Rapid multi-locus tNGS-based genotyping of *Listeria monocytogenes* by the Clear Safety™ automated platform to identify outbreak-relevant isolates

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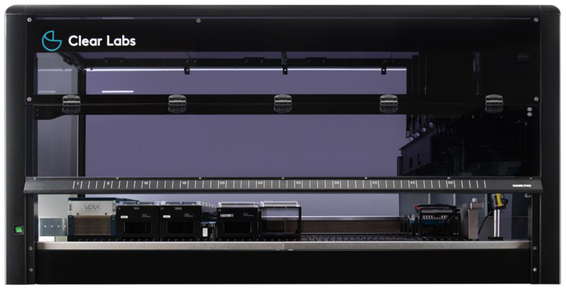
INTRODUCTION

Listeria monocytogenes is a prevalent food-borne pathogen and a leading cause of recalls in the food industry due to its severe public health risks and invasiveness in the production environment. Quickly tracking subtype patterns in *Listeria monocytogenes* is becoming increasingly necessary to get ahead of contamination events and potential outbreaks.

Current *Listeria* strain typing methods include Whole Genome Sequencing (WGS), Pulsed-field gel electrophoresis (PFGE), and Multiple loci Variable-number tandem repeat analysis (MLVA), and require colony isolation, experienced lab personnel, and several days to provide results.

The Clear Safety *Listeria* platform is an automated targeted Next Generation Sequencing (tNGS) platform which can amplify and sequence multiple loci in the *Listeria* genome to rapidly and easily speciate and strain type *Listeria* in isolates and real-world samples.

To assess the accuracy and ease of the Clear Safety *Listeria* product, *L. monocytogenes* strains associated with 3 distinct and impactful food-borne outbreaks were run on the platform amongst other samples representing similar but distinct subtypes, and different as controls.



METHODS

Culture Incubation and Preparation

Sixteen *Listeria* monocytogenes strains isolated from clinical and food sources related to three different outbreaks (Nova Scotia, 1981; Massachusetts, 1983; and California, 1985) and 9 ATCC control *Listeria spp.* strains were included in this study. All strains were sampled directly from glycerol stocks, grown in 2mL of Clear *Listeria* Media and incubated at 35°C overnight.

50µL of each overnight culture was added to 450µL of Buffer in duplicate and loaded onto the end-to-end automated system which performs cell lysis, PCR amplification of multiple *Listeria* loci, library preparation, and sequencing on an Oxford Nanopore Technologies (ONT) flow cell. Amplicon sequences were demultiplexed, quality-filtered, and mapped to a reference database through an automated workflow to confirm the species and strain type of the *Listeria* isolates.

Statistical Methods

Concordance between genotyping results and reference metadata provided at strain acquisition was evaluated using Cohen's Kappa statistic.

The Hunter-Gaston Discriminatory Index (HGDI) was used to quantify the probability that two randomly selected strains would be distinguishable based on their genotypes.

L. grayi was excluded from the statistical analyses as a *Listeria* species which is inherently difficult to type beyond the species level.

Table 1. Sample Concordance Counts Between Outbreak Collection Data and Clear Safety Listeria Results

		Clear Safety Results				
		Halifax 1981	Mass. 1983	Calif. 1985	Other	Total
Outbreak MetaData	Halifax 1981	10	0	0	0	10
	Mass. 1983	0	8	0	0	8
	Calif. 1985	0	0	12	0	12
	Other	2	2	0	14	18
	Total	12	10	12	14	48

Figure 1: Listeria spp. Tested with Metadata and Clear Safety Results

Random sample #	Genus	species	Source	Collection	Serotype	Clear Safety *Listeria* Rep 1	Clear Safety *Listeria* Rep 2
1	*Listeria*	*monocytogenes*	Clinical Isolate California 1985	USDA-ARS	4b	A	A
2	*Listeria*	*monocytogenes*	Clinical Isolate Throat Halifax, Nova Scotia 1981	USDA-ARS	4b	B	B
3	*Listeria*	*monocytogenes*	Poultry, England	Microbiologics	1/2a	D	D
4	*Listeria*	*monocytogenes*	Clinical Isolate, Massachusetts, 1983	USDA-ARS	4b	C	C
5	*Listeria*	*monocytogenes*	Clinical Isolate, Massachusetts, 1983	USDA-ARS	4b	C	C
6	*Listeria*	*grayi*	Standing corn stalks and leaves	Microbiologics	NA	N/A	N/A
7	*Listeria*	*seeligeri*	Soil	Microbiologics	NA	E	E
8	*Listeria*	*monocytogenes*	Spinal fluid from child with meningitis, Germany	Microbiologics	4b	C	C
9	*Listeria*	*monocytogenes*	Poultry, England	Microbiologics	4e	B	B
10	*Listeria*	*monocytogenes*	Jalisco Cheese, California 1985	USDA-ARS	4b	A	A
11	*Listeria*	*monocytogenes*	Clinical Isolate California 1985	USDA-ARS	4b	A	A
12	*Listeria*	*monocytogenes*	Clinical Isolate Stillborn, Halifax, Nova Scotia 1981	USDA-ARS	4b	B	B
13	*Listeria*	*monocytogenes*	Cole Slaw Halifax, Nova Scotia 1981	USDA-ARS	4b	B	B
14	*Listeria*	*monocytogenes*	Clinical Isolate, Massachusetts, 1983	USDA-ARS	4b	C	C
15	*Listeria*	*monocytogenes*	Raw Milk, Massachusetts, 1983	USDA-ARS	4b	F	F
16	*Listeria*	*welshimeri*	Decaying plant material	Microbiologics	6b	G	G
17	*Listeria*	*monocytogenes*	Human spinal fluid, Scotland	Microbiologics	2	H	H
18	*Listeria*	*monocytogenes*	Halifax, Nova Scotia 1981	USDA-ARS	4b	B	B
19	*Listeria*	*monocytogenes*	Clinical Isolate Vagina Halifax, Nova Scotia 1981	USDA-ARS	4b	B	B
20	*Listeria*	*monocytogenes*	Animal tissue, 1931	Microbiologics	4a	I	I
21	*Listeria*	*monocytogenes*	Clinical Isolate California 1985	USDA-ARS	4b	A	A
22	*Listeria*	*innocua*	Cow brain	Microbiologics	6a	J	J
23	*Listeria*	*monocytogenes*	Jalisco Cheese, California 1985	USDA-ARS	4b	A	A
24	*Listeria*	*monocytogenes*	Clinical Isolate, Massachusetts, 1983	USDA-ARS	4b	C	C
25	*Listeria*	*monocytogenes*	Clinical Isolate California 1985	USDA-ARS	4b	A	A

Key
Nova Scotia, 1981
Massachusets, 1983
California, 1985
Control Strains

Letter designations represent a longer assigned string which uniquely identifies a strain "Pattern"

RESULTS OVERVIEW

- The multi-locus tNGS genotyping method correctly identified the species of 25 *Listeria* isolates tested.
- For 24 of the *Listeria* isolates tested and typable by the system, a unique, identification number was assigned.
- Excellent concordance between outbreak collection metadata and genotyping data: Cohen's Kappa of 0.891.
- Moderately high discriminatory power of the methodology used: Hunter Gaston Discriminatory Index of 0.8369.

CONCLUSIONS

- This study highlights the capability of an end-to-end NGS platform to quickly and accurately distinguish between *Listeria* monocytogenes subtypes as well as at the species level.
- "Patterns" assigned to distinct genotypes can be leveraged to efficiently track the movement of *Listeria* contamination within a facility.

References

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For more information regarding Clear Safety™ *Listeria*, please contact us at inquiries@clearlabs.com

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