Listeria Right Now™

An enrichment-free environmental monitoring tool for *Listeria* detection providing actionable results in less than 60 minutes.
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. 1 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 re-testing
- 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.”
Neogen validation data

Listeria with and without background organisms on different surfaces:

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

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

Organisms Tested:

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

Method: Inoculated directly onto swab
NSF International study—applied research center

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

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

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 LRN 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/Conclusion

The purpose of this study was to evaluate the performance of the ANSR *Listeria Right Now* (LRN) 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.

foodsafty@neogen.com • foodsafety.neogen.com
ANSR *Listeria* Right Now is a complete system

System components: Item No. 9837
- 16-well, isothermal polymerization instrument
- ANSR *Listeria* Right Now detection software
- Three (3) heater blocks
- Vortex

Each assay kit contains: Item No. 9873
- 96 reaction tubes with internal positive control
- 96 environmental sampling swabs
- All necessary components

Simple and easy to use

1. Swab surface
2. Express swab with lysis buffer
3. Transfer solution to cluster tube and place in first heater block
4. Transfer tubes to second heater block
5. Transfer solution to reagent tubes, cap and place in reader. Hit “start”

Vectoring

The process of identifying the sources and flow of environmental pathogens is often called “vectoring.” This process typically involves a physical examination of an identified source and its surrounding area as well as specific processes such as the food production and sanitation efforts involved in the area. Through a structured series of microbiological samples, sources and vectors of contamination can be identified and any needed actions can be implemented. Because of the quick time to result of ANSR *Listeria* Right Now and its relative low cost, vectoring is easier without the need for external resources.
Neogen’s Food Safety Division
From easy-to-use lateral flow tests for numerous contaminants, to DNA-definitive assays for pathogens, Neogen offers testing products, expertise, service and support for the food industry.

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• Dehydrated culture media

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Global Headquarters:
800/234-5333 or 517/372-9200
foodsafety@neogen.com
foodsafty.neogen.com

Europe:
+44 1292 525 628
contact_uk@neogeneurope.com
Europe, Middle East and Africa Division:
+44 1292 526 093
contact_emea@neogeneurope.com
www.neogeneurope.com

Brazil:
+55 19 3935 3727
info@neogendobrasil.com.br
www.neogendobrasil.com.br

Latin America:
+52 55 5254 8235
informacion@neogenlac.com
www.neogenlac.com

China:
+86 21 6271 7013
info@neogenchina.com.cn
www.neogenchina.com.cn

India:
+91 484 230 6598
info@neogenindia.com
www.neogenindia.com

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