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Comparison of Enrichment Procedures for Fluorescent Antibody and Cultural Detection of Salmonellae in Raw Meat and Poultry

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No advantage was shown in preenriching raw meat samples for detecting salmonellae by fluorescent antibodies or culture. Trypticase soy-tryptose (Edwards and Ewing, 1972) was equal to or better than selenite-cystine as a postenrichment broth.

A fluorescent antibody (FA) method for detecting salmonellae was adopted as an Official First Action by the Association of Official Analytical Chemists (1) and fully described by Fantasia et al. (4). The method requires a postenrichment in selenite-cystine broth (pSEL) for 4 h after the selective enrichment of samples in tetrathionate broth with brilliant green dye (TET) and selenite-cystine broth (SEL). Historically, raw meat and poultry samples have not been preenriched. The present official recommendation (1) is that such samples be put into lactose broth for 18 to 24 h before the selective enrichment step or, alternatively, that the incubation time of the postenrichment broth be extended.

Preenrichment of raw meat was suggested by Edel and Kampelmacher (2) because they isolated salmonellae from more meat samples after preenrichment in buffered peptone water than upon direct inoculation into enrichments. Subsequently, Gabis and Silliker (5) recommended that preenrichment in lactose be included in the culture of raw meats, poultry, and eggs. The latter workers based their conclusions on results obtained from 21 frozen raw products.

We compared the efficiency of selenite-cystine with that of Trypticase soy-tryptose (TST; 3) as a postenrichment broth for the FA detection of salmonellae in 32 samples consisting of raw chicken livers and raw pork sausage. In addition, we compared the culture results obtained from the preenriched aliquots with those obtained from samples placed directly into the selective enrichments.

Raw samples of fresh chicken livers and pork sausage were purchased from a supermarket. The samples were blended and 25-g aliquots were placed in 225 ml of TET, SEL, and lactose broth. Tergitol 7 (sodium heptadecyl sulfate, Union Carbide, Chicago), sufficient to obtain a 1% concentration (vol/vol), was added to the sausage samples. These were incubated 24 h at 35 C. After 24 h, 1 ml of the lactose broth cultures was transferred to 9 ml each of TET and SEL and incubated for an additional 24 h.

All enrichment and postenrichment broth cultures were streaked onto brilliant green agar (Difco) for isolating salmonellae. After 24 h of incubation at 35 C, suspect colonies were confirmed by biochemical and serological procedures (3).

The postenrichment broths used for FA testing were inoculated from the 24-h TET and SEL broth cultures as follows: 2 ml each of TST and pSEL were inoculated with 0.2 ml of the selective enrichment broths. Smears for FA staining were made from TST broths after 2 h of incubation in a 37 C water bath and from the pSEL broths after 4 h of incubation. The smears were made on multiwell slides (Cel-Line Associates, Inc., Minotola, N.J.) with a 0.001-ml loop. When dry, the smears were fixed and stained with a *Salmonella* polyvalent (A-S) conjugate as described previously (6). They were examined by incident illumination; a Leitz fluorescence microscope was used that was equipped with a 50-W quartz halogen lamp as the exciting light source. A KP490 interference filter was used for primary filtration, and a K510 filter was used as the barrier filter.

The results are shown in Table 1. The maximum number of positive samples (22) was obtained from the direct enrichment of the samples in TET broth. Salmonellae were isolated from 21 of the preenriched aliquots. Direct enrichment in SEL yielded 21 positive samples, but only 19 were positive after preenrichment in lactose.

The 2-h TST broth was equally as effective as the 4-h SEL for postenrichment of samples

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TABLE 1. *Detection of salmonellae in 32 raw samples by fluorescent antibody and culture when various enrichment procedures were used*

Sample	No. of samples[a]	Culture results				Fluorescent antibody results							
				Lactose preenrichment		TET		SEL		Lactose preenrichment			
										TET		SEL	
		TET[b]	SEL[c]	TET	SEL	TST[d]	pSEL[e]	TST	pSEL	TST	pSEL	TST	pSEL
Chicken liver	2	+	+	+	+	+	+	+	+	+	−	+	+
Chicken liver	2	+	+	+	+	+	+	+	+	+	+	+	+
Chicken liver	2	−	−	−	−	−	−	+	−	+	−	+	−
Chicken liver	2	−	−	−	−	−	−	−	−	−	−	+	+
Chicken liver	1	−	−	−	−	+	+	−	−	−	+	+	+
Chicken liver	1	−	−	−	−	−	−	−	−	−	−	+	−
Chicken liver	4	−	−	−	−	−	−	−	−	−	−	−	−
Pork sausage	14	+	+	+	+	+	+	+	+	+	+	+	+
Pork sausage	2	+	+	+	−	+	+	+	+	+	+	+	+
Pork sausage	1	+	+	−	+	+	+	+	+	+	+	+	+
Pork sausage	1	+	−	+	−	+	+	+	+	+	+	+	+
Total samples positive by:													
FA		ND[f]	ND	ND	ND	23	23	24	22	24	21	28	25
Culture		22	21	21	19	22	22	21	22	17	14	16	16

[a] Total samples examined were 32.
[b] Tetrathionate with brilliant green dye.
[c] Selenite-cystine broth.
[d] Trypticase soy-tryptose broth.
[e] Postenrichment selenite-cystine broth.
[f] ND, Not done.

grown in TET broth. For samples preenriched before TET culture, the 2-h TST broth was superior to the pSEL. We noted a definite reduction in numbers of salmonellae per oil immersion field in those smears made from pSEL as compared with those made from the TST broth. This reduction in growth of salmonellae from preenriched TET samples placed in SEL for postenrichment was substantiated by culture of the postenrichment broths. Salmonellae were isolated from the pSEL broth of 14 of the 22 positive samples. Culture of the TST broth yielded salmonellae from 17 of the 22 samples.

In our study, SEL broth was not as selective as TET and allowed the growth of organisms other than salmonellae, which increased the number of false-positive FA results from the preenriched SEL samples. We had difficulty isolating salmonellae from brilliant green agar (BG) because of the overwhelming effect of the lactose fermenters. In fact, most of the salmonellae isolated were from totally green colonies picked from BG. This emphasizes the necessity of recognizing colonial morphology of salmonellae, even though the color change is not typical on BG plates. Perhaps the use of xylose lysine deoxycholate or Hektoen agar should be considered as adjunct media for isolating salmonellae.

We can see no advantage in preenriching raw meat or poultry samples. In our study, preenrichment was no more efficient and probably less efficient by both FA and culture than direct enrichment in TET or SEL. For samples preenriched before TET culture, the 2-h TST broth should be used rather than the 4-h SEL for postenrichment before FA staining.

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