Neogen® Molecular Detection Assay – *Listeria* Right Now™ (LRN)

Traditional enrichment-based testing methods can take days to provide results, delaying corrective actions and increasing the risk of cross-contamination. To address this challenge, the Neogen® Molecular Detection Assay – *Listeria* Right Now™ (MDALRN96) was designed for the rapid and specific detection of *Listeria* species on environmental surfaces without enrichment. The assay targets abundant rRNA sequences and uses loop-mediated isothermal amplification (LAMP) coupled with bioluminescence to detect *Listeria* spp. with high specificity and sensitivity. Neogen Molecular Detection Assay – *Listeria* Right Now is intended for verification and monitoring of sanitation controls and also enables rapid investigation in equipment where *Listeria* detection is recurrent. This method delivers actionable results in about 2 hours after sample collection.

The method was evaluated for sampler resistance to degradation, sampling effectiveness and recovery, qualitative detection on stainless steel, plastic and sealed concrete surfaces, sample hold time, sanitizer neutralization and tolerance, limit of detection and inclusivity/exclusivity in comparison to reference methods. The results demonstrate reliable detection of *Listeria* spp. under a range of conditions.

Right Now™ Sampler – Durability and sampling effectiveness

The LRN assay uses a kit-specific sampling device (Right Now Sampler) that is pre-moistened with a sampling solution, single-use and not interchangeable with other samplers.

Durability

Post-sampling mass loss on stainless steel, plastic and sealed concrete surfaces was <0.002 g, comparable to that of two other types of commercial foam sampling devices ($p>0.05$), confirming the sampler's durability.

Effective sampling and recovery

Stainless steel, plastic, and sealed concrete surfaces (12" × 12") were inoculated with different *Listeria* strains and sampled 10 minutes post-inoculation using the Right Now™ Sampler and Neogen® Environmental Scrub Sampler (ESS). Recovered cells were enumerated and compared across the sample collection devices, with the Right Now™ Sampler yielding counts comparable to those obtained with pre-moistened environmental samplers, demonstrating effective sampling (Table 1).

Conclusion: The Right Now Sampler is robust across common food plant surfaces and provides effective *Listeria* recovery comparable to pre-moistened Environmental Scrub Sampler.

Table 1: Effectiveness of surface sampling by Right Now™ Sampler compared to Environmental Scrub Sampler

			Right Now Sampler	Environmental Scrub Sampler	
Surface type - 12"x 12"	**Organism**	**N**	**Mean Log CFU**	**Mean Log CFU**	**Mean Log difference[a]**
Stainless steel	*L. monocytogenes*	6	1.696	1.728	-0.032
Plastic	*L. innocua*	5	1.870	1.713	0.157
Sealed concrete	*L. ivanovii*	6	1.667	1.632	0.035

[a] A mean Log_{10} difference of < 0.5 between sampling devices was considered not statistically significant

Detection

A defined sampling protocol was used to evaluate the performance of the LRN assay for qualitative detection of *Listeria* on stainless steel, plastic, and sealed concrete surfaces. Surfaces were inoculated with either low (N=40) or high (N=10) levels of various *Listeria* strains, either alone or in combination with *Enterococcus faecalis* as background microflora. Uninoculated control surfaces (N=10) were also included. Inoculated surfaces were allowed to dry for a short period (1–2 hours, 80% dry) to minimize die-off before sampling.

One set of surfaces was swabbed with Dey-Engley (DE) swabs (1"x1" areas) and DE sponges (4"x4" areas) and analyzed with the reference method, FDA BAM (Chapter 10)[1]. A parallel set of surfaces (1"x1" and 4"x4") was swabbed with Right Now Samplers and tested with the LRN assay (unpaired test). Representative results are shown in Table 2. Differences in the number of positive detections between the LRN assay and FDA BAM were analyzed using probability of detection (POD) models[2,3]. A confidence interval (95% CI dPOD) that includes zero indicates no statistically significant difference between the methods.

At high inoculum levels, LRN results showed statistical agreement with the FDA BAM method across all surface types and dimensions. At low *Listeria* inoculum levels, the LRN assay detected more positives than FDA BAM, resulting in statistically significant differences for most surfaces. This discrepancy reflects the fundamental difference in detection between the two methods: FDA BAM is culture-based and detects only viable cells, whereas LRN is a sensitive molecular assay that can detect nucleic acids from low numbers of *Listeria* spp.

Conclusion: The LRN assay provides reliable detection of low levels of *Listeria* spp. across different surface types and sampling areas.

Table 2: Surface study – Unpaired detection of *Listeria* spp. on various surface types and areas using MDALRN96 vs. FDA BAM (Chapter 10)

			MDALRN96				FDA BAM					
Matrix	Organism	Inoculation Level (CFU/surface)	N	Positives	POD	95% CI	N	Positives	POD	95% CI	Unpaired dPOD	95% CI dPOD
1x1 Stainless steel	*L. welshimeri 35897*	6.48	20	19	0.95	0.765, 1.000	20	10	0.50	0.299, 0.701	0.45	0.176, 0.657 *
		32.40	5	5	1.0	0.565, 1.000	5	5	1.0	0.565, 1.000	0	-0.434, 0.434
	Negative	0	5	0	0	0.000, 0.434	5	0	0	0.000, 0.434	0	-0.434, 0.434
4x4 Stainless steel	*L. monocytogenes* 19114	2.38	20	18	0.90	0.699, 0.972	20	6	0.30	0.145, 0.519	0.60	0.303, 0.770*
		23.80	5	5	1.0	0.565, 1.000	5	5	1.0	0.565, 1.000	0	-0.434, 0.434
	Negative	0	5	0	0	0.000, 0.434	5	0	0	0.000, 0.434	0	-0.434, 0.434
4x4 Sealed concrete	*L. ivanovii*	3.87	20	20	1.0	0.838, 1.000	20	14	0.70	0.481, 0.854	0.30	0.077, 0.519*
		38.42	5	5	1.0	0.565, 1.000	5	5	1.0	0.565, 1.000	0	-0.434, 0.434
	Negative	0	5	0	0	0.000, 0.434	5	0	0	0.000, 0.434	0	-0.434, 0.434
4x4 Plastic	*L. monocytogenes* 19114 / *E. faecalis*	4.08 / 140.47	20	15	0.75	0.531, 0.888	20	14	0.70	0.481, 0.854	0.05	-0.218, 0.309
		40.80 / 1404.67	5	5	1.0	0.565, 1.000	5	5	1.0	0.565, 1.000	0	-0.434, 0.434
	Negative	0	5	0	0	0.000, 0.434	5	0	0	0.000, 0.434	0	-0.434, 0.434

** Statistically significant difference (95% CI dPOD does not include 0)*

Neutralization effectiveness and assay tolerance to common sanitizers

The neutralization capacity of the Right Now Sampler sampling solution and the tolerance of the LRN assay to sanitizers were evaluated using representative solutions from four categories: bleach (chlorine), peroxyacetic acid (Peraside™), quaternary ammonium compounds (PI Quat 20), and a high-acid cleaner (StarSan®).

Sampling solution - neutralization and bacterial survival

The neutralization capacity of the sampling solution was evaluated using ASTM E1054:2022 - Evaluation of Inactivators of Antimicrobial Agents[4].

Right Now Samplers containing sampling solution were spiked with *Listeria* spp. (~10^3 CFU/sampler), then exposed to sanitizer solutions at concentrations exceeding typical industry use or to Phosphate Buffered Saline (PBS) as a control. Samples were plated on Tryptic Soy Agar (TSA) plates immediately after sanitizer addition and again after a 10 minute hold. After incubation, microbial counts were compared.

Microbial counts were similar between sanitizer treatments and PBS control, with no statistically significant differences ($p > 0.05$) observed over time (Table 3).

Conclusion: The sampling solution effectively neutralizes common sanitizer residues, maintaining sample integrity and ensuring consistent microbial recovery.

Table 3. Sampling solution - Neutralization effectiveness (ASTM E1054:2022)

Disinfectant	Active ingredient(s)	Active ingredient(s) concentration (ppm)	N	T0 Mean Log CFU/mL	T10 Mean Log CFU/mL	Difference	95% CI	P-value*
Bleach	Sodium hypochlorite	≈1,140	3	3.014	3.048	-0.034	-0.069, 0.002	0.057
Pi Quat 20	Alkyl dimethyl benzyl ammonium chloride, Alkyl dimethyl ethyl benzyl ammonium chloride	≈3,990	3	3.049	3.073	-0.025	-0.083, 0.034	0.274
Peraside™	Peroxyacetic acid	≈1,140	3	3.06	3.045	0.015	-0.078, 0.109	0.554
StarSan®	Dodecylbenzene sulphonic acid / Phosphoric acid	≈3,840 / ≈9,980	3	3.081	3.094	-0.013	-0.078, 0.053	0.578
Control (PBS)	n/a	n/a	3	3.003	3.031	-0.027	-0.140, 0.085	0.405

**No statistical difference (p>0.05) ; n/a : not applicable*

Tolerance to sanitizers – Assay inhibition assessment

Each sanitizer was freshly prepared and diluted according to manufacturer instructions for food-contact or non-contact surfaces, using the highest recommended concentration.

Visually clean surfaces were treated with each sanitizer or with water as a control, allowed to dry, and sampled. Samplers were then inoculated with < 50 CFU of *Listeria* and tested according to the standard LRN protocol, in parallel the same lysates were tested with Neogen Right Now Matrix Control (RNMC96) for amplification control. *Listeria* was successfully detected across all conditions, and all Matrix Controls were valid. Amplification signals were consistent between sanitizer-treated and water-treated controls, with no practical differences in detection time (time-to-peak , TTP) observed between conditions (Table 4).

Conclusion: The LRN assay demonstrated no inhibition in the presence of common sanitizers when applied according to industry practices and can be used reliably after sanitation.

Table 4. Assay tolerance to commonly used disinfectants/sanitizers

Disinfectant	Active ingredient(s) concentration (ppm)	Inoculation Level (CFU/Sampler)	N	MDA LRN Mean TTP (min)	MDA LRN StDev (min)	RNMC Mean TTP (min)	RNMC StDev (min)
Bleach	223	33	8	23.75	1.430	15.22	0.574
		0	8	n/a	n/a	15.03	0.281
Pi Quat 20	780	45	8	23.81	1.024	16.06	1.307
		0	8	n/a	n/a	16.22	0.248
Peraside™	223	33	8	24.5	0.823	15.69	0.292
		0	8	n/a	n/a	16	0.501
StarSan®	300 /780	33	8	25.44	1.725	16.53	0.388
		0	8	n/a	n/a	16.44	0.291
Water	n/a	33	8	25.31	1.736	16.22	0.410
		0	8	n/a	n/a	15.69	1.315
StDev (min)				0.76		0.49	
CV (%)				3.07		3.13	

TTP : time-to-peak ; n/a : not applicable

Sample-hold time

The effect of sample-hold time on LRN assay performance was evaluated using *Listeria monocytogenes*, *L. innocua*, and *L. ivanovii*. Right Now Samplers were directly inoculated with < 10 CFU per sampler. Two sets of samples were prepared:

- Immediate testing (T0): Samples were tested on the day of inoculation to generate same-day performance data.
- 24-hour hold (T24): Samples were stored at 4–8 °C for 24 ± 2 hours and then tested with the LRN assay to evaluate performance after storage.

Time-to-peak values of amplification signals (minutes) were compared (Table 5). No practical differences in detection were observed between samples tested immediately (T0) and those held for 24 hours (T24).

Conclusion: Post-collection samples can be stored up to 24 hours at 4–8 °C without impacting assay performance, providing flexibility for testing schedules.

Table 5. Comparison of LRN assay detection (TTP) after immediate testing and 24-hour sample-hold time

		T0			T24			
Organism	Inoculation Level (CFU/sampler)	N	Mean TTP (min)	StDev	N	Mean TTP (min)	StDev	Mean TTP Difference (min)
L. monocytogenes ATCC 45594	6.1	10	22.52	2.14	10	25.60	2.92	3.08
L. innocua ATCC 49595	5.75	8	26.44	1.82	8	27.97	1.63	1.53
L. ivanovii ATCC 19119	2.42	10	24.65	1.94	10	25.05	1.89	0.40
Negative	0	5	n/a	n/a	5	n/a	n/a	n/a

TTP : time-to-peak; n/a : not applicable

Inclusivity and Exclusivity Testing

Inclusivity: The ability of a method to detect the target analyte from a wide range of strains[5]

Sixty (60) different *Listeria* isolates were tested. Each strain was cultured overnight in Brain Heart Infusion (BHI) broth and tested according to AOAC[2] guidelines, then serially diluted prior to application to the sampler and testing with the LRN standard procedure.

Exclusivity: The lack of interference from a relevant range of non-target strains[5]

Forty one (41) different non-*Listeria* strains, including closely related organisms, were tested. Strains were cultured overnight in Tryptic Soy Broth (TSB) and inoculated at levels of >10^6CFU per sampler prior to testing following the LRN standard procedure.

Conclusion: The LRN assay is both highly inclusive for *Listeria* spp. and highly exclusive against non-target organisms.

Table 6. Inclusivity and Exclusivity results

Results	Analysis
60/60 *Listeria* strains were detected (results were "positive")*	100% Inclusivity
41/41 non-*Listeria* strains were not detected (results were "negative")	100% Exclusivity

See Appendix, Tables 1a and 1b, for list of cultures tested

** Higher LOD may occur for sensu lato species*

Limit of Detection (LOD)

The method LOD is defined as the lowest concentration at which reliable analytical results can be obtained. It may vary depending on species, strains and samples. Using defined cell preparations and direct sampler inoculation, the LRN assay demonstrated an LOD95% of 2-5 CFU/sampler.

Conclusion: The LRN assay allows detection of very low levels of *Listeria* contamination. This sensitivity is achieved without enrichment, enhancing environmental monitoring capabilities.

Overall Conclusion:

The Neogen® *Listeria* Right Now® system consistently delivers rapid and reliable detection of *Listeria* spp. on environmental surfaces. The Right Now Sampler demonstrates robust performance across a variety of surfaces, maintaining sample integrity even in the presence of common sanitizer residues. The assay is highly sensitive, capable of detecting very low levels of *Listeria* without enrichment and shows excellent inclusivity for *Listeria* strains and exclusivity against non-target organisms.

The LRN system is an efficient monitoring tool that enables timely, evidence-based safety decisions and corrective actions.

Appendix

Table 1a. Inclusive cultures

Inclusives N=60	*Listeria monocytogenes*, n=21		
	Listeria, non-*monocytogenes*, n=39	*L. aquatica* *L. fleischmanii* *L. grandensis* *L. grayi,* n=5 *L. grayi subsp. murrayi* *L. innocua,* n=12 *L. ivanovii,* n=3	*L. marthii,* n=3 *L. newyorkensis* *L. riparia* *L. rocourtiae* *L. seeligeri,* n=3 *L. welshimeri,* n=5 *L. floridensis*

N = total number of tested strains; n = number of strains tested if > 1

Table 1b. Exclusive cultures

	Family / group	**Genus / species / serotype**	
Exclusives N=41	*Enterococcaceae,* n=5	*Enterococcus faecalis,* n=3 *Enterococcus faecium* *Enterococcus durans*	
	Bacillaceae, n=12	*Bacillus atrophaeus* *Bacillus badius* *Bacillus cereus* *Bacillus circulans* *Bacillus licheniformis* *Bacillus mycoides*	*Bacillus pseudomycoides* *Bacillus pumilus* *Bacillus spizizenii* *Bacillus thuringiensis* *Bacillus weihenstephanensis* *Priestia megaterium*
	Staphylococcaceae, n=10	*Staphylococcus aureus, n=6* *Staphylococcus epidermidis, n=2* *Staphylococcus saprophyticus* *Staphylococcus schleiferi*	
	Lactobacillaceae, n=4	*Lactobacillus plantarum* *Lactiplantibacillus plantarum* *Latilactobacillus sakei* *Lacticaseibacillus casei*	
	Leuconostocaceae, n=2	*Leuconostoc mesenteroides* *Pediococcus pentosaceus*	
	Streptococcaceae, n=2	*Streptococcus agalactiae* *Streptococcus bovis*	
	Other / miscellaneous organisms, n=6	*Microbacterium testaceum* *Micrococcus luteus* *Kocuria rhizophila*	*Corynebacterium glutamicum* *Proteus mirabilis* *Brochothrix thermosphacta*

N = total number of tested strains; n = number of strains tested if > 1

References

1. United States Food and Drug Administration. (2022). Bacteriological Analytical Manual Chapter 10: Detection of *Listeria monocytogenes* in Foods and Environmental Samples, and Enumeration of *Listeria monocytogenes* in Foods.
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