P3-36

Evaluation of Idaho Technology's R.A.P.I.D.® LT *Salmonella* Food Security System in Select Foods

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ABSTRACT

Introduction: The *Salmonella* LT Food Security System (FSS) is a PCR-based detection method that rapidly and specifically identifies *Salmonella* species in food. The method take 17 h and involves: a 16 h 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.

Purpose: 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. The system was later compared to the reference method for the detection of *Salmonella* in the lettuce, ground beef and liquid egg.

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE License Number 030803

Methods: Several samples of each food type were prepared in 225 ml of media suggested by the refer ence method; 10 samples were inoculated with 1–10 CFU per 25 g, 20 samples were inoculated with 1 CFU per 25 g, and the remaining 45–25 g portion samples were left uninoculated. All were incubated for 16 h at 37°C. Samples inoculated with 1–10 CFU were pooled with negative samples post-enrichment to create 10 composite samples and tested by the *Salmonella* LT and the reference method for a side by side comparison. Samples inoculated with 1 CFU and 5 uninoculated samples were tested individually. Results: The *Salmonella* LT FSS has the same sensitivity as reference methods for cooked ham, raw chicken and chocolate in 126 samples. The system specifically identified 120 *Salmonella* strains and did not identify 29 non-*Salmonella* species. The system is robust and reproducible as demonstrated by ruggedness, lot-to-lot studies and shelf-life studies.

Significance: This PCR-based system provides reliable detection of *Salmonella* in about 17 h as opposed to 72 h for USDA and FDA BAM methods, with fewer steps and minimal sample handling.

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.

Three additional matrices were evaluated at later dates with the following serovars: *Salmonella* albony with lettuce, *Salmonella* tallahassee with ground beef and *Salmonella* muenchen with liquid eggs. 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. Samples were inoculated with liquid culture and allowed to equilibrate at 4ºC for 24 hours (for lettuce and ground beef) to 72 hours (for liquid egg). Samples were otherwise treated as in Method Comparison Study cited above.

Figure I: Workflow Pooled Samples

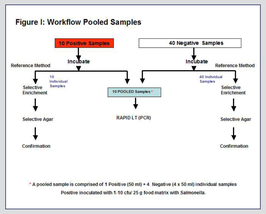
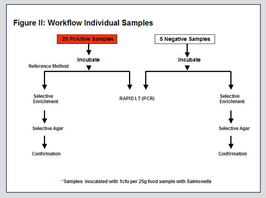


Figure II: Workflow Individual Samples



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.

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.

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.

RESULTS

METHOD COMPARISON

The results obtained with raw chicken, cooked ham, and chocolate (as well as in the additional matrices of lettuce, ground beef and liquid egg) 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, Ia, II, and IIa.

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	Pre-sumed Positive	Con-firmed Positive	Chi Square	Sensitiv-ity Rate %	False Negative	Specific-ity 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 Ia. Method Comparison Results of Additional Matrices, Individual Samples

Reference Method						Test Kit			Test Kit Performance			
Matrix	Innoculating Organism	Level	MPN CFU/25g	#. Test Portions	Positive	Presup. Positive	Confirmed Positive	Chi Square	Sensitivity Rate %	False Negative Rate %	Specificity Rate %	False Positive Rate %
Lettuce	*S. albony*	Low	<0.75 / 25g	20	6	6	6	-	100	0	100	0
		Control	0	5	0	0	0	-	-	-	-	-
Ground Beef	*S. tallahassee*	Low	0.9 / 25g	20	14	13	14	0.11	92.9	7.1	100	0
		Control	0	5	0	0	0	-	-	-	-	-
Liquid Egg	*S. muenchen*	Low	<0.75 / 25g	20	9	9	9	-	100	0	100	0
		Control	0	5	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 Neg-ative	Specificity Rate %	False Positive Rate %
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

Table IIa. Method Comparison Results of Additional Matrices, Pooled Samples

Reference Method					Test Kit			Test Kit Performance			
Matrix	Innoculating Organism	Level	MPN CFU/25g	#. Test Portions	Presup. Positive	Confirmed Positive	Chi Square	Sensitivity Rate %	False Negative Rate %	Specificity Rate %	False Positive Rate %
Lettuce	*S. albony*	Low	5.75 / 25g	10	10	10	-	100	0	100	0
Ground Beef	*S. tallahassee*	Low	2.3 / 25g	10	10	10	-	100	0	100	0
Liquid Egg	*S. muenchen*	Low	5.75 / 25g	10	10	10	-	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

SALMONELLA LT FSS PROTOCOL

Step 1:	Step 2:	Step 3:	Step 4:	Step 5:	Step 6:
Enrich individual samples	Pool post-enriched samples	Lyse bacteria and isolate DNA	Add sample to freeze-dried PCR reagent tube	Test samples on R.A.P.I.D. LT instrument	Automatic samples results. R.A.P.I.D. LT FSS software gives Positive or Negative results.
16 hours		5 minutes		30 minutes	

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 25 µL 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 evaluated samples. In additional matrix testing the *Salmonella* LT FSS had the same sensitivity as reference methods for lettuce, ground beef (92.9% sensitivity rate, 7.1% false positive rate) and liquid eggs. 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