Enhanced Recovery and Isolation of Salmonella and Listeria Using a Novel Culture-Transfer-Inoculation Device

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ABSTRACT

A novel culture-transfer-inoculation device (CTID), comprised of a fiber-foam matrix suspended in the enrichment medium and thereafter used in subsequent transfers and inoculations, was used in the analysis of foods for Salmonella using the Food and Drug Administration (FDA) method and for Listeria using the FDA and United States Department of Agriculture (USDA) procedures. In these studies, the food sample was introduced into the enrichment medium including the CTID. Following incubation, transfer from the primary enrichment culture and subsequent transfers were made by both conventional procedures and by the CTID.

Four-hundred-and-twenty-five samples were analyzed for Salmonella of which 115 were positive. Using conventional transfer and inoculation techniques, 87 positive results were observed; whereas using the CTID-modified technique, 112 positive results were obtained. The false-negative rate for the conventional transfer method was 21.7%. A 2.6% false-negative rate was observed with the CTID. The difference between conventional and CTID results were statistically significant (p<0.0160).

One-hundred samples were analyzed for Listeria using the FDA method with 32 positive results. Using the CTID 42 positives were detected from the FDA enrichments. One-hundred-and-ninety-eight samples were analyzed using the USDA method with 77 positive results. With the CTID, the same samples yielded 86 positives. No false-negatives resulted through the use of the CTID while a 10.5% to 23.8% rate was observed with the conventional transfer method. The difference between conventional and CTID results were statistically significant (p<0.0086).

These data indicate that the fiber-foam matrix of the CTID provides a micro-environment enhancing the sensitivity of the culture methods for the detection of Salmonella and Listeria.

Key Words: Salmonella, Listeria, enhanced recovery, enhanced isolation, culture-transfer-inoculation device

The observation that microorganisms preferentially attach to surfaces is an area of considerable recent interest in microbial ecology. There is a rich literature on the widespread occurrence of biofilms in various environments (3,14). Biofilms have been detected on various materials (7,8,13), on food processing equipment (4), on medical devices (3), in wastewater treatment (9), among other environmental niches. Solid surface association appears to enhance resistance to environmental stresses and increase survival.

A CTID was developed to exploit the advantages of surface association to enhance recovery of microbes, (e.g., Salmonella spp. and Listeria spp., from food and environmental samples. This idea developed from the synthesis of the concepts of surface association improving "competitiveness" and of microorganisms being more difficult to detect if stressed by environmental pressures. Microorganisms found in foods and ingredients are often stressed as a result of food processing conditions. Although non-selective pre-enrichment broths are used to encourage repair of cell injury, the cells which survive can be more difficult to isolate (2). Therefore, using surface association to a solid substrate might increase the probability of recovery of stressed cells or provide a synergistic recovery effect combining the benefits of the non-selective pre-enrichment and favorable micro-environment.

The FDA method (1) is most frequently used for the detection of Salmonella in foods in the United States. With respect to Listeria, two methods are currently used. The FDA method (1) is used for dairy products, seafood and certain environmental samples; whereas the USDA method (16) is commonly used with meat, egg, poultry products and environmental samples.

The purpose of this study was to develop a modification to each of these standard methods as a means to enhance recovery of the target organisms, namely Salmonella or Listeria. The modification consists of insertion of a fiber-foam material attached to a carrier into the pre-enrichment media, and the device is used as a means to inoculate media in the subsequent procedural steps.

MATERIALS AND METHODS

Description of culture-transfer-inoculation device

Figure 1 depicts the CTID. The rounded tip includes the solid fiber-foam surface matrix (1), which may be selected from the...
A group consisting of natural or synthetic fibers or blends, such as cotton, cotton/rayon blends, sponge, gauze or any suitable natural or synthetic solid surface or matrix to which pathogens can associate. The fiber-foam type tested was obtained from Baxter Scientific Products (A5012-7, Baxter Scientific Products, McGaw Park, IL). The CTID also included a wooden shaft (2) which suspended the CTID in a predetermined position in the container and a cap (3), which was adapted to seal the CTID ports of the container while allowing venting of gases generated during incubation. A loop side arm (4) was attached to the shaft to allow streaking an inoculum on solid agar surfaces.

**Figure 1.** Culture-transfer-inoculation device (CTID) and container lid.

Preparation of sample container for use with CTID

An appropriate liquid microbiological pre-enrichment medium is prepared, introduced into the container, lid attached and the two CTID ports capped. After sterilization, the medium is cooled to ≤35°C. Culture-transfer-inoculation devices were steam sterilized in a test tube for 15 min at 121°C and transferred aseptically to the sample container just prior to initiation of the analysis.

Source of samples

The samples used in this study were all submitted for routine testing. With respect to *Salmonella*, 25 g samples were pre-enriched in 225 ml of appropriate pre-enrichment medium (1). The only exception was spinach powder where 7.0 g samples were used. Sample weights of 25 g were used for *Listeria* testing (1,16) except in the case of the Chinese egg rolls. The entire individual egg roll, 80 to 85 g, was tested as a sample. In the case of the two exceptions, the sample to medium ratio of 1:10 was observed.

Samples tested for *Salmonella* included batter, Caesar salad, cake mixes, casein, cholesterol-free egg, coconut, collard greens, cream cheese, delicatessen chicken salad, delicatessen egg salad, delicatessen ham salad, dried egg, dried egg yolk, egg noodles, egg yolk, environmental samples, fresh whole eggs, ground nuts, half-and-half, low-fat buttermilk, meat and bone meal, non-fat dried milk, pecans, pepper, raw milk, raw ground pork, refrigerated biscuit dough, sour cream, soy flour, soy protein isolate, spinach powder, thousand island dressing, turnip greens, whole egg, whole milk and dried yeast.

Samples tested for *Listeria* included raw chicken, Chinese foods and ingredients, Gaucho beef and miscellaneous samples submitted for routine testing.

**Salmonella methodology**

A single pre-enrichment was used as the starting point for both the standard cultural (1) and CTID procedure (Fig. 2). The container lid was removed, an appropriately prepared sample was placed into the container with the medium, the lid replaced. In the CTID-modified methodology for *Salmonella* both the caps covering the CTID ports were removed and CTIDs were inserted through the ports into the medium containing the sample.
for both the conventional method and the CTID-modified method were performed in parallel as prescribed by the FDA method (1).

Sources for the media used in the *Salmonella* analysis were: TET (11706; BBL, Cockeysville, MD), SC (11606; BBL, Cockeysville, MD), XLD (12266; BBL, Cockeysville, MD), HE (12253; BBL, Cockeysville, MD), BS (4900-0212; Adam Scientific, West Warwick, RI), triple sugar iron agar (11749; BBL, Cockeysville, MD) and lysine iron agar (11363; BBL, Cockeysville, MD).

**Listeria methodology - FDA**

A single pre-enrichment was used as the starting point for both the FDA *Listeria* Enrichment Broth (LEB). One CTID was inserted through one of the ports into the medium containing the sample. The other port remained capped and was not used.

After incubation according to BAM (1), the FDA method proceeded by inoculation of Oxford (OX) Agar and Lithium chloride-Phenylenethanol-Moxalactam (LPM) Agar by streaking with a loopful of LEB. In the CTID-modified procedure the OX and LPM agars were inoculated by touching the fiber-foam matrix to the agar plates and streaking with the loop. All subsequent steps for both the conventional method and the CTID-modified method were performed in parallel as proscribed by the FDA method (1).

Sources for the media used in the FDA *Listeria* analyses were: LEB (12335; BBL, Cockeysville, MD), LPM (12337; BBL, Cockeysville, MD) and OX (CM856 and SR 140E; Oxoid, Unipath, Inc., Basingstoke, UK).

**Listeria methodology - USDA**

A single pre-enrichment was used as the starting point for both the USDA *Listeria* (16) and CTID-modified procedure (Fig. 4). A sample was placed into University of Vermont Modified *Listeria* primary enrichment broth (UVM). One CTID was inserted through one of the ports into the medium containing the sample. The other port remained capped and was not used. The UVM broth was incubated at 30°C for 24 h.

For the USDA procedure (16), 0.1 ml of the UVM primary enrichment broth was pipetted into 10 ml of Fraser and incubated at 35°C for 48 h. Following incubation, modified Oxford (mOX) Agar was inoculated by streaking with a loopful of Fraser broth.

In the CTID-modified procedure, the CTID was withdrawn from the same UVM broth and inserted into a 10 ml tube of Fraser broth. The selective-differential agar, mOX, was inoculated by touching the fiber-foam matrix to the agar plate and streaking with the loop.

All subsequent steps for both the conventional USDA method (16) and the CTID-modified method were performed in parallel as prescribed by the USDA.

Media sources for the USDA *Listeria* analyses were: UVM (12350; BBL, Cockeysville, MD), Fraser broth (UVM-12350; BBL, Cockeysville, MD; LiCl L-0505, Sigma Chemical Co.; St. Louis, MO) and mOX (12398; BBL, Cockeysville, MD).

**Statistical analysis**

The data were analyzed using linear regression (p<0.05, \(r^2>0.95\)) and t-test analysis (p<0.05) (6).

**RESULTS**

In the *Salmonella* portion of the study, a total of 425 food and environmental samples was analyzed (Table 1). Using conventional transfer and inoculation techniques (1), 87 positive results were observed; whereas using the CTID-modified technique, 112 positive results were obtained. There were three false-negative results by the CTID-modified method; 2.6% of all positive samples. The false-negative rate for the conventional transfer methods was 21.7%. The difference between conventional and CTID results were statistically significant using linear...
Table I. Performance comparison of BAM methodology to CTID modification of BAM methodology for detecting Salmonella.

<table>
<thead>
<tr>
<th>Product type (No. samples)</th>
<th>CTID+/BAM+</th>
<th>CTID+/BAM-</th>
<th>CTID-/BAM+</th>
<th>CTID-/BAM-</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENVIRONMENTAL (65)</td>
<td>6</td>
<td>5</td>
<td>0</td>
<td>54</td>
</tr>
<tr>
<td>RAW MEAT (30)</td>
<td>22</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>RAW VEGETABLES (9)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>DAIRY PRODUCTS (32)</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>28</td>
</tr>
<tr>
<td>EGG PRODUCTS (19)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>PASTA / DOUGH PRODUCTS (8)</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>DRIED, PROCESSED INGREDIENTS (104)</td>
<td>13</td>
<td>5</td>
<td>0</td>
<td>86</td>
</tr>
<tr>
<td>SALAD DRESSINGS / SAUCES (12)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>CREME SALADS (12)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>NUTS (4)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>SPICES (36)</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>MISCELLANEOUS (94)</td>
<td>35</td>
<td>7</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>Totals (425 samples total)</td>
<td>87</td>
<td>25</td>
<td>3</td>
<td>310</td>
</tr>
<tr>
<td>False-negative results</td>
<td>—</td>
<td>21.7%</td>
<td>2.6%</td>
<td>—</td>
</tr>
</tbody>
</table>

regression (p<0.05, r^2>0.95) and t-test analysis (p<0.0160) (6). Performance within food groups was always equivalent or better than the conventional method.

A total of 198 samples of various foods were analyzed by USDA cultural (16) and USDA with the CTID for *Listeria monocytogenes*. The USDA method yielded 77 positive results. Using the CTID modification, the same samples yielded 86 positives. One-hundred samples were analyzed for *Listeria* using the FDA method (1) with 32 positive results. Using the CTID, 42 positives were detected. There were no false-negative results for any analyses using the CTID. The FDA method false-negative rate was 23.8% and the USDA false-negative rate was 10.5% based on total number of positive samples. The difference between conventional USDA and CTID results were statistically significant using linear regression (p<0.05, r^2>0.95) and t-test analysis (p<0.0086)(6). The number of samples with respect to the FDA method were insufficient to analyze statistically. Performance within food groups was always equivalent or better than the conventional cultural methods.

At the same time that this work was being performed, two other matrices were tested: cotton fiber (A5008-4, Baxter Scientific Products, McGaw Park, IL) and synthetic sponge (A5010-1, Baxter Scientific Products, McGaw Park, IL). Both gave similar and markedly improved recovery of *Salmonella* (19 to 40%).

**DISCUSSION**

The primary advantage of the CTID was a statistically significant increase in method sensitivity when compared with the standard methods for isolation of *Salmonella* and *Listeria* from food and environmental samples.

A single sample in each case was enriched and analyzed in parallel using standard methods and the CTID. This difference in performance cannot be attributed to the volume of medium transferred to the selective broth(s). Use of the CTID involved transfer of only 0.2 ml (determined by weighing the CTID before and after absorption of liquid enrichment) versus 1 ml in standard *Salmonella* methods. Prior research has indicated that 1 ml volumes are adequate for transfer (5,10,17,18). Accordingly, the difference in results between the two transfer and inoculation procedures must be ascribed to the difference in the environment provided by the matrix as opposed to the environment in the surrounding medium.

Among the foods analyzed there was a large number of samples of processed foods in which one would expect stressed cells. Furthermore, there was a large number of other samples including raw foods and environmental samples where a large competing microflora would be expected. Since *Salmonella* and *Listeria* usually are present in low numbers in environments often containing higher levels of competing microorganisms, detection is difficult. Moreover, the inherent variety in physicochemical characteristics and processing methods of foods, ingredients, and feeds can affect the incidence of isolation. With respect to both processed foods and raw foods, the CTID enhanced recovery of the target organisms.

Results obtained here suggest bacteria are preferentially associating with the CTID. Whether this is
preferential attachment of Salmonella or Listeria versus background microflora is not fully known since samples were introduced into both non-selective and selective media used to isolate Salmonella and Listeria. If there were preferential surface association with the matrix, this at very least may mean enhanced growth of target microorganisms.

In addition to enhanced recovery, use of the CTID provides additional advantages including: 1) improved worker safety through reduced biohazard risk to workers from pipetting; 2) convenience, ease and speed of use from the design of the CTID; 3) potential for reducing laboratory error and time-saving by improving sample coding as the sample code is physically attached to the transfer device; and 4) potential for reduced costs due to increases in efficiency and accuracy.

Areas of prior research on bacterial attachment have focused predominantly on negative aspects of surface association and microenvironment (3,11,12,15). The focus of research on fouling, sterilization, cleaning and sanitizing has been to remove and/or inactivate microorganisms which may affect food quality and human health. The CTID exploits these same microbiological characteristics to enhance recovery and isolation of Salmonella and Listeria. The preference to associate with a surface appears to be widely found in nature. A similar pattern of enhanced recovery has been observed in additional research with Escherichia coli O157:H7 and Campylobacter (data not shown). The CTID was developed to exploit this advantage of surface association to enhance recovery of microorganisms from food and environmental samples while improving laboratory efficiency.

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REFERENCES


