

Recovering *Listeria* from a 12"x12" vs 4"x4" Surface Area with the InSite *Listeria Species* Environmental Swab

Introduction

The following study was performed by Hygiena™ to evaluate the performance of the InSite *Listeria Species* swabs for recovering *Listeria* from 12"x12" and 4"x4" environmental surfaces. In this study, stainless steel and plastic surfaces were artificially inoculated with *L. monocytogenes* then tested using the InSite *Listeria species* swabs. Results of each method were compared against one another using POD for each surface area.

The results of this study demonstrate that recovery of *Listeria* from plastic or stainless steel surfaces are equivalent regardless of the surface area sampled.

Equipment, Supplies and Reagents

- InSite *Listeria species* (ILC100)
- Dry Block Incubator (INCUBATOR)
- Hygiena Swab Tube Block-12 wells (IB003)
- Brain Heart Infusion (BHI) broth
- Modified Oxford (MOX) agar

Sample Preparation and Enrichment

L. monocytogenes (ATCC 19111) was selected to artificially inoculate stainless steel and plastic surfaces. The organism was grown overnight by suspending a single colony into 8mL of BHI broth and incubating at 35°C. Cultures were serially diluted in BHI broth to a dilution level appropriate for achieving fractional positive results. Ten stainless steel and ten plastic surfaces per surface area were inoculated with less than 10 CFU to serve as fractionally spiked samples. Inoculations were spread evenly across each surface using a sterile cell spreader. Surfaces were swabbed five times horizontally and five times vertically before incubating the swabs at 37°C for 48 hours.

Method

For the InSite *Listeria* species swab, instructions were followed according to the kit insert. After swabbing each surface, devices were activated by breaking the snap valve and squeezing the bulb expelling the broth down into the tube. Devices were incubated at 37°C for 48 hours, the color observed, and the result recorded. All samples, regardless of result, were confirmed with the reference culture method.

Results and Discussion

The results for the recovery of *Listeria* from stainless and plastic surface areas are summarized in Table 1. For stainless steel surface samples, InSite returned 6 positives when swabbing 4"x4" surfaces and 5 positives when swabbing 12"x12" surfaces. For plastic surface samples, InSite returned 7 positives when swabbing 4"x4" surfaces and 7 positives when swabbing 12"x12" surfaces. All presumptive results from InSite corresponded with the confirmation results from the reference culture method.

Table 1. <i>Listeria</i> Recovery from 4"x4" vs 12"x12" Stainless Steel and Plastic Surface Areas						
Sample Type	Strain	Surface Area	Inoculation CFU/surface	Test Portions	Presumptive Positive	Culture Positive
Stainless Steel	L. monocytogenes ATCC19111	4"x4"	6	10	6	6
		12"x12"	6	10	5	5
Plastic	L. monocytogenes ATCC19111	4"x4"	6	10	7	7
		12"x12"	6	10	7	7

The probability of detection (POD) was used as a statistical metric to summarize the results of this study in table 2. Recovering *Listeria* from stainless steel surface areas 4"x4" or 12"x12" showed no statistically significant difference (95% CL contains zero). Recovering *Listeria* from plastic surface areas 4"x4" or 12"x12" showed no statistically significant difference (95% CL contains zero).

Table 2. Comparative Method Results for Recovery of *Listeria* from 4"x4" vs 12"x12" Surface Areas

Sample Type	Inoculation CFU/swab	Test Portions	4"x4" Surface			12"x12" Surface			dPOD ^c	95% CI ^d
			X	POD ^a	95% CI	X	POD ^b	95% CI		
Stainless Steel	6	10	6	0.6	(0.31, 0.83)	5	0.5	(0.24, 0.76)	0.1	(-0.29, 0.45)
Plastic	6	10	7	0.7	(0.40, 0.89)	7	0.7	(0.40, 0.89)	0	(-0.36, 0.36)

X = Number of positive test portions

^aPOD = Number of confirmed results from 4"x4" surface positive results divided by the total number of samples

^bPOD = Number of confirmed results from 12"x12" surface positive results divided by the total number of samples

^cdPOD = Difference in recovery between 4"x4" and 12"x12" surface POD values

^d95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Conclusions

The results of this study demonstrate that with proper swabbing technique the recovery of *Listeria* from stainless steel or plastic is equivalent regardless of surface area sampled.