Clear Labs

WHITE PAPER

# Comparison of Clear Safety *Listeria* with Conventional Methodologies for *Listeria* Detection and Speciation

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## Introduction

Out of 47.8 million domestically acquired foodborne illnesses in the United States, *Listeria monocytogenes* is responsible for an estimated 1,600 illnesses and with a case mortality rate of 20%, about 260 deaths per year (1). *L. monocytogenes* is an opportunistic human foodborne pathogen that causes listeriosis in the elderly, newborn babies, unborn fetuses, immunocompromised hosts and patients suffering from viral or parasitic diseases or malignancies (2). *L. monocytogenes* is found in soil, water, and sewage as a saprophyte, and in the intestines of cattle and sheep. *Listeria* species also form biofilms that can persist in food-processing plants and contaminate food products (3). It has been reported that the presence of non-pathogenic *Listeria* species is considered an indicator for the *L. monocytogenes* species (4) and *Listeria* species detection should be included in robust environmental monitoring programs. Therefore, accurate identification of *L. monocytogenes* and *Listeria* species is important in mitigating foodborne outbreaks and for verifying the effectiveness of a pathogen control program.

The Clear Safety *Listeria* method uses automated, real-time next-generation sequencing (NGS) technology that combines DNA extraction and nucleic acid amplification with rapid sequencing. Automation reduces user error, increases repeatability and robustness, and reduces hands-on time to 0.5-1 hr, allowing technicians to perform other tasks. The Clear Safety *Listeria* method is designed for *Listeria* spp. detection, identification of *Listeria* species and Similarity Analysis® of *Listeria* strains for tracking *Listeria* in food and environmental samples (5). Clear Safety *Listeria* offers advanced high-throughput DNA sequencing technology enabling the generation of millions of sequences to simultaneously detect multiple genetic markers, allowing for *Listeria* detection, species identification and Similarity Analysis® in a single assay. The built-in redundancy that comes with detecting multiple gene targets improves accuracy and substantially reduces the chances of false negative and false positive results.

Clear Safety *Listeria* also has the ability to differentiate between live and dead target cells, which can mitigate false positives due to dead-cell DNA amplification in PCR/LAMP based methods. Real-time *Listeria* detection, speciation, and specifically Similarity Analysis® can more reliably track sources of contamination and the spread of *Listeria* through facilities, to assist customers in developing and implementing data-driven *Listeria* species mitigation strategies and amendments in Good Manufacturing Practices. The Clear Safety *Listeria* assay offers high confidence detection from primary enrichment including detection, speciation and Similarity Analysis™ without the need for colony isolation.

## Design

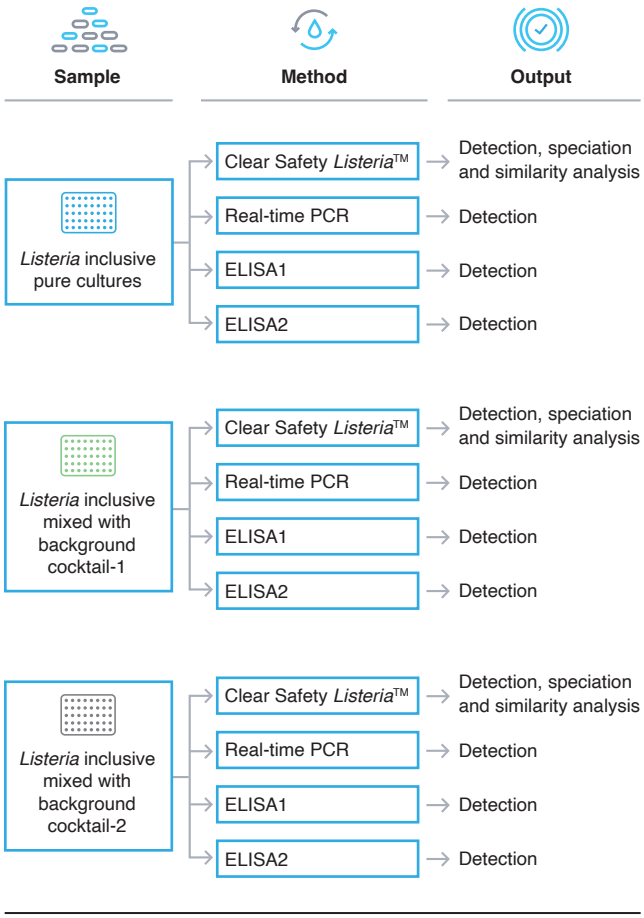
This study was performed by an independent service laboratory, which compared the performance of Clear Safety *Listeria* with a commercial qPCR method and two enzyme-linked immunosorbent assays (ELISA) methods for detection of *Listeria species*. The identification of each sample was confirmed by the gold standard culture methods described in FDA BAM Chapter 10: Listeria section G (6) along with a commercial biochemical assay kit.

Bacterial culture isolates (see Table 1) were inoculated into the 10 mL brain heart infusion broth and incubated at 35°C for 24h. All cultures were ten-fold diluted in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE) and appropriate dilutions were plated on BHI agar for enumeration. Two separate mixtures of non-*Listeria* exclusive organisms described in Table 1 were prepared by mixing equal volumes of three exclusive cultures at the concentration of 1E+07 CFU/mL are identified as cocktail #1 and cocktail #2. All *Listeria* cultures were adjusted to 2E+07 CFU/mL concentration and mixed with either TSBYE, cocktail #1, or cocktail #2 in 1:1 ratio. Samples and controls are described in Table 2. Figure 1 shows the paired study design used in this study. Aliquots of each sample were analyzed by Clear Safety *Listeria*, real-time PCR and two different ELISA systems following manufacturer's instructions.

## Table 1: List of organisms used in this study

| Category | Organism | Source | Mixture of exclusives |
|---|---|---|---|
| *Listeria* inclusives | *L. grayi* | ATCC 25401 | Not applicable |
| | *L. innocua* | ATCC 51742 | |
| | *L. ivanovii* | ATCC 49953 | |
| | *L. marthii* | ATCC BAA-1595 | |
| | *L. monocytogenes* | ATCC 35152 | |
| | *L. seeligeri* | ATCC 35967 | |
| | *L. welshimeri* | ATCC 35897 | |
| Non-*Listeria* exclusives | *Bacillus licheniformis* | ATCC 14580 | Cocktail #1 |
| | *Serratia marcescens* | ATCC 13880 | |
| | *Enterococcus faecalis* | ATCC 33186 | |
| | *Citrobacter freundii* | ATCC 43864 | Cocktail #2 |
| | *Bacillus cereus* | ATCC 130601 | |
| | *Staphylococcus aureus* | ATCC 33591 | |

## Figure 1: Paired study design




## Table 2: Test samples

| Sample ID | *Listeria* Inclusive Organism | Exclusivity Organism Mixture |
|---|---|---|
| 1 | *L. grayi* | N/A - Sterile TSBYE |
| 2 | *L. innocua* | N/A - Sterile TSBYE |
| 3 | *L. ivanovii* | N/A - Sterile TSBYE |
| 4 | *L. marthii* | N/A - Sterile TSBYE |
| 5 | *L. monocytogenes* | N/A - Sterile TSBYE |
| 6 | *L. seeligeri* | N/A - Sterile TSBYE |
| 7 | *L. welshimeri* | N/A - Sterile TSBYE |
| 8 | *L. grayi* | Cocktail #1 |
| 9 | *L. innocua* | Cocktail #1 |
| 10 | *L. ivanovii* | Cocktail #1 |
| 11 | *L. marthii* | Cocktail #1 |
| 12 | *L. monocytogenes* | Cocktail #1 |
| 13 | *L. seeligeri* | Cocktail #1 |
| 14 | *L. welshimeri* | Cocktail #1 |
| 15 | *L. grayi* | Cocktail #2 |
| 16 | *L. innocua* | Cocktail #2 |
| 17 | *L. ivanovii* | Cocktail #2 |
| 18 | *L. marthii* | Cocktail #2 |
| 19 | *L. monocytogenes* | Cocktail #2 |
| 20 | *L. seeligeri* | Cocktail #2 |
| 21 | *L. welshimeri* | Cocktail #2 |
| 22 | Sterile TSBYE | N/A - Sterile TSBYE |
| 23 | Sterile TSBYE | Cocktail #1 |
| 24 | Sterile TSBYE | Cocktail #2 |

## Results and Discussion

A total of four *Listeria* detection methods were used in this study, Clear Safety *Listeria*, a qPCR assay, ELISA-1 and ELISA-2. A panel of 7 inclusive *Listeria* species were tested individually along with a mixture competitor organisms (cocktail #1 or cocktail #2). Clear Safety *Listeria* resulted in 100% accuracy in *Listeria* detection with no false negative or false positive results. The qPCR assay also had 100% detection accuracy, but did not provide species information. ELISA-1 detected *Listeria* in 15 samples out of 21 inoculated samples resulting in a total of 6 false-negatives. ELISA-2 detected *Listeria* in 16 samples out of 21 inoculated samples resulting in a total of 5 false-negatives. Data obtained from this study is presented in Table 3.

In addition to 100% detection accuracy, Clear Safety *Listeria* provided 100% accuracy in species identification, while the commercial biochemical assay kit failed to confirm the identity of *Listeria marthii* and only identified *Listeria grayi* in two out of three samples. Additional fermentation broths were needed to confirm the species identification of *L. marthii* and *L. grayi* in those samples.

**Table 3: Comparison of Clear Safety *Listeria*, qPCR, two ELISA Assays and culture confirmation method for Listeria species**

| Sample ID | Inclusive organism | Exclusives Cocktail | Culture Confirmation | Clear Safety Listeria Screening | Clear Safety Listeria | qPCR | ELISA-1 | ELISA-2 |
|---|---|---|---|---|---|---|---|---|
| 1 | *L. grayi* | TSBYE | *L. grayi* | Positive | *L. grayi* | Presumptive | Presumptive | Presumptive |
| 2 | *L. innocua* | TSBYE | *L. innocua* | Positive | *L. innocua* | Presumptive | Presumptive | Presumptive |
| 3 | *L. ivanovii* | TSBYE | *L. ivanovii* | Positive | *L. ivanovii* | Presumptive | Negative | Presumptive |
| 4 | *L. marthii* | TSBYE | Listeria spp. | Positive | *L. marthii* | Presumptive | Presumptive | Presumptive |
| 5 | *L. monocytogenes* | TSBYE | *L. monocytogenes* | Positive | *L. monocytogenes* | Presumptive | Presumptive | Presumptive |
| 6 | *L. seeligeri* | TSBYE | *L. seeligeri* | Positive | *L. seeligeri* | Presumptive | Negative | Negative |
| 7 | *L. welshimeri* | TSBYE | *L. welshimeri* | Positive | *L. welshimeri* | Presumptive | Presumptive | Presumptive |
| 8 | *L. grayi* | Cocktail #1 | *L. grayi* | Positive | *L. grayi* | Presumptive | Presumptive | Presumptive |
| 9 | *L. innocua* | Cocktail #1 | *L. innocua* | Positive | *L. innocua* | Presumptive | Presumptive | Presumptive |
| 10 | *L. ivanovii* | Cocktail #1 | *L. ivanovii* | Positive | *L. ivanovii* | Presumptive | Negative | Negative |
| 11 | *L. marthii* | Cocktail #1 | Listeria spp. | Positive | *L. marthii* | Presumptive | Presumptive | Presumptive |
| 12 | *L. monocytogenes* | Cocktail #1 | *L. monocytogenes* | Positive | *L. monocytogenes* | Presumptive | Presumptive | Presumptive |
| 13 | *L. seeligeri* | Cocktail #1 | *L. seeligeri* | Positive | *L. seeligeri* | Presumptive | Negative | Negative |
| 14 | *L. welshimeri* | Cocktail #1 | *L. welshimeri* | Positive | *L. welshimeri* | Presumptive | Presumptive | Presumptive |
| 15 | *L. grayi* | Cocktail #2 | Listeria spp. | Positive | *L. grayi* | Presumptive | Presumptive | Presumptive |
| 16 | *L. innocua* | Cocktail #2 | *L. innocua* | Positive | *L. innocua* | Presumptive | Presumptive | Presumptive |
| 17 | *L. ivanovii* | Cocktail #2 | *L. ivanovii* | Positive | *L. ivanovii* | Presumptive | Negative | Negative |
| 18 | *L. marthii* | Cocktail #2 | Listeria spp. | Positive | *L. marthii* | Presumptive | Presumptive | Presumptive |
| 19 | *L. monocytogenes* | Cocktail #2 | *L. monocytogenes* | Positive | *L. monocytogenes* | Presumptive | Presumptive | Presumptive |
| 20 | *L. seeligeri* | Cocktail #2 | *L. seeligeri* | Positive | *L. seeligeri* | Presumptive | Negative | Negative |
| 21 | *L. welshimeri* | Cocktail #2 | *L. welshimeri* | Positive | *L. welshimeri* | Presumptive | Presumptive | Presumptive |
| 22 | Sterile TSBYE | TSBYE | Negative | Negative | Negative | Negative | Negative | Negative |
| 23 | Sterile TSBYE | Cocktail #1 | Negative | Negative | Negative | Negative | Negative | Negative |
| 24 | Sterile TSBYE | Cocktail #2 | Negative | Negative | Negative | Negative | Negative | Negative |
| Time to results (in hours) | | | 120h | 2h sample prep/12h run time | | 2h sample prep/1.5h run time* | 1h sample prep/1.5h run time* | 1h sample prep/1.5h run time* |

= Positive and species ID = Positive Listeria spp. = *Listeria* not detected * indicates time to presumptive results.

While the rapid qPCR and immunoassays could provide results in 2-4 hours, positive results are only considered presumptive since culture confirmation would be required for speciation. This would require an additional 5-7 days whereas the Clear Safety *Listeria* assay can provide confirmed results in less than 24 hours.

## Conclusion

In this study, Clear Safety *Listeria* and qPCR achieved 100% accuracy in detection while ELISA-1 (28.6% false negative rate) and ELISA-2 (23.8% false negative rate) failed to detect *Listeria* in some samples. False negative results are a severe risk to companies and public health as they can lead to the consumption of hazardous foods, thus assays for food safety testing should be carefully selected to minimize risk of false negatives. While qPCR provides rapid and accurate analyses, these results are considered presumptive and require an additional 5-7 days of culture confirmation analysis to provide actionable results with confirmed positive *Listeria* and species identification.

Comparatively, Clear Safety *Listeria* is an automated sequencing system that is able to accurately detect *Listeria* and confirm species identification 10 times faster, in a total of 10-12 hours, with an automated system that requires only 0.5-1 hours of hands-on time. Additionally, the Similarity Analysis® strain typing capabilities of the Clear Safety *Listeria* assay can match strains within a facility to aid in environmental monitoring and root cause analysis investigations. Thus, the findings of this study support the claim that the Clear Safety *Listeria* assay presents a valuable, affordable, and high throughput automated solution that can improve identification of hazardous food products and environmental monitoring to ensure the safety of the food supply.

## References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