Simultaneous Detection of *Listeria* Species and *Listeria monocytogenes* with Hygiena InSITE *L. mono* Glo for Environmental Monitoring

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Introduction

This study introduces a new test kit, Hygiena InSITE *L. mono* Glo, for the simultaneous detection of *Listeria* species and *Listeria monocytogenes* from surfaces using a combination of chromogenic and fluorogenic biochemistries in a self-contained device. InSITE *L. mono* Glo provides qualitative results for low and high levels of *Listeria* in 24 to 48 hours.

Purpose:

To demonstrate the efficiency and ease of use of InSITE *L. mono* Glo. The device will make the determination from clean sanitised surfaces where residual *Listeria* are present.

Methods:

Stainless steel coupons were spiked with serial dilutions of 20 *Listeria* species which included 10 *Listeria monocytogenes* species. The coupons were allowed to dry overnight at room temperature and then swabbed with the device. The incubation period was run at 37˚C for 24, 30 and 48 hours and the color intensity from the chromogenic substrate measured with concurrent indication of *monocytogenes* using a small inexpensive UV black light. Both 4" x 4" and 12" x 12" squares were swabbed to compare recovery from both areas.

Table 1. Direct Inoculation PoD% (n=10)

Mean CFU per 10uL[a]	PoD% 24 hour BLACK	PoD% 24 hour GLO	PoD% 30 hours BLACK	PoD% 30 hour GLO	PoD% 48 hour BLACK	PoD% 48 Hours Glo
10^7-10^8	100	100	100	100	100	100
10^6-10^7	100	100	100	100	100	100
10^5-10^6	100	100	100	100	100	100
10^4-10^5	83	100	100	100	100	100
10^3-10^4	83	75	100	100	100	100
10^2-10^3	60	50	100	100	100	100
10-10^2	33	0	83	75	83	100
1-10	0	0	12	0	12	25
<1	0	0	0	0	0	0

Table 2. 12" x 12" Surface PoD% (n=10)

Mean CFU per 1000uL[c]	PoD% 24 hour BLACK	PoD% 24 hour GLO	PoD% 30 hours BLACK	PoD% 30 hour GLO	PoD% 48 hour BLACK	PoD% 48 Hours Glo
10^7-10^8	100	100	100	100	100	100
10^6-10^7	100	100	100	100	100	100
10^5-10^6	100	100	100	100	100	100
10^4-10^5	66	75	100	100	100	100
10^3-10^4	50	50	83	75	100	100
10^2-10^3	33	50	83	50	100	100
10-10^2	16	0	50	75	83	75
1-10	0	0	16	25	50	50
<1	0	0	0	0	0	0

Table 3. 4" x 4" Surface PoD% (n=10)

Mean CFU per 1000uL[c]	PoD% 24 hour BLACK	PoD% 24 hour GLO	PoD% 30 hours BLACK	PoD% 30 hour GLO	PoD% 48 hour BLACK	PoD% 48 Hours Glo
10^7-10^8	100	100	100	100	100	100
10^6-10^7	100	100	100	100	100	100
10^5-10^6	100	100	100	100	100	100
10^4-10^5	100	100	100	100	100	100
10^3-10^4	66	75	100	100	100	100
10^2-10^3	50	25	100	100	100	100
10-10^2	16	25	66	50	100	100
1-10	0	0	12	25	50	25
<1	0	0	0	0	12	25

Results:

At 24 hours incubation dilutions, -1 through -7 were detected chromogenically at serial decreasing CFU levels from (-1) 2.8 x 10^8 CFU down to (-7) 222 CFU. The fluorescent detection of *Listeria monocytogenes* at 24 hours was from -1 down to -6th dilution at a lower mean of 2221 CFU. At 30 hours the detection limit of the test for both chromogenic and fluorometric detection dropped to 25 CFU and at 48 hours the detection level dropped to <10 CFU for both chromogenic and fluorometric detection. The Probability of Detection was also calculated at each dilution level with higher dilution levels for both species and *monocytogenes* easily detected at 24 hours and single or <10 CFUs detected at either 30 or 48 hours, depending on stress level.

Significance:

InSITE L. mono Glo is designed to make detection from surfaces easier for food manufacturers and food service retailers by combining both species and monocytogenes confirmation in one easy-to-use, rapid interpretation device. As Listeria metabolises the substrates a colour change of media will occur to indicate the presence of Listeria spp. To evaluate the presence of pathogenic Listeria, fluorescence of the test must be viewed under a simple, handheld, low-power UV light that illuminates the vivid, green fluorescence bound to the plastic test tube.

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