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ICMSF Methods Studies. XIV. Comparative Study on Recovery of *Salmonella* from Refrigerated Preenrichment and Enrichment Broth Cultures

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ABSTRACT

Twelve laboratories from 7 countries compared the productivity of refrigerated (72 h at 5 to 10°C) preenrichment and enrichment broth cultures with a standard cultural procedure for detection of *Salmonella* in 466 naturally contaminated low and high moisture foods. Refrigerated preenrichment and enrichment cultures identified 92.5 and 94.2% of contaminated samples, respectively. Variations in the ability of laboratories to successfully recover salmonellae under refrigeration test conditions were notable. Three laboratories found complete agreement between results by the standard and refrigeration test procedures and 5 additional laboratories reported >90% accuracy; lowest recovery rate for combined refrigeration results was 77%. Sensitivity of the refrigeration techniques was markedly greater with low than high moisture foods where the latter contributed all but two of the 62 false-negative results encountered in this study. Ability of individual laboratories to recover *Salmonella* from refrigerated preenrichment and enrichment broth cultures was not significantly different for given food categories. Productivity of paired enrichment-plating media differed widely with food type. Selective enrichment in tetrathionate brilliant green and plating on bismuth sulfite agar showed greatest sensitivity for isolation of *Salmonella* in high but not in low moisture foods where productivity of the 4 enrichment-plating conditions used in this study was comparable. Results on recoverability of *Salmonella* from refrigerated broth cultures concur with findings of an earlier comparative study and strongly support incorporation of this novel approach in standard cultural methods for detection of *Salmonella* in foods.

Attempts to develop rapid, sensitive and cost-efficient methods for the isolation of *Salmonella* in raw and finished food products have met with limited success (*4,10*). Standard cultural procedures usually involve overnight incubation of the sample in a non-selective (preenrichment) medium, enrichment (24 to 48 h) in a selective broth medium and plating of enrichment cultures on differential agar media (*11,12,19*). Presumptive identification of salmonellae on agar media requires a minimum of 4 d with an additional 2 to 3 d for biochemical screening and serological confirmation of isolates. Initiation of sample analysis using these standard procedures is, therefore, limited to Mondays and Tuesdays if weekend work is to be avoided and if analysis is to be interrupted only by refrigeration of incubated plating media. Ability to reliably recover *Salmonella* from preenrichment and enrichment broth cultures refrigerated for 72 h would double the number of weekdays on which analyses could be initiated (i.e., Monday to Thursday inclusively) with a concomitant increase in laboratory flexibility and productivity.

The present international comparative study extends the data base provided by a recent national comparative study (*3*) and further validates refrigeration of preenrichment and enrichment broth cultures as a reliable analytical tool.

MATERIALS AND METHODS

This 1-year study was initiated in June 1980 and involved a total of 12 laboratories, including 5 from the United States, 2 from England and one each from Canada, Greece, Israel, Netherlands and Sweden. Individual laboratories analyzed their own naturally contaminated low and high moisture food samples with bacteriological media kindly supplied by Difco Laboratories, Detroit, MI. Analytical results were submitted regularly to the coordinator on standard reporting forms. Data on samples found to be free of *Salmonella* by the standard cultural procedure involving non-refrigerated preenrichment and selective enrichment cultures and isolation on agar media were not requested but, nevertheless, provided by several laboratories.

Ability to recover *Salmonella* from refrigerated preenrichment and enrichment broth cultures was evaluated using an experimental protocol

similar to that previously described (*3*). Small quantities of frozen foods were thawed at 20 to 25°C for 60 min or at 2 to 8°C for longer periods of time with larger samples; whole turkeys were held at room temperature for up to 18 h. Analytical (25 g) samples were added to 225 ml of buffered peptone water (*12*) and homogenized in a sterile blender jar for 60 s; the preenrichment mixture was adjusted to pH 6.0 to 7.0 and incubated at 35 to 37°C for 18 to 24 h. Chocolate and other confectionery products were preenriched in 10% (wt/vol) skim milk broth with 0.002% (wt/vol) brilliant green; skim milk powder was preenriched in 0.002% (wt/vol) brilliant green water and coconut in buffered peptone water with added Tergitol 7 (0.6% vol/vol). Poultry carcasses were vigorously rinsed with 1 L of buffered peptone water, and the entire rinse fluid was incubated for preenrichment. Portions (1 ml) from each preenrichment culture were selectively enriched in 9 ml of tetrathionate brilliant green (TBG) and selenite cystine (SC) for 18 to 24 h at 43 and 35 to 37°C, respectively. The remaining preenrichment culture was refrigerated at 5 to 10°C for 72 h. A loopful of each selective enrichment culture was streaked on brilliant green sulfa (BGS) and bismuth sulfite (BiS) agar media and incubated overnight at 35 to 37°C. The remaining enrichment cultures were refrigerated for 72 h. Two to three suspect colonies from each selective plating medium were purified on MacConkey agar and screened biochemically by routine laboratory procedures. Isolates were confirmed serologically by somatic (O) and flagellar (H) agglutination reactions. A single serovar was usually reported per sample. Refrigerated preenrichment and enrichment broth cultures were further analyzed only upon presumptive evidence of *Salmonella* by the standard cultural procedure. Refrigerated cultures were discarded in the absence of *Salmonella* on plating media by the standard procedure. Following the 72-h refrigeration period, portions (1 ml) of preenrichment cultures were transferred to TBG and SC and analyzed as described above. Similarly, refrigerated enrichment broth cultures were plated directly on BGS and BiS and *Salmonella* isolates confirmed biochemically and serologically.

Statistical analysis of data included determination of lower 95% confidence limits (*6*) for recoverability of *Salmonella* by individual laboratories for each of the two refrigeration test conditions and for high and low moisture foods. McNemar's test (*6*) was also used to determine significant differences between results from each participating laboratory on productivity of refrigerated preenrichment and enrichment broth cultures of high moisture foods; data for low moisture foods were similarly analyzed. Significant differences in the performance of TBG and SC enrichment broths, and BiS and BGS agar media within each of three analytical methods (standard cultural, refrigerated preenrichment and refrigerated enrichment) were determined by the McNemar test for both high and low moisture foods. Data comparisons among the three analytical methods were not done.

RESULTS

A total of 466 naturally contaminated samples, including 291 high moisture and 175 low moisture foods, were tested (Table 1). Poultry, consisting mainly of whole chicken carcasses, accounted for 36% of all high moisture food samples. Pork meat included pork filets, skin, cheek muscles, neck bones and mince pork. Animal feeds provided 77% of all low moisture food samples. Most feed samples analyzed by U.S. laboratories were of Canadian origin. Bakery products included egg powder, cake with cream, wheat flour and bakery mix. *Salmonella*-contaminated black pepper, cloves, garlic, onion flakes and kurkum were also reported. A total of 541 isolates, representing 68 serovars and 22 untypeable cultures, were reported (Table 2). *Salmonella anatum, Salmonella montevideo, Salmonella infantis, Salmonella typhimurium, Salmonella agona* and *Salmonella senftenberg,* in that order, showed the highest frequency of isolation.

Refrigerated preenrichment and enrichment broth cultures were equally sensitive for the recovery of *Salmonella* (Table 3). Of 466 contaminated high and low moisture food samples identified by the standard cultural procedure, 431 (92.5%) were identified from refrigerated preenrichment and 439 (94.2%) from refrigerated enrichment cultures. Laboratories 3, 8 and 11 obtained complete agreement between results by the standard and refrigeration test conditions, whereas laboratories 2, 5, 7, 10 and 12 reported >90% agreement. Laboratory 1 obtained the lowest overall recovery (77%) for both refrigeration test conditions combined. Refrigeration preenrichment data reiterated the absence of false-negative results in three laboratories and a minimum recovery rate of 83% (laboratory 9). Homologous results for refrigeration enrichment conditions showed no false-negative results in 5 laboratories and a minimum recovery rate of 68% (laboratory 1).

Refrigeration of preenrichment and enrichment cultures of high moisture foods (Table 4) led to the identification of only 88 and 91%, respectively, of known positive samples. These values were lower than those obtained for high and low moisture foods combined (Table 3). The low productivity (50%) of laboratory 9 under both refrigeration conditions and of laboratory 1 with refrigerated enrichment is notable. Patterns of recovery of standard and refrigeration test conditions (Table 5) reemphasize the marginally greater sensitivity of refrigerated enrichment to that of preenrichment cultures for isolation of *Salmonella* in high moisture foods and inability of either refrigeration condition to detect 14 contaminated samples. Productivity of the refrigeration approach with low moisture foods contrasted sharply with that obtained with high moisture foods (Table 6). All but one laboratory reported successful identification of all contaminated low moisture foods; two samples of cake with cream analyzed by laboratory 6 yielded the only false-negative results. Recovery patterns for low moisture foods (Table 7) were unremarkable, attributing one false-negative result to each refrigeration condition and underlining the absence of samples positive by the standard and negative by both refrigeration conditions. The proportionately greater number of erroneous results from mince meat and catfish suggests that the physical and/or bacteriological characteristics of certain foods markedly affect the incidence of false-negative results (Table 8).

Statistical analysis of data from individual laboratories showed that differences in *Salmonella* recovery rates from refrigerated preenrichment and enrichment broth cultures for high (Table 4) and low (Table 6) moisture foods were not significant by McNemar's test (data not shown). Lower confidence limits (LCL) were calculated for results presented in Tables 5 and 7. These limits predict, at a 95% level of probability, lower bounds for the proportions of samples positive by the standard cultural method that will be identified by each of the two refrigeration test condition. For high moisture foods, LCL values ranged from 0.11 to 0.90 for both refrigeration conditions and combined laboratory values for refrigerated preenrichment (0.85) and enrichment (0.88) were similar (Table 9). The inference is that 95% of the time ($\alpha = 0.05$), no fewer than 85 and 88% of known positive samples would be detected by the refrigerated preenrichment and enrichment methods,

TABLE 1. Salmonella-*contaminated foods by reporting country.*

Food	Number of contaminated food samples							
	Canada	England[a]	Greece	Israel	Netherlands	Sweden	United States[a]	Total
High moisture								
Raw meat								
Chicken								
Whole	-[c]	18	28	-	-	-	24	70
Cut-up	1	-	-	1	-	-	2	4
Giblets	3	9	-	-	-	-	7	19
Turkey								
Whole	-	7	-	-	-	5	-	12
Pork								
Meat	1	1	-	-	-	3	15	20
Sausage	1	-	19	3	-	-	2	25
Heart/Liver	1	-	-	-	-	-	9	10
Kidney	2	-	-	-	-	-	-	2
Beef								
Meat	-	-	4	6	-	3	-	13
Heart/Liver	1	-	-	-	-	1	-	2
Mince meat	-	20	-	-	7	-	-	27
Horse meat	-	-	-	-	-	16	1	17
Others[b]	1	-	-	-	-	17	17	35
Egg products								
Liquid eggs	3	8	-	-	8	4	-	23
In-shell eggs	-	-	-	-	-	-	11	11
Vegetables	-	-	-	1	-	-	-	1
Subtotal	14	63	51	11	15	49	88	291
Low moisture								
Bakery products	-	-	-	7	1	-	3	11
Alimentary paste	1	-	1	-	-	-	1	3
Meat powder	-	8	-	-	-	-	-	8
Smoked sausage	-	-	-	-	-	-	1	1
Animal feeds	25	18	17	-	3	-	72	135
Milk powder	-	-	-	3	-	4	-	7
Chocolate	3	-	-	-	-	-	1	4
Coconut	-	-	-	-	-	-	1	1
Spices	-	1	-	4	-	-	-	5
Subtotal	29	27	18	4	4	4	79	175
Total	43	90	69	25	19	53	167	466

[a]England and United States represented by 2 and 5 laboratories, respectively.
[b]Include duck (2), wild goose (5), catfish (17), snails (1), kangaroo (4), buffalo (5), mule and donkey (1).
[c]None reported.

respectively. Homologous LCL values for low moisture foods ranged from 0.46 to 0.89, with combined laboratory values of 0.97 for both refrigeration conditions (Table 9).

The efficacy of selective enrichment and differential plating media varied with food type and experimental test condition (Table 10). With high moisture foods, TBG provided greatest recovery, irrespective of test conditions, and consistently exceeded productivity of homologous SC enrichment conditions. These differences were highly significant ($p<0.0001$). BiS or BGS plated from both selective enrichment cultures demonstrated equal sensitivity (85%). In contrast, differences in media performance were less

TABLE 2. *Incidence of* Salmonella *serovars by reporting country.*

Serovar	Number of isolations[a]							
	Canada	England[b]	Greece	Israel	Netherlands	Sweden	United States[b]	Total
S. agona		3	14		1		10	28
S. alachua							2	2
S. albany			2					2
S. anatum		9		6		20	7	42
S. anderlecht		4						4
S. arizonae							5	5
S. banana			2					2
S. bareilly			7				2	9
S. berta							1	1
S. binza			2					2
S. blockley				1				1
S. braenderup							1	1
S. brandenburg	1		1					2
S. bredeney	2	8	2			1	3	16
S. brevik						1		1
S. california							4	4
S. cambridge	1						1	2
S. cerro	2	1	1		2		11	17
S. chester						3		3
S. cubana							3	3
S. derby		4				1	16	21
S. drypool							1	1
S. eastbourne	1							1
S. eimsbuettel	1	1					6	8
S. enteritidis				6				6
S. give					2			2
S. hadar		9	2	1				12
S. havana	1		1			2	3	7
S. heidelberg		1	1				2	4
S. illinois	1							1
S. indiana		5						5
S. infantis	5		4	3	2	5	15	34
S. javiana							1	1
S. johannesburg	2						5	7
S. kentucky				1			13	14
S. livingstone		3					3	6
S. london			2				5	7
S. manila	1							1
S. mbandaka	1				4		2	7
S. minnesota							2	2
S. montevideo	7	3	18				10	38
S. muenchen	1	4				2		7
S. muenster			3				5	8
S. newhaw							2	2
S. newington						2	7	9
S. newport		4	8				5	17
S. niedstedten	1							1
S. ohio						1	2	3
S. onderstepoort						1		1
S. oranienburg	1					1	7	9

S. orion		1					3	4
S. panama		10	3			4		17
S. perth						5		5
S. rubislaw						1	1	2
S. sara							1	1
S. saint-paul	1		1				1	3
S. schwarzengrund	1	1					2	4
S. senftenberg	5	4	8			1	9	27
S. siegburg							2	2
S. sofia			5					5
S. sundsvall							1	1
S. tennessee	3	3	1				6	13
S. thomasville	1						3	4
S. thompson			2				4	6
S. toebingen							1	1
S. typhimurium	2	8	3	4	7	2	2	28
S. untypeable		10		4	3	2	3	22
S. virchow		4						4
S. worthington	1						2	3
Total	43	100	93	26	21	55	203	541

[a]Multiple serovars were reported for some samples.
[b]England and United States represented by 2 and 5 laboratories, respectively.

TABLE 3. *Recovery of* Salmonella *from refrigerated broth cultures.*

Laboratory	Total samples positive[a]	*Salmonella*-positive samples	
		Refrigerated preenrichment[b]	Refrigerated enrichment[b]
1	22	19	15
2	69	64	66
3	19	19	19
4	68	58	62
5	53	51	49
6	25	21	22
7	21	20	21
8	58	58	58
9	12	10	10
10	55	48	53
11	21	21	21
12	43	42	43
Total	466 (100)[c]	431 (92.5)	439 (94.2)

[a]Determined by the standard cultural procedure.
[b]Sample is declared positive upon recovery of *Salmonella* from any one of the four enrichment-plating conditions.
[c]Numbers in brackets are percent values.

TABLE 4. *Productivity of refrigerated broth cultures of high moisture foods*[a].

Laboratory	Total samples positive[b]	*Salmonella*-positive samples	
		Refrigerated preenrichment[c]	Refrigerated enrichment[c]
1	14	11	7
2	51	46	48
3	15	15	15
4	49	39	43
5	49	47	45
6	11	8	9
7	6	5	6
8	35	35	35
9	4	2	2
10	40	33	38
11	3	3	3
12	14	13	14
Total	291 (100)[d]	257 (88)	265 (91)

[a]See Table 1.
[b]Determined by the standard cultural procedure.
[c]See footnote b in Table 3.
[d]Numbers in brackets are percent values.

pronounced with low moisture foods. Productivities of the TBG and SC enrichment broths were significantly different ($p = 0.039$) within the standard cultural but not the two refrigeration conditions. Differences in the performance of the BiS and BGS agar media were not significant ($p > 0.05$). Use of an enrichment broth with more than one plating medium or a single plating medium with two enrichment broth media increased recovery (Table 10). Unique identification of 87 high moisture foods by TBG enrichment alone further underlines the sensitivity of the medium (Table 11). For example, of 291 contaminated high moisture foods identified by one or more of the 4 enrichment/plating conditions in the standard cultural procedure, 16 of these samples were detected only by the TBG-BiS combination and 9 other samples by TBG-BGS. Similarly, identification of 10 positive samples by SC enrichment was obtained with BiS (3) and BGS (7) only. Isolation of *Salmonella* from 27 high moisture and 11 low moisture foods by SC alone is equally notable.

Although the present study was conducted according to a rigid experimental protocol, several laboratories through their own initiative generated additional data which are

TABLE 5. Salmonella *recovery patterns in high moisture foods*[a].

Laboratory	Total samples positive	Recovery patterns			
		Standard / Refrig. preenrich.[b] / Refrig. enrich.[b]			
		+/+/+	+/−/+	+/+/−	+/−/−
1	14	7	0	4	3
2	51	44	4	2	1
3	15	15	0	0	0
4	49	37	6	2	4
5	49	45	0	2	2
6	11	7	2	1	1
7	6	5	1	0	0
8	35	35	0	0	0
9	4	2	0	0	2
10	40	32	6	1	1
11	3	3	0	0	0
12	14	13	1	0	0
Total	291 (100)[c]	245 (84)	20 (7)	12 (4)	14 (5)

[a]See Table 1.
[b]See footnote b in Table 3.
[c]Numbers in brackets are percent values.

TABLE 6. *Productivity of refrigerated broth cultures of low moisture foods*[a].

Laboratory	Total samples positive[b]	*Salmonella*-positive samples	
		Refrigerated preenrichment[c]	Refrigerated enrichment[c]
1	8	8	8
2	18	18	18
3	4	4	4
4	19	19	19
5	4	4	4
6	14	13	13
7	15	15	15
8	23	23	23
9	8	8	8
10	15	15	15
11	18	18	18
12	29	29	29
Total	175 (100)[d]	174 (99)	174 (99)

[a]See Table 1.
[b]Determined by the standard cultural procedure.
[c]See footnote b in Table 3.
[d]Numbers in brackets are percent values.

marized hereafter. Contamination levels in 8 feed samples analyzed by laboratory 1 ranged from 1 to 51 salmonellae/100 g, with a median value of 10, whereas levels in 8 samples of bulk liquid eggs were 4 to >16,000/100 ml (median = 128). Four hamburger and sausage samples tested by laboratory 6 contained 0.4 to 4.0 salmonellae/g (median = 4); levels of 1.0 to 8.0/g (median = 4) were also detected in 13 dried foods, including milk powder, cake, egg powder and spices. Five laboratories reported isolation of *Salmonella* from a total of 12 high moisture and 3 low moisture foods by the refrigeration but not the standard cultural procedure. Nine of these samples were identified from refrigerated preenrichment cultures, 4 from enrichment cultures and 2 from both refrigeration conditions (data not shown). Two laboratories compared the sensitivity of their routine cultural method with that used in the present comparative study. Laboratory 2 reported identification of 6 additional mince meat, pork sausage and animal feed samples using a modified Rappaport enrichment medium incubated overnight at 43°C (*13,14*). No samples were positive by the standard method and negative by the modified Rappaport medium. Laboratory 3 isolated salmonellae from 5 cocoa and liquid egg samples by the ISO (*12*) but not the standard cultural procedure, whereas one bone meal sample escaped detection by the ISO method. Laboratory 3 further showed that incubation of refrigerated TBG and SC for 24 h at 43°C yielded 6 additional isolates when plated on brilliant green agar. Semi-quantitative analyses by the same laboratory suggested that refrigeration of enrichment but not of preenrichment cultures decreases the number of viable salmonellae while exerting little adverse effect on numbers of competitive flora.

DISCUSSION

A recent comparative study within 6 laboratories showed that refrigeration of preenrichment and enrichment broth cultures constituted a reliable analytical approach for the detection of *Salmonella* in naturally contaminated food samples (*3*). Of 160 contaminated foods tested, 105 consisted of chicken carcasses which were cooperatively analyzed by the 6 participating laboratories. An additional 55 low and high moisture food samples were analyzed by only one of the 6 participating laboratories. Individual laboratory recovery of *Salmonella* from chicken carcasses under both refrigerated analytical conditions ranged from 74 to 100%, with a mean recovery rate of 90%. Two laboratories contributed most (19/21) of the false-negative re-

TABLE 7. Salmonella *recovery patterns in low moisture foods*[a].

Laboratory	Total samples positive	Recovery patterns			
		Standard / Refrig. preenrich.[b] / Refrig. enrich.[b]			
		+ + +	+/−/+	+/+/−	+/−/−
1	8	8	0	0	0
2	18	18	0	0	0
3	4	4	0	0	0
4	19	19	0	0	0
5	4	4	0	0	0
6	14	12	1	1	0
7	15	15	0	0	0
8	23	23	0	0	0
9	8	8	0	0	0
10	15	15	0	0	0
11	18	18	0	0	0
12	29	29	0	0	0
Total	175 (100)[c]	173 (99)	1 (0.5)	1 (0.5)	0

[a]See Table 1.
[b]See footnote b in Table 3.
[c]Numbers in brackets are percent values.

TABLE 8. *False-negative results by food type*[a].

Food category	False-negative results	
	Refrigerated preenrichment	Refrigerated enrichment
High moisture	Poultry/fowl (11/112)[b] Mince meat (7/27) Catfish (6/17) Sausage (3/25) Liquid eggs (3/23)	Poultry/fowl (10/112) Mince meat (6/27) Catfish (1/17) Sausage (2/25) Liquid eggs (4/25) Raw pork (1/20)
Low moisture	Cream cake (1/4)	Cream cake (1/4)

[a]Salmonellae recovered under standard cultural but not refrigeration conditions.
[b]Proportion of false-negative results and total number of positive samples by the standard cultural procedure.

TABLE 9. *Lower confidence limits for detection of positive samples by refrigeration test procedures.*

Laboratory	High moisture food					Low moisture food				
	Preenrichment			Enrichment		Preenrichment			Enrichment	
	n[a]	P[b]	LCL[c]	P	LCL	n	P	LCL	P	LCL
1	14	0.79	0.53	0.50	0.27	8	1.00	0.66	1.00	0.66
2	51	0.90	0.80	0.94	0.85	18	1.00	0.82	1.00	0.82
3	15	1.00	0.80	1.00	0.80	4	1.00	0.46	1.00	0.46
4	49	0.80	0.68	0.88	0.77	19	1.00	0.83	1.00	0.83
5	49	0.96	0.87	0.92	0.82	4	1.00	0.46	1.00	0.46
6	11	0.73	0.44	0.82	0.53	14	0.93	0.69	0.93	0.69
7	6	0.83	0.42	1.00	0.59	15	1.00	0.80	1.00	0.80
8	35	1.00	0.90	1.00	0.90	23	1.00	0.86	1.00	0.86
9	4	0.50	0.11	0.50	0.11	8	1.00	0.66	1.00	0.66
10	40	0.83	0.69	0.95	0.84	15	1.00	0.80	1.00	0.80
11	3	1.00	0.37	1.00	0.37	18	1.00	0.82	1.00	0.82
12	14	0.93	0.69	1.00	0.78	29	1.00	0.89	1.00	0.89
Total	291	0.88	0.85	0.91	0.88	175	0.99	0.97	0.99	0.97

[a]n = number of samples positive by the standard cultural procedure.
[b]P = proportion of samples positive by the standard cultural and refrigeration test procedures.
[c]LCL = lower 95% confidence limits.

TABLE 10. *Productivity of selective analytical conditions*[a].

Food	*Salmonella*-positive samples (%)							
	TBG BiS	TBG BGS	SC BiS	SC BGS	TBG BiS + BGS	SC BiS + BGS	TBG + SC BiS	TBG + SC BGS
High moisture (219)[b]								
Standard cultural	251	242	167	183	276	214	266	267
Refrigerated preenrichment	221	217	151	163	247	191	236	234
Refrigerated enrichment	230	214	96	137	241	155	239	240
Subtotal	702 (80)	673 (77)	414 (47)	483 (55)	764 (88)	560 (64)	741 (85)	741 (85)
Low moisture (175)[b]								
Standard cultural	162	162	151	146	172	163	167	168
Refrigerated preenrichment	156	158	151	145	163	161	167	169
Refrigerated enrichment	154	159	147	147	164	160	166	168
Subtotal	472 (90)	479 (91)	449 (86)	438 (83)	499 (95)	484 (92)	500 (95)	505 (96)
Percent total	84	82	62	65	90	75	89	89

[a]Based on results from 12 participating laboratories.
[b]Total number of *Salmonella*-contaminated samples as determined by the standard cultural procedure.

TABLE 11. *Unique isolations by selective analytical conditions.*

Food	Number of unique isolations			
	TBG BiS	TBG BGS	SC BiS	SC BGS
High moisture (291)[a]				
Standard cultural	16	9	3	7
Refrigerated preenrichment	12	14	2	5
Refrigerated enrichment	21	15	2	8
Subtotal	49	38	7	20
Low moisture (175)[a]				
Standard cultural	2	2	1	0
Refrigerated preenrichment	2	1	2	2
Refrigerated enrichment	3	0	3	3
Subtotal	7	3	6	5
Total	56	41	13	25

[a]Total number of *Salmonella*-contaminated samples as determined by the standard cultural procedure.

sults encountered with chicken samples. Analysis of other low and high moisture foods by a single laboratory yielded one false-negative result from a refrigerated preenrichment culture of porcine organ meat; overall accuracy of this laboratory was >99%. Variations in the ability of laboratories to successfully recover *Salmonella* under refrigeration conditions as noted previously (*3*) were equally prominent in the present study (Table 3). Results that deviate substantially from the norm are not uncommon in comparative, multi-lab studies. A collaborative study on the performance of 5 agar media for detection of salmonellae in artificially contaminated foods showed inconsistent trends in the ability of given analysts to reliably identify contaminated samples (*1*). Laboratory environment was also found to be a determinant in the successful isolation of *Salmonella* in low-level contaminated food samples (*21*). Although the results from 4 microbiologists analyzing naturally and artificially contaminated foods by a standardized technique were comparable when the work was conducted in a single laboratory, results from the same analysts differed significantly when the work was done in separate laboratories. Authors attributed this anomaly to possible variations in media preparations and intense activities within laboratories routinely involved in microbiological analyses.

High moisture foods challenged the sensitivity of the refrigeration approach and yielded recovery rates lower than those obtained with low moisture foods (Tables 4 and 6). Similar results were reported in an earlier study where none of 34 dried foods tested yielded false-negative results (*3*). Factors responsible for the greater recovery with low moisture foods remain obscure. Distribution of serovars in high and low moisture foods (data not shown) fails to support a serovar-dependent response. Occurrence of fewer salmonellae in low moisture foods than in raw meats and unprocessed foods (*2,3,5*) further suggests that initial levels of *Salmonella* contamination are not a determinant for recovery under refrigeration test conditions. Earlier

work showed that numbers of salmonellae in preenrichment cultures of low and high moisture foods ranged from 10^5 to 10^7 cells/ml, irrespective of serovar or incident level of contamination (*2*). Synergism between the large numbers of competitive organisms in raw products, notably psychrotrophs and their ability to survive refrigerated storage (*3,9*), may account for the observed difficulty in recovering *Salmonella* from high moisture foods.

Identification of 15 contaminated samples by refrigeration but not standard cultural procedures in 5 laboratories contrasts with earlier studies where refrigerated broth cultures failed to identify additional positive samples (D'Aoust, unpublished data). Results in the present study may reflect inability of the competitive flora in these samples to survive prolonged storage in an aqueous environment thus leading to facilitated detection of salmonellae on plating media. Involvement of a common food or foods from a restricted geographic area could not be invoked as the source of these "false-positive" results.

Data on the performance of selective enrichment-plating conditions (Table 10) further document the superiority of TBG (43°C) in combination with one or more differential plating media for the isolation of *Salmonella* in high moisture foods. Numerous studies involving different formulations of tetrathionate broth (*4*) and a variety of raw meat and unprocessed food products have found this medium to exceed the performance of selenite F (*15*), selenite cystine (*5,13*) and selenite brilliant green (*16,20*) enrichment media. Recovery of salmonellae from low moisture foods reportedly is independent of selective enrichment conditions. A comparative ICMSF study reported the absence of significant differences between TBG and SC at either 35 or 43°C for detection of *Salmonella* in dried dairy, soya and rendered animal by-products (*7*). Similar results were presented in a subsequent study involving a variety of dried foods and feed ingredients (*8*). Earlier reports (*7,16*) and our results (Table 10) suggest that use of multiple enrichment broths and agar plating media increase method sensitivity through facilitated recovery of fastidious *Salmonella* strains in the presence of large populations of nonsalmonellae. Although many standard cultural procedures advocate this analytical approach (*11,12,19*), detection of *Salmonella* in selected food types with a single enrichment medium in combination with one or more plating media is also recommended (*18*). Identification of contaminated samples, particularly high moisture foods, by unique analytical conditions (Table 11) reiterates the value of multiple enrichment and plating media for increased method sensitivity.

The present study and earlier findings (*3*) validate refrigeration of preenrichment and enrichment broth cultures as a reliable analytical procedure to increase laboratory flexibility and productivity. Efficacy of refrigeration was further underlined in a recent study on recovery of *Salmonella* from preenrichment cultures of animal feeds refrigerated for 6 d (*17*). Lack of complete agreement between results by the standard cultural and refrigeration test procedures in the present and earlier study (*3*) apparently reflect variations in laboratory performance rather than intrinsic limitations of the refrigeration approach. Occurrence of all but two false-negative results with high moisture foods (Tables 4 and 6) was fortuitous because low moisture foods, in contrast to products with a higher water content, are not usually subjected to further bactericidal treatments before consumption and, consequently, pose a major health risk when contaminated. Notwithstanding the laboratory-dependent variations of uncertain origin, our results strongly support incorporation of the refrigeration approach in standard methods for detection of *Salmonella* in foods.

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