

An Optimized *Listeria* Enrichment Media for 18 Hour Enrichment

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ABSTRACT

Introduction: *Listeria monocytogenes* is a foodborne pathogen that has been isolated from a wide variety of food matrices. Due to its slow-growing nature, laboratory testing involves long enrichment times and/or secondary enrichments, and the organism can also be outcompeted by high levels of background organisms.

Purpose: To develop and evaluate a primary enrichment medium capable of selectively enriching *Listeria* spp. in less than 20 hours for use with molecular detection platforms.

Methods: The proposed medium was tested for inclusivity by inoculation with *Listeria monocytogenes* (n=18) and other *Listeria* spp. (n=16) isolates and incubating at 37°C. To test performance with contaminated foods, eight different food matrices (raw milk, smoked salmon, bleu cheese, frozen spinach, salami, powdered milk, frozen mixed berries, and raw milk cheese) were artificially contaminated with *L. monocytogenes* or *L. seeligeri*. Organisms were heat stressed at 56°C prior to spiking into the food matrices. Spiked matrices were diluted 1:10 with prewarmed (37°C) media and incubated at 37°C. Samples were pulled at 18 and 24 hours and tested using BAC Gene *Listeria* spp. and *Listeria monocytogenes* PCR test kits. Results were culturally confirmed following ISO 11290-1:2017.

Results: All strains of *L. monocytogenes* and other *Listeria* spp. grew in the new medium, while common competing Gram positive organisms, such as *Enterococcus* and *Bacillus* spp. were inhibited. For all food matrices tested, *L. monocytogenes* and *L. seeligeri* were detected by PCR at both 18 and 24 hours and confirmed by culture methods.

Significance: These results demonstrate that BAC Gro ULTRA™ *Listeria* Broth (BULB) is a selective enrichment medium capable of producing shorter enrichment times than any commercially available media, allowing food processors and testing laboratories to achieve faster turnaround times.

Table 1: Food Matrices Tested

Matrix	Enrichment Dilution	Organism(s) Tested	Sampling Timepoints
Smoked Salmon (25g)	1:10	<i>L. monocytogenes</i> <i>L. seeligeri</i> <i>L. ivanovii</i>	16h, 18h, 24h
Frozen Mixed Berries (125g)	1:10	<i>L. monocytogenes</i> <i>L. seeligeri</i> <i>L. ivanovii</i>	16h, 18h, 24h
Frozen Spinach (125g)	1:10	<i>L. monocytogenes</i> <i>L. seeligeri</i> <i>L. ivanovii</i>	16h, 18h, 24h
Bleu Cheese (25g)	1:10	<i>L. monocytogenes</i> <i>L. seeligeri</i>	18h, 24h
Raw Milk (25g)	1:10	<i>L. monocytogenes</i> <i>L. seeligeri</i>	18h, 24h
Raw Milk Cheese (25g)	1:10	<i>L. monocytogenes</i> <i>L. seeligeri</i>	16h, 18h, 24h
Milk Powder (25g)	1:10	<i>L. monocytogenes</i> <i>L. ivanovii</i>	18h, 24h
Salami (25g)	1:10	<i>L. monocytogenes</i> <i>L. seeligeri</i>	18h, 24h

INTRODUCTION

- Listeria* are ubiquitous in nature. *L. monocytogenes* is a human pathogen and poses a health hazard, especially to immunocompromised individuals and pregnant women
- Listeria* present a challenge in food production environments due to the ability to grow in environments ranging from pH of 5.0 – 9.6 and at refrigerated temperatures
- Reference methods for *Listeria* detection, such as FDA BAM¹ and ISO 11290², require 2-step enrichment protocols that require up to 48 hours of incubation
- Many rapid screening platforms use proprietary single-stage enrichments that shorten the detection time of *Listeria* to 24 hours
- BAC Gro ULTRA™ *Listeria* Broth (BULB) has demonstrated more rapid enrichment, while maintaining selectivity, and can produce PCR detection of multiple species and strains of *Listeria* in 18 hours or less.

Table 2: Inclusivity and Exclusivity Testing

Species	# Tested	# Grown	Species	# Tested	# Grown
<i>L. monocytogenes</i>	18	18	<i>E. faecalis</i>	2	0
<i>L. seeligeri</i>	3	3	<i>B. subtilis</i>	1	0
<i>L. innocua</i>	5	5	<i>B. cereus</i>	1	0
<i>L. grayi</i>	2	2	<i>S. aureus</i>	2	2
<i>L. ivanovii</i>	3	3	<i>E. coli</i>	2	0
<i>L. welshimeri</i>	3	3			
Total <i>Listeria</i>	34	34			

METHODS

INCLUSIVITY AND EXCLUSIVITY

- Inclusivity testing was performed by inoculating 10ml tubes of BULB with 10-100 cfu of *Listeria*; exclusivity testing was performed by inoculating 10ml tubes of BULB with ~10⁴ cfu of competing organisms; growth was assessed by turbidity at 18-24 hours
- Representative strains of *Listeria* and competing organisms were inoculated into 250µl of BULB, 24LEB, UVM, and Demi-Fraser (n=8 wells per media) and incubated on the MultiScan™ plate reader. OD₆₀₀ was read every 5 minutes for 24 hours to generate growth curves of organisms in each media

FOOD MATRIX TESTING

- Food matrices were purchased from retail sources and inoculated separately with *L. monocytogenes*, *L. seeligeri*, or *L. ivanovii* at approximately 10 cfu per sample. Prior to inoculation, organisms were heat stressed and stored for 24 hours at 4°C
- Inoculated samples were enriched 1:10 with pre-warmed BULB and incubated at 37° for up to 24 hours.
- At 16, 18, and 24 hours, aliquots of the enrichment were lysed and tested using Gold Standard Diagnostics' BAC Gene *Listeria* Multiplex kit, following kit instructions³
- In parallel, a separate set of samples were inoculated and enriched using the validated proprietary media per kit instructions
- All samples were culture confirmed according to ISO 11290

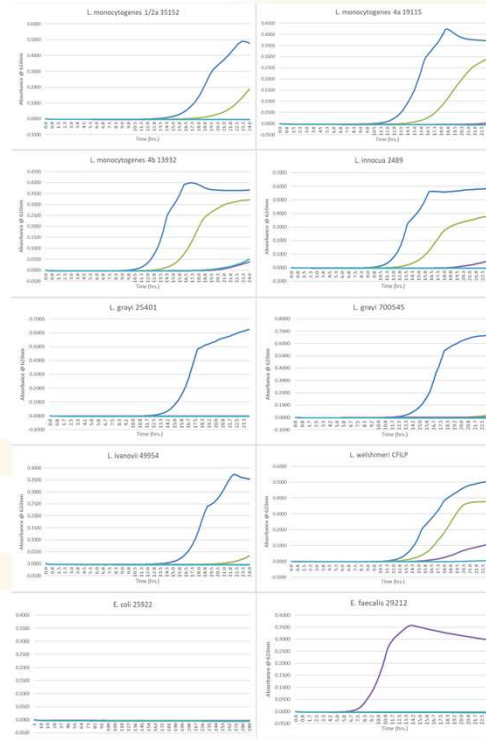


Figure 1: Growth curves of *Listeria* in various growth media

Table 3: *L. monocytogenes* PCR Results

Matrix	PCR Target	16 hr Cq Value		18 hr Cq Value		24 hr Cq Value		Culture	
		BULB	Control	BULB	Control	BULB	Control	BULB	Control
Smoked Salmon	L. mono	25.20	21.47	24.29	21.06	19.82		+	+
	L. spp.	22.49	19.31	21.97	18.72	17.42			
Frozen Salmon	L. mono	30.75	26.93	29.96	25.91	20.85		+	+
	L. spp.	27.94	23.89	27.19	22.15	18.29			
Frozen Spinach	L. mono	28.16	24.90	ND	21.85	ND		+	+
	L. spp.	25.76	22.72	38.85	19.38	35.27			
Frozen Berries	L. mono	ND	34.62	35.72	33.54	34.32		+	+
	L. spp.	37.93	32.05	33.96	30.98	31.34			
Raw Milk	L. mono		27.24	32.19	22.28	24.15		+	+
	L. spp.		25.18	29.72	20.01	21.68			
Milk Powder	L. mono		27.61	36.76	25.29	29.65		+	+
	L. spp.		24.96	32.97	22.56	27.17			
Bleu Cheese	L. mono		29.48	34.7	26.31	24.5		+	+
	L. spp.		27.79	33.1	23.35	21.89			
Raw Milk Cheese	L. mono		33.24	ND	25.18	35.59		+	+
	L. spp.		30.41	ND	22.61	32.81			

RESULTS

- BULB demonstrated 100% inclusivity for all *Listeria* strains tested (n=34) and inhibited all competitive organisms except for *S. aureus* which demonstrated weak growth at 24 hours
- Growth curves show BULB supports earlier log phase growth of *Listeria* for all isolates compared to both textbook (UVM and Demi-Fraser) and proprietary (24LEB) media; this difference is especially pronounced with *L. grayi* and *L. ivanovii*
- When used as an enrichment media with BAC Gene *Listeria* Multiplex PCR, BULB demonstrated improved detection at 18 hours compared to the current method (as shown by lower Cq values), and showed detection at 16 hours in all but one matrix (raw milk cheese) tested at that timepoint
- This data demonstrates that BULB has the potential to shorten enrichment times will providing the required sensitivity and specificity

Table 4: *L. seeligeri* PCR Results

Matrix	PCR Target	16 hr Cq Value		18 hr Cq Value		24 hr Cq Value		Culture	
		BULB	Control	BULB	Control	BULB	Control	BULB	Control
Smoked Salmon	L. spp.	20.20	17.55	18.48	14.30	15.62		+	+
Frozen Spinach	L. spp.	23.81	20.02	21.85	20.07	17.71		+	+
Frozen Berries	L. spp.	25.76	22.49	30.85	17.88	20.89		+	+
Raw Milk Cheese	L. spp.	30.99	33.09	31.74	32.45	30.44		+	+
Bleu Cheese	L. spp.		28.63	28.87	26.04	25.91		+	+
Raw Milk	L. spp.		30.68	NA	24.84	NA		+	-
Salami	L. spp.		35.89	NA	29.06	NA		+	-

Table 5: *L. ivanovii* PCR Results

Matrix	PCR Target	16 hr Cq Value		18 hr Cq Value		24 hr Cq Value		Culture	
		BULB	Control	BULB	Control	BULB	Control	BULB	Control
Smoked Salmon	L. spp.	27.47	25.43	30.44	20.30	23.54		+	+
Frozen Spinach	L. spp.	30.91	28.44	32.71	27.33	25.23		+	+
Frozen Berries	L. spp.	38.38	33.19	37.72	26.84	32.74		+	+
Milk Powder	L. spp.		29.07	32.38	20.49	26.38		+	+

CONCLUSIONS

- BAC Gro ULTRA™ *Listeria* Broth (BULB) demonstrates superior growth dynamics for all *Listeria* species tested compared to commercially available *Listeria* enrichment media
- When tested by the BAC Gene *Listeria* Multiplex PCR kit, samples enriched with BULB showed lower Cq values at 18 hours for nearly all matrices and strains tested when compared to the current validated medium
- At 16 hours, all inoculated samples tested except for *L. monocytogenes* in raw milk cheese, were detected. This suggests that with further optimization of enrichments, 16 hour enrichment prior to PCR may be possible

REFERENCES

- U.S. Food and Drug Administration. 2018. Bacteriological Analytical Manual On-line. 2018. Chapter 10, *Listeria monocytogenes*
- ISO 11290-1:2017 Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and *Listeria* spp. — Part 1: Detection method
- Instructions for Use, BAC Gro *Listeria* Multiplex. Gold Standard Diagnostics. V.3.10BX. 2023.

