Comparison of the DNA Probe to Culture Methods for the Detection of Salmonella on Poultry Carcasses and Processing Waters¹

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(Received for publication August 11, 1988)

ABSTRACT

In a series of five experiments a total of 269 broiler carcass and chill tank water samples were tested for the presence of Salmonella using the DNA probe and the standard cultural method. Carcasses were sampled using a whole carcass rinse technique. Samples consisted of pre- (48) and post-chill (103) carcasses, and pre-chill (48) and chill (70) tank water. Samples to be evaluated with the DNA probe were subjected to three preenrichment/enrichment procedures to determine the most accurate and reliable enrichment procedure to use with the DNA probe assay. Direct enrichment in Selenite Cystine followed by 24 h incubation in Gram Negative broth allowed for recovery of 0.03 salmonellae/ml from carcass rinse and from pre-chill water using either the standard cultural method or the DNA probe. Pre-enrichment in Lactose broth produced inaccurate results for pre-chill carcass and pre-chill water samples using the DNA probe assay, and may be due to extreme microbiological competition. No false positive results were obtained using the DNA probe assay for any of the four sample types.

The food industry, governmental regulatory agencies, and the consumer have a vital interest in decreasing the incidence of salmonellosis and other foodborne diseases. Contamination of chicken carcasses with Salmonella is a source of human salmonellosis and is of continuing concern to both the poultry industry and public health authorities (17). Present technology utilized in slaughtering plants cannot guarantee a salmonellae-free finished product. Because of the increased public awareness of food poisoning bacteria and increased scrutiny by regulatory agencies, development of methods for rapid and reliable detection of foodborne Salmonella is indicated (18).

Rapid methods including fluorescent antibody techniques (30,34,48,59,60), radioisotopic immunoassays (31,50,57), enrichment serology techniques (3, 5, 33, 34, 56, 57), enzyme immunoassays (39,45,58,63), membrane filter-disc-immunooimmobilization (28,41), and DNA-DNA hybridization (23,29) have been proposed for the timely screening and/or identification of various pathogenic organisms including Salmonella. Each of these methods is based on various characteristics of the organism. These rapid methods of analysis provide for the timely identification of contaminated products and reduce costs associated with storage of products pending microbiological clearance.

It is possible to obtain results within 48 h using the DNA probe. This includes the time necessary for steps that must be completed prior to performing the actual DNA hybridization assay (25,26). Use of DNA-DNA hybridization for the detection of bacteria in clinical applications has been described by several groups. Encoded enterotoxins were cloned and used to detect enterotoxigenic E. coli both in colonies and directly from stools of patients suffering from acute diarrhea (46). A similar system has been used to detect enteropathogenic E. coli in food samples (35). DNA hybridization has also been applied to the detection of Leishmania spp., hepatitis B virus, cytomegalovirus, and Epstein-Barr virus (4,6,8,64).

Cloned DNA fragments from S. typhimurium have shown high specificity for salmonellae (24). Of 10 nucleotide fragments isolated, two reacted with >350 strains of Salmonella, three reacted with more than 95%, and the remaining five reacted with less than 50% (23). DNA probes have shown sensitivities comparable to standard cultural and immunoassay techniques (23,25).

This study was designed to evaluate the DNA probe assay for use on raw chicken carcasses and poultry processing water samples. The preenrichment/enrichment methods used with the GENE-TRAK system for cooked and raw product (deboned turkey meat) samples have recently received AOAC approval (25), and have proved very effective in enhancing the growth of Salmonella in a variety of food products. However, the numbers of salmonellae and other competing organisms on the surface of the skin of a broiler carcass, or in poultry chiller water may be entirely different from that present on the food samples evaluated in the AOAC study. For this reason, three different enrichment methods were evaluated to determine which method

¹Published with the approval of the Director, Arkansas Agricultural Experiment Station.
would provide the most accurate results using the DNA probe assay for detection of Salmonella on raw broilers and in processing waters.

MATERIALS AND METHODS

In a series of five experiments, four types of poultry processing samples were evaluated using the DNA probe (GENE-TRAK Systems, Framingham, MA). These samples included pre- and post-chill carcasses and processing waters (Table 1). A standard culture method for raw poultry (16), was compared to the DNA Probe assay. A total of 269 samples were collected and evaluated using both microbiological assays. All sample types were not collected in each of the five experiments; therefore, unequal numbers of sample types appear in Table 1. Also, unequal numbers of samples within preenrichment/enrichment protocols appear because results obtained during the course of experiments warranted necessary changes in sample numbers in later experiments. In subsequent experiments of this type it would be best if sample numbers were equal in all experiments.

Pre- and post-chill broiler carcasses were obtained from one commercial poultry processing plant. The pre-chill carcasses were obtained immediately after the final on-line wash prior to entering the chill tank. The post-chill carcasses were obtained immediately post-chill prior to any handling associated with rehanging of the carcasses for subsequent draining and grading. Carcasses were selected randomly, and no attempt was made to determine if all the carcasses sampled in a given experiment were from the same flock of birds.

All carcasses were sampled by individually placing the carcass in a plastic bag to which 100 ml of sterile water was added. The bag was then vigorously shaken for 1 min, after which the rinse fluid was aseptically recovered (16). The total amount of rinse fluid recovered (usually 80-100ml) was analyzed. Processing water samples were obtained from the pre-chill tank and the chill tank. Pre-chill carcass samples and pre-chill water samples were collected only once (experiments 4 and 5, respectively). The post-chill carcass and water samples were collected in three separate experiments. The pre-chill tank was filled with slightly cooled water (15.6°C to 17.8°C) that was chlorinated at a rate of 10 to 20 ppm (free chlorine). The carcasses remained in this pre-chill tank for 15 to 20 min before entering the chill tank. The water in the chill tank was kept just above freezing and was chlorinated at a level of 20 to 50 ppm (free chlorine). Water samples were collected by dipping large sterile graduated cylinders into the tanks. The water (100 ml/sample) was dispensed into sterile 200-ml milk dilution bottles. All samples were held on ice for transportation to the laboratory and until testing was performed. This period of time did not exceed 2 h.

Culture method

Whole carcass rinse and chill tank water samples were directly enriched in concentrated (10x) Selenite Cystine (SC) (Difco; Detroit, MI) for 24 h at 37°C. Tetraphionate (TT) broth was not used in combination with SC because many researchers have demonstrated that SC is a more effective enrichment for recovering Salmonella from poultry carcasses (11,12,13,14,15,61). SC enrichment cultures were streaked on BG and Hek plates (Difco; Detroit, MI) and incubated for 24 h at 37°C. Suspect colonies were inoculated into TSI and LIA slants (Difco; Detroit, MI) and incubated for 24 h at 37°C. Presumptive isolates were confirmed serologically.

DNA probe assay

Samples to be evaluated with the DNA probe were preenriched/enriched using three different methods (Figure 1). These included: (a) addition of 1 ml of 10x SC to 10 ml of sample followed by 24 h incubation at 37°C, then transfer of 1 ml of

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**TABLE 1. Sample types and numbers for experiments conducted evaluating the GENE-TRAK DNA probe with various enrichment procedures.**

<table>
<thead>
<tr>
<th>Enrichment method</th>
<th>Pre-chill carcasses</th>
<th>Post-chill carcasses</th>
<th>Pre-chill water</th>
<th>Chill water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>Experiment No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>24</td>
<td>24</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>8</td>
<td>8</td>
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</tr>
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<td>16</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1. Cultural conditions for DNA probe detection of Salmonella on poultry carcasses and processing water.**

- **Method A**
  1. Add 10x SC to sample at 1 ml SC/10 ml sample
  2. Incubate 24 h at 37°C
  3. Add 1 ml enriched sample to 10 ml GN broth
  4. Incubate 6 h at 37°C

- **Method B**
  1. Add 10x SC to sample at 1 ml SC/10 ml sample
  2. Incubate 24 h at 37°C
  3. Add 1 ml enriched sample to 10 ml GN broth
  4. Incubate 24 h at 37°C

- **Method C**
  1. 10 ml sample added to 90 ml LB
  2. Incubate 24 h at 37°C
  3. Add 1 ml preenriched sample to 10 ml 1x SC
  4. Incubate 16-18 h at 37°C
  5. Add 1 ml enriched sample to ml GN broth
  6. Incubate 6 h at 37°C

SC = Selenite Cystine.
GN = Gram Negative Broth.
LB = Lactose Broth.
enriched sample into 10 ml of Gram-Negative (GN) broth (Difco; Detroit, MI) followed by incubation for 6 h at 37°C; (b) identical
mar's test (JO).

Experiments 4 and 5, during the preenrichment/enrichment procedures for subsequent
detection using the probe. The threshold sensitivity of the DNA
enriched sample into 10 ml of GN broth (Difco; Detroit, MI) followed by incubation for 24 h at 37°C, 1 ml preenriched
sample transferred to 10 ml 1x SC followed by incubation for
16-18 h at 37°C, then 1 ml of the SC enriched sample transferred to
10 ml of GN broth and incubated for 6 h at 37°C.

DNA probe testing of GN broth cultures was conducted
according to GENE-TRAK procedures (1). This included: 1) filtering the GN culture; 2) sequential addition and filtering of the
denaturization, neutralization, and fixative solutions; 3) addition
of the pre-hybridization solution; 4) addition of the hybridization solution and the DNA probe; 5) hybridization; 6) washing of the filters; and 7) reading of the filters with a beta detector.

DNA hybridization relies upon growth of the salmonellae
during the preenrichment/enrichment procedures for subsequent
detection using the probe. The threshold sensitivity of the DNA
probe approaches 10^{4} CFU/ml (52). The DNA probe is a single
strand of radio-labeled Salmonella DNA. The DNA of the
indigenous Salmonella is lysed and fixed onto a filter. This DNA then combines with the radio-labeled DNA in the probe,
forming a radioactive DNA hybrid that is detected with a beta
counter.

The data obtained from these experiments are presented in
Tables 2, 3, and 4. The sample types and enrichment methods
analyzed in the five experiments is presented in Table 1. In
Experiments 4 and 5, Salmonella in the samples were enumerated
using the 3-tube Most Probable Number (MPN) procedure
(2) in 1x SC. This test was analyzed statistically using McNe-
mar's test (10). This test is used when samples are related.
Because each individual sample was evaluated using the culture
method and one or more of the preenrichment/enrichment probe
procedures, the data are related. This analysis determines whether

there is a significant difference between the number of disagree­
ments (false positives and false negatives) within treatments.
The analysis was performed to compare results for each sample
obtained by the probe method vs. the culture method.

RESULTS AND DISCUSSION

A total of 269 carcass and water samples were evaluated
for the presence of Salmonella using the conventional
culture method and the GENE-TRAK DNA probe. All 48
pre-chill carcasses (except 4) yielded Salmonella (Table
2). Although no false (+) were encountered with any of
the 3 enrichment conditions, false (-) probe reactions were
obtained with methods A and C. There was a significant
difference (P<.05) between the number of false negatives
and the number of false positives for Method C. This method
produced a significantly higher number of false negatives
(10) than false positives (0). The numbers of false negatives
observed for methods A and B (1 and 0, respectively)
were not significantly different from the number of false
positives. The reason for the increased number of false
negatives with Method C could be related to the low
selectivity of the applied enrichment conditions, or to the
fact that LB enhances the growth of organisms which can
utilize lactose (51), which were undoubtedly present on
the skin surface of the carcasses. This rapid growth may
result in microbial competition that is too great for the
survival and subsequent growth of the salmonellae cells.
The value of lactose broth reportedly lies in the ability of
organisms that are capable of utilizing lactose (Salmonella
generally are not) fermenting the substrate, and thereby

| TABLE 2. Comparison of the culture method with the GENE-TRAK DNA hybridization assay using different enrichment methods and incubation times. |
|---|---|---|---|---|---|---|
| GENE-TRAK | No. | Culture+ | Culture- | Culture- | Culture+ | % Culture+ |
| enrichment | of samples | Probe+ | Probe- | Probe- | Probe+ | (% culture+ |
| methods | | | | | | false) |
| Pre-chill carcass samples | | | | | | |
| A | 16 | 15 | 0 | 0 | 1 | 6 |
| B | 16 | 16 | 0 | 0 | 0 | 0 |
| C | 16 | 6 | 0 | 0 | 0 | 63 |
| Total | 48 | 37 | 0 | 0 | 11 | 23 |
| Post-chill carcass samples | | | | | | |
| A | 39 | 16 | 0 | 0 | 0 | 13 |
| B | 36 | 34 | 0 | 0 | 0 | 0 |
| C | 8 | 8 | 0 | 0 | 0 | 0 |
| Total | 103 | 58 | 0 | 0 | 2 | 3 |
| Pre-chill water samples | | | | | | |
| A | 16 | 11 | 0 | 0 | 5 | 31 |
| B | 16 | 15 | 0 | 0 | 1 | 6 |
| C | 16 | 0 | 0 | 0 | 16 | 100 |
| Total | 48 | 26 | 0 | 0 | 22 | 46 |
| Chill water samples | | | | | | |
| A | 20 | 8 | 0 | 0 | 0 | 0 |
| B | 42 | 15 | 0 | 0 | 2 | 12 |
| C | 8 | 8 | 0 | 0 | 0 | 0 |
| Total | 70 | 31 | 0 | 0 | 2 | 6 |
| TOTAL | 269 | 152 | 80 | 0 | 37 | 13.8 |

\*Number of false positives and false negatives within a row with different superscripts differ significantly (P<.05).
causing a subsequent drop in pH which produces unfavorable growth conditions for most of the competing organisms (51). However, for samples that are heavily contaminated, preenrichment may be detrimental to isolation of salmonellae, especially if high coliform-Salmonella ratios are present (55). Research from another laboratory suggests that direct enrichment in SC is as effective as preenrichment in LB for recovery of salmonellae from broiler carcasses using conventional culture methods (12).

Method A produced two false negatives for the post-chill carcass samples (Table 2). This may possibly be due to the relatively short incubation period (6 h) in the GN broth prior to the hybridization assay. Perhaps 6 h was not long enough for the low number of salmonellae present in the samples to reach the minimum levels in enrichment broths necessary for detection (10⁷-10⁸ cfu/ml) using the probe. Several laboratories have reported increased detection with prolonged incubation of enrichment media (21,32,62). Method B produced no false negative post-chill carcasses, suggesting that direct enrichment in SC (24 h) followed by 24 h incubation in GN broth allows for adequate growth of the salmonellae organisms recovered from post-chill carcasses.

All 48 (100%) pre-chill and 58 (56.3%) of 103 post-chill carcasses were found to be contaminated (Table 2). Slaughtering operations significantly contribute to propagation of the organism (27,37). However, recent studies suggest that salmonellae contamination of broiler carcasses may be more directly related to environmental conditions during production of the birds (litter, feed, etc.) than to processing procedures (38).

The 56.3% Salmonella-contamination rate for the post-chill carcasses is somewhat higher than the 37% figure that has been suggested as the carcass-contamination average for the industry (7). However, prevalences of 79% in raw chickens in the United Kingdom (54), 15 to 48% in Canada (42,43), and similar levels in the U.S.A. (7,36) have been reported. Conversely, some reports indicate that the incidence of salmonellae contamination of various products from poultry processing plants is generally low (53). A recent study in Canada reported 2.6% contamination in beef carcasses, 4.1% in veal, 17.5% in pork, 69.1% in turkey, and 60.9% in chicken samples (40). In another study, broiler carcasses contained an average of 17 salmonellae/100 g of skin, and two out of the 40 samples evaluated harbored over 1400/100 g (47). Possible explanations for the lower rate of contamination on the post-chill carcasses vs. the pre-chill carcasses are: 1) the chilling procedure reduces the incidence of salmonellae (100% of the pre-chill carcasses were positive); 2) the organism is not being successfully recovered from post-chill carcasses using the rinse procedure (the texture of the skin is greatly changed by the chilling procedure); 3) numbers on the post-chill carcass are extremely low; or 4) a combination of these factors is occurring. Research has demonstrated that total numbers of organisms present on the surface of the carcass are reduced wherever water is used in poultry processing (27). Other work has suggested that the incidence and level of certain pathogenic organisms present on the surface of broiler carcasses are reduced by the chilling operation (37). Other recent studies have shown that residual levels of Salmonella and Enterobacteriaceae on chilled poultry carcasses is dependent on the degree of rinsing (37), carcass temperature (36), and sampling method (44).

No false positives were observed for any of the 48 pre-chill water samples. However, false negatives were observed using all three of the enrichment procedures. The number of false negatives observed using Method C (16) was significantly greater (P≤0.05) than the number of false positives (0) observed with this method. Methods A and B also produced false negatives (5 and 1, respectively); however, these numbers were not significantly different from the number of false positives (0). Again, the false negatives observed when utilizing the DNA probe were most likely due to the tremendous amount of bacteriological competition present in pre-chill water. This water was not chilled or chlorinated to the extent that would reduce survival and inhibit growth. The LB would serve to enhance growth of competitive organisms, a high percentage of which were most likely coliforms (Izat, unpublished results). Method C produced more false negatives than the other methods. This suggests that for highly contaminated samples, which would include pre-chill carcasses and pre-chill water, direct enrichment in SC was superior to preenrichment in lactose. Other researchers have found similar results (12). All pre-chill water samples harbored Salmonella. This suggested that part but not all of the Salmonella cells were washed off the carcass in the pre-chill tank such that all carcasses remain Salmonella positive by the rinse sample method. The conditions present in the pre-chill tank may allow for growth of the residual Salmonella over time.

No false positives were observed for any of the 70 chill water samples. False negatives occurred only when Method B was utilized. The number occurring (2) was not significantly different from the number of false positives (0). The reason for the two false negatives observed in method B is not fully understood. This method had produced the lowest number of false negatives for the other three sample types evaluated. The most likely explanation is that numbers of Salmonella were extremely low, producing levels during enrichment that were below the minimum needed for detection with the DNA probe. Thirty-three (31 positives + 2 false negatives) of the 70 chill water samples (47%) were positive for Salmonella. Incidence in the pre-chill water was 100%; therefore, a reduction was observed. This reduction was most likely due to the increased chlorination levels and decreased temperature (0 to 2°C) associated with the chill tank. Researchers have demonstrated that a significant reduction in the bacterial population on carcasses and equipment can be achieved by utilizing 20 ppm chlorine in poultry processing water (20). However, recent studies have reported that Salmonella cells can survive 100 ppm chlorine for 1 h in the chill (9). Additional studies have found that 200 ppm chlorine treatment for 10 min is effective in eliminating Salmonella.
when fewer than 1000 Salmonella organisms have been inoculated onto the carcass (19).

For post-chill carcasses and chill water, lactose preenrichment (Method C) (Table 2) did not produce unfavorable results with the DNA probe. However, the lactose preenrichment did not appear beneficial over direct enrichment in SC, except for the two false negatives observed in the chill water utilizing Method B and the 2 false-negative results with post-chill carcasses in Method A (Table 2). Studies have suggested and FDA recommends preenrichment in LB is beneficial for recovery of salmonellae from food products that have been subjected to some type of physical stress (22,49). Undoubtedly, salmonellae that have been present in the chill water for any period of time would be damaged or stressed.

Indigenous salmonellae on 16 pre-chill carcass rinse samples were enumerated using the MPN procedure (2) (Table 3). MPN samples were not preenriched in lactose. One of the carcasses evaluated harbored only 0.03 salmonellae/ml rinse fluid. The probe assay indicated that this carcass rinse sample was positive for Salmonella using samples from enrichment methods B and C. This finding was not surprising and suggests that for extremely low levels, preenrichment and/or extended incubation periods in a selective media are necessary for detection. However, at some of the higher levels (11.0 and 15.0) the probe was unable to detect salmonellae when Method C was utilized.

When pre-chill carcasses harbored Salmonella cells at rates of 0.23, 0.93, 4.60, and >11.0 salmonellae/ml of rinse, all three methods were capable of producing positive results with the DNA probe (Table 3). However, method C failed to detect some positive samples. These results indicate that for broiler carcasses with low levels of salmonellae, Method B was the most reliable. At levels greater than 0.23 salmonellae/ml, methods A and B produced accurate and reliable results.

**TABLE 3. Most probable number (MPN) of Salmonella in rinse sample from pre-chill carcasses.**

<table>
<thead>
<tr>
<th>MPN Salmonellae/ml in 100 ml water</th>
<th>Culture Method # samples positive</th>
<th>GENE-TRAK enrichment methods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>0.03</td>
<td>1/1</td>
<td>nd/1</td>
</tr>
<tr>
<td>0.23</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>0.43</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>0.93</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>4.60</td>
<td>4/4</td>
<td>4/4</td>
</tr>
<tr>
<td>11.00</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>&gt;11.00</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>15.00</td>
<td>1/1</td>
<td>nd/1</td>
</tr>
<tr>
<td><strong>TOTAL SAMPLE (+)</strong></td>
<td>16</td>
<td>15/16</td>
</tr>
</tbody>
</table>

| Total false negatives | 1/16 | 0/16 | 10/16 |
| % False Negatives | 6 | 0 | 63 |

1The number of samples positive/number of samples tested.
2Number false negatives with different superscripts differ significantly (P<.05).
3S.A. Mozola not detected.

Sixteen pre-chill water samples were also evaluated for levels of salmonellae in the original sample using the MPN procedure (Table 4). Method C was not capable of detecting salmonellae in any of the 16 samples. This suggested that there was a tremendous amount of competition in the pre-chill water and that the salmonellae simply could not compete and therefore never reached levels necessary for detection using the DNA probe, or that the applied enrichment procedures were not highly selective. Method B produced the most accurate and reliable results for the extremely low levels of organisms present in these samples.

The results from these experiments suggest that the GENE-TRAK DNA hybridization assay was as accurate as the conventional culture method for detecting Salmonella in rinse samples from raw poultry carcasses and in poultry chiller waters. Method B (direct enrichment in SC followed by incubation in GN broth for 24 hours) was superior to methods A and C in detecting extremely low levels of the organism. The results indicate that preenrichment in LB was not necessary and can produce inaccurate false negative results.

**ACKNOWLEDGMENTS**

This study was supported in part by GENE-TRAK Systems. We greatly acknowledge the technical assistance and consultation of Dr. Mark A. Mozola.

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