**INTRODUCTION**

The Salmonella LT Food Safety System (FSS) is a PCR-based detection method that rapidly and specifically identifies Salmonella species in food. Thermocycling takes only 30 minutes, and the entire procedure takes only 1.5 hours from sample collection to results. For Salmonella FSS, little or no DNA amplification (polymerase chain reaction (PCR)) in the Idaho Technology R.A.P.I.D. LT instrument, internal amplification controls, and automatic serial dilution/registry by software. Samples can be tested individually or in two samples can be pooled for an increased throughput. The Salmonella LT FSS was validated for sensitivity, specificity, ruggedness, and suitability of organisms for DNA isolation studies, in which Salmonella was spiked into cooked ham, raw chicken, and chocolate and compared to reference methods.

**METHODS**

**METHOD COMPARISON**

The Salmonella LT FSS was evaluated with three food types: ham, chicken, and chocolate and compared to reference methods. Each food type was divided into two portions. One portion of the food type was not inoculated. The second portion was inoculated in a large batch to provide enough samples for testing by both the Salmonella LT FSS and the reference method. Both inoculated and un inoculated batches were tested in the same manner. The inoculum concentrations were selected in order to result in 1-10 CFU of Salmonella per 25 g food sample for pooled samples, and 1-15 CFU of Salmonella per 25 g food sample for individual samples. Cooked ham and raw chicken samples were inoculated with liquid culture and allowed to equilibrate at 4°C for 48-72 hours. The chocolate samples were melted, inoculated with culture, allowed to harden at room temperature, and equilibrated at room temperature for two weeks. Each food matrix was inoculated with a different Salmonella enterica serovar. The following serovars were used: Salmonella Enteritidis with cooked ham, Salmonella Typhimurium with raw chicken; and Salmonella Senftenberg with chocolate. These serovars have been responsible for food-borne illness or associated with recent outbreaks.

**RESULTS**

**METHOD COMPARISON**

The results obtained with raw chicken, cooked ham, and chocolate show that the Salmonella LT FSS is as effective as the reference method and leads to faster results. Results are summarized in tables I and II.

**SPECIFICITY**

A total of 123 strains of Salmonella species were evaluated. At least 50 of these strains were isolated from food related sources. All strains were then incubated in Nutrient Broth overnight. Approximately 10-50 CFU were added to 250 μL of BPH and 10 μL of BGM media (because BPH is a plastic surface and has some form of chocolate). Samples were processed according to the protocols for Salmonella LT FSS. In addition, a total of 30 non-Salmonella bacterial species were evaluated, including closely related taxa. Organisms were incubated in Nutrient Broth overnight (24-48 hours) and tested with sample processing and DNA amplification portions of the Salmonella LT FSS.

**RUGGEDNESS AND REAGENT VARIATION**

Two different Salmonella enterica serovars (Typhimurium and Heidelberg) and one non-Salmonella organism (E. coli O157:H7) were tested. For each organism, five samples were prepared and evaluated for each ruggedness parameter. Organisms were initially grown in LB and incubated overnight (16 hours). After incubation, approximately 10-50 CFU were added to 250 μL BPH. The samples were tested individually.

**DISCUSSIONS**

The Salmonella LT FSS had the same sensitivity as reference methods for cooked ham, raw chicken and chocolate in 126 samples. The system specifically identified 121 Salmonella strains, a 97% correct identification rate for Salmonella species. The system is robust and reproducible as demonstrated by ruggedness, lot to lot and shelf life studies.

**COMPARISON TO REFERENCE METHODS**

Chocolate samples were difficult to spike at the correct inoculum level because Salmonella died during the spiking (adding bacteria to hot melted chocolate), drying, or equilibration steps (sitting two weeks at room temperature). The number of slides varied from batch to batch as well. Several batches of chocolate were testedd preliminary to attempt to achieve the desired proportion of positive and negative samples. Results from the three batches spiked at a similar inoculum level for individual samples were presented here. Batch A was spiked slightly low (<120 positive, Table I) and Batch B slightly high (>120 positive, Table II) but are both very close to 1 CFU per 25 g Batch C was tested with optimal recovery results.

**INCLUSIVITY AND EXCLUSIVITY**

The Salmonella LT FSS is highly specific and was able to detect 121 out of 121 strains tested in the inclusivity panel. It did not detect out of the 30 bacteria tested in the exclusivity panel. Each Salmonella strain, 121 of 121 strain by the inclusivity panel, was tested grown in Parisite pea-water or grown in rose by dry with brilliant green and tested. Out of the 121 strains, 119 were positive in both combinations. One of the negatives was associated with a low inoculum level, and the other with a bad solid culture due to a noisy amplification curve.

**RUGGEDNESS AND REAGENT VARIATION**

The Salmonella LT FSS is robust and reproducible as demonstrated by the ruggedness, lot to lot and shelf life studies. The ruggedness study demonstrated that the system produced consistent results even with variability in reagent preparation time and sample volumes pipetted. The lot to lot and shelf life study demonstrated that the Salmonella LT FSS gave consistent results with several lots of reagents produced at different times.

**CONCLUSIONS**

This PCR-based system provides reliable detection of Salmonella in about 17 hours as opposed to 72 hours for USDA and FDA BAM methods, with fewer steps and minimal sample handling. The data presented demonstrated that the Salmonella LT FSS is equivalent to current USDA and FDA BAM official methods used to detect low numbers of organisms. Thus, the Salmonella LT FSS is able to detect a single bacterium in a 25 gram food sample. Sensitivity and specificity were 100% compared to reference methods.

- The Salmonella LT FSS represents a significant improvement over standard methods in a number of ways.
- The Salmonella LT FSS is significantly faster, providing results in about 17 hours as opposed to 72 hours for the USDA and FDA BAM methods.
- The R.A.P.I.D LT can perform real-time PCR and provide automated results in 30 minutes after enrichment and sample processing.
- Results are easier to interpret than standard methods because the software gives a "Positive" or "Negative" answer.
- The Salmonella LT FSS is easy to use with fewer steps (such as a single enrichment) and minimal sample handling.

**REFERENCES**