

# Verification of a Commercial Real-Time PCR Shiga-toxin Producing *E. coli* (STEC) Detection Method on Individually Quick-Frozen Vegetable Matrices

Erica Miller<sup>1</sup>, Christopher Crowe<sup>2</sup>, J. David Legan<sup>3</sup>  
Eurofins Microbiology Laboratories, Inc. <sup>1</sup>. Louisville KY, <sup>2</sup>. Salinas, CA. <sup>3</sup>. 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 STEC in produce. However, validations for other vegetable matrices are sparse, particularly for frozen matrices which can severely reduce initial temperatures of enrichment broths.

**PURPOSE:** To verify the ability to detect STEC 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 *Escherichia coli* O111 at ca. 30 cfu/sample. All samples were enriched with 1,125 mL of BACGro™ modified tryptic soy broth (mTSB) and were incubated within the AOAC validated enrichment time window of 10-24 hrs. Following incubation, samples were tested for STEC 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 result for STEC for each of the 23 matrices, giving a total of 161 presumptive 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 for all 23 matrices in this study.

**SIGNIFICANCE:** These data demonstrate that this method can reliably detect STEC organisms in a wide range of frozen vegetable matrices.

## INTRODUCTION

- Fresh produce commodities have been associated with numerous outbreaks and recalls due to *Salmonella* or STEC contamination.<sup>1,2</sup>. 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 each inoculated with ca. 30 CFU of *E. coli* O111 in accordance with matrix verification protocols taken from the US microbiological method validation guidelines<sup>3</sup>.

**ENRICHMENT:** All samples were diluted in 1,125 mL of prewarmed (42°C) modified Tryptic Soy Broth. Enriched samples were incubated for 17 ± 7 hours at 42°C.

**PCR:** A 30 µL aliquot of each sample was added to 70 µL of BACGene lysis buffer. Lysis and PCR using the BACGene STEC 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 mTSB.

Samples were incubated at 37°C for 17 ± 7 hours.

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

## REFERENCES

1. Food and Drug Administration. "Outbreak Investigation of Salmonella Typhimurium: BrightFarms Packaged Salad Greens" Food and Drug Administration, 16 October 2021, <https://www.fda.gov/food/outbreaks-foodborne-illness/outbreak-investigation-salmonella-typhimurium-brightfarms-packaged-salad-greens-july-2021>.
2. Marshall, K. E., Hexemer, A., Seelman, S. L., Fatica, M. K., Blessington, T., Hajmeer, M.,Gieraltowski, L. (2020). Lessons Learned from a Decade of Investigations of Shiga Toxin–Producing *Escherichia coli* Outbreaks Linked to Leafy Greens, United States and Canada. *Emerging Infectious Diseases*, 26(10), 2319-2328. <https://doi.org/10.3201/eid2610.191418>.
3. 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	STEC DETECTION
Blanched potatoes	7/7
Black beans	7/7
Butternut squash	7/7
Corn, bell pepper, and poblano pepper	7/7
Corn, carrots, and peas	7/7
Corn, carrots, peas, and green beans	7/7
Corn	7/7
Dark red kidney beans	7/7
Grilled corn	7/7
Grilled sweet potatoes	7/7
Garbanzo beans	7/7
Great northern bean	7/7
Light red kidney beans	7/7
Multi-colored cauliflower	7/7
Organic spinach	7/7
Organic corn, peas, and carrots	7/7
Peas	7/7
Pinto beans	7/7
Poblano peppers	7/7
Small red kidney beans	7/7
Sweet potatoes	7/7
White corn and poblano peppers	7/7

## RESULTS

For all 23 matrices tested, all 7 inoculated replicates were detected by the STEC Multiplex PCR assay.

## CONCLUSIONS

BACGene STEC Multiplex PCR reliably detects the presence of Shiga-Toxin producing organisms in IQF vegetables, even if samples are tested frozen.

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



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