Comparison of Fluorescent-Antibody Methods and Enrichment Serology for the Detection of Salmonella

H. K. Mohr, H. L. Trenk, and M. Yeterian

Technical Center, General Foods Corporation, White Plains, New York 10602

Received for publication 24 October 1973

Four rapid methods for detection of Salmonella, (i) the conventional fluorescent-antibody (FA) technique, (ii) a rapid direct FA technique, (iii) microcolony FA, and (iv) enrichment serology (ES), were compared with conventional cultural procedures. A total of 347 subsamples representing 16 different food prototypes, alleged to be naturally contaminated with Salmonella, were analyzed. From these samples, 52 were found to contain Salmonella by cultural methods. Conventional FA identified all 52 culturally positive samples, ES identified 51, microcolony FA identified 48, and the rapid FA method identified 34. The number of false-positive samples for each procedure was: ES-selenite, 7; tetrathionate, 8; rapid FA, 26; microcolony FA, 33; conventional FA-selenite, 27; tetrathionate, 26. Tetrathionate enrichment was found to be superior to selenite for Salmonella recovery from most foods, but the concurrent use of both media allowed maximum recovery.

The demand for routine analysis for the detection of Salmonella in food and feeds has increased significantly in the last few years. As a result of this increased testing, research has been directed toward the development of faster and/or more sensitive methods to detect these organisms. The use of the fluorescent-antibody technique (FA) is not new, and recently two modified FA procedures for the detection of Salmonella have been described (5, 12). In addition, an enrichment serology (ES) method (1, 2, 10) has been evaluated and reported for use as a quality control measure in bacteriological laboratories.

The primary objective of this study was to compare five different methods for the detection of Salmonella in food and feeds. These methods were: (i) the conventional FA technique; (ii) a rapid direct technique described by Insalata et al. (5); (iii) the microcolony technique as described by Thomason (12); (iv) the ES technique as proposed by Sperber and Deibel (10); and (v) the standard cultural methods (13), with minor modifications. The procedure referred to as "conventional FA technique" is a modification of direct FA staining as described by numerous investigators (3, 6, 9). The experimental design used allowed a comparison of the time for pre-enrichment (7 versus 24 h) and an evaluation of the efficiency of tetrathionate and Selenite-F selective enrichments.

MATERIALS AND METHODS

Food and feed samples suspected of being naturally contaminated with Salmonella were obtained from various sources. The products, number of lots, and subsamples analyzed are presented in Table 1. Two sources of commercially available FA antisera were used throughout this work: FA Salmonella poly antiserum (11) (Difco, Detroit, Mich.) diluted 1:4 with sterile saline, and Salmonella Fluoro-Kit (4) (Clinical Sciences Inc., Whippany, N.J.) prepared as directed by the manufacturer. No differences between antisera were noted.

Pre-enrichment. Samples (25 g) of each food or feed prototype were pre-enriched in 225 ml of FAS broth (Difco) which was tempered at $35 \pm 2$ C. Milk products were pre-enriched in brilliant green water as recommended in the Bacteriological Analytical Manual (13). Incubation was at $35 \pm 2$ C for 24 h, with the exception of the rapid FA technique for which the incubation time was 7 h (5). Incubation was without agitation.

Selective enrichment. After incubation, the pre-enrichments were shaken and allowed to settle for about 5 min. In the rapid FA technique, 50 ml was transferred to 450 ml of prewarmed $35 \pm 2$ C Selenite-F broth (BBL) and incubated for 16 to 18 h at $35 \pm 2$ C without agitation. For all other tests, 2-ml portions of the pre-enrichment broth were transferred to 18 ml of Selenite-F broth and 18 ml of tetrathionate broth, respectively, and incubated at the same temperature for 24 h.

 Elective enrichment. Selenite-F broth was agitated and allowed to settle for 5 min for the rapid FA technique. Two milliliters was then withdrawn from
TABLE 1. Recovery of salmonellae from food with five methods*

| Food prototype          | No. of lots | No. of subsamples | Rapid FA (selenite) | Conventional FA | Micro-colony FA (tetra-thionate) | ES | Cultural |
|------------------------|------------|-------------------|---------------------|-----------------|---------------------------------|----|----------|
| Meat meal              | 4          | 60                | 6                   | 10-10           | 9                               | 6  | 8        | 8 10      |
| Dog food               | 2          | 40                | 5                   | 6-7             | 5                               | 6  | 7        | 7 7       |
| Pasta                  | 2          | 30                | 1                   | 1-2             | 1                               | 1  | 2        | 1 2       |
| Nonfat dry milk        | 1          | 30                | 0                   | 0               | 1                               | 0  | 1        | 0 1       |
| Buttermilk solids      | 2          | 30                | 1                   | 1               | 1                               | 1  | 1        | 1 1       |
| Whole egg powder       | 2          | 30                | 0                   | 0               | 0                               | 0  | 0        | 0 0       |
| Coconut                | 1          | 20                | 19                  | 19              | 19                              | 19 | 19       | 19 19     |
| Cereal-spice mix       | 1          | 20                | 1                   | 1               | 1                               | 1  | 1        | 1 1       |
| Fresh chicken          | 2          | 20                | 0                   | 0               | 0                               | 0  | 0        | 0 0       |
| Cereal mix             | 1          | 15                | 0                   | 0               | 0                               | 0  | 0        | 0 0       |
| Baker's cheese         | 1          | 10                | 0                   | 0               | 0                               | 0  | 0        | 0 0       |
| Frozen frog legs       | 1          | 10                | 0                   | 0               | 0                               | 0  | 0        | 0 0       |
| Fish meal              | 1          | 10                | 0                   | 0               | 0                               | 0  | 0        | 0 0       |
| Whey sox mix           | 1          | 10                | 0                   | 1               | 1                               | 0  | 1        | 1 0       |
| Potato mix (wet)       | 1          | 7                 | 1                   | 4               | 4                               | 4  | 4        | 4 4       |
| Egg yolk powder        | 1          | 5                 | 0                   | 5               | 5                               | 5  | 5        | 5 5       |

* Number of samples confirmed positive.

the top third of the culture, transferred to 18 ml of tempered FAS broth, and incubated for 5 h at 35 ± 2 C. For conventional FA analysis, 1 drop from Selenite-F and 1 drop from tetrathionate broth were transferred separately into 3 ml of Trypticase soy broth (TST) (7) and were incubated at 35 ± 2 C for 3 h or until visible growth was obtained. This elective enrichment medium for conventional FA was suggested by Wallace H. Andrews, Jr., of the Food and Drug Administration. The use of an elective enrichment in FA analysis resulted in cleaner slides with less background debris.

For the enrichment serology technique, 1 drop from each selective enrichment culture was added to M broth and incubated for 6 h at 35 ± 2 C in a water bath. Serological analysis was performed as recommended by Sperber and Deibel (10).

FA staining procedure. A loopful (2 mm) of the appropriate elective enrichment broth was placed onto the Fluoro-Kit slides, and they were stained by using the materials and methods specified by the supplier. If it is desired to observe nonfluorescent cells, FA Rhodamine counterstain (Difco) may be applied for 1 min after FA staining and then slides are rinsed for 30 s with distilled water. All nonfluorescent cells would be stained red.

For the microcolony technique, a 2-mm loopful from tetrathionate enrichments was placed onto brilliant green agar plates in such a manner as to coincide with the prepared areas on the Fluoro-Kit slides. The plates were incubated for 3 h at 35 ± 2 C. After this time, impression smears were made by placing the slides on the agar plates and exerting slight pressure. Slides were removed, allowed to air-dry, and were then stained by the recommended method.

Microscopy examination. Slides were examined on a Leitz Ortholux microscope equipped with incident-light fluorescence using exciting blue filters KP490 (FITC) and dichroic beam splitters with a K495 built-in suppression filter and 510 suppression filter slide. The light source was an Osram HBO-200 mercury arc burner equipped with a transmission heat filter (2 mm KGl) and a red suppression filter (4 mm BG38).

Smears were examined by using a dry ×40 objective and ×16 ocular. A ×54 oil fluorite objective was used for more detailed examination of smears to confirm the presence of attached flagella. Approximately 100 microscopic fields were scanned on each slide. One or more strongly fluorescing rods with discernable lumen in 10 or more fields was considered as positive.

Cultural methods. Selective enrichments of Selenite-F and tetrathionate broths were streaked on XLD agar (Difco) and heetcon enteric (HE; Difco) agar (8). XLD and HE agars were incubated at 35 ± 2 C for 24 h. In our laboratory, we found XLD and HE to be as good or better than Salmonella-Shigella agar and brilliant green agar for the isolation of Salmonella. Typical colonies were picked to triple sugar iron agar and lysine iron agar, and cultures exhibiting a presumptive positive Salmonella characteristic were confirmed serologically.

Subcultures in TST broth from Selenite-F and tetrathionate were incubated at 35 ± 2 C for 7 h and then streaked on XLD and HE agar. The experimental procedures are illustrated schematically in Fig. 1.

RESULTS

Out of 347 subsamples, 52 were positive by the cultural method employed in this study. Five of the isolates were not recovered from selenite enrichment but were recovered from tetrathionate enrichment. One of these five cultures was isolated from tetrathionate only after the additional elective enrichment in TST broth. This isolate would have been missed by
routine cultural methods. Another isolate did not grow in tetrathionate broth but was recovered from selenite broth (Table 1). The conventional FA method correctly identified 48 positive samples from selenite enrichment and 52 from tetrathionate enrichments. The rapid FA method correctly identified 34 positive samples.

Microcolony FA detected 48 of the 52 positive samples. The ES method gave 43 and 50 positive results from selenite and tetrathionate, respectively. Since the ES method calls for analysis of both selective enrichments, the method detected a total of 51 positives (Table 1).

In the following discussion, a false-negative result is defined as a culturally positive sample which was negative for Salmonella by either FA or ES methods. A false-positive result is defined as a culturally negative sample which exhibited a presumptive positive test for Salmonella by either FA or ES methods.

The rapid FA procedure gave 18 false-negative results compared to 4 false negatives for conventional FA from selenite enrichment. No false-negative results were obtained with the conventional FA technique using tetrathionate enrichment. Microcolony FA resulted in four false negatives.

The enrichment serology method showed nine and two false negatives from selenite and tetrathionate, respectively. Since the method calls for analysis of both enrichments, the use of the ES technique resulted in only one false negative (Table 2).

False-positive results were found with all methods. However, the number was quite different for the various procedures. The lowest was ES, with seven and eight from selenite and tetrathionate, respectively. Conventional FA showed 27 and 26 from selenite and tetrathionate. The microcolony method resulted in 33 false positives, and rapid FA had 26 (Table 2).

**DISCUSSION**

Any screening procedure for Salmonella must be as sensitive as standard cultural methods. This means the procedure should not result in any false negatives. However, as a practical quality control method, it should also not produce excessive false-positive results, since these require lengthy and costly cultural confirmation.

The rapid FA method resulted in 18 false negatives. This was probably due to the shortened pre-enrichment phase. When the same samples were analyzed by conventional FA using 24-h pre-enrichment followed by selenite selective enrichment, only four false negatives were obtained. Since the rapid FA technique relies on selenite enrichment, 5 of the 18 false-negative results can be explained by the failure of the Salmonella in those samples to grow in selenite. The conventional FA procedure using tetrathionate selective enrichment resulted in no false negatives. In one sample Salmonella was not recovered from tetrathionate culturally, yet the conventional FA produced a positive test. This positive result was confirmed by the
use of the elective TST enrichment, which is not part of the AOAC method. The number of Salmonella cells in the tetrathionate enrichment was too low to be detected on streak plates, but cells were found in FA smears. The false-positive rate found with conventional FA using the TST elective enrichment is comparable to that reported without the use of elective enrichment (5).

In the microcolony technique, 24-h pre-enrichment was combined with tetrathionate enrichment, resulting in four false negatives. Some of these false negatives may have resulted from excessive growth on the brilliant green agar elective enrichment, creating a "quenching" of fluorescence on the slides. Reducing the incubation time of the brilliant green agar plates may overcome this problem. The microcolony technique did not offer any time saving over other methods.

Microscopy examination of all FA smears was facilitated by the use of incident light illumination. Fluorescence was extremely bright and easily seen under \(\times640\) magnification. Since no dark-field condenser was needed, adjustment or oiling of the substage condenser was not necessary, thereby saving time and avoiding the possibility of inaccurate adjustment which would reduce brightness of fluorescence.

The ES method using both enrichments gave only one false-negative result. The amount of growth in the elective M-broth enrichment was very scant in this instance and may have been the cause of this single failure. The need for a longer incubation time for M broth has been recently demonstrated (1).

In the opinion of the authors the ES technique, using 6-h elective enrichment, is the preferred Salmonella screening method. Incubation of the elective enrichment must be extended in cases where culture turbidity is insufficient for serological testing. The ES method is simpler to perform, gives fewer false-positive results, requires less costly equipment, and requires significantly less technical expertise. The ES results found in this study are similar to those seen by Sperber and Deibel (10) and more recently by Boothroyd and Baird-Parker (1).

Conventional FA would be the method of second choice. It has the advantage of detecting low numbers of Salmonella cells from elective enrichment broth. This sensitivity was demonstrated by the absence of false negatives when tetrathionate enrichment was used. However, due to numerous false positives, additional cultural confirmation would be required.

The rapid FA procedure was found to be inadequate because incubation of the pre-enrichment broth for 6 h was insufficient. Fourteen additional Salmonella-positive samples were found when pre-enrichment incubation was extended to 24 h.

If only one selective enrichment broth is to be used, then tetrathionate would be the broth of choice. However, in these studies limiting the enrichment to just tetrathionate would have missed two positive samples that were isolated from selenite broth.

Studies are currently under way to evaluate the use of antibiotics and elevated temperatures for enhancement of Salmonella isolation.

LITERATURE CITED
1. Boothroyd, M., and A. C. Baird-Parker. 1973. The use of enrichment serology for salmonella detection in human foods and animal feeds. J. Appl. Bacteriol. 36:165–173.
2. Fantasia, L. D., W. H. Sperber, and R. H. Deibel. 1969. Comparison of two procedures for detection of Salmonella in food. Appl. Microbiol. 17:540–541.
3. Georgala, D. L., and M. Boothroyd. 1965. Further evaluation of rapid immunofluorescence technique for detecting salmonellae in meat and poultry. J. Appl. Bacteriol. 28:421–425.
4. Insalata, N. F., W. G. Dunlap, and C. W. Mahnke. 1973. Evaluation of the salmonellae Fluoro-Kit for fluorescent-antibody staining. Appl. Microbiol. 25:292–294.
5. Insalata, N. F., C. W. Mahnke, and W. G. Dunlap. 1972. Rapid, direct fluorescent-antibody method for the detection of salmonellae in food and feeds. Appl. Microbiol. 24:645–649.
6. Insalata, N. F., S. J. Schulte, and T. H. Berman. 1967. Immunofluorescence technique for detection of salmonellae in various foods. Appl. Microbiol. 15:1145–1149.
7. Journal of the 1971 Association of Official Analytical
Chemists. Changes in official methods. J. Ass. Offic. Anal. Chem. 54:496.
8. King, S., and W. I. Metzger. 1968. A new plating medium for isolation of enteric pathogens. II. Comparison of hektone enteric agar with SS and EMB agar. Appl. Microbiol. 16:579-581.
9. Schulte, S. J., J. S. Witzeman, and W. M. Hall. 1968. Immunofluorescent screening for salmonella in foods: comparison with culture methods. J. Ass. Anal. Chem. 51:1334-1338.
10. Sperber, W. H., and R. H. Deibel. 1969. Accelerated procedure for Salmonella detection in dried foods and feeds involving only broth cultures and serological reactions. Appl. Microbiol. 17:533-539.
11. Thomason, B. M. 1971. Preparation and testing of polyvalent conjugates for fluorescent-antibody detection of salmonellae. Appl. Microbiol. 22:876-884.
12. Thomason, B. M. 1971. Rapid detection of Salmonella microcolonies by fluorescent antibody. Appl. Microbiol. 22:1064-1069.
13. U. S. Department of Health, Education, and Welfare. 1972. Bacteriological analytical manual. Food and Drug Administration, Washington, D. C.