Performance Evaluation of InSite *Listeria* (IL050/IL100) and New InSite *Listeria* (ILC050/ILC100)

Introduction

InSite *Listeria* is a screening test for *Listeria* spp., intended for the use on food contact surfaces and food processing equipment after cleaning to detect the presence of *Listeria* species. Insite Listeria is a self-contained swab device containing a selective growth medium to inhibit most non-*Listeria* microorganisms and provide nutrients to support growth of *Listeria*. Indicator compounds turn broth from yellow to black utilizing B-glucosidase enzyme production produced by *Listeria* species. A darkening of the media to grey/black after 24-48 hours at 37°C indicates a presumptive positive result for *Listeria* spp.

A new InSite *Listeria* Species device has been developed to improve selectivity reducing the proportion of presumptive positives from non-Listeria that can survive and grow in the current InSite *Listeria*. This study was designed to evaluate the performance of detecting *Listeria* species as well as selectivity against non-*Listeria* species.

Equipment, Supplies and Reagents

- InSite *Listeria*- IL050
- New InSite *Listeria* – ILC050
- Hygiena™ Digital Dry Block Incubator for maintaining temperatures at 37 ± 2°C – INCUBATOR2
- Hygiena Swab Tube Block for Model No: INCUBATOR2 – IB001
- Brain heart infusion broth (BHI)
- Buffered Peptone Water (BPW)

Table 1. Inclusivity Panel for *Listeria* species

Sample No.	Genus species
1	*L. monocytogenes* ATCC 19115
2	*L. monocytogenes* ATCC 19118
3	*L. monocytogenes* ATCC 7644
4	*L. monocytogenes* ATCC BAA-751
5	*L. monocytogenes* ATCC 13932
6	*L. innocua* NCTC 11288
7	*L. innocua* ATCC 33090
8	*L. welshimeri* ATCC 35897
9	*L. seeligeri* ATCC 35967
10	*L. ivanovii* ATCC 19119

Table 2. Exclusivity Panel for Non-*Listeria* species [a]			
Sample No.	**Genus species**	**Sample No.**	**Genus species**
1	*Klebsiella pneumoniae*	13	Unknown ID #1
2	*Bacillus licheniformis*	14	*Bacillus licheniformis*
3	*Pseudomonas fragi*	15	*Proteus vulgaris*
4	*Bacillus licheniformis*	16	Unknown ID #2
5	*Enterococcus faecalis*	17	*Bacillus pumilus*
6	*Carnobacterium maltaromaticum*	18	*Proteus vulgaris*
7	*Carnobacterium maltaromaticum*	19	*Bacillus pumilus*
8	*Bacillus pumilus*	20	*Enterococcus faecalis*
9	*Micrococcus luteus*	21	*Streptomyces cinnamoneus*
10	*Streptomyces cinnamoneus*	22	*Carnobacterium maltaromaticum*
11	*Klebsiella pneumoniae*	23	*Bacillus pumilus*
12	*Bacillus licheniformis*	24	*Bacillus pumilus*

[a] Confirmed Non-*Listeria* Organisms Isolated from Industrial Environmental samples. ID using Hygiena RiboPrinter® System

Sample Preparation and Enrichment

Inclusivity Panel – Inclusivity cultures were enriched by adding a colony from a TSA plate to BHI broth for 24 hours producing an overnight culture. Each culture was then serially diluted 1:10 to produce a concentration between 10^3-10^4CFU/mL. 100 µL were spread onto TSA plates to perform a purity check and confirm final CFU levels. For each suspension 10µL was pipetted onto the top of duplicate swabs producing a concentration of 10^1-10^2 CFU per swab. Devices were activated and incubated at 37°C for 48 hours.

Exclusivity Panel – Exclusivity cultures were enriched by adding a colony from a TSA plate to BHI broth for 24 hours producing an overnight culture. Each Culture was then serially diluted 1:10 to -1, -2, and -3. 100 µL were spread onto TSA plates to perform a purity check and confirm final CFU levels. For each suspension neat to -3, 10µL was pipetted onto the top of swab buds. Devices were activated and incubated at 37°C for 48 hours.

Method

For each device, the media was visually inspected for color change from yellow/amber to black/grey after 48hrs. Results were recorded as presumptive positive or negative for all samples. All samples were confirmed according to BAM chapter 10, Detection of *Listeria* monocytogenes in Foods and Environmental Samples (2).

Results and Discussion

InSite *Listeria* and New InSite *Listeria* devices demonstrated a presumptive positive color change for all Listeria species tested (Table 3). This is in line with the historical AOAC RI certification for InSite media and the AOAC RI certification of New InSite *Listeria* (1,3).

The exclusivity results of InSite *Listeria* and New InSite *Listeria* were compared using probability of detection (POD) and difference in POD (dPOD) (Table 4). Of the non-*Listeria* samples tested, both Insite *Listeria* and New InSite *Listeria* detected non-*Listeria* species at a CFU per swab greater than 1000 CFU per swab. As the concentration of non-*Listeria* species increased the presumptive positive POD increases for both devices. The statistical analysis demonstrated a significant difference between the two tests when each test contained greater than 10^3 CFU per swab.

Table 3. Inclusivity Results IL50 vs ILC050 [bc]

Sample No.	Genus species	InSite Listeria (IL50) Result [a]	New InSite Listeria (ILC050) Result [a]
1	*L. monocytogenes* ATCC 19115	+	+
2	*L. monocytogenes* ATCC 19118	+	+
3	*L. monocytogenes* ATCC 7644	+	+
4	*L. monocytogenes* ATCC BAA-751	+	+
5	*L. monocytogenes* ATCC 13932	+	+
6	*L. innocua* NCTC 11288	+	+
7	*L. innocua* ATCC 33090	+	+
8	*L. welshimeri* ATCC 35897	+	+
9	*L. seeligeri* ATCC 35967	+	+
10	*L. ivanovii* ATCC 19119	+	+

[a] Chromogenic color change result

[b] 10^1-10^2 CFU per swab

[c] N=2 for each sample

Table 4. Exclusivity Results IL050 vs ILC050										
Sample Type	**CFU/Test Portion**	**N**	**InSite *Listeria* (IL050)**			**New InSite *Listeria* (ILC050)**			**dPOD**	**95% CI**
			X	**POD_{IL}**	**95% CI**	**X**	**POD_{ILC}**	**95% CI**		
Non-Listeria Species	$3.8x10^7$	4	4	1.00	0.51, 1.00	1	0.25	0.00, 0.70	0.75	0.51, 0.30
	$4.5x10^6$	11	9	0.82	0.52, 0.95	4	0.36	0.15, 0.65	0.45	0.37, 0.30
	$4.0x10^5$	19	13	0.68	0.46, 0.85	4	0.21	0.09, 0.43	0.47	0.38, 0.41
	$4.0x10^4$	24	12	0.50	0.31, 0.69	2	0.08	0.02, 0.26	0.42	0.29, 0.43
	$4.1x10^3$	20	6	0.30	0.15, 0.52	2	0.10	0.03, 0.30	0.20	0.12, 0.22
	$3.6x10^2$	13	0	0.00	0.00, 0.23	0	0.00	0.00, 0.23	0.00	0.00, 0.00
	$2.7x10^1$	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	0.00, 0.00

CFU/Test portion = Average CFU per swab
N = Number of test portions
X = Number of positive test portions
POD_{IL} = IL50 method positive results divided by the total number of test portions
POD_{ILC} = ILC050 method positive results divided by the total number of test portions
dPOD = Difference between IL50 and ILC050 POD values
95% CI = If the confidence interval of dPOD does not contain zero, then the difference is statistically significant at the 5% level

Conclusions

The results of this study demonstrate that the overall performance of the new InSite *Listeria* (ILC050) outweighs the performance of InSite *Listeria* (IL050). Both devices were able to detect a variety of different *Listeria* species within 48 hours of enrichment. New InSite *Listeria* was able to better suppress the growth of a wide concentration of Non-*Listeria* per swab resulting in fewer presumptive positives. This increased selectivity reduced the number of presumptive positives from non-*Listeria* that would ultimately confirm negative when further confirmation procedures are followed.

References

1. Calderon, D., Familiari, N., and Meighan, P., InSite *L. mono* Glo for Detection of *Listeria* species and Listeria monocytogenes from Environmental Surfaces, AOAC® *Performance Tested*℠ certification number 121902
2. U.S. Food and Drug Administration (2017) *FDA Bacteriological Analytical Manual,* Chapter 10, Detection of *Listeria monocytogenes* in Foods and Environmental Samples, and Enumeration of *Listeria monocytogenes* in Foods
3. Yurttas, H.C., Maher,J.,Danter, W., Bargoo, L., Brown, M., Olstein, A., and Feirtag, J., Evaluation of the PDX-LIB, AOAC® *Performance Tested*℠ certification number 040501

keydiagnostics

T: 02 8212 4074 F: 02 9423 6992
info@keydiagnostics.com.au
www.keydiagnostics.com.au
PO Box 1038, Gymea, NSW, 2227