P2-07

Evaluation of Idaho Technology's R.A.P.I.D.® LT *Listeria* Food Security System in Select Foods and on Environmental Surfaces

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ABSTRACT

INTRODUCTION
The *Listeria* LT Food Security System (FSS) is a PCR-based detection method that rapidly and specifically identifies *Listeria* species (*monocytogenes*, *innocua*, *seeligeri*, *welshimeri*, and *ivanovii*) in food and on environmental surfaces. Thermo-cycling takes only 30 minutes, and the entire procedure takes only 25-29 hours. The method involves: a single 24-28 hour sample enrichment, bacterial lysis to release DNA, DNA amplification by PCR in the Idaho Technology R. A.P.I.D. LT instrument, internal amplification controls, and automatic result interpretation by the software. Samples can be tested individually or pooled.

PURPOSE
The *Listeria* LT FSS was evaluated for sensitivity, specificity, ruggedness, and stability of reagents for an AOAC evaluation study, in which *Listeria* was spiked into turkey deli meat and Mexican-style cheese, and onto ceramic tile, food-grade stainless steel, and plastic environmental surfaces and compared to reference methods.

METHODS
Several samples of each food type and environmental surface were tested for *Listeria* with the *Listeria* LT FSS, MPN analysis, and the reference method tests. The samples were inoculated at levels to result in samples (25g for food and 4x4 inch for surfaces) with approximately 1 CFU of *Listeria* after equilibration. Oxoid ONE broth was used for food and BLEB was used for environmental surfaces. Samples were incubated at 30°C for 24-26 hours. Samples were tested side-by-side with the reference method individually, and then pooled and tested.

RESULTS
The *Listeria* LT FSS is equivalent to the reference methods for turkey deli meat, Mexican-style cheese, ceramic tile, food-grade stainless steel, and plastic in a total of 120 samples. The system detected 54 *Listeria* isolates from all five target species, including 17 different serotypes and none of 31 non-*Listeria* species were detected. The system is robust and reproducible as demonstrated by ruggedness, lot-to-lot and shelf life studies.

SIGNIFICANCE
This PCR-based system provides reliable detection of *Listeria* in about 25-29 hours as opposed to 72 hours for USDA and FDA BAM methods, with fewer steps and minimal sample handling.

- **Easy to use PCR instrument**
  - Freeze-dried reagents/no proprietary media
  - Designed for minimally trained users
- **Accurate and specific pathogen testing**
  - Double specificity (primer + probe)
  - Extensive scientific support & expertise
- **Timely results**
  - Shortened enrichment times
  - PCR in under an hour

ISSUE

Current methods can take approximately 3 days to identify *Listeria* species in food and environmental samples. The goal of the *Listeria* LT FSS is to provide a sensitive and robust system that is faster than currently available detection systems.

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METHODS

METHOD COMPARISON
Food
The *Listeria* LT FSS was evaluated with two food types; turkey deli meat and Mexican-style soft cheese 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 the *Listeria* LT FSS, MPN analysis, 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 approximately 1 CFU of *Listeria* per 25g food sample (fractional positive levels, 5-15 positives out of 20 replicates) after equilibration. Samples were inoculated with liquid culture and allowed to equilibrate at 4°C for 48-72 hours. Each matrix was inoculated with a different *Listeria* species: *L. welshimeri* (ATCC 35897) for Mexican-style soft cheese and *L. monocytogenes* (ATCC 13932) for turkey deli meat.

Environmental
Three surfaces, ceramic tile, food-grade stainless steel, and plastic were inoculated (40 of each). For each surface, 25 uninoculated samples were prepared. Inoculation levels were selected to result in fractionally positive results (5-15 positives out of 20 replicates tested) by at least one of the methods (FSS or reference method). The inoculated surface samples were allowed to dry at room temperature for 16-24 hours before sampling with a sterile sponge. Each surface was inoculated with a different *Listeria* species: *L. seeligeri* (ATCC 35967), *L. ivanovii* (ATCC 19119), or *L. innocua* (ATCC 33090). One set of environmental surface samples from plastic was also inoculated with a 10x higher concentration of *Enterococcus faecium* in the background.

For each matrix, 25 samples (20 inoculated and 5 uninoculated) were prepared, enriched, plated, and evaluated according to the reference method (FDA BAM Eighth edition for cheese (1) and USDA MLG for turkey deli meat and environmental samples (2)). For each matrix, 40 samples (20 inoculated, 5 uninoculated, and 15 uninoculated for pooling) were prepared and enriched in Oxoid ONE broth for the food samples and basal Buffered *Listeria* Enrichment Broth (BLEB) for environmental samples. The cheese and environmental samples were incubated for 24-26 hours, the turkey deli meat samples were incubated for 26-28 hours. All samples were incubated at 30°C.

The inoculated FSS samples were tested individually and then pooled and tested (see figure 1A and 1B). Each pooled food sample was prepared by combining one 50mL aliquot of an incoulated sample with four separate 50mL aliquots from four uninoculated samples. This creates a 250mL wet composite or pooled sample (see Figure 1B). Each pooled environmental sample was prepared by combining one 20mL aliquot of an inoculated sample with four separate 20mL aliquots from four uninoculated samples. This creates a 100mL wet composite or pooled sample (see Figure 1B). Five uninoculated samples were tested individually by the reference method and five by the *Listeria* LT FSS. All positive and negative samples were confirmed by appropriate reference methods.

After the required 1mL aliquot for PCR and the 50mL aliquot for pooling were removed the samples were returned to the incubator to incubate for a total of 48 hours at 30°C. An aliquot from the enrichment was streaked to Modified Oxford agar (MOX), FDA and USDA procedures were followed for the remainder of the confirmation.

Most Probable Number (MPN) quantification was conducted on the day that analysis of test samples was initiated for the food matrices (there is no MPN method for environmental samples). The MPN is calculated according to the FDA BAM 8th edition, Appendix 2 (1).

Figure 1A: Flowchart of the AOAC Evaluation Method Comparison Study

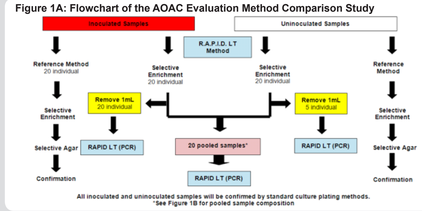


Figure 1B: Pooled Sample Composition

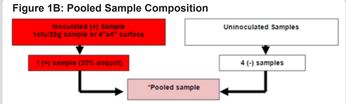


Table I. Method Comparison Results, Food Samples

Matrix	Inoculating Organism	Level	MPN/25g[a]	Samples	*Listeria* LT positive	Confirmed positive	Reference positive	χ^2[b]	False Negative	False Positive
Soft cheese	*Listeria welshimeri*	Low	1.85	20	16	16	15	0.14	0	0
		Pooled	0.37	20	16	16	15	0.14	0	0
		Control	0	5	0	0	0	-	-	-
Turkey deli meat	*Listeria monocytogenes*	Low	3.75	20	15	15	17	0.61	0	0
		Pooled	0.75	20	15	15	17	0.61	0	0
		Control	0	5	0	0	0	-	-	-

[a]MPN = Most Probable Number: colony-forming units in a 25g sample. Pooled MPN calculated by dividing individual MPN by 5.
[b]Mantel-Haenszel Chi-square for comparison to reference samples.

Table II. Method Comparison Results, Surface Samples

Matrix	Inoculating Organism	Level	Samples	*Listeria* LT positive	Confirmed positive	Reference positive	χ^2[a]	False Negative	False Positive
Ceramic	*Listeria seeligeri*	Low[b]	20	3	3	5	0.61	0	0
		Pooled	20	3	3	5	0.61	0	0
		Control	5	0	0	0	-	-	-
Plastic	*Listeria innocua*	Low[b]	20	13	13	14	0.11	0	0
		Pooled	20	13	13	14	0.11	0	0
		Control	20	0	0	0	-	-	-
	Listeria innocua and *E. faecium*	Low[b]	20	7	7	5	0.46	0	0
		Pooled	20	7	7	5	0.46	0	0
		Control	5	0	0	0	-	-	-
Stainless Steel	*Listeria ivanovii*	Low[b]	20	10	10	11	0.10	0	0
		Pooled	20	8	10	11	0.88	20	0
		Control	5	0	0	0	-	-	-

[a]Mantel-Haenszel Chi-square for comparison to reference samples.
[b]Spiked at fractional levels. Most probable number (MPN) cannot be calculated for surface samples.

Table III. Method Comparison Results, Overall Results

	Relative Sensitivity	False Negative	False Positive
Individual Samples	96%	0%	0%
Pooled Samples	93%	3%	0%

Table IVa. Inclusivity Summary

	Organism	Serotypes Verified
Detected	*Listeria monocytogenes*	1/2a, 1/2b, 1/2c, 3a, 3b, 4a, 4ab, 4b, 4c, 4d, 4e, 7
	Listeria innocua	6a, 6b
	Listeria seeligeri	1/2b
	Listeria welshimeri	6a, 6b
	Listeria ivanovii	5

Table IVb. Exclusivity Summary

Organisms Not Detected		
Bacillus cereus	*Propionibacterium freundenreichii*	*Erysipelothrix rhusiopathiae*
Brochothrix thermocphacta	*Proteus vulgaris*	*Kurthis gibsonii*
Citrobacter braakii	*Rhodococcus equi*	*Lactobacillus plantarum*
Corynebacterium amycolatum	*Shigells flexneri*	*Micrococcus luteus*
Escherchia coli including serotypes O55, O145, O157	*Staphylococcus xylosus*	*Pantoea agglomerans*
Enterococcus faecalis	*Bacillus mycoides*	*Proteus hauseri*
Enterococcus malodoratus	*Carnobacterium gallinarum*	*Pseudomonas aeruginosa*
Klebsiella pneumonia	*Citrobacter freundii*	*Salmonella enteriditis*
Lactobacillus delbruckii subp. lactis	*Corynebacteriun bovis*	*Staphylococcus aureus*
Listeria grayi	*Enterobacter sakazakii*	*Streptococcus pneumoniae*
Morganella morgani	*Enterococcus faeceum*	

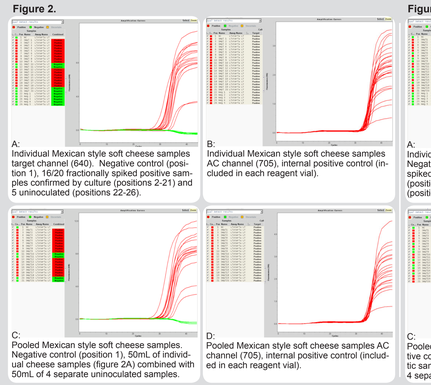
Table V. Ruggedness Study Results

	Parameter 1	Parameter 2	Parameter 3	Parameter 4
	Storage time of enriched samples	Sample volume in bead tube	Storage time of bead tubes after lysis	Reagent preparation time
Listeria monocytogenes ATCC 13932	0 h: 5/5 (+)	2.5 µL: 5/5 (+)	0 h: 5/5 (+)	0 h: 5/5 (+)
	2 h: 5/5 (+)	5 µL: 5/5 (+)	2 h: 5/5 (+)	1 h: 5/5 (+)
	8 h: 5/5 (+)	10 µL: 5/5 (+)	4 h: 5/5 (+)	2 h: 5/5 (+)
	24 h: 5/5 (+)		24 h: 5/5 (+)	4 h: 5/5 (+)
Listeria monocytogenes ATCC 43256	0 h: 5/5 (+)	2.5 µL: 5/5 (+)	0 h: 5/5 (+)	0 h: 5/5 (+)
	2 h: 5/5 (+)	5 µL: 5/5 (+)	2 h: 5/5 (+)	1 h: 5/5 (+)
	8 h: 5/5 (+)	10 µL: 5/5 (+)	4 h: 5/5 (+)	2 h: 5/5 (+)
	24 h: 5/5 (+)		24 h: 5/5 (+)	4 h: 5/5 (+)
E. faecium	0 h: 5/5 (-)	2.5 µL: 5/5 (-)	0 h: 5/5 (-)	0 h: 5/5 (-)
	2 h: 5/5 (-)	5 µL: 5/5 (-)	2 h: 5/5 (-)	1 h: 5/5 (-)
	8 h: 5/5 (-)	10 µL: 5/5 (-)	4 h: 5/5 (-)	2 h: 5/5 (-)
	24 h: 5/5 (-)		24 h: 5/5 (-)	4 h: 5/5 (-)

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

	Lot 1: 359308	Lot 3: 605408	Lot 4: 109808
Organism	Expires 02 Oct 08 Age: 6 months	Expires 14 Nov 08 Age: 4 months	Expires 17 Feb 09 Age: 1 months
Listeria monocytogenes ATCC 13932	5/5 (+)	5/5 (+)	5/5 (+)
Listeria monocytogenes ATCC 43256	5/5 (+)	5/5 (+)	5/5 (+)
E. faecium	5/5 (-)	5/5 (-)	5/5 (-)

Figure 2.



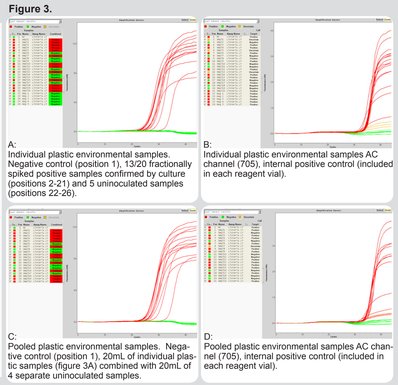
A: Individual Mexican style soft cheese samples target channel (640). Negative control (position 1), 16/20 fractionally spiked positive samples confirmed by culture (positions 2-21) and 5 uninoculated (positions 22-26).

B: Individual Mexican style soft cheese samples AC channel (705), internal positive control (included in each reagent vial).

C: Pooled Mexican style soft cheese samples. Negative control (position 1), 50mL of individual cheese samples (figure 2A) combined with 50mL of 4 separate uninoculated samples.

D: Pooled Mexican style soft cheese samples AC channel (705), internal positive control (included in each reagent vial).

Figure 3.



A: Individual plastic environmental samples. Negative control (position 1), 13/20 fractionally spiked positive samples confirmed by culture (positions 2-21) and 5 uninoculated samples (positions 22-26).

B: Individual plastic environmental samples AC channel (705), internal positive control (included in each reagent vial).

C: Pooled plastic environmental samples. Negative control (position 1), 20mL of individual plastic samples (figure 3A) combined with 20mL of 4 separate uninoculated samples.

D: Pooled plastic environmental samples AC channel (705), internal positive control (included in each reagent vial).

SPECIFICITY
A total of 54 strains of *Listeria* species were evaluated for detection by the *Listeria* LT. Twenty seven of these strains were isolated from food related sources. Organisms were initially incubated in Brain Heart Infusion (BHI) for at least 24 hours. Approximately 10-50 CFU were added to 250mL of BLEB and Oxoid ONE Broth with supplements. Samples were processed according to the food protocols for *Listeria* detection. In addition a total of 31 non-*Listeria* bacterial species and *Listeria* grayi were evaluated, including closely related taxa. Organisms were initially incubated in BHI for at least 24 hours and tested with sample processing and DNA amplification portions of the *Listeria* LT FSS protocol.

RUGGEDNESS AND REAGENT VARIATION
Two different *L. monocytogenes* and one non-*Listeria* species organism (*E. faecium*) were tested from pure culture. For each organism, five samples were prepared and evaluated for each ruggedness parameter. The strains were grown for 24 hours in basal BLEB for *L. monocytogenes* and LB for *E. faecium*. Approximately 10-50 CFU of each *L. monocytogenes* strain was added to 250mL of basal BLEB. Undiluted *E. faecium* was added to Buffered Peptone Water (BPW). Samples were individually processed according to the *Listeria* LT FSS protocol.

Ruggedness Parameters Tested
- Storage time of enriched samples prior to cell lysis. Enriched samples were tested immediately and after storage times (at 4°C) of 2, 4, and 24 hours.
- Volume of sample added to bead tube for cell lysis. Varied volume of cultured sample added to the bead tube. Sample volumes of 2.5µL, 5µL (correct volume), and 10µL were evaluated.
- Storage time of lysed sample in the bead tube prior to PCR analysis. Samples were tested immediately and after storage times (at 4°C) of 2, 4, and 24 hours.
- Reagent preparation time. Processed sample was added to reagent vials along with reconstitution buffer and run on the instrument immediately or after sitting at room temperature for 1, 2, or 4 hours.

Reagent Variation
Three different lots of Idaho Technology *Listeria* LT freeze-dried PCR reagents were evaluated (one to six months old). Stability and lot-to-lot variation were evaluated simultaneously. Five samples of each organism were evaluated using each lot.

RESULTS

METHOD COMPARISON
Sponsor lab results for turkey deli meat, Mexican-style soft cheese, and stainless steel, ceramic, and plastic environmental surfaces show that the *Listeria* LT FSS is as effective as the reference method at detecting *Listeria* in all samples tested. Statistical analysis using the Mantel-Haenszel Chi square calculation determined that the two methods are not significantly different (less than 3.84). Method comparison results are summarized in Tables I – III.

SPECIFICITY
Of the 54 *Listeria* strains tested.
- All 54 strains were detected in BLEB.
- Five strains did not grow in the Oxoid ONE Broth after 24 hours of enrichment without any food present. These strains were detected if grown for 48 hours or with Mexican-style soft cheese present (24-28 hours). See Table IVa.
- None of the 31 non-*Listeria* species, or 4 *L. grayi* strains, were detected by the *Listeria* LT FSS. Results summarized in Table IVb.

RUGGEDNESS AND REAGENT VARIATION
None of the parameters tested led to a negative result; results are summarized in Table V. All of the reagent lots performed equivalently, results are summarized in Table VI.

CONCLUSION

The *Listeria* LT FSS is equivalent to current reference methods used to detect low levels of *Listeria* in food and on environmental surfaces in individual samples. Pooled samples gave similar results; however, there is no post-enrichment pooling reference method so there is no direct comparison to a reference method.

The *Listeria* LT FSS represents a significant improvement over standard methods in a number of ways:
- The *Listeria* LT FSS is significantly faster, providing results in as little as 25-27 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 *Listeria* LT FSS is easy to use, with a single enrichment and minimal sample handling, including freeze-dried PCR reagents.

REFERENCES
(1) United States Food and Drug Administration, FDA Bacteriological Analytical Manual http://www.cfsan.fda.gov/~ebam/bam-10/html
(2) United States Department of Agriculture/Food Safety Inspection Services, Microbiological Laboratory Guidelines http://www.fsis.usda.gov/PDF/MLG_8_06.PDF