

Verification of a Commercial Real-Time PCR *Listeria monocytogenes* and *Listeria* species Detection Method on Individually Quick-Frozen Vegetable Matrices

Erica Miller¹, Christopher Crowe², J. David Legan³
Eurofins Microbiology Laboratories, Inc. ¹. Louisville KY, ². Salinas, CA. ³. Madison, WI.

ABSTRACT

INTRODUCTION: With increasing concerns and rising numbers of product recalls associated with pathogens in produce, most major PCR testing platforms are validated for detection of *Listeria* in fresh produce. However, validations for other produce matrices are sparse, particularly for frozen matrices which can severely reduce initial temperatures of enrichment broths.

PURPOSE: To verify the ability to detect *Listeria monocytogenes* and *Listeria* species in frozen vegetable matrices utilizing real-time PCR.

METHODS: Twenty-three different frozen vegetable matrices were tested, including cauliflower, carrots, various vegetable medleys, and chopped spinach: each with seven replicates (125g) inoculated with *Listeria monocytogenes* at ca. 7.5 cfu/sample. All samples were enriched with 1,125 mL of BACGro Ultra *Listeria* Broth™ (BULB) and incubated at 37°C for 21 ± 3 hours. Following incubation, samples were tested for *Listeria monocytogenes* and *Listeria* spp. using BACGene™ real-time PCR kits according to the manufacturer’s instruction. All proprietary media and tests kits were supplied by Gold Standard Diagnostics™.

RESULTS: All seven replicates returned a presumptive positive PCR result for *Listeria monocytogenes* and *Listeria* spp. positive PCR results. The FDA microbiological method validation guidelines require 7/7 (or 19/20) presumptive results to consider a method extended to a new matrix, with no statistical analysis necessary. This standard was met 46 times in this study for both *Listeria* spp. and *Listeria monocytogenes*.

SIGNIFICANCE: These data demonstrate that this method can reliably detect *Listeria monocytogenes* and *Listeria* spp. in a wide range of frozen vegetable matrices.

INTRODUCTION

- Fresh produce commodities have been associated with numerous outbreaks and recalls due to *Listeria monocytogenes* contamination ¹. Freezing is not considered a lethality step.
- During the IQF process, different aids are used that could be inhibitors to the PCR assay.
- Most pathogen test methods validate fresh produce but not IQF produce.
- Here we describe a method evaluating frozen vegetables acquired from a processing plant to ensure reliable detection and no interference with the PCR assay.

MATERIALS AND METHODS

MATRICES: 23 different IQF produce items were supplied by the manufacturer: blanched potatoes, black beans, butternut squash, carrots, (corn, bell pepper, and poblano pepper blend), (corn, carrots, and peas), (corn, carrots, peas, and green beans), corn, dark red kidney beans, grilled corn, grilled sweet potatoes, garbanzo beans, great northerner beans, light red kidney beans, multi-colored cauliflower, organic spinach, (organic corn, peas, and carrots), peas, pinto beans, poblano peppers, small red kidney beans, sweet potatoes, and white corn and poblano pepper blend.

INOCULATION: Seven frozen replicates (125g) of each matrix were inoculated with ca. 7.5 CFU of *Listeria monocytogenes* in accordance with matrix verification protocols taken from the US microbiological method validation guidelines².

ENRICHMENT: All samples were diluted in 1,125 mL of prewarmed (37°C) BACGro Ultra *Listeria* Broth™. Enriched samples were incubated for 21 ± 3 hours at 37°C.

PCR: A 30 µL aliquot of each sample was added to 70 µL of BACGene lysis buffer. Lysis and PCR using the BACGene *Listeria monocytogenes* and BACGene *Listeria* spp. PCR kit was performed according to manufacturer’s instructions. PCR was run on a BioRad CFX Deepwell or Agilent AriaMx thermocycler.



Vegetables weighed out at 125g and enriched with 1,125 mL of BULB.

Samples were incubated at 37°C for 21 ± 3 hours.

Samples underwent the lysis procedure according to manufacturer’s instruction and proceeded to PCR.

REFERENCES

1. Centers for Disease Control and Prevention. (2024, August 9). *Listeria outbreak linked to Leafy Greens - February 2023*. Centers for Disease Control and Prevention. <https://www.cdc.gov/listeria/outbreaks/monocytogenes-02-23/index.html>
2. US Food and Drug Administration. *Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds*, Edition 3.0, Oct. 2019.

MATRIX	LISTERIA MONOCYTOGENES DETECTION	LISTERIA SPECIES DETECTION
Blanched potatoes	7/7	7/7
Black beans	7/7	7/7
Butternut squash	7/7	7/7
Corn, bell pepper, and poblano pepper	7/7	7/7
Corn, carrots, and peas	7/7	7/7
Corn, carrots, peas, and green beans	7/7	7/7
Corn	7/7	7/7
Dark red kidney beans	7/7	7/7
Grilled corn	7/7	7/7
Grilled sweet potatoes	7/7	7/7
Garbanzo beans	7/7	7/7
Great northern bean	7/7	7/7
Light red kidney beans	7/7	7/7
Multi-colored cauliflower	7/7	7/7
Organic spinach	7/7	7/7
Organic corn, peas, and carrots	7/7	7/7
Peas	7/7	7/7
Pinto beans	7/7	7/7
Poblano peppers	7/7	7/7
Small red kidney beans	7/7	7/7
Sweet potatoes	7/7	7/7
White corn and poblano peppers	7/7	7/7

RESULTS

For all 23 matrices tested, all 7 inoculated replicates were detected by the *Listeria* spp. and *Listeria monocytogenes* and PCR assay.

CONCLUSIONS

BACGene *Listeria monocytogenes* and *Listeria* spp. PCR reliably detects the presence of *Listeria* in IQF vegetables, even if samples are tested frozen

No interference with PCR detection was observed from any of the matrices tested.



Presented at IAFP 2025
P-120