A Research Note

Evaluation of a Colorimetric DNA Hybridization Test for Detection of Salmonellae in Meat and Poultry Products

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ABSTRACT

A commercially available colorimetric DNA hybridization test was compared with the USDA/FSIS conventional culture method for detection of salmonellae in naturally contaminated meat and poultry products and inoculated ground beef samples. All samples which were Salmonella-positive by the culture method were also positive by the DNA probe assay. There were no false-negative or false-positive results by the colorimetric DNA hybridization test, which was slightly more sensitive than the culture method.

Rapid detection of Salmonella in foods has been a subject of research during the past 30 years. Accelerated Salmonella detection procedures would allow the food industry to reduce warehousing costs and permit increased testing of both food ingredients and final products, especially those with a relatively short shelf life. Salmonellae in foods often are injured sublethally and are low in number in comparison with the total bacterial flora (3). Conventional culture methods of analysis involve the use of one or more selective broths, usually preceded by nonselective enrichment. Selective enrichment broths must be streaked onto plates of one or more selective/differential agar media, and colonies resembling salmonellae must be confirmed by various biochemical and serological tests. Conventional culture methods require a minimum of 4 d for identification of negative samples, and 6 to 7 d for identification of Salmonella-positive samples (5,14).

Recent developments in the rapid detection of salmonellae in foods include the use of DNA hybridization tests (7,8). Data published by several laboratories have shown that the GENE-TRAK DNA hybridization test for detection of Salmonella (GENE-TRAK Systems, Framingham, MA) is a reliable method for detection of salmonellae in foods (6,9,10,12,13). The major disadvantage of the test is that laboratories must obtain a license from the United States Nuclear Regulatory Commission to use the radioisotope $^{32}$P in the hybridization test. GENE-TRAK Systems has subsequently developed a second-generation DNA hybridization test called the Colorimetric GENE-TRAK Salmonella Assay. The assay employs Salmonella-specific DNA probes and a colorimetric (instead of radioisotopic) detection system for the detection of Salmonella species in food samples following broth culture enrichment. Detection is based on the hybridization of a polydeoxyadenylic (poly-dA) tailed "capture" probe and a fluorescein labeled detector probe to the Salmonella ribosomal RNA (rRNA). The two probes hybridize to adjacent regions on the same target rRNA molecule. This target complex is captured onto a polystyrene "dipstick" coated with polydeoxythymidylic acid (poly-dT) and detected colorimetrically using an antifluorescein antibody conjugate of horseradish peroxidase (2,3,15).

The present study was undertaken to evaluate the Colorimetric GENE-TRAK Salmonella Assay and to compare results obtained by culture procedures used in our laboratory for detection of salmonellae in meat and poultry products with the DNA probe results.

MATERIALS AND METHODS

Bacterial strains

Forty-three Salmonella serotypes, representing 36 different O groups (see Table 1), from our culture collection were grown in trypticase soy broth (TSB; BBL, Cockeysville, MD) at 35°C overnight. Ten non-Salmonella cultures from our collection were also grown as described above: Aeromonas hydrophila, Escherichia coli (H,S positive), E. coli (H,S negative), Citrobacter freundii, Enterobacter cloacae, E. aerogenes, Klebsiella pneumoniae, Hafnia alvei, Proteus vulgaris, and Serratia marcescens. All 53 broth cultures were then tested according to the instructions provided with the GENE-TRAK Salmonella Assay (1).

Naturally contaminated meat/poultry samples

Protein concentrates, beef jerky, and 17 of the ground beef samples were obtained from federally inspected meat processing establishments. Chicken parts, chicken livers, pork sausage, and two ground beef samples were purchased at local retail stores. The dry protein concentrates were held at room temperature for approximately 3 months until time of analysis; all other samples from federally inspected establishments were held frozen. Retail samples were held in the refrigerator and analyzed the day of purchase.
Inoculated ground beef

The ground beef used for preparation of inoculated samples was obtained from a federally inspected meat processing establishment. Prior to inoculation, the meat was Salmonella-negative with a total aerobic plate count (35°C) of approximately 10^3 per gram. Twenty-five gram samples of the meat were formed into balls and inoculated in the center with low (0.1-1/gm), medium (1-10/gm), or high (10-100/gm) levels of one of six Salmonella serotypes: S. typhimurium, S. montevideo, S. newport, S. panama, S. anatum, and S. cerro. The inoculated samples were held frozen a minimum of 1 week prior to analysis.

Cultural method

All samples were preenriched in lactose broth (BBL) containing 0.6% (wt/vol) Tergitol 7 (LAC). A 75-g sample of protein concentrate was sprinkled onto the surface of 350 ml of LAC in a sterile quart Mason jar. The jar was capped and the sample allowed to soak undisturbed for 30 min. The sample was then mixed well and adjusted to pH 7.0. The cap was replaced loosely and the culture was incubated for 20-24 h at 35°C. All other products were also preenriched in LAC. A 25-g sample was added to 225 ml LAC and blended in an Osterizer blender for 2 min. The pH then was adjusted to 7.0 and the sample incubated as described above. After incubation, 0.5-ml portions of all LAC cultures were removed and selectively enriched in 10-ml volumes of tetrathionate broth (TT; BBL; Hainza and Damon, 11). The TT broth cultures were incubated for 18-24 h at 43°C, streaked onto xylose lysine deoxycholate (XLD; Difco, Detroit, MI) and brilliant green sulfonates (BGS; Difco) agar plates, and further analyzed according to procedures described in the USDA/FSIS “Microbiology Laboratory Guidebook” (14). Salmonella isolates were sent to the USDA National Veterinary Services Laboratories, Ames, IA, for serotyping.

Colorimetric DNA probe assay

The same LAC preenrichments described above were used for the colorimetric DNA probe assay. However, a separate set of selective enrichments was inoculated from the LAC for the probe assay. One-ml portions of the overnight LAC cultures were transferred to 10 ml of selenite cystine broth (SC; Difco) and to 10 ml of TT broth (11). The selective enrichment broths of all samples, except the protein concentrates, were incubated for 16-18 h at 35°C. Following incubation, each selective enrichment broth was mixed using a vortex mixer. One ml was transferred from each selective enrichment to a separate 10-ml tube of gram-negative broth (GN; Difco). The GN cultures were incubated at 35°C for 6 h prior to performance of the colorimetric DNA probe assay. The selective enrichments of the protein concentrates were incubated at 35°C for 6 h prior to transfer to GN. The GN cultures were then incubated for 12-18 h at 35°C prior to assay. In addition, the protein concentrate selective enrichments were returned to 35°C for an additional 18 ± 2 h.

GN cultures were analyzed for Salmonella according to the manufacturer’s instructions included in the Colorimetric GENE-TRAK Salmonella Assay kit (1). This method requires about 3 h to complete and was performed as follows. Two hundred fifty microliters were removed from each of the two GN broth cultures per sample and pooled in a 12 x 75-mm glass test tube. One-half ml of both a positive and negative control solution was added to the appropriate tubes. A lysis solution was added to each tube; after a 5 min incubation at room temperature, a neutralization solution was added to each tube. Next the tubes were covered with aluminum foil and incubated in a 65°C water bath for 15 min. Then a Salmonella probe solution was added to each tube followed by incubation for an additional 15 min at 65°C. The probe solution contained a poly-dA tailed “capture” probe and a fluorescein labeled detector probe to Salmonella rRNA. The next step was the addition of a polystyrene dipstick coated with poly-dT capture reagent to each tube. The tubes with the dipsticks again were incubated in the waterbath at 65°C for 1 h. The dipsticks were then removed from the tubes and washed sequentially with gentle shaking for 1 min each, first in a 65°C wash solution basin, then in a room temperature wash solution basin. The dipsticks were blotted on absorbent paper and were then incubated for 20 min at room temperature in a second set of tubes containing a solution of antifluorescein horseradish peroxidase conjugate. Following another two wash steps, the dipsticks were incubated for 20 min at room temperature in a third set of tubes containing substrate-chromogen solution. The dipsticks were removed and discarded, and the color reaction in the chromogen solution was terminated by the addition of a stop solution to each tube. Color was measured in a GENE-TRAK Photometer at a wavelength of 450 nm. A tube with an absorbance value less than or equal to 0.10 indicated the presence of Salmonella in the test sample. An assay producing an absorbance value greater than 0.10 indicated the absence of Salmonella in the test sample.

Samples found positive by the DNA probe assay were confirmed culturally by streaking a loopful of the GN cultures or the selective enrichments to XLD and BGS agars and continuing with biochemical and serological identification of presumptive Salmonella isolates using standard procedures (14).

RESULTS AND DISCUSSION

All Salmonella serotypes (Table 1) used in our study were detected by the GENE-TRAK Salmonella Assay. Pure broth cultures of 43 different Salmonella serotypes, representing 36 different O Groups were all strongly positive when tested with the colorimetric DNA probe assay. Additional serotypes, also listed in Table 1, were isolated from naturally contaminated meat and poultry products and from inoculated ground beef samples which were Salmonella-positive by the colorimetric DNA probe assay. Pure broth cultures of ten non-Salmonella species gave negative results by the DNA probe assay.

Table 2 shows a summary of the results obtained by the USDA/FSIS culture method and the colorimetric DNA probe assay for detection of Salmonella in 118 samples of meat and poultry products. All 63 samples which were Salmonella-positive by the culture method were also positive by the DNA probe assay. A total of 51 samples was Salmonella-negative by both methods. There were no false-negative or false-positive results by the DNA probe assay. Four samples which were negative by the USDA/FSIS culture method were positive by the Colorimetric GENE-TRAK Salmonella Assay. All four samples were confirmed positive by isolation of Salmonella from the GN broth cultures. Therefore, the colorimetric DNA probe assay was slightly more sensitive than the culture method. Proteus overgrowth on the XLD and BGS agars contributed to our failure to isolate Salmonella from these four samples by the USDA/FSIS culture method.

Based upon these results, the GENE-TRAK Salmonella Assay appeared to be an effective screening procedure for rapid detection of salmonellae in meat and poultry products. The major advantage of the colorimetric DNA probe assay is that large numbers of samples can be screened fairly rapidly for salmonellae. DNA probe test results can be obtained late on day 3 or by noon on day 4, a savings of 1-3 d over the conventional cultural methods. However,
TABLE 1. Salmonella serotypes which tested positive with the Colorimetric GENE-TRAK Salmonella Assay in this study.

<table>
<thead>
<tr>
<th>Serotypes tested in pure culture</th>
<th>Serotypes isolated from meat/poultry samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Naturally contaminated</td>
</tr>
<tr>
<td>S. paratyphi A (A)</td>
<td>S. agona (B)</td>
</tr>
<tr>
<td>S. typhimurium (B)</td>
<td>S. typhimurium (B)</td>
</tr>
<tr>
<td>S. jerkio (B)</td>
<td>S. heidelberg (B)</td>
</tr>
<tr>
<td>S. montevideo (C)</td>
<td>S. reading (B)</td>
</tr>
<tr>
<td>S. denver (C)</td>
<td>S. mbondaka (C)</td>
</tr>
<tr>
<td>S. newport (C)</td>
<td>S. thompson (C)</td>
</tr>
<tr>
<td>S. glostrup (C)</td>
<td>S. johannesburg (R)</td>
</tr>
<tr>
<td>S. durban (D)</td>
<td>S. lethe (S)</td>
</tr>
<tr>
<td>S. panama (D)</td>
<td>S. kampala (T)</td>
</tr>
<tr>
<td>S. dublin (D)</td>
<td>S. berkeley (U)</td>
</tr>
<tr>
<td>S. anatum (E)</td>
<td>S. niarenbe (V)</td>
</tr>
<tr>
<td>S. newington (E)</td>
<td>S. deversoir (W)</td>
</tr>
<tr>
<td>S. thomasville (E)</td>
<td>S. kaolack (X)</td>
</tr>
<tr>
<td>S. schoenberg (E)</td>
<td>S. dahlem (Y)</td>
</tr>
<tr>
<td>S. senfenberg (E)</td>
<td>S. greenside (Z)</td>
</tr>
<tr>
<td>S. aberdeen (F)</td>
<td>S. treforest (51)</td>
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<tr>
<td>S. worthington (G)</td>
<td>S. utrecht (52)</td>
</tr>
<tr>
<td>S. florida (H)</td>
<td>S. ucle (54)</td>
</tr>
<tr>
<td>S. vancouker (I)</td>
<td>S. tranora (55)</td>
</tr>
<tr>
<td>S. morotai (J)</td>
<td>S. manombo (57)</td>
</tr>
<tr>
<td>S. memphis (K)</td>
<td>S. betioky (59)</td>
</tr>
<tr>
<td>S. ghana (L)</td>
<td></td>
</tr>
</tbody>
</table>

1LETTERS/NUMBERS IN PARENTHESES INDICATE SALMONELLA O GROUP.

TABLE 2. Comparison of the USDA culture method with the Colorimetric GENE-TRAK Salmonella Assay for detection of Salmonella in meat and poultry products.

<table>
<thead>
<tr>
<th>Test Product</th>
<th>No. samples analyzed</th>
<th>Culture + Probe +</th>
<th>Culture - Probe -</th>
<th>Culture + Probe -</th>
<th>Culture - Probe -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein concentrates</td>
<td>20</td>
<td>12</td>
<td>7</td>
<td>0</td>
<td>1*</td>
</tr>
<tr>
<td>Ground beef</td>
<td>19</td>
<td>1</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Inoculated ground beef</td>
<td>38</td>
<td>36</td>
<td>2*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chicken muscle/skin</td>
<td>16</td>
<td>6</td>
<td>8</td>
<td>0</td>
<td>2*</td>
</tr>
<tr>
<td>Chicken livers</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1*</td>
</tr>
<tr>
<td>Pork sausage</td>
<td>20</td>
<td>5</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Beef jerky</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Totals</td>
<td>118</td>
<td>63</td>
<td>51</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

2Samples inoculated with S. anatum (0.7 or 3.2/gm).
3USDA culture method negative; GENE-TRAK Salmonella Assay confirmed positive.
4Proteus overgrowth is a possible reason for failure to isolate Salmonella from these samples by the USDA culture method.

all DNA probe positive samples should be confirmed culturally. Application of this quicker method of Salmonella detection in laboratories monitoring food production would permit more rapid release of Salmonella-negative finished product and might encourage increased monitoring of critical control points.

An Association of Official Analytical Chemists (AOAC) collaborative study involving 11 laboratories was completed, and the Colorimetric GENE-TRAK Salmonella Assay was recently granted Official First Action status by the AOAC as an analytical method for detection of Salmonella in food products (4).

REFERENCES

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Siragusa and Nelsen, con't. from p. 123


