Journal of Food Protection, Vol. 53, No. 7, Pages 562-565 (July 1990)

Limited Sensitivity of Short (6 h) Selective Enrichment for Detection of Foodborne *Salmonella*

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(Received for publication November 21, 1989)

ABSTRACT

Short (6 h) enrichment under five different selective conditions adversely affected the isolation of *Salmonella* from pre-enriched samples of naturally contaminated foods. Of the 109 high moisture and 18 low moisture foods found to contain salmonellae following conventional (24 h) enrichment, combined results of the abbreviated enrichment procedures identified only 99 (90.8%) and 13 (72.2%) contaminated samples, respectively. The productivities of tetrathionate brilliant green (TBG_{43}) and Muller-Kauffman tetrathionate ($MKTBG_{43}$) broths consistently exceeded that obtained with the modified Rappaport (RV_{43}), tetrathionate brilliant green (TBG_{35}), and selenite cystine (SC_{35}) media after 6 and 24 h of incubation. Semi-quantitative analyses of growth under all enrichment conditions indicated that short (6 h) enrichment negatively affected method sensitivity through reduced numbers of *Salmonella* colonies and heavy growth of nonsalmonellae on bismuth sulfite (BSA) and brilliant green sulfa (BGS) plating media. These findings raise concerns on the dependability of commercial diagnostic schemes that incorporate abbreviated (6 h) enrichment in TBG_{35} and/or SC_{35} in their analytical protocol.

Standard cultural procedures for the isolation of salmonellae in foods are laborious and require a minimum of 4d to obtain presumptive evidence of contamination (*4,11,17,18*). The economics of storage of raw and processed foods pending microbiological clearance have underscored the need for more rapid and reliable methods. Recent attempts at method brevity involving serological or enzyme-linked immunosorbent techniques have generally focused on selective enrichment cultures as test material (*4,6*). These novel techniques have reduced by a single day the amount of time required to obtain presumptive results by standard cultural procedures (*4*). Although reduction of the common (16-24 h) pre-enrichment period to 6-8 h would have greatly accelerated sample analysis, the approach was found to yield unacceptably high levels of false-negative results (*1,3*). Alternately, successful replacement of the conventional (16-24 h) enrichment of pre-enriched samples for a shorter (6 h) period of incubation would yield presumptive results with a temporal efficiency equal to that offered by commercial analytical schemes but without the added costs for custom reagents, equipment, or diagnostic kits (*4,6*).

The present study examines the reliability of short (6 h) selective enrichment for the more rapid isolation of *Salmonella* in pre-enriched foods and food ingredients.

MATERIALS AND METHODS

Naturally contaminated high moisture (483) and low moisture (28) foods were obtained as a result of federal monitoring or compliance activities, or were purchased at local retail outlets. Most raw meat and poultry samples were obtained at retail.

Samples were examined for *Salmonella* by a standard cultural procedure involving pre-enrichment for 24 h at 35°C in nonselective broth media, and selective enrichment in tetrathionate brilliant green (TBG_{43}) and selenite cystine (SC_{35}) for 24 h at 43°C and 35°C, respectively. Enrichment cultures were streaked on bismuth sulfite (BSA) and brilliant green sulfa (BGS) agar media and incubated for 16-18 h at 35°C. Suspect colonies were screened biochemically on triple sugar iron (TSI) and lysine iron (LI) agar media and confirmed serologically using polyvalent and single grouping somatic (O) and flagellar (H) antisera (*11*). Concurrently, other enrichment media including Muller-Kauffman tetrathionate ($MKTBG_{43}$) distributed by Oxoid Ltd. (CM343), modified Rappaport (RV_{43}) medium (20) prepared in our laboratory and stored in the refrigerator for 1 to 4 weeks pending use, and TBG incubated at 35°C (TBG_{35}) were inoculated in parallel with the standard TBG_{43} and SC_{35} using replicate 1.0 ml portions of the same pre-enrichment culture. All enrichment cultures were plated on BSA and BGS after 6 h of incubation and then incubated for an additional 18 h before streaking on a second set of agar media.

The sensitivity and selectivity of each enrichment medium were assessed after 6 and 24 h incubation using a semi-quantitative score scheme for *Salmonella*-positive samples only. Growth of *Salmonella* on BSA and BGS from each enrichment condition was estimated according to the following sensitivity scale: 1 = presumptive salmonellae in the first quadrant; 2 = salmonellae in quadrants 1 and 2; 3 = salmonellae in quadrants 1, 2, and 3; 4 = salmonellae in all quadrants. BSA and BGS scores for each enrichment condition were then combined into a single value. A similar approach was used to measure the selectivity of each enrichment condition as reflected in the prevalence of nonsalmonella on homologous BSA and BGS media. The following

scale of selectivity was applied to *Salmonella* positive samples: 1 = 0-25% of surface growth is nonsalmonellae; 2 = 26-50% prevalence of nonsalmonellae; 3 = 51-75%, 4 = 76-100%; 5 = presence of nonsalmonellae but absence of *Salmonella*; 6 = plating medium devoid of bacterial growth. Further insight into the selectivity of enrichment conditions was obtained from a percent estimate of black (nonsalmonellae) mimics on BSA in both *Salmonella* positive and negative samples. This estimate was calculated as the percent ratio of plates showing mimicry to the total number (511) of test samples.

RESULTS AND DISCUSSION

Of 511 foods tested (Table 1), 127 (24.9%) were found to contain *Salmonella* by at least one of the test conditions. Combined results on the productivity of five enrichment media after 6 and 24 h of incubation showed that short (6 h) enrichment of pre-enrichment cultures adversely affected method sensitivity. The overall sensitivity of abbreviated enrichment was 88.2% with false-negative rates

TABLE 1. *Detection of* Salmonella *in foods.*

Food	No. Samples Tested	No. Samples Positive	Serovar
High Moisture			
Chicken			
Whole Carcass	45	38	S. agona (1) S. albany (1); S. brandenburg (1); S. haardt (3); S. hadar (7); S. heidelberg (2); S. infantis (10); S. muenchen (1); S. saint-paul (2); S. schwarzengrund (1); S. stanley (2); S. thompson (4); S. typhimurium (3).
Cut-up	14	11	S. blockley (1); S. haardt (1); S. hadar (2); S. heidelberg (1); S. infantis (1); S. muenchen (2); S. stanley (1); S. thompson (1); S. typhimurium (1).
Giblets	37	14	S. albany (1); S. eimsbuettel (1); S. hadar (3); S. heidelberg (3); S. infantis (1); S. muenchen (1); S. typhimurium (2); Salmonella untypable (2).
Nuggets	1	1	S. infantis.
Turkey			
Whole carcass	4	4	S. albany (1); S. arizonae (1); S. senftenberg (2).
Giblets	1	0	
Burgers	4	1	S. saint-paul
Other Poultry[a]	18	6	S. agona (1); S. enteritidis (1); S. montevideo (1); S. muenchen (2); S. saint-paul (1).
Pork			
Sausages	50	7	S. brandenburg (4); S. infantis (1); S. saint-paul (1); S. worthington (1).
Giblets	26	2	S. nienstedten (1); Salmonella untypable (1).
Minced Meat	10	3	S. brandenburg (1); S. indiana (1); S. typhimurium (1).
Cut up	6	0	
Other[b]	5	0	
Beef			
Giblets	15	2	S. brandenburg (1); S. hadar (1).
Minced Meat	8	0	
Miscellaneous Meat[c]	5	1	S. virchow
Marine Foods			
Crustaceans[d]	155	5	S. aberdeen (1); S. arizonae (1); S. infantis (1); S. lohbruegge (1); S. stanley (1).
Mollusks[e]	15	1	S. sundsvall.
Fish[f]	18	1	S. potsdam.
Egg Products			
Shell Eggs	12	0	
Whole Egg (Liquid)	15	8	S. alachua (1); S. brandenburg (1); S. cerro (1); S. heidelberg (2); S. montevideo (1); S. saint-paul (1); S. schwarzengrund (1).
Egg Albumin (Liquid)	8	1	S. cerro.
Egg Yolk (Liquid)	10	3	S. cerro (2); S. saint-paul (1).
Cheese	1	0	
Subtotal	483	109	

TABLE 1 (con't.)

Low Moisture			
Chocolate			
Novelties	3	2	S. napoli (2).
Chips	1	1	S. senftenberg.
Cocoa Beans	2	0	
Coconut	2	0	
Egg Powder	2	0	
Pasta	5	4	S. infantis (2); S. mbandaka (2).
Spices			
Black Pepper	11	9	S. give (1); S. glostrup (3); S. madelia (1); S. morehead (2); S. new-brunswick (1); S. san diego (1).
Turmeric	1	1	S. anatum.
Tea	1	1	Salmonella untypable.
Subtotal	28	18	
TOTAL	511	127	

[a]Duck (10), quail (2), and cornish hen (6).
[b]Fermented sausages (4) and pork burger (1).
[c]Frog legs (3) and lamb chops (2).
[d]Shrimp (142), lobster (6), octopus (3), squid (3), and crab (1).
[e]Scallops (8), oysters (3), periwinkles (2), snails (1), and conch (1).
[f]Freshwater (5) and marine (11) fish, and prepared fish balls (2).

of 9.2 and 27.8% for high and low moisture foods, respectively (Table 2). The reduced incubation period variously affected the productivity of individual enrichment media (Table 3). Although TBG_{43} and $MKTBG_{43}$ produced the highest levels of *Salmonella* recovery in both food categories, appreciable numbers of false-negative results were associated with each of the five enrichment conditions. The 6 h enrichment period apparently precipitated unfavorable ratios of competitive flora to salmonellae that challenged the selectivity of BSA and BGS resulting in low recoveries of *Salmonella* (Table 4). The markedly higher incidence of nonsalmonellae on plating media after short (6 h) enrichment supports this view (Table 5). Our findings are not inconsistent with earlier reports on the sensitivity of abbreviated enrichment. Low numbers of false-negative results were reported with reduced (6-8 h) enrichment of pre-enriched foods in Muller-Kauffman tetrathionate and in Rappaport-Vassiliadis (RV) media (*2,14,16*). In contrast, only 19 (45%) of 42 contaminated environmental samples and 20 (26%) of 76 river water samples were detected following 6 h enrichment in RV medium (*15,19*).

TABLE 2. *Reliability of short (6 h) selective enrichment.*

Food	No. samples Tested	No. samples positive[a] (%) 6 h	24 h
High moisture	483	99 (91)	109 (100)
Low moisture	28	13 (72)	18 (100)
TOTAL	511	112 (88)	127 (100)

[a]Based on combined results of five selective enrichment conditions.

TABLE 3. *Detection of* Salmonella *with different enrichment media.*

Food	No. samples Recovery pattern[a] (6 h/24 h) +/+	+/-	-/+	-/-	Percent recovery 6 h	24 h
High moisture (109)[b]						
TBG_{35}	84	3	13	9	80	89
TBG_{43}	98	0	8	3	90	97
$MKTBG_{43}$	98	0	9	2	90	98
RV_{43}	83	0	16	10	76	91
SC_{35}	76	1	20	12	71	88
Low moisture (18)[b]						
TBG_{35}	7	0	8	3	39	83
TBG_{43}	11	0	7	0	61	100
$MKTBG_{43}$	9	0	8	1	50	94
RV_{43}	8	0	2	8	44	56
SC_{35}	8	0	6	4	44	78

[a]Detection of salmonellae on BGS and/or BSA constitutes a positive result.
[b]Total number of positive samples by combined enrichment conditions.

The present study also underscored major differences in the selectivity of enrichment media under standard (24 h) incubation periods (Table 3). Inability of any of the five enrichment conditions to identify all positive samples underlines the diagnostic benefits of using more than one enrichment condition in food analyses (*5,6*). It is of equal interest that TBG_{35} and SC_{35} which are widely used in the United States and other countries identified only 97 (89%) and 96 (88%) of the contaminated high moisture foods. Homologous results with low moisture foods showed even lower levels of recovery. The generally greater sensitivity of the other three enrichment conditions likely stemmed from incubation at an elevated (43°C) temperature. In fact, ancillary data suggest that enrichment at 43°C facilitated

TABLE 4. *Productivity of selective plating media.*

Enrichment condition	No. of positive samples			
	BSA		BGS	
	6 h	24 h	6 h	24 h
High moisture (109)[a]				
TBG_{35}	74	90	77	89
TBG_{43}	92	98	85	103
$MKTBG_{43}$	93	97	91	106
RV_{43}	70	94	74	92
SC_{35}	65	84	69	89
Subtotal	394	463	396	479
Low moisture (18)[a]				
TBG_{35}	7	15	3	9
TBG_{43}	11	18	8	16
$MKTBG_{43}$	9	17	6	16
RV_{43}	8	10	5	10
SC_{35}	8	14	5	9
Subtotal	43	74	27	60
TOTAL	437	537	423	539

[a]Total number of positive samples by combined enrichment conditions.

the isolation of foodborne salmonellae through greater *Salmonella* densities and reduced populations of competitive flora and *Salmonella* mimicry on plating media (Table 5). These findings concur with the body of scientific literature which is replete with evidence on the merits of selective enrichment at elevated (41-43°C) temperatures (*1,6*). It is therefore not surprising that this highly selective condition enjoys widespread acceptance in standard cultural methods (*11,12,13*). Performance assessment of RV_{43} in this study was based on a standardized 1.0 ml transfer volume of pre-enrichment culture into 9.0 ml of enrichment broth. Earlier reports underlined the limited selectivity of RV_{43} and need for a 1:100 inoculum ratio for maximum sensitivity (*19,20*). The low density of salmonellae and high levels of background flora on RV-inoculated plating media (Table 5) apparently arose from use of a 1:10 inoculum ratio in the present study. More recent work in our laboratory on the interaction between transfer volume and enrichment incubation periods showed greater productivity and selectivity of RV_{43} when inoculated at the optimal 1:100 ratio (unpublished data).

TABLE 5. *Semi-quantitative growth characteristics on plating media.*

Enrichment condition	Index Score[a]					
	Salmonella[b]		Competitive flora[b]		Mimicry on BSA[c]	
	6 h	24 h	6 h	24 h	6 h	24 h
TBG_{35}	685	792	986	895	10.3	4.5
TBG_{43}	691	919	738	575	10.7	2.9
$MKTBG_{43}$	690	938	659	536	7.0	2.7
RV_{43}	511	798	1036	851	18.5	12.8
SC_{35}	513	757	1028	818	10.5	5.3

[a]Scores for growth on BGS and BSA were combined.
[b]Perfect scores for *Salmonella* and competitive flora are 1016 and 254, respectively.
[c]Percent occurrence of BSA plates with black (nonsalmonellae) colonies.

The present study clearly established the limited sensitivity of short (6 h) enrichment of pre-enriched food samples. Furthermore, it raises legitimate concerns on the dependability of commercial diagnostic schemes that have incorporated abbreviated enrichment in their analytical protocol (*7,8,9.10*), and whose equivalence was established through comparisons with TBG_{35} and SC_{35}-dependent standard procedures of reduced sensitivity (Table 3).

ACKNOWLEDGMENTS

The authors gratefully acknowledge the collaboration of the National Enteric Reference Center (Health and Welfare Canada) in serotyping *Salmonella* isolates. The technical assistance of D. Warburton was greatly appreciated.

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ACKNOWLEDGMENTS

The authors gratefully acknowledge the laboratory assistance of Catherine Barkate and Patti Wiese.

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are located very close to the cutoff line. Raw milk samples normally contain low levels of *Enterobacteriaceae* and this was observed during our analysis of raw milk. This observation resulted in a clustering of data points in the low plate count and high DT area. On the graph in Fig. 6 the lowest plate counts observed (10 or <10 CFU/ml) correspond with a large amount of variation on the DT axis (7.8 - 15 h). Some of the samples which produced these delayed DT's may actually be stressed *Enterobacteriaceae* or non-*Enterobacteriaceae* microorganisms. This does not effect the accuracy of the results since the DT's produced by these cultures are above the cutoff threshold, and therefore, these samples are classified correctly.

Although use of a maximum conductance change of 200 microsiemens did discriminate in this study between samples containing *Enterobacteriaceae* and those which did not, it does not appear necessary to use this characteristic in routine quality control analyses (pass/fail tests similar to the one described in this study) since non-*Enterobacteriaceae* cultures which do produce a DT in the selective medium evaluated produce DT's which are significantly delayed. Results of this study show that levels of *Enterobacteriaceae* can be automatically detected in raw milk with results obtainable within 6 - 12 h at levels of 500 - <10 CFU/ml, respectively.

ACKNOWLEDGMENTS

The authors wish to thank Milk Marketing, Inc., 8257 Dow Circle, Strongsville, OH 44136 for supplying the milk samples used throughout this study.

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