

Listeria LT Food Security System for the R.A.P.I.D.[®] LT

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Summary

The *Listeria* LT Food Security System (FSS) is a rapid detection method for *Listeria* species that combines sensitive, rapid, real-time polymerase chain reaction (PCR) with minimal sample preparation. The PCR process takes less than 1 hour and the entire procedure 25–29 hours. The method involves a 24–28 hour sample enrichment using commercially available media, sample processing by cell lysis to release DNA, rehydration of freeze-dried assay reagents, DNA amplification (PCR) in the Idaho Technology (Salt Lake City, Utah, USA) R.A.P.I.D. LT instrument, and automatic result interpretation by the R.A.P.I.D. LT FSS software.

The AOAC Research Institute certified the *Listeria* LT FSS as a Performance-Tested Method in January 2009 (Certificate No. 010901). Idaho Technology, Inc. (ITI) developed the *Listeria* LT FSS to enable fast, simple, inexpensive testing for *Listeria*-contaminated foods and surfaces. *Listeria* LT is the second product in the FSS to receive AOAC certification. The *Salmonella* LT FSS was certified in March 2008 for select foods. To qualify for AOAC approval, the *Listeria* FSS was evaluated for sensitivity, specificity, ruggedness, and stability of reagents.

This report describes the evaluation of the *Listeria* LT FSS compared to reference methods on three surfaces (stainless steel, ceramic, and plastic) and in two foods (turkey deli meat and soft cheese).

Method

See *Figure 1* for method workflow.

Enrichment

The sensitivity of PCR allows for a shorter enrichment time than standard detection methods. One colony-forming unit (CFU) of *Listeria* in 25 g of food, or collected from a surface, can be detected after only 24–28 hours of enrichment using commercially available media (Buffered *Listeria* Enrichment Broth [BLEB], or Oxoid ONE broth) at 30°C. There is no re-growth step required.

Sample Processing

DNA is extracted from the *Listeria* bacteria by mechanical cell lysis. Bead tubes for mechanical lysis are contained in the *Listeria* LT test kit. The bacteria are lysed directly after enrichment and diluted to remove PCR inhibitors before amplification.

Freeze-dried Reagents

PCR is simple with the R.A.P.I.D. LT FSS because all PCR components are included in easy-to-use freeze-dried reagent vials contained in the *Listeria* LT test kit. Freeze-dried reagents are stored at room temperature.

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The processed sample is used to rehydrate freeze-dried assay reagents with a buffer, and the mix is added directly to a PCR vessel.

PCR

Real-time DNA amplification occurs in the Idaho Technology R.A.P.I.D. LT instrument. *Listeria* LT has two levels of specificity. PCR primers specifically amplify *Listeria* DNA that is then detected by specific fluorescent probes that only detect *Listeria*. Thus, fluorescence detection only occurs if a *Listeria* target is amplified. The freeze-dried reagents also contain an internal amplification control to ensure that amplification is successful. The R.A.P.I.D. LT instrument is faster than traditional PCR instruments (less than 1 hour versus 3 hours) because it uses air thermocycling to heat and cool the sample.

Final Result

Results are reported by the R.A.P.I.D. LT FSS software as “positive” or “negative.” The final result is determined in as little as 25 hours from the beginning of enrichment.

Validation Results

The *Listeria* LT FSS is at least as sensitive as reference methods with samples spiked at low inoculum levels. In independent lab results with turkey deli meat, *Listeria* LT method detection was significantly better than the reference method at a very low inoculum level (0.38 MPN/25 g). Out of 150 samples evaluated in the sponsor laboratory, 64 samples were positive with the *Listeria*, LT and 67 were positive with the reference method.

The FSS also proved to be robust and reproducible. The ruggedness study demonstrated that the system produced consistent results with variability in reagent preparation time and sample volumes. Lot-to-lot and shelf-life studies demonstrated that the *Listeria* LT produced consistent results with several lots of reagents produced at different times.

Method Comparison for Surface Samples

The *Listeria* LT FSS is as sensitive as the reference method for surface samples at low inoculum levels. Surface results are summarized in Table 1. The *Listeria* LT FSS was compared to reference methods from the USDA Microbiology Laboratory Guidebook (MLG; 1). Three surfaces (ceramic tile, food-grade stainless steel, and plastic) were inoculated, each with a different *Listeria* species. For each surface, 40 pieces were inoculated, 20 species for each method, and 5 were not inoculated for controls. Inoculation levels were selected to result in fractionally positive results (approximately 1 CFU per 4-in. square) by at least one of the methods (FSS or reference method). The inoculated surfaces were allowed to dry at room temperature for 16 to 24 hours before sampling. One set of plastic samples was inoculated simultaneously with *Listeria innocua* and a higher background concentration of *Enterococcus faecium*. All samples were confirmed by standard plating methods.

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Method Comparison for Food Samples

The *Listeria* LT FSS is at least as sensitive as the U.S. Department of Agriculture (USDA) and Food and Drug Administration (FDA) reference methods at low inoculum levels. Food sample results are summarized in Table 2. The *Listeria* LT FSS was compared to reference methods from the FDA Bacteriological Analytical Manual (BAM; 2) and USDA MLG (1). Two food types were inoculated (turkey deli meat and Mexican-style soft cheese), each with a different *Listeria* species. Each food was divided into two portions. One portion was not inoculated for negative controls. The second portion was inoculated for the *Listeria* LT FSS (20 samples), most probably number (MPN) analysis, and reference method tests (20 samples). Inoculation levels were selected to result in 25 g food samples with approximately 1 CFU *Listeria* (fractional positive results, 5–15 positive for one test method) after equilibration. Inoculated and uninoculated portions were allowed to equilibrate at 4°C for 48–72 hours. All samples were confirmed by standard plating methods.

Inclusivity and Exclusivity

The *Listeria* LT FSS was able to detect all 54 *Listeria* isolates from five species tested in the inclusivity panel (see summary of isolates tested in Table 3). It did not detect 31 out of the 31 bacteria species tested in the exclusivity panel, consisting of closely related non-*Listeria* species and *Listeria grayi* (see Table 3). Each strain was grown in BLEB and Oxoid ONE broth.

Conclusions

The *Listeria* LT FSS is as sensitive as the USDA and FDA methods at low inoculum levels in food and on environmental surfaces with individual and pooled samples. An independent laboratory verified that the *Listeria* LT FSS is comparable to or better than the reference methods. *Listeria* LT detected all 54 *Listeria* isolates tested from all five target species, including 17 different serotypes. It detected none of the 31 non-*Listeria* species, or *Listeria grayi*, which the method does not detect.

The *Listeria* LT FSS is a significant improvement over standard methods in a number of ways. It provides results in 24–29 hours compared to about 4 days for the USDA and FDA reference methods. The R.A.P.I.D. LT performs real-time PCR and provides automated results in about an hour (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 very few steps and minimal sample handling.

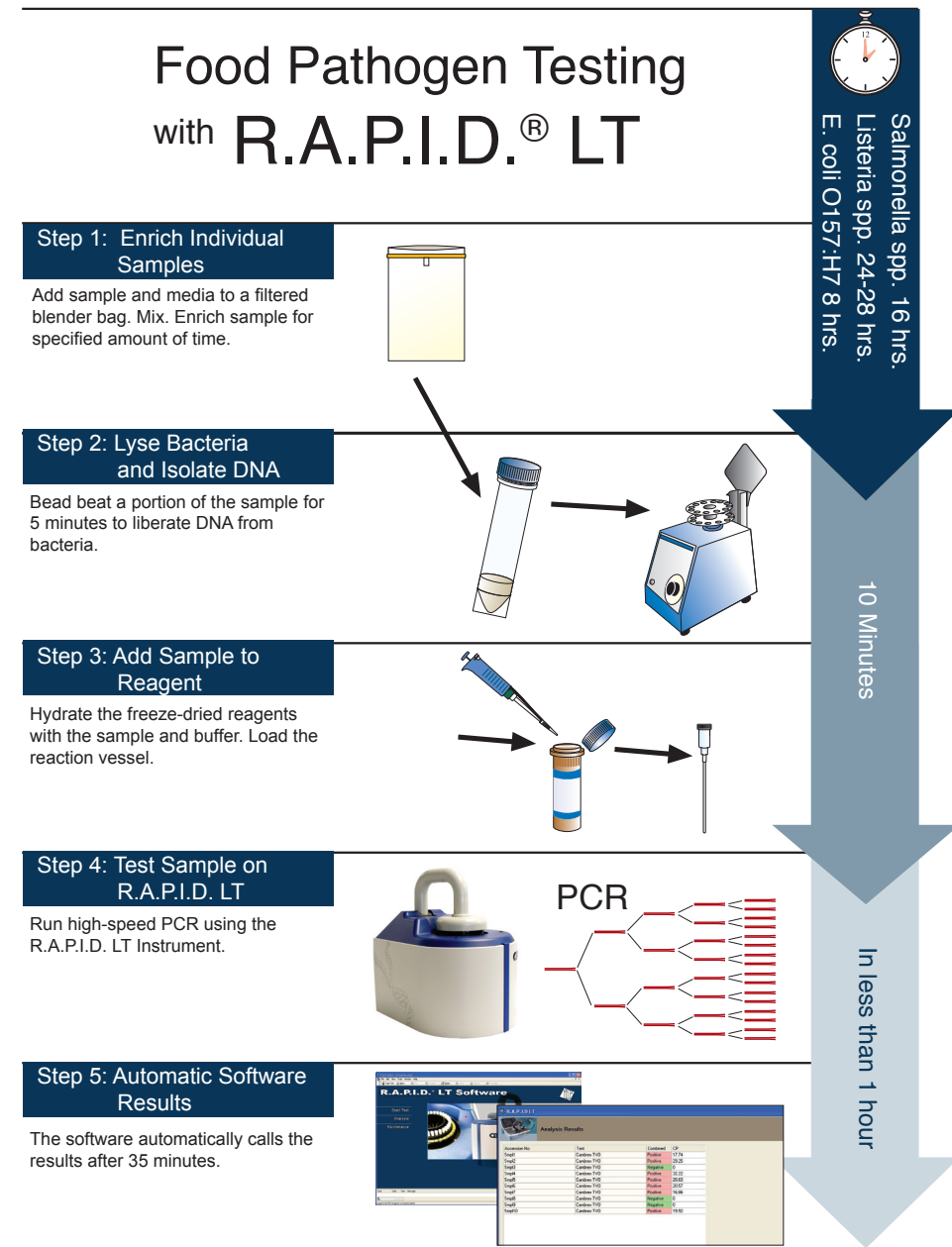
Based on results of the validation study, the *Listeria* LT FSS for the detection of *Listeria* in Mexican-style soft cheese, turkey deli meat, and on stainless steel, ceramic tile, and plastic was adopted as AOAC Performance-Tested Method No. 010901.

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Figure 1: *Listeria* LT FSS method workflow



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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_04.pdf
3. FDA BAM Eighth edition, revision A, Chapter 10 for cheese
4. USDA MLG Chapter 8 for turkey deli meat and environmental samples

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Table 1. 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	<i>Listeria seeligeri</i>	Low ^b	20	3	3	5	0.61	0	0
		Control	5	0	0	0	-	-	-
Plastic	<i>Listeria innocua</i>	Low ^b	20	13	13	14	0.11	0	0
		Control	5	0	0	0	-	-	-
	<i>Listeria innocua</i> and <i>E. faecium</i>	Low ^b	20	7	7	5	0.46	0	0
		Control	5	0	0	0	-	-	-
Stainless steel	<i>Listeria ivanovii</i>	Low ^b	20	10	10	11	0.10	0	0
		Control	5	0	0	0	-	-	-
Stainless steel ^c	<i>Listeria ivanovii</i>	Low ^b	20	13	13	15	0.46	0	0
		Control	5	0	0	0	-	-	-

^aMantel-Haenszel Chi-square for comparison to reference samples.

^bSpiked at fractional levels. Most probable number (MPN) cannot be calculated for surface samples.

^cIndependent laboratory results.

Table 2. Method Comparison Results: Food Samples

Matrix	Inoculating organism	Level	MPN/25 g ^a	Samples	Listeria LT positive	Confirmed positive	Reference positive	χ^2 ^a	False negative, %	False positive, %
Soft cheese	<i>Listeria welshimeri</i>	Low	1.85	20	16	16	15	0.14	0	0
		Control	0	5	0	0	0	-	-	-
Turkey deli meat	<i>Listeria monocytogenes</i>	Low	3.75	20	15	15	17	0.61	0	0
		Control	0	5	0	0	0	-	-	-
Turkey deli meat ^c	<i>Listeria monocytogenes</i>	Low	0.38	20	15	18	0	23.4	17	0
		Control	0	5	0	0	0	-	-	-

^aMPN = Most probable number: colony-forming units in a 25 g sample. Pooled MPN calculated by dividing individual MPN by 5.

^bMantel-Haenszel Chi-square for comparison to reference samples.

^cIndependent laboratory results

Table 3. Inclusivity and exclusivity summary

	Organism	Serotypes verified
Detected	<i>Listeria monocytogenes</i>	1/2a, 1/2b, 1/2c, 3a, 3b, 4a, 4ab, 4b, 4c, 4d, 4e, 7
	<i>Listeria innocua</i>	6a, 6b
	<i>Listeria seeligeri</i>	1/2b
	<i>Listeria welshimeri</i>	6a, 6b
	<i>Listeria ivanovii</i>	5
Not detected	<i>Bacillus cereus</i>	<i>Bacillus mycoides</i>
	<i>Brochothrix thermosphacta</i>	<i>Carnobacterium gallinarum</i>
	<i>Citrobacter braakii</i>	<i>Citrobacter freundii</i>
	<i>Corynebacterium amycolatum</i>	<i>Corynebacterium bovis</i>
	<i>Escherichia coli</i> , including serotypes O55, O145, O157	<i>Enterobacter sakazakii</i>
	<i>Enterococcus faecalis</i>	<i>Enterococcus faecium</i>
	<i>Enterococcus malodoratus</i>	<i>Erysipelothrix rhusiopathiae</i>
	<i>Klebsiella pneumoniae</i>	<i>Kurthia gibsonii</i>
	<i>Lactobacillus delbrueckii subsp. lactis</i>	<i>Lactobacillus plantarum</i>
	<i>Listeria grayii</i>	<i>Micrococcus luteus</i>
	<i>Morganella morganii</i>	<i>Pantoea agglomerans</i>
	<i>Propionibacterium freundenreichii</i>	<i>Proteus hauseri</i>
	<i>Proteus vulgaris</i>	<i>Pseudomonas aeruginosa</i>
	<i>Rhodococcus equi</i>	<i>Salmonella enteritidis</i>
	<i>Shigella flexneri</i>	<i>Staphylococcus aureus</i>
<i>Staphylococcus xylosum</i>	<i>Streptococcus pneumoniae</i>	