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Inadequacy of Small Transfer Volume and Short (6 h) Selective Enrichment for the Detection of Foodborne *Salmonella*

J.-Y. D'AOUST*, A. M. SEWELL, and E. DALEY

Food Directorate, Health Protection Branch, Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

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ABSTRACT

The propensity of short (6 h) selective enrichment combined with a preenrichment to enrichment transfer volume ratio of 1:100 to provide greater method brevity could not be demonstrated. Inoculation of tetrathionate brilliant green (35 and 43°C), Rappaport-Vassiliadis (43°C), and selenite cystine (35°C) enrichment broths (9.0 ml) with 0.1 ml of preenrichment culture and incubation for 6 h identified, respectively, 107 (84.9%), 104 (82.5%), 112 (88.9%), and 113 (89.7%) of the 126 contaminated samples detected in the present study; homologous results with the 1.0-ml transfer volume showed a marginal increase in sensitivity. Recoveries of foodborne salmonellae with the standard (24-h) period of selective enrichment were generally transfer volume-independent and consistently exceeded that obtained with 6-h enrichment cultures. Results further underlined the importance of enrichment at an elevated (43°C) temperature for the effective repression of competitive microflora, and the facilitated isolation of *Salmonella* on plating media.

The prevalence of *Salmonella* in the natural environment and in the agricultural food sectors has underlined the need for more rapid analytical procedures equivalent in sensitivity and specificity to the standard cultural methods of analysis (*10,11,21*). Attempts at method brevity have focused mainly on reducing the period of incubation of preenrichment and enrichment broth cultures. It soon became apparent that short (4-8 h) periods of preenrichment did not provide sufficient time for the repair of stressed or injured *Salmonella* cells encountered in raw and in processed foods (*2,5,8*). For example, the inability of short preenrichment to resuscitate injured salmonellae, and the high ratio of native microflora to salmonellae frequently found in foods and food ingredients, preempted the successful recovery of *Salmonella* from approximately 50% of contaminated samples (*6*). Other studies examining the growth kinetics of *Salmonella* in nonselective broth media (*17,24*), and the detection of foodborne salmonellae by different cultural (*13,14,16,25*) and fluorescent antibody techniques (*15*) also found short preenrichment to be counterproductive. Concurrent studies on the reliability of short (6-8 h) selective enrichment in tetrathionate brilliant green, selenite cystine, and Rappaport-Vassiliadis (RV_{43}) media also questioned the validity of this novel analytical approach (*1,7,18,22*). Several commercial diagnostic kits based on DNA probe, ELISA and hydrophobic membrane filtration technologies utilize a short (6-h) period of enrichment for the detection of foodborne *Salmonella* (*5*). Although comparative studies have generally established their equivalence with a standard cultural method (*21*), such validations against a cultural method of reduced sensitivity (*5,7*) may indicate that both the standard method and implicated diagnostic kits endure a similar lack of sensitivity. The issue of abbreviated methodology for the timely identification of contaminated foods has been the subject of several reviews (*4,5,8*).

The present study compares the productivities of four enrichment media inoculated with two different volumes of preenrichment culture (0.1 and 1.0 ml) and incubated for 6 and 24 h.

MATERIALS AND METHODS

Naturally contaminated high moisture foods (270) were generally obtained from local retail markets, whereas most low moisture foods (90) became available as a result of federal monitoring or compliance activities.

Food samples (100-g) were preenriched in an appropriate nonselective broth medium for 16-18 h at 35°C (*10*). Replicate portions (1.0-ml) of each preenrichment culture were inoculated into separate tubes of enrichment broths (9.0 ml) consisting of tetrathionate brilliant green (TBG_{35} and TBG_{43}), selenite cystine (SC_{35}), and Rappaport-Vassiliadis (RV_{43}). A second set of enrichment broths was inoculated with 0.1-ml portions of the same preenrichment culture. After 6 h of incubation, each of the eight enrichment cultures were plated on bismuth sulfite (BSA) and brilliant green sulfa (BGS) agar media; plates were incubated for 16-18 h at 35°C. All broth cultures were reincubated at the appropriate temperatures for a total period of enrichment of 24 h, and then plated on BSA and BGS as previously described. Suspect colonies were screened biochemically on triple sugar iron and lysine iron agars and confirmed serologically with polyvalent and single grouping somatic (O) antisera (*10*).

The sensitivity and specificity of the four enrichment conditions were assessed semiquantitatively on the basis of growth densities of presumptive salmonellae and nonsalmonellae on plat-

ing media after 6 and 24 h of selective enrichment. The rating scales used in the calculation of index scores for *Salmonella* and competitive flora were previously described (*7*). This rating scheme was applied to the 126 *Salmonella*-contaminated samples identified in this study.

RESULTS AND DISCUSSION

Of 360 food and animal feed samples tested, 126 (35.0%) were found to be contaminated by one or more of the test conditions evaluated in this study (Table 1). The combined results from TBG_{43}, TBG_{35}, SC_{35}, and RV_{43} enrichment cultures identified fewer positive samples after 6 h than after 24 h of incubation (Table 2). The limited sensitivity of short (6 h) selective enrichment is well documented (*1,7,18,22*) and likely stems from the imposed temporal constraints on the selective action of enrichment conditions. This situation not only leads to a minimal repression of the high numbers of competing microorganisms transferred from the preenrichment cultures but also hinders the subsequent isolation of *Salmonella* on plating media (*3,6,19,23*). The anticipated synergism between short (6 h) enrichment and small (0.1-ml) transfer volume for the more rapid yet reliable detection of foodborne salmonellae could not be demonstrated in the present study and actually led to the identification of only 117 (92.9%) of the 126 positive samples (Table 2). The equivalent sensitivity of the 1.0- and 0.1-ml transfer volumes after 24 h of selective enrichment was predictable and recalled the results of an earlier study on the growth kinetics of foodborne *Salmonella* under different conditions of preenrichment (*6*). Such facilitated isolation of salmonellae likely arose from a net increase in the number of *Salmonella* cells and concomitant decrease in populations of competitive microflora during the standard (24-h) enrichment period (*20*). Although a 1.0-ml transfer volume (1:10 preenrichment to enrichment ratio), and 18-24-h periods of selective enrichment are widely used in standard cultural procedures (*10,11,21*), the limited selectivity of RV_{43} has predicated the use of a larger (1:100) transfer volume ratio for the reliable detection of foodborne *Salmonella* (*9,11,22*). Such a limitation may not be restricted to RV_{43} but may also apply to other analytical schemes. Interestingly, a recent study demonstrated that inoculation of different enrichment media (10 ml) with 1.0-ml portions of 10^{-2} to 10^{-4} dilutions of a preenrichment culture identified more artificially contaminated minced meat samples than that obtained with the undiluted preenrichment culture (*12*).

Results on the productivity of individual enrichment conditions (Table 3) underlined the greater sensitivity of TBG_{43} under all test conditions except with 6 h of enrichment and 0.1-ml transfer volume where SC_{35} identified 113 (89.7%) contaminated samples. The higher levels of recovery attained with RV_{43} and TBG_{43} reiterate the propensity of selective enrichment at an elevated temperature for increased method sensitivity. Earlier reports also showed that the choice of enrichment medium and period of incubation were determinants in the recovery of *Salmonella* from foods and from environmental samples (*5,7,8*). The superiority of the TBG_{43} enrichment condition was further reflected in the growth characteristics of *Salmonella* and competitive flora on plating media (Table 4). Under standard conditions of enrichment (1.0-ml transfer volume and 24-h incubation period), TBG_{43} yielded the greatest density of *Salmonella* cells and the lowest incidence of nonsalmonellae on agar media. The reduction of the competitive index score from 612 (1.0 ml) to 340 (0.1 ml) with RV_{43} (24 h) further documents the limited selectivity of this medium, and its transfer volume-dependent recovery of *Salmonella* (Table 3 and *22,25*).

The present study indicated that combination of short (6 h) selective enrichment and small (0.1-ml) transfer volume for greater method brevity could not be applied without compromising method sensitivity. Results confirmed the determinant role of selective enrichment at elevated temperatures in repressing competitive microflora and providing for increased detection of foodborne *Salmonella* through facilitated isolation on plating media.

TABLE 1. *Detection of* Salmonella *in foods and animal feeds.*

Food	No. of samples		
	Tested	Positive[a]	Somatic grouping (No. strains)[b]
High moisture			
Chicken			
Whole carcasses	38	25	B(14); C_1(3); C_2(7); E(1).
Cut-up	23	12	B(3); C_1(4); C_2(5).
Giblets[c]	40	24	B(13); C_1(5); C_2(4); E(1); untypeable (1).
Nuggets	1	0	NA[e]
Other poultry[d]	5	2	B(1); C_2(1).
Pork			
Sausages (raw)	69	4	B(4).
Giblets	20	0	NA
Minced meat	17	1	B(1).
Retail-cuts	9	0	NA
Beef/veal			
Minced meat	13	2	C_1(2).
Liver	12	0	NA
Retail-cuts	4	0	NA
Sausages (fermented)	3	1	C_1(1).
Lamb			
Minced meat	2	1	K(1).
Chops	1	0	NA
Kidney	1	0	NA
Miscellaneous[f]	12	5	B(1); C_2(1); D(1); 061(1); untypeable (1).
Subtotal	270	77	
Low moisture			
Animal feeds	71	37	B(5); C_1(14); C_2(2); D(1); E(11); G(1); R(2); untypeable (1).
Spices[g]	11	6	B(1); C_2(1); E(2); untypeable (2).
Pasta	3	3	C_1(3).
Chocolate	3	1	M(1).
Albumen	1	1	B(1) .
Drink mix	1	1	C_1(1).
Subtotal	90	49	

[a] Based on combined analytical results.
[b] Isolated strains reported according to their somatic (0) antigenic grouping.
[c] Chicken liver, heart, and gizzard.
[d] Duck (1), turkey burger (2), and breast (2).
[e] Not applicable.
[f] Froglegs (8), snails (2), turtle water (1), and cheese (1).
[g] Black pepper (6), basil (2), caraway seed (2), and turmeric (1).

TABLE 2. *Transfer volume-dependent recovery of foodborne* Salmonella.

Food	Enrichment period (h)	No. of positive samples (%)[a]	
		Transfer volume	
		1.0 ml	0.1 ml
High moisture (77)[b]	6	74 (96.1)	73 (94.8)
	24	76 (98.7)	75 (97.4)
Low moisture (49)	6	45 (91.8)	44 (89.8)
	24	49 (100.0)	49 (100.0)
Total (126)	6	119 (94.4)	117 (92.9)
	24	125 (99.2)	124 (98.4)

[a] Based on the combined results from TBG_{43}, TBG_{35}, SC_{35}, and RV_{43} enrichment cultures.
[b] Total number of positive samples identified by one or more analytical conditions.

TABLE 3. *Effect of transfer volume on the productivity of enrichment media.*

Enrichment media	Incubation period (h)	No. of positive samples (%)[a]	
		Transfer volume	
		1.0 ml	0.1 ml
TBG_{35}	6	111 (88.1)	107 (84.9)
	24	117 (92.9)	119 (94.4)
TBG_{43}	6	117 (92.9)	104 (82.5)
	24	124 (98.4)	124 (98.4)
SC_{35}	6	108 (85.7)	113 (89.7)
	24	116 (92.1)	117 (92.9)
RV_{43}	6	111 (88.1)	112 (88.9)
	24	119 (94.4)	122 (96.8)

[a] Combined analytical results identified a total of 126 contaminated samples.

TABLE 4. *Sensitivity and specificity of enrichment conditions.*[a]

Enrichment media	Incubation period (h)	Index score[b]			
		Salmonella		Competitive flora	
		1.0 ml	0.1 ml	1.0 ml	0.1 ml
TBG_{35}	6	742	570	773	737
	24	836	912	754	587
TBG_{43}	6	702	464	721	708
	24	961	941	516	404
SC_{35}	6	635	643	803	787
	24	846	868	694	703
RV_{43}	6	662	556	794	666
	24	881	946	612	340

[a] Based on the combined results for 126 contaminated high and low moisture foods.
[b] Best possible scores for *Salmonella* and competitive flora were 1008 and 252, respectively.

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