

# Evaluation of two Real-Time BAX® PCR Assays for the Detection of Genus *Listeria* species and *Listeria monocytogenes*

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## INTRODUCTION:

Detection of *Listeria* species and *L. mono* in food/environmental samples is of increasing importance highlighted by rising numbers/severity of food safety recalls and outbreaks in recent years. According to the ProMED website, the 2017-18 South African listeriosis outbreak involving polony, a processed meat, was the worst foodborne illness outbreak ever recorded causing 180 deaths and over 1,000 infections.<sup>1</sup> Due to the ever-growing need to develop faster techniques to detect Genus *Listeria* and *L. mono* in the food supply, two real-time PCR assays were evaluated in a sensitivity and collaborative study.

## PURPOSE:

This study, performed by ADRIA labs (Quimper, France), aimed to evaluate two real-time PCR methods ability to detect Genus *Listeria* and *Listeria monocytogenes* in a broad range of foods and industrial production environmental samples to obtain AFNOR Certification according to NF Validation rules. Assay sensitivity, relative level of detection, inclusivity and exclusivity were assessed in pure culture and in food and environmental surface enrichments relative to ISO standard 16140-2:2016. Collaborative study was completed at a separate time.

## METHOD:

Lysates of artificially spiked and naturally contaminated food/environmental enrichments from 6 sample categories (n=442 *Listeria* sp.; n=462 *L. mono*) in 24 LEB Complete media were prepared (see Table 1). Test kit results were compared to independently spiked or naturally contaminated samples, enriched per ISO. 50 target and 30 non-target strains of Genus *Listeria* and *L. mono* were tested during inclusivity/exclusivity studies. The inclusivity portion tested 10 cells/225 mL of 24 LEB Complete; the exclusivity portion tested pure cultures at ≥10<sup>5</sup> CFU/ml.

Table 1. Distribution per tested Category and Type for *L. monocytogenes* study

Category		Type		Positive samples	Negative samples	Total
1	Composite foods / Ready-to-eat and ready-to-reheat	a	Ready-to-eat	16	10	26
		b	Ready-to-reheat	13	19	32
		c	Confectionaries, pastries and egg products	9	13	22
		Total		38	42	80
2	Meat Products	a	Raw products (frozen or fresh)	10	13	23
		b	Meat based products ready to reheat	12	11	23
		c	Raw and cooked delicatessen	18	22	40
		Total		40	46	86
3	Milks & Dairy products	a	Raw milk cheeses	8	12	20
		b	Other products based on raw milk	14	12	26
		c	Heat treated products	9	13	22
		Total		31	37	68
4	Vegetables	a	Raw products (fresh and frozen)	12	16	28
		b	Pre-cooked vegetables, vegetables under modified atmosphere	9	14	23
		c	RTE, RTRH	10	11	21
		Total		31	41	72
5	Seafood and fishery products	a	Raw products (fresh and frozen)	7	15	22
		b	Cured & smoked	25	17	42
		c	RTE, Ready to reheat	14	12	26
		Total		46	44	90
6	Environmental Samples	a	Dusts and Residues	9	11	20
		b	Cleaning and Process Waters	10	16	26
		c	Surface samples	12	8	20
		Total		31	35	66
Total protocol 1				174	206	380
Total protocol 2				43	39	82
All categories				217	245	462

## RESULTS:

Of the five food and one environmental categories tested in this unpaired study, the BAX® Real-Time L mono method showed 39 positive deviations (PD) and 37 negative deviations (ND) for the overall categories. (Table 2). The BAX® Real-Time Genus *Listeria* method showed 35 PD and 38 ND for the overall categories (Table 3).

For both methods, the (ND - PD) calculated values are lower or equal to the acceptability limits (AL) for each of the individual categories, and for the overall 6 categories. The Relative Levels of Detection (RLOD) are all lower than the AL fixed at 2.5 for the unpaired data study, whatever the matrix/strain pairs. The inclusivity and exclusivity testing gave the expected results for the 50 appropriate target strains and the 30 non-target strains.

## SIGNIFICANCE:

In 2016, there were 2555 confirmed Listeriosis case, with infants below 1 year and the elderly population over 64, have the highest rates of infection (1.3 and 1.6 per 100, 000 cases respectively).<sup>2</sup> These statistics is a reminder of how important it is to have a rapid and dependable food safety testing method in place to prevent these transmissions to our most vulnerable populations.

Table 2. Interpretation of results between BAX® RT *L. mono* and reference methods (based on confirmed alternative test results)

	Category	Type		PA	NA	PD	ND	PPND	PPNA
1	Composite foods / Ready-to-eat and ready-to-reheat	a	Ready-to-eat	6	9	4	6	0	1
		b	Ready-to-reheat	8	18	3	2	0	1
		c	Confectionaries, pastries and egg products	5	12	1	3	0	1
		Total		19	39	8	11	0	3
2	Meat Products	a	Raw products (frozen or fresh)	5	12	2	2	1	1
		b	Meat based products ready to reheat	9	11	3	0	0	0
		c	Raw and cooked delicatessen	15	22	2	1	0	0
		Total		29	45	7	3	1	1
3	Milks & Dairy products	a	Raw milk cheeses	5	12	1	2	0	0
		b	Other products based on raw milk	11	12	1	2	0	0
		c	Heat treated products	3	13	3	3	0	0
		Total		19	37	5	7	0	0
4	Vegetables	a	Raw products (fresh and frozen)	9	16	1	2	0	0
		b	Pre-cooked vegetables, vegetables under modified atmosphere	3	14	3	3	0	0
		c	RTE, RTRH	7	10	1	2	0	1
		Total		19	40	5	7	0	1
5	Seafood and fishery products	a	Raw products (fresh and frozen)	3	15	2	2	0	0
		b	Cured & smoked	20	17	5	0	0	0
		c	RTE, Ready to reheat	8	12	2	4	0	0
		Total		31	44	9	6	0	0
6	Environmental Samples	a	Dusts and Residues	8	11	1	0	0	0
		b	Cleaning and Process Waters	8	16	0	2	0	0
		c	Surface samples	8	8	4	0	0	0
		Total		24	35	5	2	0	0
Total protocol 1				106	201	32	35	1	5
Total protocol 2				35	39	7	1	0	0
All categories				141	240	39	36	1	5

PA- Positive Agreement  
NA- Negative Agreement  
PD- Positive Deviation  
ND- Negative Deviation  
PPND- Presumptive Positive/Negative Deviation  
PPNA- Presumptive Positive/Negative Agreement

Table 3. Interpretation of results between BAX® RT Genus *Listeria* and reference methods (based on confirmed alternative test results)

Category		Type		PA	NA	PD	ND	PPND	PPNA
1	Composite foods / Ready-to-eat and ready-to-reheat	a	Ready-to-eat	5	10	3	6	0	1
		b	Ready-to-reheat	6	14	4	3	0	1
		c	Confectionaries, pastries and egg products	4	11	2	2	0	1
		Total		15	35	9	11	0	3
2	Meat products	a	Raw products (frozen or fresh)	11	10	2	2	0	0
		b	Meat based products ready to reheat	9	10	3	0	0	0
		c	Raw and cooked delicatessen	15	14	0	2	0	0
		Total		34	35	5	4	0	0
3	Milk & dairy products	a	Raw milk cheeses	6	13	2	2	0	0
		b	Other products based on raw milk	8	10	0	2	0	0
		c	Heat treated products	7	10	1	2	0	0
		Total		21	33	3	6	0	0
4	Vegetables	a	Raw products (fresh and frozen)	8	11	1	2	0	0
		b	Pre-cooked vegetables, vegetables under modified atmosphere	5	11	4	4	0	0
		c	RTE, RTRH	6	12	2	0	0	0
		Total		19	34	7	6	0	0
5	Seafood and fishery products	a	Raw products (fresh and frozen)	5	14	1	2	0	0
		b	Cured & smoked	24	16	4	3	0	0
		c	RTE, Ready to reheat	10	11	1	4	0	0
		Total		39	41	6	9	0	0
6	Environmental Samples	a	Dusts and Residues	5	11	3	1	0	0
		b	Cleaning and Process Waters	10	15	1	1	0	0
		c	Surface samples	8	11	1	0	0	0
		Total		23	37	5	2	0	0
Total protocol 1				113	184	31	33	0	3
Total protocol 2				38	31	4	5	0	0
All categories				151	215	35	38	0	3

PA- Positive Agreement  
NA- Negative Agreement  
PD- Positive Deviation  
ND- Negative Deviation  
PPND- Presumptive Positive/Negative Deviation  
PPNA- Presumptive Positive/Negative Agreement

This study aimed to evaluate the performance the BAX® Real-Time Genus *Listeria* and BAX® Real-Time *L. monocytogenes* methods in a broad range of food and environmental samples. Both BAX® Real-Time *Listeria* assays fulfilled all the EN ISO 16140-2:2016 and AFNOR technical rules (Draft Revision 6) and were able to obtain AFNOR certification according to NF validation rules (QUA 18/09-01/19 and QUA 18/10-01/19).

1. <https://www.promedmail.org>  
2. [https://ecdc.europa.eu/sites/portal/files/documents/AER\\_for\\_2016-listeriosis.pdf](https://ecdc.europa.eu/sites/portal/files/documents/AER_for_2016-listeriosis.pdf)

