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Direct Fluorescent-Antibody Technique for the Microbiological Examination of Food and Environmental Swab Samples for Salmonellae

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Comparative studies of a modified fluorescent-antibody procedure and the 5 to 7 day method used by the Association of Official Analytical Chemists for the detection of *Salmonella* were made on 151 samples of wheat products and 183 swab samples. The agreement between the two methods for the 334 samples tested was 92.5%. Food samples yielded 94.7% agreement, whereas the swab samples yielded 90.7% agreement. There were 7.5% false positives for the total number of samples tested. No false negatives were obtained by using the fluorescent-antibody method.

The fluorescent-antibody (FA) technique introduced by Coons et al. in 1942 (2) as a rapid serological method for detecting microorganisms is now widely used in microbiology. Many attempts have been made to develop techniques for the detection of salmonellae with the FA procedure. Thomason and Wells (12) applied the FA technique for the detection of salmonellae by using pure cultures. The use of the FA technique for the detection of salmonellae in foods has been reported by Arkhangelskii and Kartoshova (1), Silliker et al. (11), Georgala and Boothroyd (4, 5), Haglund et al. (6), Fantasia (3), Insalata et al. (8), and others.

In this study, a combination of the Association of Official Analytical Chemists (AOAC) standard cultural method (9) and an FA method (7) was used to screen wheat products and swab samples collected from in-process equipment in the wheat handling operation.

MATERIALS AND METHODS

Samples. The samples consisted of 151 wheat products and 183 swab samples. For each swab sample, a sterile swab from a tube containing 10 ml of lactose broth (Difco) was used to swab 10 square inches (64.5 cm^2) of equipment, food contact surfaces, or surrounding areas.

AOAC cultural method. The conventional AOAC cultural method was followed through the selective enrichment phase so that a direct comparison of the FA results could be made with the standard AOAC method for the detection of salmonellae. Samples were pre-enriched in lactose broth for 24 h at 35 ± 2 C. A 1:10 ratio of sample to broth was always maintained. After incubation of the pre-enriched samples, a 2-ml amount was transferred to 18 ml of selenite-cystine broth, which was incubated for 24 h at 35 ± 2 C. After incubation, the selenite-cystine broth cultures were streaked onto Brilliant Green, SS, and XL agar plates (Difco) for evaluation by the AOAC method.

Pooling phase. Occasionally, to minimize the number of FA slides that had to be read in 1 day, a pooling phase was included (10). At the same time of the AOAC pre-enrichment transfer to selenite-cystine, a maximum of five pre-enrichment broths were also pooled by transferring an additional 2 ml from each of the five pre-enriched samples into a single selenite-cystine broth. A 1:10 ratio of sample to broth was still maintained.

Elective enrichment phase. After incubation of the selenite-cystine broth cultures from which an FA slide was desired, 2 ml was transferred to 18 ml of preheated (35 ± 2 C) FAS broth (Difco) and incubated for 5 h in a 35 ± 2 C still-water bath.

The procedure used for FA slide preparation, FA staining, and microscope examination were identical to those described by Insalata et al. (7).

The antiserum used was FA *Salmonella* Poly (Difco; lot RX 16214), which is a fluorescein-conjugated polyvalent anti-*Salmonella* globulin. It is prepared from motile organisms representative of somatic groups A through S, including O factors 1 through 25, 27, 28, 30, 34 through 41, 46, and Vi. The flagellar spectrum includes H antigen a through i, k through p, r through u, w through z, z_4, z_6, z_{10}, z_{13}, z_{15}, z_{23}, z_{24}, z_{27}, z_{28}, z_{32}, z_{35}, z_{38}, z_{42}, and 1, 2, 5, 6, and 7.

After the slides were prepared from the elective enrichment broth, the FA cultural (FAC) confirmation procedure was performed by streaking a 3-mm loopful on selective media plates as outlined in the AOAC method.

RESULTS AND DISCUSSION

Table 1 compares the FA slide results with the FAC results against the standard AOAC method.

Of the 151 wheat products tested, 19 FA slide positives were found. Eleven of these were confirmed positive by the AOAC cultural method or the FAC method, or both. Therefore, eight (5.3%) of the FA slide-positive samples were not detected by either of the cultural methods; thus, the slides were considered false positives.

In one instance in which five 2-ml amounts from five pre-enriched samples were pooled into one 90-ml selenite-cystine broth, four of the individual enriched samples were negative and one was positive when run by AOAC methodology. However, the pooled selenite-cystine broth culture failed to recover salmonellae. This failure of the pooled broth to recover salmonellae may indicate that the 2-ml amount of pre-enriched samples which is transferred should be increased in volume, or it may be necessary in instances of low numbers of salmonellae to pool fewer samples. Wheat samples (132) were negative for both the cultural and FA slide methods. The FA technique yielded 94.7% agreement with the AOAC method. No culturally positive wheat samples were recorded as negative by the FA method.

The 183 swab samples analyzed yielded 42 FA slide positives, of which 25 were confirmed positive by using the AOAC or FAC methods, or both. Seventeen (9.3%) of the FA slide-positive samples were negative by using either of the cultural procedures. It is possible that the swab samples, because of the exposure to concomitant microflora sharing the same antigens as salmonellae, may produce more false-positive results than are obtained when certain food samples are analyzed. Swab samples (141) were negative for both the FAC and FA slide methods. The FA technique yielded 90.7% agreement with the AOAC method. Again, no culturally positive swab samples were recorded as negative by the FA method.

The percent correlation of the FA method as compared to the AOAC method on the total 334 samples tested was 92.5%. The total false-positive results obtained in this study was 7.5%. In most instances, the samples recorded as FA slide positive showed large numbers of fluorescing organisms. In no instance did the FA method fail to detect a sample proved salmonella positive by cultural methods.

This study has demonstrated the high degree of correlation of the FA technique in comparison to the 5 to 7 day AOAC method, when applied in the screening of samples contaminated with salmonellae. Also of significance is the degree of success which has been demonstrated by the FA method when applied against swab samples representing the sanitary indices of a process.

TABLE 1. *Summary of the FA slide results*[a]

Types of samples	Total no. of samples with antiserum	No. of FA slide trials in agreement with cultural confirmation				FA slide false positives	% Agreement	% Slide positives
		AOAC+ FAC+ slide+	AOAC− FAC− slide−	AOAC+ FAC− slide+	AOAC− FAC+ slide+	AOAC− FAC− slide+		
Wheat products	151	10	132	1	0	8	94.7	5.3
Swab products	183	24	141	1	0	17	90.7	9.3
Summary with all samples	334	34	273	2	0	25	92.5	7.5

[a] Abbreviations: AOAC+, any sample producing an isolate that exhibited cultural, biochemical, and unconjugated serological reactions typical for salmonellae in accordance with the AOAC published method. AOAC−, any sample confirmed as salmonellae negative by AOAC published methodology. FAC+, any fluorescent-antibody salmonellae broth elective enrichment (from which the slides are made) confirmed as containing salmonellae by AOAC published methodology. FAC−, any fluorescent-antibody salmonellae broth elective enrichment negative for salmonellae by AOAC published methodology. Slide +, any smear showing rods of (i) proper morphology (with or without attached flagella) under darkfield and ultraviolet light, and (ii) a 3+ or 4+ degree of fluorescence under ultraviolet light. Slide−, any smear not conforming to the above; FA slide agreement occurs when (i) a positive slide is confirmed by an AOAC positive or FAC positive, or both, (ii) when the FA slide and AOAC and FAC methods are negative concurrently. FA slide false positive, any slide positive which cannot be culturally confirmed. FA slide false negative, any instance in which the slide was negative and the AOAC culture or FAC method, or both, yielded salmonellae.

The advantages of the application of the FA technique are reflected in shorter test times of 52-h elapsed time, shorter storage times for final products awaiting clearance, and the ability to rapidly determine the effectiveness of sanitation clean-up procedures in industrial processes to maintain good manufacturing practices.

This study has also demonstrated that pooling suspect samples is possible to permit larger numbers to be tested simultaneously by this method. For this work, one trained laboratory technician was capable of preparing and examining 15 sets of FA slides daily, representing a maximum of 75 individual product or environmental samples. This figure may be increased if the work is segmented into a sample and slide preparation phase performed by one technician and the microscopy examination phase performed by a second technician.

An undetermined number of false positives encountered while using the FA technique were caused by the nonspecific staining of a strain of *Enterobacter agglomerans* identified by B. M. Thomason of the Center for Disease Control. To reduce the number of false positives, there are several aspects of the procedure that require further development. These include: (i) increasing the specificity and sensitivity of the antiserum, (ii) cultural improvement, and (iii) definition of observations made when using the microscope in the diagnostic phase, to minimize subjective interpretations.

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