

R.A.P.I.D.[®] LT Food Security System for *Salmonella* Detection in Select Foods



Validation Summary

Abstract

The R.A.P.I.D. LT Food Security System (FSS) includes a method for *Salmonella* detection that is fast and specific. The *Salmonella* LT FSS is designed as a qualitative detection method for *Salmonella* in food. This report describes the evaluation of the *Salmonella* LT FSS method, compared to standard methods, in cooked ham, chicken and chocolate. The method involves a series of sequential steps: sample collection, enrichment, sample processing which includes cell lysis to release DNA, DNA amplification (polymerase chain reaction (PCR)) in the Idaho Technology R.A.P.I.D. LT instrument, and automatic result interpretation by the R.A.P.I.D. LT FSS software. Two alternative procedures for sample preparation are available. One method involves cell concentration with the MATRIX MicroScience PATHATRIX[®] System, while the other involves a dilution step to remove PCR inhibitors prior to amplification (referred to as PCR-only). The PATHATRIX is useful to isolate *Salmonella* from dirty food backgrounds that may interfere with detection. Samples can be tested individually or by pooling up to five samples for increased throughput at a lower cost. Results from a sponsor laboratory and an independent laboratory demonstrated that the *Salmonella* LT FSS detection is as sensitive as the USDA Microbiology Laboratory Guidebook (MLG) and FDA Bacteriological Analytical Manual (BAM) methods at low inoculum levels of 1 colony forming unit (CFU)/25 g of food sample (chicken, cooked ham and chocolate), and that the system is sensitive even with sample pooling. All 121 *Salmonella* species tested were detected and 30 non-*Salmonella* species were not detected.

Method

The *Salmonella* LT FSS is a rapid detection method that couples real-time PCR with a short enrichment time. Real-time PCR specifically amplifies target DNA which is detected by fluorescent probes. All required PCR components including target-specific fluorescent probes are included in freeze-dried reagent vials for ease of use. The sensitivity of PCR allows for a shorter enrichment time than standard detection methods. Enrichment uses traditional media (Buffered Peptone Water or Non-fat Dry Milk). One CFU of *Salmonella* in 25 g of food can be detected after just 16 hours of enrichment at 37° C. The R.A.P.I.D. LT instrument has an advantage over traditional PCR instruments because it uses air thermo cycling to heat and cool the sample (within a closed glass reaction vessel) instead of a metal block, allowing for a more rapid heating and cooling. The final result is determined within about 17 hours. Results are reported as “Positive” or “Negative”.

Inoculation - 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. 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 4°C 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.

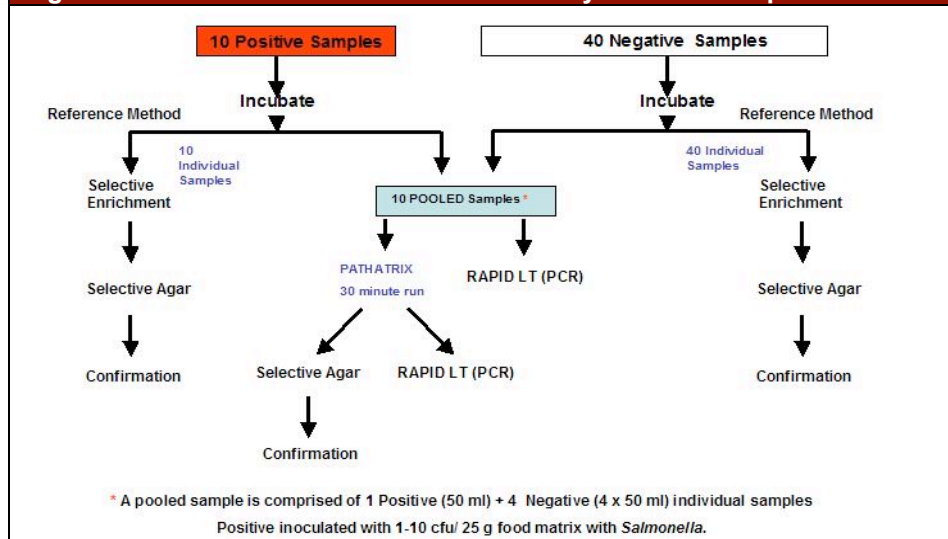
Reference Method - The *Salmonella* LT FSS was compared to FDA BAM methods (1) and USDA (MLG) methods (2). For the PATHATRIX protocol, the reference method was conducted with beads plated on selective agar.

Enrichment - The reference method and the *Salmonella* LT FSS use the same primary enrichment protocol for ham, chicken, and chocolate so that the same sample can be used for both methods. Following a 16-hour incubation at 37° C, the samples were analyzed with one (PCR-only) or both (PCR-only and PATHATRIX with PCR) *Salmonella* FSS protocols. The same samples were also tested with the appropriate reference method protocol, which includes additional incubation time.

Pooled Samples - A total of 10-pooled samples were prepared from the 50 individual samples. 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. 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. All samples were tested individually via the reference method. The samples were also tested in a pooled format using both of the protocols (PCR-only and PATHATRIX with PCR) for the R.A.P.I.D. LT Food Security System for *Salmonella* Detection. 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 PCR-only protocol for the *Salmonella* LT FSS. Individual samples were not evaluated with the PATHATRIX protocol. Figure 2 summarizes the workflow.

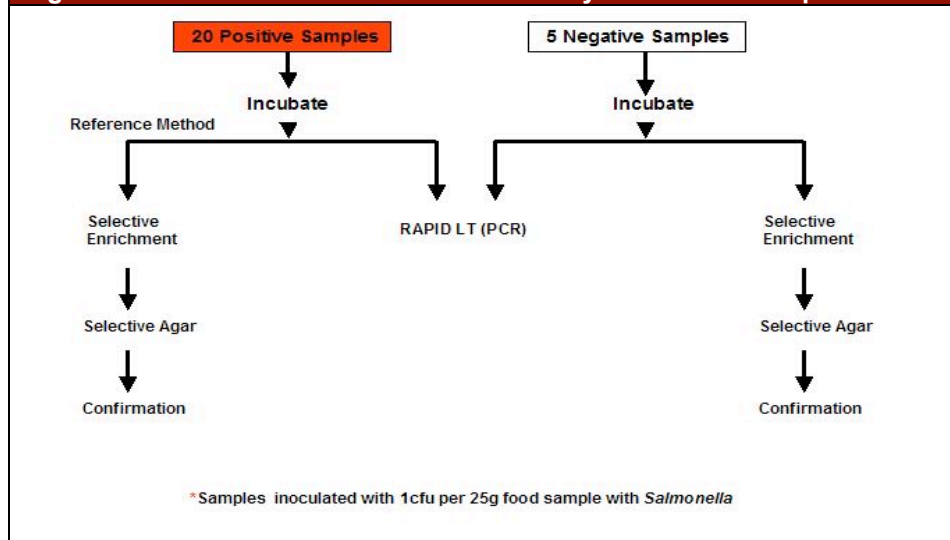
Figure 1: Overview of AOAC Validation Study: Pooled Samples



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Figure 2: Overview of AOAC Validation Study: Individual Samples



Results

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 below.

Table I. Method Comparison Results: Pooled Samples

					Reference Method	Test kit			Test Kit Performance			
Matrix	Inoculating organism	Protocol	MPN CFU/25g	No. test portions	Positive	Presump. Positive	Confirmed Positive	Chi square	Sensitivity rate, %	False negative	Specificity rate, %	False positive
Raw Chicken	<i>Salmonella</i> Typhimurium	PCR	18.8	10	10	10	10	-	100	0	100	0
		PTX	18.8	10	10	10	10	-	100	0	100	0
Cooked Ham	<i>Salmonella</i> Enteritidis	PCR	5.8	10	10	10	10	-	100	0	100	0
		PTX	5.8	10	10	10	10	-	100	0	100	0
Chocolate	<i>Salmonella</i> Senftenberg	PCR	10.8	10	9	9	9	-	100	0	100	0
		PTX	10.8	10	9	9	9	-	100	0	100	0

PTX = PATHATRIX with PCR protocol

Table II. Method Comparison Results: Individual Samples

					Reference Method	Test kit			Test Kit Performance			
Matrix	Inoculating organism	Level	MPN CFU/25g	No. test portions	Positive	Presump. Positive	Confirmed Positive	Chi square	Sensitivity rate, %	False negative	Specificity rate, %	False positive
Raw Chicken	<i>Salmonella</i> Typhimurium	Low	<0.8	20	13	13	13	-	100	0	100	0
		Control	0	5	0	0	0	-	-	-	-	-
Cooked Ham	<i>Salmonella</i> Enteritidis	Low	<0.8	20	7	7	7	-	100	0	100	0
		Control	0	5	0	0	0	-	-	-	-	-
Chocolate	<i>Salmonella</i> 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	-	-	-	-	-

Inclusively 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 in four ways, grown in buffered peptone water and tested with the PCR-only or PATHATRIX protocol, or grown in nonfat dry milk with brilliant green and tested with the PCR-only or PATHATRIX protocol. Out of the 121 strains, 111 were positive in all four combinations, while the remaining were positive for at least three combinations. Most of the negatives were grown in milk using the PATHATRIX protocol (8/10), one was associated with a low inoculum level, and the other with a bad software call due to a noisy amplification curve. Eight *Salmonella* strains (out of 121) were not detected when grown in NFDM and tested with the PATHATRIX. The same strains were detected when grown in NFDM without the PATHATRIX and worked with the PATHATRIX when grown in BPW. It is possible that the NFDM media interferes with the binding of the PATHATRIX *Salmonella* beads with only those eight strains. However, three of those strains were tested from different sources that were detected, so the cause may have been operator error.

Conclusion

The *Salmonella* LT FSS is as sensitive as the USDA and FDA BAM methods at low inoculum levels of 1 CFU/25 g of food sample, with individual and pooled samples. Out of 230 samples evaluated, 126 samples were positive with both *Salmonella* LT and the reference method. Sensitivity and specificity were 100% for the *Salmonella* LT FSS with individual or pooled samples, and with PATHATRIX for pooled samples. False positive and false negative rates were 0%. *Salmonella* LT detected all 105 *Salmonella* serotypes evaluated. It detected none of the 30 non-*Salmonella* species tested. 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

- U.S. Food and Drug Administration, FDA *Bacteriological Analytical Manual*, <http://www.cfsan.fda.gov/~ebam/bam-5.html>
- United States Department of Agriculture/Food Safety Inspection Services *Microbiological Laboratory Guidelines*, http://www.fsis.usda.gov/PDF/MLG_4_03.pdf

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