



One Health Diagnostics™

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INTRODUCTION:

MicroSnap® Surface Express *Listeria* and *L. mono* GLO is a screening test for *Listeria* species differentiating between *Listeria monocytogenes* by green fluorescence, intended to be used for environmental monitoring in food processing environments. Bioluminescent output from the media is considered presumptive positive for *Listeria* species when the predetermined threshold is reached at any time point between 12 and 24 hours and the green fluorescence of the media confirms presence of *L. mono* in bioluminescence positive devices.

MicroSnap Surface Express *Listeria* and *L. mono* Glo tests contain a proprietary formula of real-time viability substrate, antibiotics, growth enhancers and bioluminescent substrate compounds. Antibiotics inhibit most non-*Listeria* microorganisms while growth enhancers provide recovery nutrients to support growth of sub-lethally injured *Listeria*. Substrate compounds produce an intense bioluminescent output by *Listeria* species. Verification of *L. mono* is shown by green fluorescence under UV light due to presence of the diagnostic enzyme, phospholipase C.

BAX® System Q7

Powerful and precise molecular pathogen detection



PURPOSE:

This study aims to evaluate how many CFU are needed to obtain a positive result (>30 RLU) in MicroSnap Surface Express *Listeria* and *L. mono* GLO at 14 hours of incubation. Additionally, it was important to assess the possibility of interference of the MicroSnap Surface Express broth with the BAX® System PCR chemistry. Moreover, a vital question to answer is whether BAX System can detect *Listeria* faster than MicroSnap Surface Express, and can it be used as a confirmation step for positive and/or negative MicroSnap Surface Express *Listeria* and *L. mono* GLO results.

REGISTERED TRADEMARKS:

BAX® is a registered trademark of Hygiena for its line of equipment, reagents and software used to analyze samples for microbial contamination.

EnSURE® is a registered trademark of Hygiena.

MicroSnap® is a registered trademark of Hygiena.

Direct BAX® System Q7 PCR Confirmation of Presumptive Positive Results after Rapid Screening for *Listeria* with MicroSnap® Surface Express *Listeria* and *L. mono* GLO Devices

BAX® System Q7

BAX® System X5

foodproof®

microproof®

METHODS:

Overnight cultures of *Listeria* spp. were prepared from a single colony of pure culture in TSB and incubated at 37 °C and serially diluted in MRD to achieve levels 10⁴-10² CFU/ml. 10 µL of each dilution was spiked on the swab of MicroSnap Surface Express *Listeria* and *L. mono* GLO device in five replicates per dilution. This was repeated with 4 sets of swabs, each set read at one time point. 24 LEB broth was used as a reference, 3 mL was inoculated with 10 µL of each dilution level of *Listeria* in five replicates per dilution. Readings of the swabs were taken at 12, 14, 16 and 22 hours of incubation in EnSURE® Touch. Vials with the 24 LEB broth were visually observed for turbidity. Aliquots of each method at each time point were taken and processed using the BAX System Q7 ready reference method for Real-Time Genus *Listeria* and *L. mono* kits and 24E Genus *Listeria* and *L. mono* kits.

Organism	NCTC	BAX Q7 KIT
<i>Listeria grayi</i> ssp. <i>grayi</i>	10815	2019, 2003
<i>Listeria innocua</i>	11288	2019, 2003
<i>Listeria ivanovii</i> ssp. <i>londoniensis</i>	12701	2019, 2003
<i>Listeria welshimeri</i>	11857	2019, 2003
<i>Listeria seeligeri</i>	11289	2019, 2003
<i>Listeria monocytogenes</i>	10357	2019, 2003, 2005, 2002
<i>Listeria monocytogenes</i>	13372	2005, 2002
<i>Listeria monocytogenes</i>	7973	2005, 2002

Table 1. Demonstrates which organisms were tested in which BAX System Kit. BAX System kits listed by product code: KIT2019 Real-Time Genus *Listeria*, KIT2003 Genus *Listeria* 24E, KIT2005 Real-Time *L. mono*, KIT 2002 *L. mono* 24E.

DISCUSSION:

Overall, the assay worked well in answering the aims that were set out. In the majority of *Listeria* tested, detection occurred at 16 hours. There were a few species where a signal appeared at 14 hours although the inoculum was 10² CFU/10 µL. However, this was not true of all species. Some species with a higher inoculum were only detected after 16 or even 22 hours. This is likely due to the metabolism of the particular microorganism and its readiness to use the substrate in the broth of the MicroSnap Surface Express device. In almost all cases, the BAX System Q7 assays immediately detected *Listeria* after 12 hours of enrichment. This is unsurprising as PCR methods are very sensitive. The claim is that the BAX System Q7 *Listeria* kits are able to detect 10⁴ CFU after enrichment which equates to 50 cells spiked into the lysis buffer¹. Initially, the concern was that the MicroSnap Surface Express *Listeria* and *L. mono* GLO broth may inhibit the PCR reaction; however, this was confirmed to not be the case. *Listeria grayii* NCTC10815 was the only one not to be detected using the BAX System Q7 assay, but this had already been observed in product development and an AOAC validation study².

The results are indicative that the BAX System Q7 *Listeria* kits can easily be used to confirm positive or negative MicroSnap Surface Express *Listeria* and *L. mono* GLO results, without fear of chemistry interference. This method has a big advantage over the standard ISO detection method which takes up to 96 hours to confirm presence or absence of *Listeria*. With the MicroSnap Surface Express *Listeria* and *L. mono* GLO/ BAX System Q7 method, confirmatory results can be obtained in as little as 20 hours.

REFERENCES:

1. BAX System Q7 (package insert) Hygiena LLC, Camarillo California, (2012). Accessed (2022); [ins-bax-q7-assay-listeria-monocytogenes-rt.pdf \(brandfolder.io\)](#)
2. Wallace, Morgan, Mrozinski, Peter, Tice, George, Andaloro, Bridget, White, Kirk, Davis, Eugene and Wagner, Winona., Evaluation of the DuPont™ BAX System Test Kit for Detection of *Listeria* spp. From Food and Environmental Surfaces, AOAC-RI *Performance Tested*SM Certification Number 050903.

RESULTS:

GENUS LISTERIA/L.MONO 24E KITS															
<i>L. welshimeri</i> NCTC 11857				<i>L. seeligeri</i> NCTC 11289				<i>L. grayii</i> NCTC 10815				<i>L. innocua</i> NCTC 11288			
Time Point	Spiked	MSX /5	PCR /5	Time Point	Spiked	MSX /5	PCR /5	Time Point	Spiked	MSX /5	PCR /5	Time Point	Spiked	MSX /5	PCR /5
12 Hours	6	0	5	12 Hours	161	5	5	12 Hours	20	0	0	12 Hours	790	0	5
	0.6	0	4		16.1	0	5		2	0	0		79	0	5
14 Hours	0.06	0	2	14 Hours	1.6	0	1	14 Hours	0.2	0	0	14 Hours	8	0	3
	6	0	5		161	4	5		20	0	0		790	2	5
16 Hours	0.6	0	5	16 Hours	16.1	4	5	16 Hours	2	0	0	16 Hours	79	0	5
	0.06	0	1		1.6	0	3		0.2	0	0		8	0	2
22 Hours	6	5	5	22 Hours	161	5	5	22 Hours	20	0	0	22 Hours	790	5	5
	0.6	4	4		16.1	5	5		2	0	0		79	3	5
1(a)	0.06	3	3	1(b)	1.6	2	2	1(c)	0.2	0	0	1(d)	8	0	3

<i>L. ivanovi</i> NCTC 12701				<i>L. monocytogenes</i> NCTC 10357				<i>L. monocytogenes</i> NCTC 13372				<i>L. monocytogenes</i> NCTC 7973			
Time Point	Spiked	MSX /5	PCR /5	Time Point	Spiked	MSX /5	PCR /5	Time Point	Spiked	MSX /5	PCR /5	Time Point	Spiked	MSX /5	PCR /5
12 Hours	137	0	5	12 Hours	30	0	5	12 Hours	227	0	5	12 Hours	653	0	5
	14	0	5		3	0	5		23	0	5		65	0	5
14 Hours	1	0	1	14 Hours	0.3	0	2	14 Hours	2	0	3	14 Hours	7	0	0
	137	0	5		30	0	5		227	0	5		653	0	5
16 Hours	14	0	5	16 Hours	3	0	5	16 Hours	23	0	5	16 Hours	65	0	5
	1	0	1		0.3	0	3		2	0	3		7	0	4
22 Hours	137	2	5	22 Hours	30	5	5	22 Hours	227	5	5	22 Hours	653	5	5
	14	0	5		3	0	5		23	1	5		65	0	5
1(e)	1	0	4	1(f)	0.3	0	1	1(g)	2	0	4	1(h)	7	0	5
	137	5	5		30	5	5		227	5	5		653	5	5
	14	5	5		3	5	5		23	5	5		65	5	5
	1	0	0		0.3	0	2		2	5	5		7	3	5

GENUS LISTERIA/L.MONO Real-Time KITS															
<i>L. welshimeri</i> NCTC 11857				<i>L. seeligeri</i> NCTC 11289				<i>L. grayii</i> NCTC 10815				<i>L. innocua</i> NCTC 11288			
Time Point	Spiked	MSX /5	PCR /5	Time Point	Spiked	MSX /5	PCR /5	Time Point	Spiked	MSX /5	PCR /5	Time Point	Spiked	MSX /5	PCR /5
12 Hours	40	0	5	12 Hours	111	5	5	12 Hours	21	0	5	12 Hours	400	0	5
	4	0	5		11	0	5		2	0	5		40	0	5
14 Hours	0.4	0	3	14 Hours	1	0	2	14 Hours	0.2	0	0	14 Hours	4	0	4
	40	0	5		111	5	5		21	0	5		400	5	5
16 Hours	4	0	5	16 Hours	11	3	5	16 Hours	2	0	5	16 Hours	40	0	5
	0.4	0	2		1	0	2		0.2	0	2		4	0	3
22 Hours	40	5	5	22 Hours	111	5	5	22 Hours	21	1	5	22 Hours	400	5	5
	4	0	5		11	5	5		2	0	5		40	3	5
2(a)	0.4	0	3	2(b)	1	1	2	2(c)	0.2	0	0	2(d)	4	0	4
	40	5	5		111	5	5		21	5	5		400	5	5
	4	4	5		11	4	5		2	3	3		40	5	5
	0.4	1	1		1	1	2		0.2	1	1		4	2	4

<i>L. ivanovi</i> NCTC 12701				<i>L. monocytogenes</i> NCTC 10357				<i>L. monocytogenes</i> NCTC 13372				<i>L. monocytogenes</i> NCTC 7973			
Time Point	Spiked	MSX /5	PCR /5	Time Point	Spiked	MSX /5	PCR /5	Time Point	Spiked	MSX /5	PCR /5	Time Point	Spiked	MSX /5	PCR /5
13 Hours	74	0	5	12 Hours	40	0	5	12 Hours	108	0	5	12 Hours	460	0	5
	7	0	5		4	0	5		11	0	5		46	0	5
14 Hours	0.7	0	3	14 Hours	0.4	0	2	14 Hours	1	0	3	14 Hours	5	0	2
	74	0	5		40	0	5		108	0	5		460	0	5
16 Hours	7	0	5	16 Hours	4	0	5	16 Hours	11	0	5	15 Hours	46	0	5
	0.7	0	4		0.4	0	4		1	0	2		5	0	3
22 Hours	74	1	5	22 Hours	40	5	5	22 Hours	108	5	5	22 Hours	460	1	5
	7	0	5		4	0	5		11	1	5		46	0	5
2(e)	0.7	0	3	2(f)	0.4	0	0	2(g)	1	0	2	2(h)	5	0	5
	74	5	5		40	5	5		108	5	5		460	5	5
	7	5	5		4	5	5		11	5	5		46	5	5
	0.7	3	4		0.4	3	4		1	3	4		5	2	5

Table 1(a-h) and 2(a-h). Summary of the gathered results. The spiked column shows the number of CFU spiked in 10 µL on the swab, calculated from 100 µL TSA spread plates. The MicroSnap Surface Express (MSX) /5 column shows the number of "positive" swabs, out of 5 replicates, which produced signal above the pre-set 30 RLU threshold. PCR/5 is the number of positive wells from the PCR reaction, each well corresponds to the MicroSnap Surface Express swab. Tables numbered "1a" and "2a" are displaying the results of the same microorganism processed on two separate days; tables numbered #1 show the 24E kit results and tables numbered #2 show the real-time kit results.

The majority of *Listeria* were detected at 16 hours of incubation; however, the exact CFU at which this happened varied depending on the species of *Listeria*, with anything from 20 to 600 CFU/10 µL being observed.

There were a couple of exceptions. *L. seeligeri* NCTC 11289 was detected as early as 12 hours at 160 CFU/10 µL, 14 hours at 16 CFU/10 µL and 16 hours at 2 CFU/10 µL in the MicroSnap Surface Express device. *Listeria innocua* NCTC 11288 was detected at 14 hours at 790 CFU/10 µL and 16 hours at 79 CFU/10 µL in the MicroSnap Surface Express device.

All *Listeria* tested were detected as early as 12 hours at 10³ and 10² CFU/10 µL spike levels across all BAX System Q7 kits used.

Fractional detection occurred between 1-10 CFU/10 µL which pushed detection to 16 or 22 hours in most cases in both MicroSnap Surface Express devices and BAX System Q7 kits.

An anomaly was detected with *Listeria grayii* NCTC 10815 which did not produce a positive result in the BAX Q7 Genus *Listeria* 24E KIT2003.