Neogen®'s ANSR®

Listeria Right Now™

NEOGEN®

Less Than an Hour? How is This Possible?

The ANSR® *Listeria* Right Now™ system is able to detect very low numbers of *Listeria spp.*, including *L. monocytogenes*, from environmental samples without enrichment. The system employs an isothermal, amplified nucleic acid-based reaction to target rRNA. Amplification occurs through a polymerization mechanism by a specific endonuclease. Detection occurs in real-time using a fluorescent, molecular beacon.

Ribosomal RNA is present in much greater numbers in *Listeria* cells than the traditional DNA target (~1000–10,000 copies per cell vs. one copy per cell for DNA). This can result in a 1,000–10,000 fold increase in target analyte concentration.

The isothermal reaction within the instrument produces a constant cycle of molecular replication producing analyte copies much more quickly than traditional PCR reactions which run through a series of heating and cooling cycles.

Summary

Significantly more targets with a significantly faster cycle time = significantly faster results.

Less Than a 60 Minute Total Time-to-result Means Everything Has Changed

Now You Can

- Use *Listeria* monitoring as a process control
- Find a potential problem quickly — fix the potential issue by cleaning and retesting
- Conduct investigations in near real-time after positives
- Perform vectoring more easily
- Be more flexible and proactive with your environmental testing program

No enrichment with an easy-to-use system means you can conduct *Listeria* environmental testing without "growing pathogens."

PERFORMANCE TESTE
AOAC
RESEARCH INSTITUT
LICENSE NUMBER 081802

Neogen® Validation Data

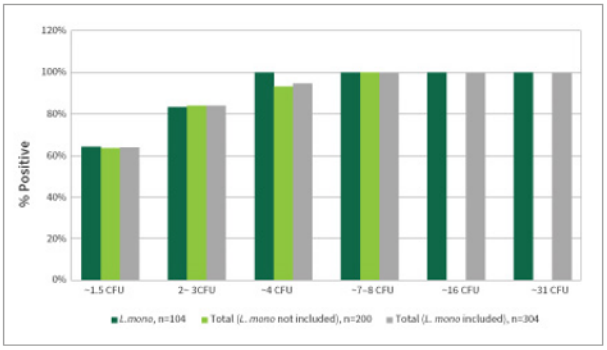
Listeria with and without background organisms on different surfaces:

Surface Type	Trial	*Listeria* CFU/Swab	N	ANSR *Listeria* Right Now +	Culture +	dPOD (95% CI)
Stainless steel	Lm (4b) only	1	15	3	2	0.07 (-0.21, 0.34)
		2	15	10	7	0.20 (-0.14, 0.48)
		2438	5	5	5	0 (-0.43, 0.43)
		0	5	0	0	0 (-0.43, 0.43)
Stainless steel	Lm (4b) + background	1.8	20	8	7	0.05 (-0.23, 0.32)
		1800	5	5	5	0 (-0.43, 0.43)
		0	5	0	0	0 (-0.43, 0.43)
Plastic	*L. innocua* + background	2.3	20	9	9	0 (-0.28, 0.28)
		2250	5	5	5	0 (-0.43, 0.43)
		0	5	0	0	0 (-0.43, 0.43)
Sealed concrete	*L. welshimeri* + background	1.2	20	6	11	-0.25 (-0.5, 0.05)
		1550	5	5	5	0 (-0.43, 0.43)
		0	5	0	0	0 (-0.43, 0.43)
Ceramic tile	Lm (1/2a) + background	1.93	20	14	9	0.25 (-0.05, 0.50)
		1930	5	5	5	0 (-0.43, 0.43)
		0	5	0	0	0 (-0.43, 0.43)

dPOD (95% CI) = difference between the candidate method and reference method calculated as a Probability of Detection with a 95% confidence interval. The ANSR *Listeria* Right Now kit is designed using swabs, not sponges for sampling in order to get the proper sample concentration into the assay. ANSR *Listeria* Right Now has been tested on surfaces with residual cleaning agents. The residual cleaning agents had no effect on the assay. ANSR *Listeria* Right Now is an environmental test and due to sample homogeneity, matrix effects, and representative sample volume, it is not intended for use with food products.

Limit of Detection

4 CFU per swab with 95% confidence



Method: Inoculated directly onto swab

Organisms Tested:

- *L. monocytogenes*
- *L. innocua*
- *L. welshimeri*
- *L. grayi*
- *L. ivanovii*
- *L. seeligeri*

NSF International Study — Applied Research Center

Environmental surface study results for *Listeria monocytogenes* and background organisms on stainless steel.

Level	Theoretical Inoculum (CFU/swab)	Sample Number	LRN Positive	% LRN Positive	Culture Positive	% Culture Positive
Negative	0	5	0	0%	0	0%
Positive	2.4E+4	5	5	100%	5	100%
L1	3	15	14	93%	9	60%
L2	9	15	15	100%	15	100%
L3	22.5	15	15	100%	15	100%

Note: The table presents the results for the environmental surface study using a challenge inoculum of *L. monocytogenes* plus a consortium of competing organisms. Three different inoculation levels were evaluated on the stainless steel carriers: level 1 = 3 CFU, level 2 = 9 CFU, and level 3 = 22.5 CFU (theoretical CFU/swab). At level 1, the detection rates for *Listeria* Right Now and the reference enrichment-based culture method were 93% and 60%, respectively. At levels 2 and 3, the detection rates for ANSR *Listeria* Right Now and the reference enrichment-based culture method were 100%. No false negatives, false positives, or invalids were observed during this study. The data illustrates that under the conditions employed in this study ANSR *Listeria* Right Now is as sensitive as the enrichment-based culture reference method for detection of *L. monocytogenes* on a stainless steel surface.

Summary and Conclusion

The purpose of this study was to evaluate the performance of the ANSR *Listeria* Right Now assay for the detection of *Listeria spp.* in environmental swabs without a prior enrichment process.

After allowing the inoculum to partially dry (50%), surface samples were collected using semi-paired swabs. One swab was tested by the ANSR *Listeria* Right Now assay and the other swab was enriched by the culture method. The swab for the culture method was enriched overnight at 37°C in growth medium and an aliquot plated on to agar plates for detection on the following day. In the ANSR *Listeria* Right Now test, the entire collected contents of the swab were subjected to sample processing and testing on the same day.

No false negatives, false positives, or invalids were observed during this study. The evaluation determined that under the conditions employed in this study, the enrichment-free *Listeria* Right Now method is as sensitive as the enrichment-based culture reference method for detection of *L. monocytogenes* on a stainless steel surface.

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