immune response to the somatic antigen of *Salmonella enteritidis*. J. Exp. Med. **122**:483–504.
7. **Landy, M., and W. P. Weidanz.** 1964. Natural antibodies against gram-negative bacteria, p. 275–290. *In* M. Landy and W. Braun (ed.), Bacterial endotoxins. Institute of Microbiology, Rutgers State University, New Brunswick, N.J.
8. **Maggio, E. T. (ed.).** 1980. Enzyme immunoassay. CRC Press, Inc., Boca Raton, Fla.
9. **March, S. C., I. Parikh, and P. Cuatrecasas.** 1974. A simplified method for cyanogen bromide activation of agarose for affinity chromatography. Anal. Biochem. **60**:149–152.
10. **Nossal, G. J. V., G. L. Ada, and C. M. Austin.** 1964. Immunogenic properties of flagella, polymerized flagellin and flagellin in the primary response. Aust. J. Exp. Biol. Med. Sci. **42**:283–294.
11. **Poelma, P. L., and J. H. Silliker.** 1976. *Salmonella*, p. 301–328. *In* M. L. Speck (ed.), Compendium of methods for the microbiological examination of foods. American Public Health Association, Inc., Washington, D.C.
12. **Polin, R. A., and R. Kennett.** 1980. Use of monoclonal antibodies in an enzyme immunoassay for rapid identification of group B *Streptococcus* types II and III. J. Clin. Microbiol. **11**:332–336.
13. **Sperber, W. H., and R. H. Deibel.** 1969. Accelerated procedure for *Salmonella* detection in dried foods and feeds involving only broth culture and serological reactions. Appl. Microbiol. **17**:533–539.
14. **Swaminathan, B., and J. C. Ayres.** 1980. A direct immunoenzyme method for the detection of salmonellae in foods. J. Food Sci. **45**:352–355, 361.
15. **Swaminathan, B., J. C. Ayres, and J. E. Williams.** 1978. Control of nonspecific staining in the fluorescent antibody technique for the detection of salmonellae in foods. Appl. Environ. Microbiol. **35**:911–919.
16. **Thomason, B. M.** 1976. Fluorescent antibody detection of salmonellae, p. 329–343. *In* M. L. Speck (ed.), Compendium of methods for the microbiological examination of foods. American Public Health Association, Inc., Washington, D.C.
17. **Thomason, B. M.** 1981. Current status of immunofluorescent methodology for salmonellae. J. Food Prot. **44**:381–384.
18. **Voller, A., D. Bidwell, and A. Bartlett.** 1976. Microplate enzyme immunoassays for the immunodiagnosis of viral infections, p. 506–512. *In* N. R. Rose and H. Friedman (ed.), Manual of clinical immunology, 1st ed. American Society for Microbiology, Washington, D.C.
19. **Voller, A., D. Bidwell, and A. Bartlett.** 1979. The enzyme-linked immunosorbent assay (ELISA). Nuffield Laboratories of Comparative Medicine, The Zoological Society of London, London.
20. **Yolken, R. H., and P. J. Stopa.** 1979. Enzyme-linked fluorescence assay: ultrasensitive solid-phase assay for detection of human rotavirus. J. Clin. Microbiol. **10**:317–321.