C-FoodSSS019

The New R.A.P.I.D.® LT Salmonella Food Security System for Rapid Detection in Select Foods

David Nielsen, Haleigh Millward, Elijah Powell, Stephanie Thatcher, and Mike Powers, Idaho Technology Inc., 390 Wakara Way, Salt Lake City, UT 84108.

CONTACT INFORMATION
Haleigh Millward
Haleigh@idahotech.com
801-736-6354

INTRODUCTION

The Salmonella LT Food Security System (FSS) is a PCR-based detection method that rapidly and specifically identifies Salmonella species in food. Thermo-cycling takes only 30 minutes, and the entire procedure takes only 17 hours. The method involves: a 16 hour sample enrichment, bacterial lysis to release DNA, DNA amplification (polymerase chain reaction (PCR)) in the Idaho Technology R.A.P.I.D. LT instrument, internal amplification controls, and automatic result interpretation by software. Samples can be tested individually or five samples can be pooled for an increased throughput. The Salmonella LT FSS was evaluated for sensitivity, specificity, ruggedness, and stability of reagents for an AOAC evaluation study, in which Salmonella was spiked into cooked ham, raw chicken, and chocolate and compared to reference methods.

ISSUE

Current methods can take up to 72 hours to identify Salmonella. The goal is a faster system than current detection systems available.

METHODS

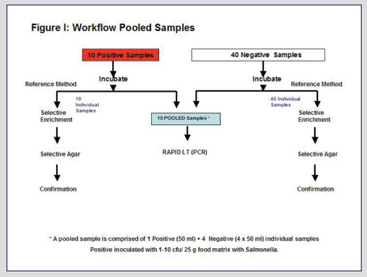
METHOD COMPARISON

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 uninoculated batches were handled 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 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 4ºC for 48-72 hours. The chocolate samples were melted, inoculated with liquid 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.

Pooled Samples

A total of 50 samples per food type were prepared for primary enrichment in the recommended broth according to the reference method. Ten samples were inoculated with a low level of target organism, 1-10 CFU per 25 g food sample, while 40 samples were not inoculated. All samples were tested individually via the reference method. A 50 mL aliquot from each of the individual positive samples was combined with a 50 mL aliquot from each of the four individual negative samples to create a 250 mL wet composite, or pooled sample. A total of 10 pooled samples were prepared from the 50 individual samples. Pooled samples were not evaluated using the reference method. Figure 1 summarizes the workflow.

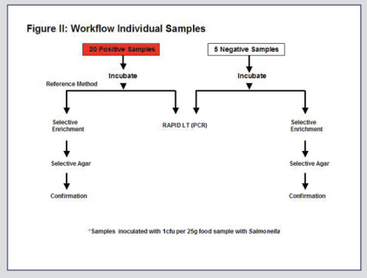
Figure I: Workflow Pooled Samples



Individual Samples

A total of 25 samples per food type were prepared for primary enrichment in the recommended broth according to the reference method. Twenty samples were inoculated with a low level of target organism, 1 CFU per 25 g food sample, while 5 samples were not inoculated. All samples were tested via the reference method and with the protocol for the Salmonella LT FSS. Figure 2 summarizes the workflow.

Figure II: Workflow Individual Samples



SALMONELLA LT FSS PROTOCOL

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 at detecting Salmonella in all foods tested. Results are summarized in tables I and II.

Table I. Method Comparison Results, Individual Samples

			Reference Method			Test Kit			Test Kit Performance			
Matrix	Inoculating organism	Level	MPN CFU/25g	# of Test Portions	Positive	Presumed Positive	Confirmed Positive	Chi Square	Sensitivity Rate %	False Negative	Specificity Rate %	False Positive
Raw Chicken	Salmonella Typhimurium	Low	<0.8	20	13	13	13	-	100	0	100	0
		Control	0	5	0	0	0	-	-	-	-	-
Cooked Ham	Salmonella Enteritidis	Low	<0.8	20	7	7	7	-	100	0	100	0
		Control	0	5	0	0	0	-	-	-	-	-
Chocolate	Salmonella Senftenberg	High-B	10.8	20	17	17	17	-	100	0	100	0
		Low-C	0.9	20	9	9	9	-	100	0	100	0
		Low-A	<0.8	20	1	1	1	-	100	0	100	0
		Control	0	15	0	0	0	-	-	-	-	-

Table II. Method Comparison Results, Pooled Samples

		Reference Method			Test Kit			Test Kit Performance			
Matrix	Inoculating organism	MPN CFU/25g	# of Test Portions	Positive	Presumed Positive	Confirmed Positive	Chi Square	Sensitivity Rate %	False Negative	Specificity Rate %	False Positive
Raw Chicken	Salmonella Typhimurium	18.8	10	10	10	10	-	100	0	100	0
Cooked Ham	Salmonella Enteritidis	5.8	10	10	10	10	-	100	0	100	0
Chocolate	Salmonella Senftenberg	10.8	10	9	9	9	-	100	0	100	0

SPECIFICITY

Of the 123 Salmonella strains tested:
- Two did not grow in the inoculum. Therefore, 121 strains were tested.
- A total of 119 strains were detected
- One was a bad software call (amplified but called negative), the other was spiked low.

Of the non-Salmonella bacteria, one species (Acetobacter aceti) did not grow in the inoculum. None of the remaining 29 non-Salmonella species were detected.

RUGGEDNESS AND REAGENT VARIATION

None of the parameters tested led to a negative result. Results are summarized in Table III. All of the reagent lots performed equivalently. Results are summarized in Table IV.

Table III. Ruggedness Study Results

Organism	Sample volume in bead tube	Reagent preparation time (minutes) (0, 1, 2, 4 hours)
Salmonella Typhimurium	5 µL: 5/5 positive	0 hr.: 5/5 positive
	10 µL: 5/5 positive	1 hr.: 5/5 positive
	25 µL: 5/5 positive	2 hr.: 5/5 positive
		4 hr.: 5/5 positive
Salmonella Heidelberg	5 µL: 5/5 positive	0 hr.: 5/5 positive
	10 µL: 5/5 positive	1 hr.: 5/5 positive
	25 µL: 5/5 positive	2 hr.: 5/5 positive
		4 hr.: 5/5 positive
E. coli	5 µL: 5/5 negative	0 hr.: 5/5 negative
	10 µL: 5/5 negative	1 hr.: 5/5 negative
	25 µL: 5/5 negative	2 hr.: 5/5 negative
		4 hr.: 5/5 negative

Table IV. Shelf-life and Lot to Lot Study Results

Organism	Lot 1: 308407	Lot 1: 308407	Lot 2:311207	Lot 3: 320507
	Expires 24 Jan 08 Age: 3 months	Expires 24 Jan 08 Age: 6 months	Expires 11 Feb 08 Age: 2 months	Expires 10 Apr 08 Age: 0 months
Salmonella Typhimurium	5/5 positive	5/5 positive	5/5 positive	5/5 positive
Salmonella Heidelberg	5/5 positive	5/5 positive	5/5 positive	5/5 positive
E. coli	5/5 negative	5/5 negative	5/5 negative	5/5 negative

SPECIFICITY

A total of 123 strains of Salmonella species were evaluated. At least 50 of these strains were isolated from food related sources. Organisms were initially incubated in Nutrient Broth overnight. Approximately 10-50 CFU were added to 250 mL of BPW and NFDM + BG media (because BPW is used for chicken and ham samples and NFDM for chocolate). Samples were processed according to the protocols for Salmonella LT FSS. In addition a total of 30 non-Salmonella bacteria species were evaluated, including closely related taxa. Organisms were initially incubated in Nutrient Broth overnight (22-24 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 mL BPW. The samples were tested individually.

Ruggedness Parameters Tested
- Volume of sample added to the bead tube for cell lysis. Varied volume of cultured sample added to the bead tube. Sample volumes of 5 µL (correct volume), 10 µL, and 25µL were evaluated.
- Reagent preparation time. Processed sample was added to reagent vials along with reconstitution buffer and put on the instrument immediately or after sitting at room temperature for 1, 2 or 4 hours.

Reagent Variation

Three different lots of Idaho Technology Salmonella LT freeze-dried PCR reagents were evaluated at different points in shelf-life. One lot was at the beginning of the reagent shelf-life, one in the middle and one close to the end. Stability and lot-to-lot variation were evaluated simultaneously.

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 and did not identify 30 non-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 level of death varied from batch to batch as well. Several batches of chocolate were tested preliminarily to attempt to achieve the desired proportion of positive and negative samples. Results from the three batches spiked at or near the appropriate level for individual samples are presented here. Batch A was spiked slightly low (1/20 positive, Table I) and Batch B slightly high (17/20 positive Table I) 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 30 out of the 30 bacteria tested in the exclusivity panel. Each Salmonella strain, of 121 in the Inclusivity panel, was tested grown in buffered peptone water or grown in nonfat dry milk 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 software call 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 demonstrate that the Salmonella LT FSS is equivalent to current USDA and FDA BAM official methods used to detect low levels of Salmonella in food. A low level of contaminating organism is 1 CFU / 25 g of food, which means that the system can 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 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

(1) U.S. Food and Drug Administration, FDA Bacteriological Analytical Manual, http://www.cfsan.fda.gov/~ebam/bam-5.html

(2) United States Department of Agriculture/Food Safety Inspection Services Microbiological Laboratory Guidelines, http://www.fsis.usda.gov/PDF/MLG_4_03.pdf