Comparison of culture, polymerase chain reaction (PCR), TaqMan Salmonella, and Transia Card Salmonella assays for detection of Salmonella spp. in naturally-contaminated ground chicken, ground turkey, and ground beef

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Abstract

Four types of assays were evaluated for the detection of Salmonella spp. in retail ground chicken (86 packages), ground turkey (104 packages), and ground beef (54 packages). Two 25 g samples from each package were separately subjected to pre-enrichment in buffered peptone water for 20 h at 37 °C followed by enrichment in Rappaport Vassiliadis (RV) broth for 20 h at 42 °C. The RV enrichments were plated onto Rambach agar, Rainbow Agar Salmonella, and XLT4 agar, and were also tested by a PCR assay targeting the Salmonella invA gene, as well as by the TaqMan® Salmonella PCR assay. Additionally, the RV enrichments were tested using the Transia Card Salmonella® immunoassay. Results showed that 16.8, 24.0, 28.8, and 26.4% of turkey samples were positive for Salmonella spp. by culture, PCR, TaqMan PCR, and Transia Card Salmonella assays, respectively. Eighteen, 28.5, 35.5, and 34.9% of chicken samples were positive by culture, PCR, TaqMan PCR, and Transia Card Salmonella assays, respectively, and 6.5, 6.5, 6.5, and 18.5% of ground beef samples were positive by the four assays, respectively. Analysis of the data using the kappa statistic showed that there was substantial to excellent agreement between the PCR and TaqMan PCR assays and between the PCR and culture assays (kappa coefficients ranging from 0.67 to 0.87), while there was poor to fair agreement between the results of the Transia Card Salmonella assay and the other methods (kappa coefficients ranging from 0.28 to 0.32). Overall, results showed that the PCR-based assays were more sensitive than the culture method, and the culture and PCR-based assays were more specific than the immunoassay for detection of Salmonella in ground chicken, turkey, and beef due to the occurrence of false positive results using the immunoassay.

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1. Introduction

Nontyphoidal Salmonella spp. cause an estimated 1.34 million cases of food-borne illness and 553 deaths per year in the US [1]. Salmonella spp. are found in the gastrointestinal tracts of a wide range of animals, thus contact with animals and foods of animal origin are frequent causes of salmonellosis [2,3]. Furthermore, seafood and produce are also documented vehicles of transmission of Salmonella to humans [3–6]. Due to the relatively high prevalence of Salmonella spp. in meat, poultry, and other foods [7], as well as the high incidence of disease caused by these organisms, rapid, sensitive, and reliable methods for detection of Salmonella in foods are needed to reduce the occurrence of salmonellosis. To address the problem of Salmonella contamination of raw meat and poultry products, the USDA Food Safety and Inspection Service (FSIS) issued the ‘Pathogen Reduction; Hazard Analysis and Critical Control Point (PR/HACCP) Systems; Final Rule’ on July 25, 1996 setting Salmonella performance standards that the food industry must meet [8]. Testing results of 735, 3,192, and 50,515 randomly-collected ground chicken, ground turkey, and ground beef samples,
respectively, collected by the USDA FSIS from January 26, 1998 to December 31, 2000 from large, small, and very small establishments showed decreased *Salmonella* prevalences compared to prevalences as determined from the nationwide baseline studies conducted before the PR/HACCP was implemented [9].

It is essential that methods for detection of *Salmonella* and other pathogens in foods have the ability to detect low levels of pathogens that are healthy, as well as those that are stressed/injured due to conditions in the food and/or during food processing. Conventional culture-based methods that rely on enrichment of the food sample and plating onto selective agar media may not be as sensitive as immunologic- or genetic-based ‘rapid methods’ [10]. Immunologic methods rely on binding of an antigen on the bacterium to a specific monoclonal or polyclonal antibody, and several immunoassay-based systems and devices are commercially available for detection of a variety of food-borne pathogens including *Salmonella* [11,12]. The DNA of the pathogen is the target for nucleic acid-based systems such as probe hybridization or the polymerase chain reaction (PCR). Methods based on the PCR such as the fluorogenic 5' nuclease assay offer the advantages of high specificity and sensitivity, and a number of PCR-based kits are commercially available for testing of food or other types of samples for the presence of *Salmonella* or other pathogens [13–15].

The objective of this study was to compare a traditional culture method, PCR-based methods, and a commercially-available immunochromatographic assay for detection of *Salmonella* spp. in retail ground chicken, ground turkey, and ground beef.

2. Materials and methods

2.1. Enrichment of ground chicken, turkey, and beef samples

Packages of ground chicken (*n* = 86), ground turkey (*n* = 104), and ground beef (*n* = 54) were purchased from local grocery stores from July 1999 to October 2001. Samples were processed on the day of purchase or after 1 day of storage at 4 °C. The procedure used for enrichment and detection of *Salmonella* in the ground meat products is shown in Fig. 1. Two 25 g samples were removed aseptically from each package of meat and added to 225 ml of sterile buffered peptone water (BPW; GENE-TRAK Systems, Hopkinton, MA) in 500 ml volume flasks and were incubated for 22 h at 37 °C at 150 rpm. The secondary enrichment consisted of adding 500 µl of the BPW enrichment to 9.5 ml of Rappaport Vassiliadis (RV; GENE-TRAK Systems) broth, and the tubes were incubated for 22 h at 42 °C at 150 rpm.

2.2. Detection and isolation of *Salmonella* spp. on selective media

The enrichments were diluted in sterile 0.1% peptone (Difco, Detroit, MI) and plated using a Spiral Plater (Model

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Fig. 1. Procedure used for detection of *Salmonella* in ground chicken, ground turkey, and ground beef.

Two samples (25 g each) of ground chicken, turkey, and beef from each package

1. **Added 225 ml of BPW**
2. **37°C for 22 h, 150 rpm**
3. **Transferred 500 µl of BPW enrichment to 9.5 ml of RV broth**
4. **42°C for 20 h, 150 rpm**
5. **Plated onto Rambach agar, Rainbow Agar *Salmonella*, and XLT4 agar**
6. **1 ml - heated at 100°C for 20 min**
7. **1 ml - DNA extraction using PrepMan reagent**
8. **Confirm isolates by the PCR (invA gene)**
9. **Transia Card *Salmonella***
10. **1) TaqMan *Salmonella* PCR**
11. **2) PCR (invA gene)**
D. Spiral Biotech, Bethesda, MD) onto Rambach® agar
(EM Science, Gibbstown, NJ), Rainbow® Agar Salmonella
(Biolog, Hayward, CA), and XLT4 agar (XLT4 Agar Base
containing the XLT4 agar supplement, 7-ethyl-2-methyl-4-
undecanol hydrogen sulfate, sodium salt) (Difco). The
plates were incubated at 37 °C for 20 h. Black colonies on
Rainbow Agar Salmonella and XLT4 agar, and red colonies
on Rambach agar were picked and mixed in 50 μl of sterile
water. The bacteria were lysed at 99 °C for 10 min in a
GeneAmp PCR System 9600 thermal cycler (PE Applied
Biosystems, Foster City, CA), then confirmed as Salmonella
by the PCR by amplifying a portion of the invA gene as
described below. Selected isolates were sent to the
Salmonella Reference Center, University of Pennsylvania,
New Bolton Center, Kennett Square, PA for serotyping.

2.3. DNA extraction and detection of Salmonella by the PCR
and TaqMan® Salmonella assays

One milliliter of the RV enrichment was centrifuged
16,000 × g for 2 min, and DNA extraction was performed
on the cell pellet using the PrepMan reagent (PE Applied
Biosystems) following the manufacturer’s instructions. The
PCR mixture for amplification of the Salmonella invA gene
consisted of 1 μM of primers INVAF and INVVAR,
5'-CGGTGGTTTTAAGCGTACTCTT-3' and 5'-CGAAT-
TATGCTCCACAAGGTTA-3', respectively, [16] 20 mM
Tris–HCl, pH 8.4, 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM
(each) of the 4 deoxynucleotide triphosphates, and 1.25 U
Taq DNA polymerase (PCR Reagent System; Invitrogen,
Carlsbad, CA). Forty-five microliters of the PCR mixture
were added to 5 μl of template DNA, and the PCR was
performed on a GeneAmp thermal cycler. The thermal
cycling protocol consisted of 94 °C for 2 min, followed by
35 cycles of 94 °C for 20 s, 57 °C for 1 min, and 72 °C for
1 min, with a final extension of 72 °C for 10 min. The PCR
yielded a 796 bp product, visualized following electrophore-
sis on 1.5% agarose gels that were stained with ethidium
bromide. Additionally, 5 μl of template DNA were used for
the PCR employing the TaqMan Salmonella PCR Ampli-
fication/Detection Kit (PE Applied Biosystems) following
the procedure recommended by the manufacturer.

2.4. Detection of Salmonella by the Transia Card
Salmonella® assay

One milliliter of the RV enrichment was heated at 100 °C
for 20 min, and 4 drops were placed into the sample well of
the Transia Card Salmonella test device (GENE-TRAK
Systems, Hopkinton, MA). After incubation at room
temperature for 10 min, the results were recorded as positive
if two reddish-purple lines appeared, one in the test window
and one in the control window, and as negative if a line
appeared only in the control window.

2.5. Statistical analyses

The results from the four assays were analyzed to test the
agreement among methods. The four methods were treated
as ‘raters’, and the simple kappa statistic was calculated to
test how well the methods agreed in classifying the samples
as positive or negative [17]. In general, values of kappa
larger than 0.75 are indicative of excellent agreement
beyond chance, whereas values of kappa between 0.40 and
0.75 are indicative of fair to good agreement beyond chance.

3. Results and discussion

The enrichment protocol used for detection of Salmonella
in retail ground chicken, turkey, and beef samples
consisted of a non-selective enrichment in BPW to allow
recovery of injured/stressed Salmonella [18], followed by a
secondary selective enrichment in RV broth. Studies have
shown that overall recovery of Salmonella from foods and
animal feed using RV medium for enrichment was
comparable to or higher than recovery using tetrathionate
or selenite cysteine broths [19–21]. Interestingly, in one
study, RV medium prepared from individual ingredients
yielded higher numbers of Salmonella-positive samples
compared to commercially-prepared RV medium [19].
Furthermore, enrichment in RV at 42 °C was superior to
37 °C when fecal or food samples were pre-enriched in
BPW or other media [19,21]. The increased recovery of
Salmonella in RV at 42 °C compared to 37 °C is due to an
increased inhibition of background microflora at the higher
temperature [19–21].

Dilutions of the RV enrichments were plated onto
Rainbow Agar Salmonella, XLT4 agar, and Rambach
agar. Rambagh agar is based on the metabolism of propylene
glycol by Salmonella, and typical colonies appear bright
red [22]. On Rainbow Agar Salmonella and XLT4 agar,
Salmonella appear as black colonies due to the production
of H₂S. The XLT4 medium was the most suitable for detection/
isolation of Salmonella by the culture method compared to
Rainbow Agar Salmonella or Rambach agar. After incu-
bation for 20 h at 37 °C, the colonies on XLT4 were smaller
than those on Rambach agar or Rainbow Agar Salmonella,
thus there was less crowding from background colonies,
rendering isolation of typical black colonies easier. The PCR
results showed that only Salmonella colonies were black on
XLT4 agar, whereas isolation of Salmonella from Rainbow
Agar Salmonella was more problematic, since bacteria other
than Salmonella also formed black colonies. In addition,
non-Salmonella bacteria also formed dark pink to red
colonies on Rambach agar. Thus XLT4 agar was sufficiently
selective, and made it possible to easily differentiate
Salmonella from other bacteria. This is in agreement with
results of a study by Miller et al. [23] showing that XLT4
inhibited Proteus, Pseudomonas, Providencia, and many
other non-salmonellae, in addition to providing good
differentiation between Salmonella spp. and Citrobacter. In all cases, Salmonella was isolated from XLT4 agar, except in one case (ground turkey sample) in which Salmonella was isolated from both Rambach agar and Rainbow Agar Salmonella, but not from XLT4 agar.

Since two 25 g portions were tested from each package, and on several occasions, only one of the samples was positive (discussed below), each 25 g sample was analyzed as a separate sample. Thus, there were 488 total samples tested. Results showed that 16.8, 24.0, 28.8, and 26.4% of the turkey samples were positive for Salmonella spp. by culture, PCR, TaqMan Salmonella PCR, and Transia Card Salmonella assays, respectively. Eighteen, 28.5, 35.5, and 34.9% of the chicken samples were positive by culture, PCR, TaqMan Salmonella PCR, and Transia Card Salmonella assays, respectively, and 6.5, 6.5, 6.5, and 18.5% of the ground beef samples were positive by the four assays, respectively. For 17 out of 84 of the ground chicken packages, only one of the two samples produced a positive result by one or more of the assays used, while the other sample from the package produced negative results with all four assays. For six of the 17 packages, only the Transia Card Salmonella assay gave a positive result. For 12 out of 104 of the ground turkey packages, only one of the two samples produced a positive result by one or more of the assays tested, while the other sample from the package produced negative results for all four assays. For one of the 12 packages, only the Transia Card Salmonella assay produced a positive result. And finally, in two out of 54 ground beef packages, a positive result was obtained for one sample by one or more of the assays tested, while the other sample from the package produced negative results for all four assays. Both of these samples were positive for Salmonella by the Transia Card Salmonella assay. These results indicate that Salmonella are not homogeneously distributed within ground beef and ground poultry, and that more than one sample or a sample larger than 25 g may need to be tested to obtain a positive result.

In agreement with the results in the current study, a number of reports have demonstrated that the PCR is as sensitive or more sensitive than the traditional culture methods and allows for more rapid detection of pathogens from food and fecal samples [13,14,24–26]. Furthermore, an advantage of the TaqMan-based PCR assay or real-time PCR systems is the absence of post-PCR sample handling, preventing potential contamination due to PCR product carryover, and resulting in more rapid analysis and higher throughput capability. The PCR results for 12 of the samples of ground chicken or turkey are shown in Fig. 2. Samples in lanes 11 (sample No. 119; positive for the invA gene product) and 12 (sample No. 120; negative) were from enrichments from two ground chicken samples removed from the same package. Sample No. 119 produced positive results for the PCR (invA), TaqMan PCR, and the Transia card assays, but was negative by culture. Sample No.120 was negative by all four assays.

In Table 1, the frequency or the number of times that a particular combination of assay results was obtained is shown. For example, for combination number 1, there were 290 samples in which all types of assays evaluated produced a negative result (0, 0, 0, 0). For combination number 11 (1, 1, 1, 1), there were 43 samples in which the four assays were simultaneously positive, and for combination number 10, there were 26 samples in which only the cultural method, PCR, and TaqMan PCR gave positive results, while results for the Transia Card Salmonella assay were negative (1, 1, 1, 0). By summarizing the data in this manner, it is readily apparent that differences in sensitivity and specificity among different types of detection methods exist.

In Tables 2–4, the kappa values for each two by two table pairing of the four methods for the results for the chicken, turkey, and beef samples, respectively, are

<table>
<thead>
<tr>
<th>Observed combination</th>
<th>Cultural method</th>
<th>PCR Salmonella</th>
<th>TaqMan Salmonella</th>
<th>Transia Card Salmonella</th>
<th>Frequency</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>290</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
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</tr>
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<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
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</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
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<td>7</td>
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<td>1</td>
<td>1</td>
<td>1</td>
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<td>0</td>
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<td>0</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>43</td>
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</table>

Total number of samples: 488

The two samples tested from each package were recorded as separate samples, thus results are of 488 samples rather than of the 244 packages of meat.

a 0 = Negative result.

b 1 = Positive result.
shown. Overall, analysis of the results for three types of foods using the simple kappa statistic showed excellent agreement between the PCR and TaqMan Salmonella PCR assays, and good to excellent agreement between the PCR and the cultural methods. The agreement between the TaqMan Salmonella PCR and the cultural method was good but not as strong. The agreement between the PCR and TaqMan Salmonella PCR was excellent. There was poor agreement between the Transia Card Salmonella assay and the other three assays, indicating that the Transia Card Salmonella assay produced false positive or negative results in many cases. The data do not show differences in performance of the four methods for detection of Salmonella based on the type of meat.

A report describing serotyping results of Salmonella isolates recovered from cattle, swine, and poultry carcasses and from raw ground product samples collected from different establishments by the USDA FSIS from June 1997 to August 1998 prior to implementation of the PR/HAACP Systems final rule, showed that Salmonella Heidelberg, Kentucky, Schwarzengrund, and Infantis were the most common isolated from raw ground chicken, with Heidelberg being the most common from both carcasses and raw product [27]. Salmonella Hadar, Heidelberg, Agona, and Senftenberg were the most common serotypes isolated from turkey carcasses, and Hadar, Agona, Muenster, and Senftenberg were the most common isolated from raw ground turkey, with Hadar being the most common isolated from carcasses and raw product. Salmonella Montevideo was the most common serotype isolated from cattle carcasses and also the most common isolated from raw ground beef. Bailey et al. [28] found that Salmonella Senftenberg was a predominant serotype isolated from chicken hatchery samples and from the processing plant. They concluded that hatchery disinfection should be an important component of intervention programs for broiler production operations. The serotypes of selected isolates recovered in the current study from XLT4 agar plates are shown in Table 5. Salmonella Heidelberg, Typhimurium, and Kentucky were serotypes isolated from chicken in

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Kappa statistic values showing agreement between the cultural method, the PCR (invA gene), TaqMan Salmonella, and Transia Card Salmonella assays for ground chicken</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultural method</td>
<td>PCR</td>
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<td>Cultural method</td>
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</tr>
<tr>
<td>PCR</td>
<td>0.6824*</td>
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<tr>
<td>TaqMan Salmonella</td>
<td>0.6103*</td>
</tr>
<tr>
<td>Transia Card Salmonella</td>
<td>0.2643*</td>
</tr>
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</table>

* Indicates that kappa is significantly (p < 0.05) nonzero.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Kappa statistic values showing agreement between the cultural method, the PCR (invA gene), TaqMan Salmonella, and Transia Card Salmonella assays for ground turkey</th>
</tr>
</thead>
<tbody>
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<td>Cultural method</td>
<td>PCR</td>
</tr>
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<td>Cultural method</td>
<td>–</td>
</tr>
<tr>
<td>PCR</td>
<td>0.7333*</td>
</tr>
<tr>
<td>TaqMan Salmonella</td>
<td>0.6496*</td>
</tr>
<tr>
<td>Transia Card Salmonella</td>
<td>0.3160*</td>
</tr>
</tbody>
</table>

* Indicates that kappa is significantly (p < 0.05) nonzero.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Kappa statistic values showing agreement between the cultural method, the PCR (invA gene), TaqMan Salmonella, and Transia Card Salmonella assays for ground beef</th>
</tr>
</thead>
<tbody>
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<td>Cultural method</td>
<td>PCR</td>
</tr>
<tr>
<td>Cultural method</td>
<td>–</td>
</tr>
<tr>
<td>PCR</td>
<td>1.0000*</td>
</tr>
<tr>
<td>TaqMan Salmonella</td>
<td>1.0000*</td>
</tr>
<tr>
<td>Transia Card Salmonella</td>
<td>0.3042*</td>
</tr>
</tbody>
</table>

* Indicates that kappa is significantly (p < 0.05) nonzero.
agreement with Schllosser et al. [27], and Salmonella Schwarzengrund and Heidelberg were isolated from turkey. None of the isolates was Salmonella Senftenberg. Only a few isolates from the ground products were serotyped; therefore a larger study is warranted to determine Salmonella serotypes associated with different types of meat.

In conclusion, several different serotypes of Salmonella were isolated from retail ground chicken, turkey, and beef samples. Overall, results showed that the PCR-based assays were more sensitive than the culture method, and the culture and PCR-based assays were more specific than the immunoassay for detection of Salmonella in these foods. The poor specificity of the immunoassay is likely due to poor performance of the antibody used in the device. The percentage of positive samples obtained by the TaqMan Salmonella PCR assay was 35.5, 28.8, and 6.5 for the ground chicken, ground turkey, and ground beef, respectively. The overall prevalence of Salmonella-positive ground chicken, ground turkey, and ground beef samples collected from establishments of all sizes from January 26, 1998 to December 31, 2000 was 14.4, 29.7, and 3.7%, respectively, [9] which are somewhat lower than results obtained in the current study for ground chicken and ground beef using the TaqMan Salmonella assay. Rose et al. [9] tested one 25 g sample collected prior to packaging, and the enrichment was screened using a commercially-available immunoassay then confirmed by culture. In the current study, in a number of cases, only one of the two samples from each package was positive for Salmonella by any of the assays employed. Thus to increase the probability of finding a positive sample it is important to test a portion larger than 25 g and/or to take portions from multiple sites in the food. Use of PCR-based systems may also enhance the ability to detect Salmonella in foods.

References


