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 (TBG₄₃) and Muller-Kauffman tetrathionate (MKTBG₄₃) broths consistently exceeded that obtained with the modified Rappaport (RV₄₃), tetrathionate brilliant green (TBG₃₃), and selenite cystine (SC₃₅) 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 salmonellae colonies and heavy growth of nonsalmonellae on bismuth sulfite (BSA) and brilliant green sulfua (BGS) plating media. These findings raise concerns on the dependability of commercial diagnostic schemes that incorporate abbreviated (6 h) enrichment in TBG₄₃ and/or SC₃₅ 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 (TBG₄₃) and selenite cystine (SC₃₅) for 24 h at 35°C and 35°C, respectively. Enrichment cultures were streaked on bismuth sulfite (BSA) and brilliant green sulfua (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 (MKTBG₄₃) distributed by Oxoid Ltd. (CM343), modified Rappaport (RV₄₃) medium (20) prepared in our laboratory and stored in the refrigerator for 1 to 4 weeks pending use, and TBG incubated at 35°C (TBG₄₃) were inoculated in parallel with the standard TBG₄₃ and SC₃₅ 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 nonsalmonellae on homologous BSA and BGS media.
SENSITIVITY OF SHORT SELECTIVE ENRICHMENT FOR SALMONELLA

A 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.

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

JOURNAL OF FOOD PROTECTION, VOL. 53, JULY 1990
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<sub>43</sub> and MKTBG<sub>43</sub> 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).

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<sub>35</sub> and SC<sub>35</sub> 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.

<table>
<thead>
<tr>
<th>Enrichment condition</th>
<th>No. of positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BSA</td>
</tr>
<tr>
<td></td>
<td>6 h 24 h 6 h 24 h</td>
</tr>
<tr>
<td>High moisture (18)</td>
<td></td>
</tr>
<tr>
<td>TBG&lt;sub&gt;35&lt;/sub&gt;</td>
<td>74 90 77 89</td>
</tr>
<tr>
<td>TBG&lt;sub&gt;43&lt;/sub&gt;</td>
<td>92 98 85 103</td>
</tr>
<tr>
<td>MKTBG&lt;sub&gt;43&lt;/sub&gt;</td>
<td>93 97 91 106</td>
</tr>
<tr>
<td>RV&lt;sub&gt;43&lt;/sub&gt;</td>
<td>70 94 74 92</td>
</tr>
<tr>
<td>SC&lt;sub&gt;35&lt;/sub&gt;</td>
<td>65 84 69 89</td>
</tr>
<tr>
<td>Subtotal</td>
<td>394 463 396 479</td>
</tr>
<tr>
<td>Low moisture (18)&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>TBG&lt;sub&gt;35&lt;/sub&gt;</td>
<td>7 15 3 9</td>
</tr>
<tr>
<td>TBG&lt;sub&gt;43&lt;/sub&gt;</td>
<td>11 18 8 16</td>
</tr>
<tr>
<td>MKTBG&lt;sub&gt;43&lt;/sub&gt;</td>
<td>9 17 6 16</td>
</tr>
<tr>
<td>RV&lt;sub&gt;43&lt;/sub&gt;</td>
<td>8 10 5 10</td>
</tr>
<tr>
<td>SC&lt;sub&gt;35&lt;/sub&gt;</td>
<td>8 14 5 2</td>
</tr>
<tr>
<td>Subtotal</td>
<td>43 74 27 60</td>
</tr>
<tr>
<td>TOTAL</td>
<td>437 537 423 539</td>
</tr>
</tbody>
</table>

<sup>a</sup>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 enrichment conditions. The low density of salmonella and high levels of background flora on RV-inoculated plating media (Table 5) apparently arose from use of a low 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 low 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 low 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 low inoculum ratio for maximum sensitivity (19,20).

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

<table>
<thead>
<tr>
<th>Enrichment condition</th>
<th>Index Score&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Salmonella&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>6 h 24 h 6 h 24 h</td>
</tr>
<tr>
<td>TBG&lt;sub&gt;35&lt;/sub&gt;</td>
<td>685 792 986 895 10.3 4.5</td>
</tr>
<tr>
<td>TBG&lt;sub&gt;43&lt;/sub&gt;</td>
<td>691 919 738 575 10.7 2.9</td>
</tr>
<tr>
<td>MKTBG&lt;sub&gt;43&lt;/sub&gt;</td>
<td>690 938 659 536 7.0 2.7</td>
</tr>
<tr>
<td>RV&lt;sub&gt;43&lt;/sub&gt;</td>
<td>511 798 1036 851 18.5 12.8</td>
</tr>
<tr>
<td>SC&lt;sub&gt;35&lt;/sub&gt;</td>
<td>513 757 1028 818 10.5 5.3</td>
</tr>
</tbody>
</table>

<sup>b</sup>Scores for growth on BGS and BSA were combined.
<sup>d</sup>Perfect scores for Salmonella and competitive flora are 1016 and 254, respectively.
<sup>c</sup>Percent occurrence of BSA plates with black (nonsalmonellae) colonies.

Ingham et al., cont. from p. 567

ACKNOWLEDGMENTS

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

REFERENCES


Cousins and Mariott, cont. from p. 570

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 affect 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.

REFERENCES