Rapid Two Day Isolation of Salmonella Using Single Selective Enrichment and Brilliance™ Salmonella Agar

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Abstract:
Salmonella is a gram-negative, rod-shaped, motile bacterium with a widespread occurrence in animals, especially in poultry and swine. The most common food sources contaminated with Salmonella are poultry, beef, pork, eggs, and fresh produce (1). Detection is critical as Salmonella infections cause an estimated 1.3 million human illnesses and 400 deaths annually in the United States (2) It has been reported an infectious dose of as low as 17 cells may cause infection(3). The data presented from this study describes the evaluation of Oxoid ONE Broth-Salmonella and Brilliance™ Salmonella chromogenic media as a detection method for Salmonella in ground beef, ground chicken, lettuce, shrimp, and shell eggs. The selected foods were inoculated with Salmonella serovars at levels ranging from low (1 colony forming unit (CFU)/25g) to high (1.1 CFU/g) and analyzed for growth.

The method was also evaluated for inclusivity using multiple Salmonella serovars (n=102) from various sources. Exclusivity testing was performed using closely related bacterial species (n=30) to determine the specificity of the described method. Overall, when compared to the standard USDA and FDA reference methods there was no significant difference in sensitivity between Brilliance™ Salmonella and the standard reference method. However, when Oxoid ONE Broth-Salmonella was used as the enrichment step prior to plating on Brilliance™ Salmonella, the time to detection was reduced from 3-4 days to 2 days. For the inclusivity study, 98 Salmonella serovars grew on Brilliance™ Salmonella. For the exclusivity study, 30 non-Salmonella spp. were tested to determine the specificity of the described method and only one, Enterobacter sakazukii, demonstrated typical growth on the chromogenic medium, but only when not enriched in ONE Broth-Salmonella prior to plating.

Introduction:
Brilliance™ Salmonella is a novel chromogenic medium for the detection and identification of Salmonella spp. in food. When used in conjunction with Oxoid ONE Broth-Salmonella it reduces Salmonella detection time from 3-4 days to 2 days. This study evaluated and compared the performance of the Salmonella Rapid Culture method to the reference U.S. Department of Agriculture-Food Safety Inspection Service (USDA/FSSIS) and U.S. Food and Drug Administration's Bacteriological Analytical Manual (FDA/RAM) methods for the detection of Salmonella spp. in raw ground beef, ground chicken, lettuce, shrimp, and shell eggs. Results demonstrate the Salmonella Rapid Culture method to be superior to the reference methods in incubation time and ease of identification.

Materials and Methods:
Method Comparison
Ground beef, ground chicken, lettuce, shrimp, and shell eggs were inoculated with approximately 1 CFU of each strain and allowed to equilibrate per AOAC instructions. Side by side samples were set up and run using Oxoid ONE Broth-Salmonella/Brilliance™ Salmonella and the appropriate FDA/USDA reference for each strain. The method was also evaluated for inclusivity using multiple Salmonella serovars (n=102) from various sources. Exclusivity testing was performed using closely related bacterial species (n=30) to determine the specificity of the described method. Overall, when compared to the standard USDA and FDA reference methods there was no significant difference in sensitivity between Brilliance™ Salmonella and the standard reference method. However, when Oxoid ONE Broth-Salmonella was used as the enrichment step prior to plating on Brilliance™ Salmonella, the time to detection was reduced from 3-4 days to 2 days. For the inclusivity study, 98 Salmonella serovars grew on Brilliance™ Salmonella. For the exclusivity study, 30 non-Salmonella spp. were tested to determine the specificity of the described method and only one, Enterobacter sakazukii, demonstrated typical growth on the chromogenic medium, but only when not enriched in ONE Broth-Salmonella prior to plating.

Table 1: Method Comparison

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Incubation time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxoid ONE Broth</td>
<td>98%</td>
<td>99%</td>
<td>3-4</td>
</tr>
<tr>
<td>Brilliance™</td>
<td>98%</td>
<td>99%</td>
<td>2</td>
</tr>
</tbody>
</table>

Conclusions:
This method comparison evaluation of the Salmonella Rapid Culture Method (Oxoid ONE Broth-Salmonella/Brilliance™ Salmonella) clearly demonstrated that this method is equivalent to the USDA/FSSIS reference method for the detection and presumptive identification of Salmonella spp. at spiked levels ranging from in ground beef, ground chicken, lettuce, shrimp, and shell eggs. In addition, the Salmonella Rapid Culture method was found to be superior to the reference method in incubation time and ease of identifying typical colonies. The inclusivity data demonstrated that the Salmonella Rapid Culture method (Oxoid ONE Broth-Salmonella/Brilliance™ Salmonella) detected essentially all species and serovars of Salmonella tested. The exclusivity data confirmed that the Salmonella Rapid Culture method was able to discriminate Salmonella spp. from non-Salmonella microorganisms. Lot-to-lot comparability and stability data along with the ruggedness data verified that the Salmonella Rapid Culture method was robust and can provide reproducible results over a range of culture conditions (time and temperature).

References: