APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1990, p. 924–927
0099-2240/90/040924-04$02.00/0

Vol. 56, No. 4

Salmonella-TEK, a Rapid Screening Method for *Salmonella* Species in Food

LIEVE S. G. VAN POUCKE

Laboratory of Pharmaceutical Microbiology and Hygiene, State University of Ghent, Harelbekestraat 72, 9000 Ghent, Belgium

Received 26 September 1989/Accepted 8 January 1990

A micro-enzyme-linked immunosorbent assay (micro-ELISA) using the Salmonella-TEK screen kit was tested for the detection of *Salmonella* spp. in pure cultures as well as in 30 artificially contaminated food samples and in 45 naturally contaminated food samples. Different raw, fleshy foods and processed foods were used as test products. The artificially contaminated minced meat samples were preenriched in buffered peptone water, and after incubation, different selective enrichment broths were tested. The micro-ELISA optical density values after enrichment and isolation of the different broths were very analogous. The quickest method to detect *Salmonella* spp. in different foods is to enrich them with Salmosyst broth, which reduces the total analysis time to 31 h. The Salmonella-TEK kit for *Salmonella* spp. provides a promising test for the detection of *Salmonella* antigens in food even when they are present at a low concentration (1 to 5 CFU/25 g). The cross-reaction of the anti-*Salmonella* antibodies, especially to other gram-negative bacteria, is nil.

Salmonella organisms are gram-negative rods which belong to the family *Enterobacteriaceae*. This group contains the etiologic agents of food-borne salmonellosis as well as the agents that cause typhoid and paratyphoid fever. *Salmonella* infection is responsible for 10 to 15% of acute gastroenteritis cases. Eggs, poultry, meat, meat products, and chocolate are the most common sources of food-borne salmonellosis (3, 8, 9, 11, 13, 17, 19).

Salmonella infection results from the ingestion of food or water containing sufficient numbers of these bacteria to reach and invade the small intestine. Clinical symptoms include acute gastroenteritis, bacteremia with or without localized extraintestinal infection, typhoid-like fever, and an asymptomatic carrier state.

The detection of *Salmonella* in food and foodstuff is often difficult. Salmonellae may be sublethally injured and may be present in low numbers compared with similar, closely related organisms (3). Thus, they are difficult to culture in sufficient numbers for easy identification. Furthermore, conventional culture and serologic identification techniques (7, 21) are lengthy and require costly handling and storage of food during testing.

Enzyme immunoassays and enzyme-linked immunosorbent assays (ELISAs) have recently been developed to detect salmonellae in food (1, 4–6, 10, 12, 15, 16, 18) and in clinical specimens (2). The Salmonella-TEK screen kit (Organon Teknika) incorporates monoclonal antibodies to provide an excellent means of detecting the presence of *Salmonella* spp. in food.

Different enrichment methods were tested in order to determine as soon as possible whether the food samples were contaminated with *Salmonella* spp. By decreasing the time required to screen food products, this test provides an economical alternative to conventional methods.

The Salmonella-TEK screen kit was routinely used in the microbiological food analysis.

MATERIALS AND METHODS

Chemicals. All reagents needed for the detection of *Salmonella* species with the micro-ELISA test system were supplied by the Salmonella-TEK kit from Organon Teknika N.V.

Tryptic soy broth, tryptic soy agar (TSA), tetrathionate broth (TetB), and selenite cystine broth (SCB) were commercial products from Difco Laboratories. Rappaport (RB) and Salmosyst were obtained from Merck & Co., and M-broth was prepared as described by Sperber and Deibel (20). Buffered peptone water (BPW) contained peptone, 10 g; sodium chloride, 5 g; disodium hydrogen phosphate ($Na_2HPO_4 \cdot 12\ H_2O$), 9 g; potassium dihydrogen phosphate (KH_2PO_4), 1.5 g; and water, 1 liter (pH 7.5 ± 0.1). Phosphate-buffered saline contained Na_2HPO_4, 1.25 g; $NaH_2PO_4 \cdot H_2O$, 0.18 g; NaCl, 8.59 g; and water, 1 liter (pH 7.5 ± 0.2). Peptone physiological saline (PPS) was prepared from 9 g of NaCl and 1 g of Difco Proteose Peptone in 1 liter of water.

Test organisms. Three *Salmonella* species, *Salmonella typhimurium* ATCC 14028, *Salmonella typhi* 54136, and *Salmonella paratyphi* QC 1985/3, were used for inoculation into foods. Individual *Salmonella* species were inoculated in 10 ml of tryptic soy broth from stock TSA slants and incubated for 24 h at 35°C. Following incubation, cells were centrifugated at 4,000 × *g* for 10 min, the supernatant fluid was discarded, and the cell pellets were suspended in 10 ml of sterile BPW. The number of viable salmonellae per gram of inoculum was then estimated by the direct surface plate count procedure. Decimal dilutions of bacteria were prepared in PPS, and counts were done by using pour plates of TSA at 35°C for 2 days. Each developed colony was assumed to have grown one viable unit.

Preparation of inoculated foods. The following method was used for preparation of inoculated test products. A suspension of the sterile BPW with the *Salmonella* pellets was added to 60 g of each test product contained in a sterile plastic bag, and the contents were mixed by shaking with a Stomacher 80 for 15 to 30 min to produce a "seed." These seed inocula were used to test the different selective enrichment media and were measured by the micro-ELISA test system. Different foods obtained from local stores were used as test products; they included raw meat, chicken, egg, fish,

and processed food such as chocolate and nonfat dry milk (5).

Analysis of food samples. Enrichment and isolation procedures for raw, fleshy foods were different than those for processed foods.

The 30 artificially contaminated minced meat samples (seed) were preenriched in BPW. The use of this nonselective medium is an appropriate preenrichment technique and gives very good results (14). Briefly, 25 g of the seed was homogenized in 225 ml of BPW and then preenriched by incubation for 24 ± 2 h at 35°C. After this preenrichment, different selective enrichment broths, TetB, SCB, RB, and Salmosyst, were tested. Samples of the preenrichment broth were transferred to TetB and SCB, as described in the Food and Drug Administration's Bacteriological Analytical Manual, and also to RB. The incubation times and temperatures were 18 to 24 h at 43°C for TetB and 18 to 24 h at 35°C for SCB and RB. An enrichment with Salmosyst was also tested, but here the preenrichment was 6 to 8 h at 35°C in the Salmosyst broth base, which contains only nutrients, electrolytes, and pH-regulating substances (25 g of seed per 225 ml of broth), with consecutive enrichment in selective broth (1 selective supplement tablet per 10 ml of preenrichment broth) for 18 to 22 h at 35°C. After incubation, 0.5 ml from each selective enrichment was transferred into a single 10-ml tube of sterile M-broth containing novobiocin (10 μg/ml) and incubated at 43°C for 4 to 6 h. The selective enrichment broths and the M-broth were reincubated and retained for possible cultural confirmation of enzyme immunoassay-positive samples. Following incubation, 10 ml of the M-broth culture was pipetted into a centrifuge tube and centrifuged at 3,000 × *g* for 20 min. The supernatant was discarded, and the pellet was suspended in 1.0 ml of phosphate-buffered saline. The pellet was vortexed to mix thoroughly and was heated in boiling water for 20 min. The heated extract was cooled to 25 to 37°C prior to testing or was stored at 4°C for no more than 3 days.

Preenrichment procedures for the isolation of *Salmonella* spp. in processed foods was identical to that for raw, fleshy food, namely 25 g of processed food per 225 ml of BPW and 18 to 24 h of incubation at 35°C. Portions (1 ml) of the preenrichment broth were transferred to the selective enrichment broths, TetB, SCB, and RB, prewarmed to 35°C as described in the Food and Drug Administration's *Bacteriological Analytical Manual for Foods* (7). These broths were incubated for 6 to 8 h at 35°C. The enrichment with Salmosyst was identical to that described for fleshy food. After incubation, 1.0 ml of the TetB mixtures was transferred to a 10-ml tube of sterile M-broth without novobiocin and labeled as "M-broth Tet." One milliliter of SCB was transferred to a second 10-ml tube of sterile M-broth and labeled as "M-broth SC." For the RB as well as for the Salmosyst, 1.0 ml was transferred to 10 ml of M-broth. All M-broths and remaining TetB, SCB, RB, and the Salmosyst broths were incubated at 35°C for 14 to 18 h. Following incubation, the M-broth Tet and M-broth SC tubes were mixed and 0.5 ml was removed from each M-broth culture and placed in the same glass screw-top test tube. The remaining M-broths, TetB, and SCB were retained for cultural confirmation of enzyme immunoassay-positive samples. The combined M-broths and 1.0 ml of the M-broths from RB and Salmosyst were heated in a boiling water bath for 20 min. The samples were cooled to 25 to 37°C prior to testing or were stored at 4°C for no more than 3 days.

Salmonella-TEK ELISA test system. Specifically, polystyrene micro-ELISA wells are coated with monoclonal antibodies to *Salmonella* antigens. With the addition of test samples (in duplicate) or appropriate controls containing *Salmonella* organisms (positive control [PC]), immune complexes are formed through the interaction of *Salmonella* antigen in the sample and the specific *Salmonella* monoclonal antibodies bound to the well. Also, two negative controls were included. The negative control consists of a lyophilized antigen, nonreactive for *Salmonella* species. After an incubation of 30 min at 37°C, the samples and controls were aspirated and the wells were washed three times to remove unreacted specimens. Subsequently, a peroxidase-labeled antibody (conjugate) that binds to the *Salmonella* antigen portion of the immune complex was added; the mixture was incubated for 30 min at 37°C and washed six times to remove unreacted conjugate. Peroxidase substrate (3,3'-5,5'-tetramethylbenzidine) was added to the wells, and the plates were kept at room temperature for 30 min for color development. A blue color, which turned yellow when the reaction was stopped with Stop solution (2 N sulfuric acid), was produced. This color development indicates the presence of *Salmonella* antigen in the sample tested.

The absorbance or optical density (OD) at a wavelength of 450 nm was measured on a microplate reader MPR A4 (Euro-Genetics). The whole ELISA can be done in about 2 h.

Sensitivity of the ELISA procedure. Studies with *S. typhimurium* were conducted to determine the level of sensitivity of the ELISA procedure. Serial decimal dilutions of overnight M-broth cultures were made in PPS. A viable-cell count was made by plating appropriate dilutions in TSA at 35°C for 2 days. Concomitantly, 100-μl amounts of dilutions of 10^1 through 10^9 CFU/ml were dispensed into microdilution plate wells and subjected to micro-ELISA analysis.

Specificity of the ELISA procedure. Seven gram-negative bacteria (*Escherichia coli*, *Proteus vulgaris*, *Proteus mirabilis*, *Yersinia enterocolitica*, *Shigella sonnei*, *Citrobacter freundii*, and *Pseudomonas aeruginosa*) were tested to control the specificity of the monoclonal antibody of the kit. These species were inoculated in 10 ml of tryptic soy broth from stock TSA slants and were incubated for 24 h at 35°C. Decimal dilutions of bacteria were prepared in PPS, and counts were done by using pour plates of TSA at 35°C for 2 days. First, 100 μl of a dilution of 10^7 CFU/ml of PPS was dispensed into microdilution plate wells and subjected to micro-ELISA analysis. Second, these bacteria were also enriched in RB by the same protocol as was done for the food samples contaminated with *Salmonella* spp. A 100-μl portion of these suspensions was also subjected to micro-ELISA analysis.

Calculation and interpretation. The mean of the negative controls was calculated ($NC\bar{x}$). This value should be less than 0.300. The PC value must be greater than 0.700. All controls must be within these limits for the test to be valid. If the controls were not within these limits the run was repeated.

If the absorbance of a sample was greater than or equal to the cutoff value (equal to $NC\bar{x} + 0.250$), it was presumed to be positive for the presence of *Salmonella* species. The cutoff value is a criterion that is used to conclude whether a sample is positive, and these results should be confirmed by culture. Samples of each broth should be streaked onto selective agars. Negative ELISA readings indicate that the sample does not contain detectable levels of *Salmonella* antigens by this test method, and no further testing is required.

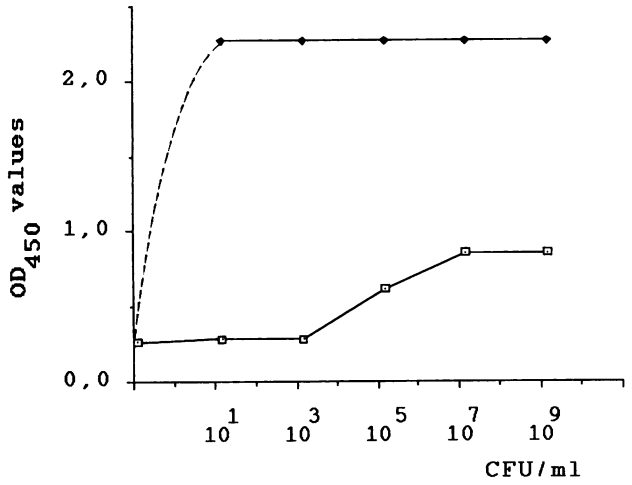

FIG. 1. Dose responses of the micro-ELISA test system for *S. typhimurium* in PPS culture (□) and after enrichment in Rappaport broth (■). The low concentration of *S. typhimurium* after enrichment was not tested and is illustrated by the dashed line.

TABLE 1. Specificity of monoclonal antibodies for *Salmonella* species tested with seven other gram-negative bacteria[a]

Bacteria used (10^7 CFU/ml)	OD_{450} values		Result
	In PPS	After enrichment	
Salmonella typhimurium	0.816	2.229	+
Salmonella typhi	0.894	2.268	+
Salmonella paratyphi	0.501	0.726	+
Proteus vulgaris	0.267	0.329	−
Proteus mirabilis	0.318	0.356	−
Escherichia coli	0.302	0.321	−
Pseudomonas aeruginosa	0.319	0.320	−
Yersinia enterocolitica	0.333	0.359	−
Shigella sonnei	0.321	0.382	−
Citrobacter freundii	0.318	0.362	−
$NC\bar{x}$	0.237		
PC	1.981		
Cutoff (= $NC\bar{x}$ + 0.250)	0.487		

[a] The OD values of these bacteria were measured immediately in a PPS dilution and after enrichment of the bacteria in RB.

RESULTS AND DISCUSSION

To evaluate the Salmonella-TEK kit, two negative controls and a PC are necessary. The values of both negative controls must be smaller than 0.300. Values greater than or equal to 0.300 indicate problems with the wash procedure, and the run must be repeated. Even when the PC value is smaller than 0.700, the run must also be repeated. If the $NC\bar{x}$ and the PC are within these limits, the OD values of the samples can be accepted. The results concerning the sensitivity threshold of the micro-ELISA test system obtained by using *S. typhimurium* are presented in Fig. 1. The OD value for the average of the negative controls was 0.237, and the cutoff value was 0.487 (0.237 + 0.250). The sensitivity threshold was 10^5 CFU/ml when the cultures in PPS were analyzed, or 10^4 cells per microdilution well. This level of sensitivity is 1 order of magnitude lower than that reported by other workers (1, 15). After enrichment in a selective enrichment broth (RB), the sensitivity threshold value decreased to 10 CFU/ml or less but these low concentrations of *Salmonella* spp. per milliliter were not tested and are shown by the dashed line in Fig. 1.

The cross-reactivity of the antibody (specificity of the micro-ELISA test system) is shown in Table 1. The OD values of a dilution in PPS of the *Salmonella* species were higher than those of the cutoff value (0.487). For *S. paratyphi*, the concentration of 10^7 CFU/ml was necessary to give a positive value. The monoclonal antibodies show a greater affinity for *S. typhimurium* and *S. typhi* than for the species *S. paratyphi*. Similar specimens inoculated with or containing various species other than *Salmonella* spp. were negative by this test. The OD values of these seven gram-negative species were lower than the cutoff value even after enrichment. Hence, we can conclude that there is no cross-reactivity with the monoclonal antibodies for *Salmonella* spp. with these bacteria when the dilutions were made in PPS and immediately subjected to the micro-ELISA analysis, as well as after a preenrichment and enrichment in the selective RB.

Thirty different artificially contaminated foods were tested by the micro-ELISA test system. Raw meat, chicken, eggs, and fish were enriched according to the protocol for raw, fleshy food, and chocolate and dry milk were tested by the method for processed food.

All of the samples were first tested for *Salmonella* spp. by using conventional methods. All of them were negative. Simultaneously, we made a seed inoculum from each sample as described in the preparation of inoculated foods. The total counts of the seed inocula were obtained by a direct surface count procedure and were performed before and after contamination with the *Salmonella* spp. The values of the CFU of *Salmonella* spp. per 25 g of seed inoculum are illustrated in Table 2. Each seed inoculum was isolated in the four different selective enrichment broths. The micro-ELISA OD values after the enrichment and isolation of the different broths were very analogous; the mean OD values are shown in Table 2. For each blank sample, a preenrichment and a selective enrichment in TetB were also performed, as was done for the micro-ELISA test procedure. The values of these measurements are also shown in Table 2, and all of these results were lower than the cutoff value, which means that they were all negative. The seed inocula were all positive, independent of the selective enrichment broth used. To reduce the analysis time, we preferred to make the enrichment and the isolation by means of the Salmosyst broth. The time needed for the isolation of *Salmonella* spp. was reduced to 31 h (29 h in enrichment broths plus 2 h for the ELISA), as compared with 45 h by the current method.

TABLE 2. *S. typhimurium* detection after enrichment of different artificially contaminated food types (seed) in different selective enrichment broths

Food tested	OD_{450} values					CFU of *Salmonella* spp./25 g of seed inocula
	Inoculated seed in:				Uninoculated seed in TetB	
	TetB	SCB	RB	Salmosyst		
Meat	2.233	2.180	2.226	2.192	0.366	1×10^2
Chicken	2.231	2.197	2.248	2.214	0.370	7×10^1
Egg	2.237	2.150	2.167	2.218	0.218	5×10^0
Fish	2.232	2.160	2.203	2.211	0.301	1×10^2
Chocolate	2.211		1.914	1.995	0.387	6×10^1
Dry milk	2.192		2.118	1.947	0.289	2×10^2

A total of 45 samples were tested with the micro-ELISA screen kit after enrichment in RB and Salmosyst broth. Of these samples, 4% yielded positive results on the presence of *Salmonella* spp., even after enrichment in RB as well as in Salmosyst broth. The positive samples were confirmed by conventional methods.

Conclusion. In this study we have shown that the Salmonella-TEK kit for *Salmonella* spp. provides a promising test for the detection of *Salmonella* antigens, even when they are present at low concentrations in food samples. The cross-reaction of the anti-*Salmonella* antibodies, especially to other gram-negative bacteria, is very low. The quickest method to detect *Salmonella* spp. in different foods or foodstuff is by enrichment with Salmosyst broth, which reduced the total analysis time to 31 h.

ACKNOWLEDGMENT

We thank Rosa Aerts for excellent technical assistance.

LITERATURE CITED

1. **Anderson, J. M., and P. A. Hartman.** 1985. Direct immunoassay for detection of salmonellae in foods and feeds. Appl. Environ. Microbiol. **49:**1124–1127.
2. **Araj, G. F., and T. Das Chugh.** 1987. Detection of *Salmonella* spp. in clinical specimens by capture enzyme-linked immunosorbent assay. J. Clin. Microbiol. **25:**2150–2153.
3. **Bryan, F. L., M. J. Fanelli, and H. Riemann.** 1979. Salmonella infections. *In* H. Riemann and F. L. Bryan (ed.), Food-borne infections and intoxications, 2nd ed. Academic Press, Inc., New York.
4. **Eckner, K. F., R. S. Flowers, B. J. Robison, J. A. Mattingly, D. A. Gabis, and J. H. Silliker.** 1987. Comparison of Salmonella Bio-Enza Bead immunoassay method and conventional culture procedure for detection of salmonella in foods. J. Food Prot. **50:**379–385.
5. **Flowers, R. S., M. J. Klatt, S. L. Keelan, B. Swaminathan, W. D. Gehle, and H. E. Chandonnet.** 1989. Fluorescent enzyme immunoassay for rapid screening of Salmonella in foods: collaborative study. J. Assoc. Off. Anal. Chem. **72:**318–325.
6. **Flowers, R. S., M. J. Klatt, B. J. Robison, J. A. Mattingly, D. A. Gabis, and J. H. Silliker.** 1987. Enzyme immunoassay for detection of salmonella in low-moisture foods: collaborative study. J. Assoc. Off. Anal. Chem. **70:**530–535.
7. **Food and Drug Administration.** 1984. Bacteriological analytical manual for foods, 6th ed. Food and Drug Administration, Washington, D.C.
8. **Hayes, R. P.** 1985. Food microbiology and hygiene, p. 24–34. Elsevier Science Publishing, Inc., New York.
9. **Hockin, J. C., J.-Y. D'Aoust, D. Bowering, J. F. Jessop, B. Khanna, H. Lior, and M. E. Milling.** 1989. An international outbreak of Salmonella nima from imported chocolate. J. Food Prot. **52:**51–54.
10. **Ibrahim, G. F., M. J. Lyons, R. A. Walker, and G. H. Fleet.** 1985. Rapid detection of salmonellae by immunoassays with titanous hydroxide as the solid phase. Appl. Environ. Microbiol. **50:**670–675.
11. **Jay, M.** 1978. Modern food microbiology, 2nd ed. D. Van Nostrand Reinhold, New York.
12. **Krysinski, E. P., and R. C. Heimsch.** 1977. Use of enzyme-labeled antibodies to detect *Salmonella* in foods. Appl. Environ. Microbiol. **33:**947–954.
13. **Lammerding, A. M., M. M. Garcia, E. D. Mann, Y. Robinson, W. J. Forward, R. D. Truscott, and F. Tittiger.** 1988. Prevalence of salmonella and thermophilic campylobacter in fresh pork, beef, veal and poultry in Canada. J. Food Prot. **51:**47–52.
14. **Leusden, F. M., M. van Schothorst, and H. J. Beckers.** 1982. The standard Salmonella isolation method. *In* J. Corry, D. Roberts, and F. Skinner (ed.), Isolation and identification methods for food poisoning organisms. Academic Press, Inc., New York.
15. **Mattingly, J. A., and W. D. Gehle.** 1984. An improved enzyme immunoassay for detection of salmonella. J. Food Sci. **49:** 807–809.
16. **Minnich, S. A., P. A. Hartman, and R. C. Heimsch.** 1982. Enzyme immunoassay for detection of salmonellae in foods. Appl. Environ. Microbiol. **43:**877–883.
17. **Mossel, D. A. A.** 1988. Impact of foodborne pathogenes on today's world and prospects for management. Anim. Hum. Health **1:**13–23.
18. **Robison, B. J., C. I. Pretzman, and J. A. Mattingly.** 1983. Enzyme immunoassay for which a myeloma protein is used for detection of salmonellae. Appl. Environ. Microbiol. **45:**1816–1821.
19. **Rubin, R. H., and L. Weinstein.** 1977. Salmonellosis: microbiologic, pathologic and clinical features. Stratton Intercontinental Medical Book Corp., New York.
20. **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.
21. **Williams, S. (ed.).** 1984. Official methods of analysis, 14th ed., p. 963–971. Association of Official Analytical Chemists, Washington, D.C.