Development of a Solid-Phase Fluorescence Immunoassay for the Detection of *Salmonella* in Raw Ground Turkey

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**ABSTRACT**

A solid-phase fluorescence sandwich immunoassay was developed to detect *Salmonella* spp. and tested for sensitivity on pure cultures as well as on spiked and naturally contaminated raw ground turkey. The solid support was a soft-glass capillary tube to which a polyclonal antibody against *Salmonella* spp. was adsorbed. The adsorbed antibody captured cellular antigens from the sample solution. The same polyclonal antibody was also biotinylated and, in combination with an avidin-Cy5 dye conjugate, served to allow detection of the immunologically captured cells. The minimum detectable number of *Salmonella* cells spiked into ground turkey was approximately 2.5 CFU/25 g. Fifty-one raw ground turkey samples were tested for the presence of *Salmonella* spp. using both this immunoassay and a modified *Bacteriological Analytical Methods* enrichment method. Fluorescence intensity values were normalized as a ratio of the sample fluorescence to the fluorescence of a standard. Fluorescence intensity ratios of 0.50 or greater were scored as *Salmonella* positive. The solid-phase fluorescence immunoassay yielded 10% false positives and no false negatives when results were compared with those from the modified enrichment method. Isolates recovered from the samples that produced false-positive results were identified as *Citrobacter freundii* and *Enterobacter cloacae*, both of which are known to cross-react with the antiserum used.

Key words: *Salmonella*, fluorescence immunoassay, ground turkey, solid-phase immunoassay

*Salmonella* spp. are gram-negative rods which are the etiologic agents responsible for food-borne outbreaks of salmonellosis as well as typhoid and paratyphoid fever. Salmonellosis has occurred following the consumption of contaminated eggs, poultry, meat, and chocolate (2, 12, 14, 22). The detection of *Salmonella* spp. in foodstuffs is often difficult. *Salmonella* cells may be sublethally injured or present in low numbers, which in combination with competing microflora makes their detection difficult (22).

Conventional methods for the detection of *Salmonella* spp. in foods require multiple enrichments followed by selective plating; even then the results are only presumptive identifications. Confirmational tests must also be conducted to positively identify the suspect colonies (8). Immunoassays have been developed in an attempt to decrease the time and effort involved in standard microbiological detection methods. Several fluorescence-based immunoassays have been developed for the detection of *Salmonella* spp. Many of these assays utilize antibodies against the target organism that are conjugated to an enzyme or a fluorescent dye. For such formats, the presence of an antigen results in the formation of a complex consisting of the antigen and the detection antibody. This antigen-antibody complex can then be detected directly by measuring the intensity of fluorescence of the dye or indirectly using a fluorogenic substrate (1, 6, 8, 13, 16). There are also many biosensors that utilize antibodies coupled to a variety of solid supports such as silica chips, microtiter wells, and capillary tubes (4, 19, 21). Many biosensors use field-effect transistors coated with an antibody or enzyme to determine the presence of certain antigens or chemical molecules. For example, the spoilage of improperly sterilized milk can be determined using an l-lactate biosensor that produces a current when lactic acid is present (20). Piezoelectric immunosensors have been developed; one specific for *S. typhimurium* detects an amperometric change when the bacterial cells bind to the sensor-conjugated antibody (18). Optical biosensors are also able to detect specific antigens or chemicals both directly and indirectly using fluorescence (3, 11, 15). Fiber-optic probes have been coupled with an antibody to capture a specific antigen. Detection of the antigen can then be accomplished by determining the change in absorbance or reflectance on the probe surface or by the incorporation of an antibody with a fluorescing label (3). In addition to the direct detection of antigens, many optical biosensors have been developed to detect chemicals indirectly. One example involves the detection of l-lactic acid by a fiberoptic oxygen probe which is coated with lactic acid oxidase. This biosensor determines the lactic acid concentration by measuring the enzymatic consumption of oxygen (lactate + O\(_2\) → pyruvate + H\(_2\)O\(_2\)) via the dynamic quenching of the fluorescence of a dye by molecular oxygen (7). Capillary tubes can also be used as the solid support for an immunoassay. One such capillary-tube enzyme immunoassay has been developed to detect herpesvirus (19). This particular immunoas-

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say utilizes an enzyme-substrate system which produces a fluorescent product.

We have developed a solid-phase fluorescence immunoassay which utilizes Cy5, a fluorescent cyanine dye, for detection and soft-glass capillary tubes as the solid support. Quantitation of the cyanine dye present in the capillary tube is determined by a fluorometer. A diode laser provides the required excitation (λ = 635 nm). To demonstrate the utility of such a format, we have developed a sandwich immunoassay for the detection of Salmonella spp.

**MATERIALS AND METHODS**

**Bacterial strains and antigen preparation**

The clinical Salmonella and non-Salmonella isolates used in this study are listed in Table 1. Strains were stored frozen at −80°C in gram negative (GN) broth (Difco Laboratories, Detroit, MI) with 15% glycerol. The frozen strains were streaked onto XLT4 (XLD agar (Difco) containing 0.46% Tergitol 4 (Sigma Chemical Co., St. Louis, MO)) plates and individual colonies were inoculated into 10 ml of GN broth and grown for 8 h at 37°C. Pure cultures were grown in GN broth at 37°C to an OD600 of 1.5, concentrated 2× by centrifuging 100 μl of the culture at 12,000 × g for 5 min and resuspending the pellet in 50 μl of phosphate-buffered saline (PBS, pH 7.4), and boiled for 10 min before testing. The positive control standard for the solid-phase fluorescence immunoassay consisted of a Salmonella-positive broth culture recovered from a ground turkey sample and prepared as described above. A commercial preparation of heat-killed S. typhimurium (KPL, Gaithersburg, MD) was used as the antigen for the capillary wall coating.

**Instrumentation**

The fluorometer was designed and built in-house at Idetek Corporation (Sunnyvale, CA) to measure fluorescence from glass capillary tubes. The excitation source was a diode laser (λ = 635 nm, Toshiba Corporation, Tokyo, Japan). The laser beam was scanned across the capillary tube, with the detection optics positioned to capture the off-axis fluorescence. The output of the silicon p-type-insulated-n-type (P-I-N) diode detector was amplified, converted from an analog to a digital signal, and processed with a 486/33 PC running the software DSSCAN (Idetek). The fluorescence units were measured using the peak height for each capillary.

**Solid support**

The solid support for the solid-phase fluorescence immunoassay was a 6.5 cm soft-glass capillary tube (i.d. 0.65 mm, o.d. 1 mm) (Drummond, Broomall, PA). Capillary tubes were incubated in a 1.0% Aquasil solution (Pierce, Rockford, IL) for 1 min, rinsed with distilled water, blown dry with compressed air, and baked for 5 min at 120°C.

**Competition assay**

The Aquasil-treated tubes were incubated in a solution of heat-killed S. typhimurium at 0.1 mg (dry weight) of bacterial cells per ml of PBS for 2 h at 4°C. The tubes were then rinsed with PBS, blown dry, and blocked with 1% bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, MO), 10% sucrose, and 0.2% Tween 20 (Sigma) in 1× PBS solution for 2 h at room temperature.

In order to detect the capillary tube-bound antigen, biotinylated anti-Salmonella antiserum (KPL) was incubated in the tube. The antibody was biotinylated using biotin hydrazide (Sigma) as previously described by O'Shannessy et al. (17). A Cy5-streptavidin conjugate (excitation 650 nm, emission 667 nm) (Biological Detection Systems, Pittsburgh, PA) was subsequently used for the detection of the bound antigen-biotinylated antibody complex.

The competition assay was performed as follows. A 20-μl solution of 1× PBS, 0.2% Tween 20, 0.1 mg of biotinylated anti-Salmonella antiserum per ml, 90 pmol of Cy5-streptavidin, and 10 μl of 2× concentrated sample culture was incubated at room temperature for 30 min. This solution was incubated in the capillary tube for an additional 30 min at room temperature. The tube was then rinsed with 2.0 ml of PBS, blown dry, and scanned with the laser detection system.

**Antibody sandwich assay**

Capillary tubes were first treated with Aquasil as described above and then coated with the KPL polyclonal anti-Salmonella antiserum at 0.1 mg/ml in for 2 h at 4°C, rinsed with PBS, blown dry, and blocked with a solution consisting of 1% BSA, 10% sucrose, and 0.2% Tween 20 in PBS for 2 h at room temperature. The tubes were then rinsed with PBS, blown dry, and stored at 4°C for up to 2 months.

The optimized protocol for the sandwich assay was as follows. A 20-μl solution of 1× PBS with 0.2% Tween 20 containing 10 μl of 2× concentrated sample culture was incubated in the capillary tube for 30 min at room temperature. The tube was rinsed with 1.0 ml of PBS and blown dry. A 1× PBS-0.2% Tween 20 solution containing 0.1 mg/ml of the biotinylated S. typhimurium antibody and 90 pmol of Cy5-avidin was incubated in the capillary tube for an additional 30 min at room temperature. The tube was then rinsed with 2.0 ml of PBS, blown dry, and scanned with the laser detection system.

**Analysis of turkey samples**

Fifty-one market-obtained turkey samples were analyzed for Salmonella spp. using the solid-phase fluorescence immunoassay. The sensitivity of the assay was determined by inoculating various numbers of Salmonella 739102 serogroup C1 into 25 g of ground turkey. Cells for inoculation were taken from XLT4 plates and grown in GN broth at 37°C for 8 h. Serial dilutions to 10−7 were made using sterile 0.85% saline solution. Cell numbers were determined by plating 100 μl of the dilution suspension on XLT4 agar plates in triplicate. Aliquots (100 μl) from the three greatest dilutions, correlating to 10, 5, and 2.5 CFU/25-g sample were injected into several locations within the 25-g sample. Samples were kept under refrigeration for 10 min to allow the absorption of the liquid inoculum. After inoculation, the samples were cultured and tested as follows. Preenrichment and selective enrichment were conducted according to the modified AOAC method (10). The 25-g sample was added to 225 ml of lactose broth (Difco) with 0.1% Triton X-100 (Sigma) and treated for 30 s in a Stomacher 400 (Seward Medical, London). The homogenized mixture was then incubated at 37°C for 24 h. After this preenrichment, 1 ml of the culture was inoculated into 9 ml of tetrahionate broth (Difco) and incubated at 37°C for 24 h. Following the selective enrichment, enrichment cultures were plated on to XLT4 medium and incubated at 37°C for 48 h, according to the modified AOAC guidelines. Suspect Salmonella colonies were then identified using API 20E test strips (bioMerieux Vitek, Hazelwood, MO).

For the solid-phase fluorescence immunoassay, 1 ml was removed from the 24-h tetrahionate broth culture, inoculated into 9 ml of GN broth, and incubated at 37°C for 12 h. The GN culture (1.0 ml) was then centrifuged at 3,000 × g for 4 min. The pellet was resuspended in 500 μl of PBS and boiled for 10 min. A 10-μl aliquot of this suspension was then used for the antibody sandwich detection assay.
RESULTS

Assay development

The solid-phase fluorescence immunoassay was initially tested in an antigen down competition format. In this format, the test antigen in the sample and the antigen on the capillary surface competes for the antibody in solution. Figure 1 shows the average fluorescence intensity due to increasing antibody-Cy5 concentrations bound to the antigen-coated capillary. The concentration of antibody ranged from 0.4 to 400 μg/ml, which corresponds to a fluorescence intensity range of 169 to 1229. The resulting curve is biphasic, probably because of the polyclonal nature of the antisera. There is a rapid increase in the fluorescence intensity up to 50 μg/ml of antibody; probably the high-affinity antibody binds rapidly to a point at which saturation occurs.

In order to increase the signal strength, biotin was conjugated to the antibody and used in conjunction with a streptavidin-Cy5 conjugate. The combination of 40 μg/ml of the biotin-antibody conjugate and 30 pmol of Cy5-streptavidin gave a fluorescence intensity of 917. This was a 45% increase in signal over the 40 μg/ml of the Cy5-antibody conjugate, which had a fluorescence intensity of 627. The biotin-antibody conjugate was also tested at a concentration of 100 μg/ml with 30 pmol of Cy5-streptavidin. This increase in antibody concentration resulted in a fluorescence intensity that was 58% higher (1454 versus 917). Increasing concentrations of Cy5-streptavidin (30 pmol, 60 pmol, 90 pmol, 120 pmol) tested with the biotinylated antibody (100 μg/ml) resulted in fluorescence intensities of 1160, 1315, 1602, and 1650 respectively.

The antibody sandwich assay was developed using a streptavidin-Cy5 conjugate and 30 pmol of Cy5-streptavidin. The combination of 40 Ilg/ml of antibody and Cy5-streptavidin and placed in the biotin-antibody conjugate, and 90 pmol of Cy5-streptavidin.

Three blocking solutions (1% BSA with 10% sucrose; 1% nonfat dry milk [NFDM] in PBS; and 5% NFDM in PBS) were tested with and without 0.2% Tween 20 for their ability to reduce background fluorescence on the antigen-coated tube. The presence of 0.2% Tween 20 reduced the background for all three solutions by nearly 20%. The signal-to-noise ratios for 1% BSA-10% sucrose, 1% NFDM, and 5% NFDM, all with 0.2% Tween 20, were 4.44:1, 3.60:1, and 3.78:1, respectively, under test conditions. The 1% BSA-10% sucrose solution was used as the blocking reagent for both the antigen down competition assay and the antibody sandwich assay throughout this study.

Two clinical Salmonella isolates, strain 763400 (sero-group E) and strain 761516 (sero-group Cl) from P. McDonough (New York State College of Veterinary Medicine), the S. typhimurium positive control from KPL, Inc., and two E. coli isolates from raw ground beef, strains N5A and D6-T, from C. A. Batt (Department of Food Science, Cornell University), were used to test the antigen down competition assay (see Table 1). The S. typhimurium positive control produced a baseline fluorescence level of 350 when used as the free antigen in solution. Both Salmonella isolates, as well as the E. coli isolates, generated fluorescence intensity values greater than 1500. In order to determine why the anti-Salmonella antibody was not completely bound to the sample antigen, thus preventing it from binding to the antigen-coated capillary wall, the capillary tubes were coated with heat-killed strain 763400 and tested with several other Salmonella serogroups. Fluorescence intensities are shown in Figure 2. The fluorescence intensity for strain 763400 was 308; however, for other strains tested it ranged from 495 to 1293. These results demonstrate the nonoverlapping heterogeneity in the antigenic makeup of various Salmonella strains.

The antibody sandwich assay was developed using a 0.1-mg/ml concentration for the capture antibody coat. The

![FIGURE 1](http://meridian.allenpress.com/jfp/article-pdf/59/9/922/1666205/0362-028x-59_9_922.pdf)

**FIGURE 1.** Effect of Cy5-antibody concentration on fluorescence intensity. Various concentrations of Cy5 labelled anti-Salmonella antibody were incubated in capillary tubes coated with heat-killed S. typhimurium; the tubes were washed and scanned as described in the Materials and Methods.

![FIGURE 2](http://meridian.allenpress.com/jfp/article-pdf/59/9/922/1666205/0362-028x-59_9_922.pdf)

**FIGURE 2.** Antigen down competition assay of various Salmonella strains using Salmonella strain 763400 to coat the glass capillary tube. Heat-killed Salmonella were incubated with biotinylated anti-Salmonella antibody and Cy5-streptavidin and placed in the antigen-coated capillary tubes. The tubes were then washed and scanned as described in the Materials and Methods.
SOLID-PHASE FLUORESCENCE IMMUNOASSAY FOR SALMONELLA

Detection antibody and Cy5-streptavidin concentrations (0.1 mg/ml and 90 pmol, respectively) were the same for this assay as for the antigen down competition assay. Pure cultures of various Salmonella and E. coli strains were grown to an OD$_{600}$ > 2.0 and 2 µl of each were tested by the antibody sandwich format. The results from these tests are shown in Table 1. The two E. coli isolates produced fluorescence intensity scores of 839 and 787 while scores from the Salmonella isolates ranged from 1238 to 3900. Capillary tubes coated with the capture antibody and blocking solution were stored dry at 4°C for up to 5 months with no loss of reactivity.

Cy5-avidin was later used due to a change in the Cy5-streptavidin supply lot. The molar ratio of Cy5 to streptavidin for the second lot (2.1:1) was considerably lower than the original molar ratio of 4.6:1. Fluorescence intensities dropped by an average of 35% when the second lot of antibody was used to retest samples. Several concentrations of Cy5-avidin were tested for the antibody sandwich assay: the optimal concentration found was 30 pmol per reaction.

Sensitivity of sandwich immunoassay

Ground turkey samples were spiked with Salmonella 763400 to determine the minimum detectable levels achievable with the solid-phase fluorescence immunoassay. All of the samples were taken from the same package of ground turkey. A 25-g sample was initially tested by both the modified enrichment method and the solid-phase fluorescence immunoassay. The sample was Salmonella negative by the modified enrichment method and resulted in a fluorescence intensity ratio of 0.40 for the solid-phase fluorescence immunoassay. Ratios are calculated based upon the fluorescence intensity for a test sample divided by the fluorescence intensity of the standard. The standard was from an initial Salmonella-positive turkey sample; it had been heat killed and stored frozen. The use of a standard is necessary for reference since new reagent lots will affect the relative fluorescent intensity. Inoculum colony-forming units were determined by plating in triplicate 100 µl of 2 x 10$^{-7}$, 1 x 10$^{-7}$, and 5 x 10$^{-8}$ dilutions, which represented approximately 10, 5, and 2.5 CFU, respectively. Results from the spiked samples are presented in Figure 3. Spiked samples from these were tested in duplicate using both the modified enrichment method and the solid-phase fluorescence immunoassay. The presence of Salmonella was confirmed by the modified enrichment method in all 6 of the spiked samples. The fluorescence intensity ratio obtained for the unspiked sample was 0.42 and ranged from 0.62 to 1.24 for the spiked samples, which initially contained approximately 2.5 CFU and 10 CFU, respectively.

Food sample analysis

A total of 51 ground turkey samples were obtained from local markets. Salmonella spp. were detected by the modified enrichment presumptive plating method with API strip confirmation in 17 out of the 51 samples tested (Fig. 4). These 17 samples produced fluorescence intensity ratios ranging from 0.52 to 1.32. There were 9 samples that generated fluorescence intensity ratios of 0.45 or greater that were Salmonella spp. negative according to the modified enrichment method. The microorganisms in these samples were characterized to determine if any cross-reacted with the antisera used, accounting for the relatively high fluorescence intensity ratios. Seven of these samples contained non-sulfur-reducing Citrobacter freundii and the remaining 2 contained Enterobacter cloacae (Fig. 4), both of which cross-react with the polyclonal KPL antiserum. Pure cultures of all 9

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<th>TABLE 1. Bacterial strains and their fluorescence intensities tested by the solid-phase fluorescence sandwich immunoassay</th>
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<td><strong>Bacterial strain</strong></td>
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<td>Escherichia coli N-5A</td>
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*Salmonella* strains were received from P. McDonough College of Veterinary Medicine, Cornell University, Ithaca, NY). *E. coli* strains were isolated from raw ground beef in our laboratory. nd, not determined.

![Detection sensitivity of the solid-phase immunoassay in a sandwich format](https://example.com/detection_sensitivity.png)

**FIGURE 3.** Detection sensitivity of the solid-phase immunoassay in a sandwich format. Ground turkey samples (25 g) were inoculated with Salmonella strain 763400 and cultured in lactose broth for 24 h. After enrichment in tetraionate broth for 24 h and in GN broth for 12 h, cultures were heated and then assayed in a sandwich format. Fluorescence intensity ratios were calculated by dividing the sample fluorescence intensity by the fluorescence intensity of a Salmonella-positive standard. The enrichment cultural method was used for the presumptive identification of Salmonella.
Salmonella spp. in market-obtained ground turkey samples using the sandwich format solid-phase fluorescence immunoassay and enrichment protocol. Market-obtained ground turkey 25-g samples were cultured as described. Heat-killed broth cultures were then assayed in the sandwich format. Fluorescence intensity ratios were calculated by dividing the sample fluorescence intensity by the fluorescence intensity of a Salmonella-positive standard. The modified enrichment cultural method was used for the presumptive identification of Salmonella. Bacterial isolates from samples that produced a fluorescence intensity ratio of greater than 0.45 for the solid-phase fluorescence immunoassay were identified as Salmonella, Citrobacter, or Enterobacter species by API test strips and are indicated within the figure.

isolates were tested with the solid-phase fluorescence immunoassay and resulted in fluorescence intensity ratios of 0.85 or greater (data not shown). The other 25 samples that were Salmonella spp. negative by the modified enrichment presumptive test generated fluorescence intensity ratios of <0.45 (Fig. 4). Two samples were initially presumed to be positive for Salmonella spp. according to the modified enrichment plating method due to the presence of black colonies. These samples generated fluorescence intensity ratios of 0.39 and 0.40. Identification of suspect colonies from each of these samples by API test kits revealed that the suspect colonies were sulfur-reducing C. freundii, which are not known to cross-react with the antiserum. The time required to complete the solid-phase fluorescence immunoassay of the turkey samples was 62 h.

FIGURE 4. Detection of Salmonella spp. in market-obtained ground turkey samples using the sandwich format solid-phase fluorescence immunoassay and enrichment protocol. Market-obtained ground turkey 25-g samples were cultured as described. Heat-killed broth cultures were then assayed in the sandwich format. Fluorescence intensity ratios were calculated by dividing the sample fluorescence intensity by the fluorescence intensity of a Salmonella-positive standard. The modified enrichment cultural method was used for the presumptive identification of Salmonella. Bacterial isolates from samples that produced a fluorescence intensity ratio of greater than 0.45 for the solid-phase fluorescence immunoassay were identified as Salmonella, Citrobacter, or Enterobacter species by API test strips and are indicated within the figure.

DISCUSSION

A solid-phase fluorescence immunoassay specific for Salmonella spp. was developed and tested for both the antibody sandwich and antigen down competition formats. The antigen down competition format produced variable results among different strains of Salmonella. Differences in antigenic composition and density probably account for the variable response. Therefore, the antibody sandwich format was developed and tested with 13 clinical Salmonella strains. The results presented in Table 1 show that the levels of fluorescence intensity vary from one strain to the next. The cultures were grown to an OD₆₀₀ equivalent to 2.0, so the cell numbers for each strain should have been similar. The fluorescence intensity ranged from 1238 to 3900, which indicates that there was a varying amount of labelled antibody bound to the captured cells. These results, in addition to the antigen down competition format results, indicate that there is a difference in the number and reactivity of antigens among Salmonella strains.

We also determined the fluorescence intensities produced by a Cy5-labelled detection antibody as well as by the combination of a biotinylated detection antibody and Cy5-avidin conjugate. The fluorescence intensity was 45% higher for the biotin-antibody/Cy5-streptavidin combination. This was the expected result since every antibody-bound biotin molecule would capture one avidin molecule and each avidin was labelled with approximately 4.6 Cy5 molecules.

It is necessary to include a Salmonella-positive standard for each set of samples tested due to day to day variations, e.g., ambient temperature and new reagent aliquots or lots. The fluorescence intensity was determined for the standard with each group of test samples to allow for a comparison between samples tested over a period of time with different reagent lots. Fluorescence intensity ratios were calculated by dividing the test sample fluorescence by the standard sample fluorescence.

After optimization, the solid-phase fluorescence immunoassay was tested in comparison to the modified enrichment method for the detection of Salmonella spp. in ground turkey. The AOAC-approved initial enrichment and selection steps were employed for the solid-phase fluorescence immunoassay. The additional enrichment in GN broth allowed for cell growth after tetrathionate broth enrichment. Initial testing of cultures directly from the tetrathionate broth resulted in false-negative results, possibly due to the presence of inhibitory substances.

The lowest number of Salmonella cells detectable by the solid-phase fluorescence immunoassay in ground turkey was approximately 2.5 CFU/25-g sample. This level of sensitivity is comparable to if not better than most current rapid methods. For example, the minimum detectable level for the Salmonella-Tek assay (22) is 100 CFU/25 g of meat (22). The BioEnzabead enzyme immunoassay (5), which was tested on naturally contaminated samples and pure cultures, identified every sample that was Salmonella spp. positive by the standard cultural method. However, this technique produced a very high rate of false positives (46%) (5). Studies on pure cultures of non-salmonella strains revealed a high rate of cross-reactivity with C. freundii. Other rapid methods such as the 1-2 Test and magnetic immuno-polymerase chain reaction assay have comparable sensitivities ranging from 1 to 5 CFU/25-g sample (9, 23). However, the 1-2 Test had a false negative rate of 4% and the immuno-polymerase chain reaction was tested on spiked samples only.

A modified form of the enrichment method (10) for the presumptive identification of Salmonella spp. in ground turkey samples was used as a standard for comparison of the solid-phase fluorescence immunoassay. All of the modified enrichment method positive samples had fluorescence intensity ratios of 0.52 or greater. Isolates were recovered from negative samples that produced fluorescence ratios of greater than 0.45. Each of these modified enrichment method
negative samples contained either C. freundii or Enterobacter cloacae, which (according to the manufacturer) cross-react with the antiserum used. If samples containing cross-reactive isolates are excluded, all of the modified enrichment method Salmonella spp. negative samples fall below fluorescence ratios of 0.45, while all of the positive sample fluorescence ratios are above 0.52. This gap between positive and negative samples suggests a threshold for a positive fluorescence ratio of 0.50.

A total of 17 out of 51 ground turkey samples were contaminated with Salmonella cells. Each of these 17 samples resulted in a positive test result by both the modified enrichment method and the solid-phase fluorescence immunoassay. This immunoassay produced no false-negative results. The false-positive results generated by this immunoassay are due to inherent limitations of the commercially prepared antiserum and do not suggest any flaws specific to the immunoassay itself.

The Salmonella-specific fluorescence capillary tube immunoassay is a model system for capillary tube-based fluorescence immunoassays. This type of assay has many benefits when compared to the standard methods, which require plating of cultures and identification of individual colonies. The solid-phase fluorescence immunoassay allows presumptive identification of Salmonella spp. in less time than the standard cultural methods, although the positive identification of Salmonella with cultural methods would be required. These benefits include the requirement for small amounts of reagents (20 µL), short assay reaction times (1 h total), quantification of the fluorescence signal, and the ability to test multiple samples with a single capillary cartridge. The reaction time for this assay is much less than that required for the 1-2 Test (8 h). The need for a small amount of reagents and the fast reaction times are the direct result of the high surface area-to-volume ratio (19). For example, a microtiter well with a diameter of 6 mm, a height of 2 mm, and a volume of 60 µL would have a surface-to-volume ratio of 0.66:1. The capillary tubes used for this study (diameter 0.3 mm, length 65 mm, volume 25 µL) have a surface-to-volume ratio of 4.9:1. This provides for a very large reactive surface in a very small total volume, thus decreasing the distance between free antibody and the captured antigen and thereby decreasing the time for antigen-antibody binding.

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