

Update on Preenrichment and Selective Enrichment Conditions for Detection of *Salmonella* in Foods

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ABSTRACT

The search for short and sensitive cultural methods for detection of *Salmonella* in foods has met with limited success. Short (3-8 h) incubation of non-selective enrichment media do not provide conditions for effective resuscitation of stressed or injured salmonellae and result in unacceptably high numbers of false-negative results. Isolation of *Salmonella* is not dependent on the nutritional value of preenrichment media; simple media such as lactose and nutrient broths are equally reliable as highly nutritive sterility testing media. The need for detergents in non-selective enrichment of fatty foods and use of preenrichment transfer volumes greater than 1 ml is not indicated. Although selective enrichment in tetrathionate brilliant green (TBG) broth at an elevated temperature (43°C) increases method sensitivity, use of Mueller-Kauffmann TBG under similar analytical conditions may be inhibitory to *Salmonella*. Refrigeration of preenrichment and selective enrichment broth cultures has been used successfully to provide greater analytical flexibility by increasing the number of days on which analyses can be initiated without engendering weekend work.

Standard cultural procedures for isolation of *Salmonella* in foods are time-consuming and require a minimum of 4-5 days to obtain presumptive evidence of *Salmonella* contamination (1,26,28,51). These procedures generally include preenrichment of food samples in a non-selective broth medium, enrichment in a selective broth medium, isolation of presumptive colonies on differential plating media, biochemical screening and serological confirmation of presumptive isolates. Preenrichment in a non-selective broth medium provides for the non-inhibited growth of indigenous bacterial flora and the resuscitation and proliferation of stressed or injured salmonellae to detectable levels (35). Selective enrichment inhibits growth of non-salmonellae and facilitates isolation of *Salmonella* on differential plating media (17). Selective agar media commonly used for presumptive identification of *Salmonella* respond to lactose and/or sucrose utilization except bismuth sulfite agar, which reacts to bacterial production of hydrogen sulfide (7). The increasing incidence of *Salmonella* biotypes that ferment lactose (3,18) or show urease activity (19) underlines the growing limitations of biochemical profiles as a reliable taxonomic tool, and the

importance of serological confirmation of isolates with somatic and flagellar antisera.

This paper reviews the significance of different preenrichment and selective enrichment conditions in the isolation of *Salmonella* from foods.

NON-SELECTIVE ENRICHMENT [PREENRICHMENT]

Several preenrichment media of varying nutritive value are used in standard cultural procedures (Table 1). Although lactose broth and buffered peptone water (BPW) are preferred by U.S. and international agencies, respectively, agreement can be found in the use of brilliant green water for preenrichment of nonfat dried milk and dried whole milk and of reconstituted nonfat dried milk with added brilliant green for chocolate and other confectionery products. Lack of unanimity in the choice of a non-selective enrichment medium apparently stems from few detailed studies on productivity of preenrichment media under different experimental conditions. The performance of Clausen, EE (Mossel), Eugon, GN, lactose, nutrient, and tergitol-7 broths

TABLE 1. Media for non-selective enrichment.

Agency/Group	Medium
AOAC (2) and APHA (1)	Lactose Brilliant green water
FDA (51)	Lactose Tryptic soy Brilliant green water Powdered milk with brilliant green Nutrient
HPB (26)	Nutrient Brilliant green water Trypticase soy Powdered milk with brilliant green
ICMSF (28)	Lactose Buffered peptone water Brilliant green water Powdered milk with brilliant green
ISO (29,30)	Buffered peptone water Ringer's with brilliant green
NAS (42)	Lactose Brilliant green water
USDA (50)	Lactose

(Table 2) was recently compared and, except for EE broth, found to be equally effective for isolation of *Salmonella* in naturally contaminated low and high moisture foods (9, and Table 3). These findings indicate that the nutrient level of preenrichment media is not a determinant for effective isolation of *Salmonella* from foods. Similar recoveries have been reported with BPW and lactose broth enrichment of egg products (55), BPW and tryptone soya broth enrichment of minced meat (52), and BPW, reconstituted nonfat dried milk with added brilliant green, trypticase soy, and lactose broth enrichment of milk chocolate (60). Although similar in productivity, lactose and nutrient broths identified fewer naturally contaminated gelatin samples than tryptic soy broth (43).

Short preenrichment incubation periods have been evaluated for their ability to abbreviate standard cultural procedures without adversely affecting method sensitivity. Data supporting use of short incubation periods have been obtained with *Salmonella*-infected foods and animal feeds preenriched with continuous shaking for 7 h in a modified M broth (27). Good recovery of heat-injured salmonellae was also reported for milk powder preenriched for 2-6 h in BPW (54). Preenrichment of dry foods for 6 h in shallow layers of tryptone soya peptone broth was superior to overnight incubation in lactose broth for recovery of *Enterobacteriaceae* (40). Evidence of the limited reliability of short incubation periods is equally of interest. Studies on isolation of injured salmonellae from foods showed that resuscitation for up to 6 h in lactose or tryptone soya broths was insufficient for repair and active growth of the organisms (53). Preenrichment for 24 h rather than 7 h in FAS broth (Difco) markedly increased the immunofluorescent detection of *Salmonella* in food and animal feeds (37). Recent work on the efficacy of seven preenrichment culture media showed that short (6 h) incubation periods are unreliable and produce unacceptably high numbers of false-negative results (9, and Table 3). Similar results were obtained with fish and shellfish preenriched for 3 h

TABLE 3. Recovery of *Salmonella* with different preenrichment media.^{1,2}

Medium	Salmonella-positive samples Incubation time	
	6 h	24 h
Clausen	15	23
EE (Mossel)	8	17
Eugon	15	22
GN	14	22
Lactose	15	23
Nutrient	14	23
Tergitol-7	11	23

¹A total of 23 positive samples examined.

²Replicate food samples (100 g) were preenriched in 900 ml of each culture medium; preenrichment cultures were selectively enriched in TBG₄₃ and SC₃₅ and plated on BiS and BGS agar media.

in lactose broth (10).

Standard methods generally recommend use of 0.5-1.0% anionic Tergitol-7 (sodium heptadecyl sulfate) or other suitable detergents for effective recovery of *Salmonella* in fatty foods (Table 4). Presumably surfactants liberate salmonellae trapped in lipid micelles into the culture medium where the organisms grow to detectable levels. Although Tergitol-7 increased the recovery of *Salmonella* from pork sausages (21,38), other studies have found this agent to be of little or no diagnostic value and to inhibit *Salmonella* and other *Enterobacteriaceae* (12,39,45). Preliminary results (D'Aoust, unpublished) on recovery of *Salmonella* from naturally contaminated fatty foods preenriched in nutrient broth supplemented with 3-10% (w/v) surfactant fail to support use of these agents to increase method sensitivity (Table 5). Each contaminated food was detected under all analytical conditions except for a single false-negative result with Myrj 52S; salmonellae populations in supplemented and control preenrichment cultures ranged from 10⁶-10⁸ cells/ml with median counts of 10⁴-10⁵ cells/ml.

TABLE 2. Composition of selected preenrichment media.

Broth medium	Ingredients		Recommended for
	Extract	Saccharide	
Clausen	Tryptone Peptone Yeast extract	Dextrose Glycerol	Sterility testing
EE (Mossel)	Tryptose	Dextrose	<i>Enterobacteriaceae</i>
Eugon	Peptone	Dextrose	Sterility testing
GN	Peptone	Dextrose Mannitol	<i>Salmonella-Shigella</i>
Nutrient	Peptone Beef extract	None	General purpose
Lactose	Peptone Beef extract	Lactose	Coliforms
Tergitol-7	Peptone Yeast extract	Lactose	<i>Salmonella</i> and enteric bacilli

TABLE 4. *Detergents in non-selective enrichment.*

Agency/Group	Detergent	Concentration (%)
FDA (51)	Tergitol-7	1.0
	Triton X-100	To initiate foaming
HPB (26)	Tergitol-7	0.6
ICMSF (28)	Tergitol-7	0.2-1.0
ISO (29)	Tergitol-7	1.0
NAS (42)	Tergitol-7	0.6
USDA (50)	Tergitol-7	0.5-1.0

TABLE 5. *Detergents for recovery of Salmonella in fatty foods.^{1,2}*

Detergent ²	Number of positive samples	Log ₁₀ <i>Salmonella</i> /ml preenrichment culture		
		Low	High	Median
Arlacel 80 + Tween 60	20	0	7	4
MYRJ 52S	19	0	8	4
Tergitol-7	20	3	7	5
Triton X-100	20	2	8	5
Tween 80	20	1	7	4
Nutrient broth control	20	0	7	4

¹A total of 20 naturally contaminated foods analyzed.

²Replicate food samples (100 g) were preenriched in nutrient broth containing 3% (w/v) detergent except Arlacel 80 + Tween 60 where 10% (w/v) was used. Preenrichment cultures were selectively enriched in TBG₄₃ and SC₃₅ and plated on BiS and BGS agar media.

The amount of preenrichment culture transferred into selective enrichment medium (transfer volume) differs with standard methods (Table 6). In a recent study on the efficacy of different preenrichment conditions, transfer volume-dependent recovery was observed only with short (6 h) preenrichment incubation periods; at 24 h of incubation, 10 ml and 1 ml transfer volumes were equally sensitive (Table 7). No significant differences in recoveries were reported upon examination of the effect of 1-ml and 2-ml transfer volumes on isolation of salmonellae in artificially contaminated dry foods (44). Limited data also indicate that 1-ml transfer volumes can readily recover *Salmonella* from 1500-g samples of dry foods preenriched in 13.5 litres of broth (48). Clearly, transfer volumes greater than 1 ml do not increase method sensitivity and substantially increase analytical costs through use of greater volumes of selective enrichment media.

SELECTIVE ENRICHMENT

Direct enrichment (no preenrichment) of clinical specimens in selective broth media is not uncommon, and the approach has been adopted for raw or highly contaminated food products (1,51). It is important to note that the bacterial characteristics of these two types of samples differ markedly; in clinical samples, large populations of physiologically active organisms facilitate detection, whereas comparatively few, inactive salmonellae may contaminate food samples. Detection of

TABLE 6. *Preenrichment transfer volumes.*

Agency/Group	Sample (g)/culture volume	Transfer volume (ml)
AOAC (2) and APHA (1)	25/225	1.0
FDA (51)	25/225	1.0
HPB (26)	25/225	1.0
ICMSF (28)	25/225	1.0
ISO (29)	25/225	10.0
NAS (42)	25/225	1.0
USDA (50)	25/225	0.5
	Large composite	10.0

TABLE 7. *Effect of preenrichment transfer volume on recovery.^{1,2}*

Medium	Incubation time (h)	Salmonella-positive samples		
		Transfer volume (ml)		
		10	1.0	0.1
Clausen	6	19	15	7
	24	23	23	23
EE (Mossel)	6	10	8	4
	24	17	17	17
Eugon	6	19	15	9
	24	22	22	22
GN	6	19	14	5
	24	22	22	22
Lactose	6	20	15	8
	24	23	23	22
Nutrient	6	21	14	5
	24	23	23	23
Tergitol-7	6	18	11	5
	24	23	23	22
TOTAL:	6	126	92	43
	24	153	153	151

¹A total of 23 positive samples examined.

²See Table 3.

Salmonella in raw meats and sewage materials was substantially greater with preenrichment than with direct enrichment (Table 8). Direct enrichment of food samples heavily contaminated with competitive flora is not justified and may lead to false-negative results.

The multiplicity of time-temperature regimens and selective enrichment broths used in standard cultural procedures is notable (Table 9). In addition to the tetrathionate and selenite cystine (SC) broths, selenite brilliant green (4,5,49) and Rappaport (57,59) enrichment media have also been used in food analysis. Several formulations of tetrathionate broths, which differ widely in composition and selectivity, are currently in use (Table 10). These include TBG, the Mueller (41) tetrathionate broth as modified by Kauffman (33); TT, the formulation of Hajna (23); Tet, as formulated by Rolfe (47); MK TBG, the Mueller-Kauffman tetrathionate broth widely used in Europe (14,24,31,58) and marketed by Oxoid Limited. The tetrathionate ($S_4O_6^{2-}$)

TABLE 8. *Efficacy of preenrichment and direct enrichment.*

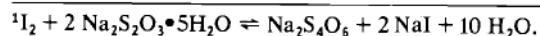
Sample material	Preenrichment/ enrichment	Percent recovery by		Reference
		Preenrichment	Direct enrichment	
Minced meat (artificially)	BPW/MK TBG ₄₃	90	27	Edel (15)
Minced meat (naturally)	BPW/MK TBG ₄₃	83	56	
Meat	LAC/TBG ₃₅	91	43	Gabis (20)
	LAC/SC ₃₅	91	62	
Sewage material	BPW/MK TBG ₄₃	100	55	Grunnett (22)
Sewage water	BPW/MK TBG ₄₃	100	51	Harvey (24)

TABLE 9. *Selective enrichment conditions in standard methods.*

Agency/Group	Media	Incubation time (h)
AOAC (2) and		
APHA (1)	TBG ₃₅ , SC ₃₅	24 ± 2
FDA (51)	TBG ₃₅ , SC ₃₅	24 ± 2
HPB (26)	TBG ₄₃ , SC ₃₅	24 ± 2
ICMSF (28)	TBG ₄₃ , SC ₄₃	24
ISO (29)	MK TBG ₄₃ , SC ₃₅	24 and 48
NAS (42)	TBG ₃₅ , SC ₃₅	18-24
USDA (50)	TT ₃₅	18-24

TABLE 10. *Characteristics of tetrathionate¹ enrichment broths.*

Characteristic	TBG	TT (Hajna)	TET (Rolfe)	MK TBG
I ₂ (g/l)	6.0	5.0	11	4.0
KI (g/l)	5.0	8.0	13.75	5.0
Molarity I ₂ = S ₄ O ₆ ⁼	0.024	0.020	0.043	0.016
Excess S ₂ O ₃ ⁼ (moles)	0.072	0.11	NONE	0.17
Brilliant green (g/l)	0.01	0.01	0.01	0.01
Bile salts (g/l)	1.0	0.5	NONE	5.0
CaCO ₃ (g/l)	10.	25.0	50.0	45.0



anion constitutes the principal selective agent in these enrichment media and is most effective at a concentration of 0.03 M (34). The high S₄O₆⁼ content and absence of bile salts to neutralize the toxicity of brilliant green (36) in the Rolfe formulation underlines the probable toxicity of this medium to salmonellae. Differences in CaCO₃ content (Table 10) further indicate the limited ability of some formulations to neutralize tetrathionate reductase-linked acid production (34). The excess of S₂O₃⁼ which may be inhibitory to salmonellae (34), and the greater bile salts content of the MK TBG formulation may account, in part, for the reported toxicity of this medium with pure *Salmonella* cultures and raw meat samples (Table 11).

Incubation temperature markedly affects productivity of selective enrichment broths by suppressing growth of competing microorganisms but not *Salmonella*. Since the first report of increased recovery of salmonellae from clinical specimens using selenite broth incubated at 43 C (25), several studies involving different analytical schemes and sample material have shown the net

TABLE 11. *Toxicity of the MK tetrathionate enrichment medium.*

Sample material	Percent recovery		Reference
	35-37 C	43 C	
Heat-injured cultures ²	90	< 1	Corry (6)
Pure cultures ²	60	30	Vassiliadis (58)
Pork sausage ²	ND ³	6	Roberts (46)
Meat filtrate ²	36	44	Kafel (31)
Pork sausage ¹	ND ³	64	Vassiliadis (57)
Pure cultures ²	86	72	van Schothorst (56)

¹With non-selective preenrichment.

²Direct enrichment.

³ND = Not done.

advantage of incubation at elevated temperatures (Table 12). Although international organizations such as the I.C.M.S.F. and ISO recommend high-temperature incubation of enrichment broths, federal agencies and health associations in the United States have not yet opted for this approach in their analytical procedures (Table 10).

Standard methodology recommends incubation of selective enrichment broths for 18-24 h (Table 9); however, incubation periods greater than 24 h are used (29,30) and, on occasion, have been found to yield greater numbers of *Salmonella*-positive samples irrespective of enrichment conditions (Table 13). Claims of increased recovery with prolonged incubation originate mainly from European laboratories using the inhibitory MK

TABLE 12. *Efficacy of selective enrichment at elevated temperatures*

Sample material	Enrichment	Percent recovery		Reference
		35-37 C	43 C	
Animal feed	SBGS ²	58	84	Carlson (5)
Feces	TET (Rolfe) ²	66	75	Edel (13)
	Selenite F ²	64	74	
	TET (Oxoid) ²	74	98	
Pork sausage	TBG ²	43	57	Morris (38)
Red meat	SC ¹	76	92	Silliker (49)
	SBGS ¹	82	91	
	TET ¹	64	81	
	TBG ¹	64	86	
Sewage water	MK TBG ²	38	96	Grunnett (22)

¹With non-selective enrichment.

²Direct enrichment.

TBG at 43 C. Some of our preliminary work with food samples preenriched in nutrient broth suggests that such increased recovery is not dependent on *Salmonella* growth (8). *Salmonella* counts in TBG₄₃ and SC₃₅ broths incubated for 16-48 h ranged between 10⁵-10⁶ cells/ml after 16 h of incubation with a one log maximum reduction in counts after 48 h. Increased recovery of salmonellae with prolonged incubation presumably results from differential death rate kinetics; competitive flora, because of their greater sensitivity to elevated temperatures and toxicity of selective enrichment media, die off more rapidly, thus facilitating recovery of *Salmonella* on selective plating media.

The need for increased laboratory efficiency and flexibility in analytical procedures led us to consider refrigeration of preenrichment and enrichment broth cultures for 72 h as a means of increasing the number of days on which sample analyses could be initiated without engendering weekend work. Presently, preenrichment of food samples is restricted to Mondays and Tuesdays to obtain, the following Friday, presumptive evidence of *Salmonella* on selective agar media. With the refrigeration approach, enrichment and preenrichment broth cultures of samples analyzed on Wednesday and Thursday, respectively, would be refrigerated on Friday for the weekend and sample analysis resumed the following Monday. A recent evaluation of this novel approach showed that recovery of *Salmonella* from low and high moisture foods under standard and refrigerated conditions were identical, except for a single contaminated pork liver sample which could not be identified with the refrigerated preenrichment culture (Table 14). Six laboratories participated in the analysis of poultry samples; two of these laboratories contributed 19 of the 21 false-negative results obtained with this food product

TABLE 14. Recovery from refrigerated preenrichment and enrichment broth cultures.¹

Food	Salmonella-positive samples		
	Standard cultural	Refrigerated preenrichment	Refrigerated enrichment
Poultry	105	93 (90)	96 (91)
High moisture	21	20 (95)	21 (100)
Low moisture	34	34 (100)	34 (100)
Total	160 (100)	147 (92)	151 (94)

¹Percent values are in brackets.

(11). The encouraging results obtained with this analytical scheme, and the need for greater method flexibility has led to an international I.C.M.S.F. evaluation of the refrigeration approach.

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TABLE 13. Recovery with prolonged incubation of selective enrichment media.

	Sample material	Preenrichment/enrichment	Percent recovery after			
			24 h	48 h	72 h	96 h
<i>Preenrichment</i>						
Edel (15)	Minced meat (artificially)	BPW/MK TBG ₄₃	90	95		
	Minced meat (naturally)	BPW/MK TBG ₄₃	83	92		
Edel (16)	Animal feed	BPW/MK TBG ₄₃	95	100		
Harvey (24)	Sewage water	BPW/MK TBG ₄₃	64	100		
Vassiliadis (57)	Pork sausage	BPW/MK TBG ₄₃	52	64		
<i>Direct enrichment</i>						
Carlson (5)	Animal feed	NA ¹ /SBGS ₃₇	58	53		58
		NA/SBGS ₄₃	84	87		82
Kampelmacher (32)	Minced meat	NA/MK TBG ₄₃	75	100		
Edel (14)	Minced meat (artificially)	NA/MK TBG ₄₃	99	100		
	Feces (artificially)	NA/MK TBG ₄₃	80	99		
Edel (15)	Minced meat (artificially)	NA/MK TBG ₄₃	27	52		
	Minced meat (naturally)	NA/MK TBG ₄₃	56	69		
Grunnett (22)	Sewage water	NA/MK TBG ₄₃	60	80	95	100
Kafel (31)	Meat filtrate	NA/MK TBG ₃₇	36	34		
		NA/MK TBG ₄₃	44	59		

¹NA = Not applicable.

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