Matrix Validation of 450 g Romaine Lettuce for the Detection of *E. coli* O157:H7 and *Salmonella* Using Hygiena's BAX® System

Deja Latney[1],Julie Weller[1] and Nona Childress[2]
1. Hygiena®, 2 Boulden Circle, New Castle, DE, 19720
2. PrimusLabs™, 2810 Industrial Pkwy, Santa Maria ,CA ,93455

BAX® System Q7 | BAX® System X5 | foodproof® | microproof®

INTRODUCTION:

Leafy greens, such as romaine lettuce, are high-risk foods that are continuously implicated in foodborne outbreaks and associated illnesses. When elevated risk factors are present, the California Leafy Greens Marketing Agreement (LGMA) has stated that growers must sample larger quantities of product per acre to minimize risk.

Current industry standards for pathogen analysis require testing 375 g samples. Regardless of sample size, rapid methods need to be able to detect very low levels of contamination. Thus, with the increase in required sample sizes, it is imperative to keep up with industry demands.

PURPOSE:

To accommodate the need for testing larger sample sizes being added by LGMA, this study evaluated 450 g samples of romaine lettuce with two real-time PCR assays for the detection of *E. coli* O157:H7 and *Salmonella*.

REGISTERED TRADEMARKS

METHODS:

Romaine lettuce was dually inoculated with *E. coli* O157:H7 and *Salmonella* at two levels; a low level to achieve 25 – 75% positives and a high level to achieve 100% positives. Additional uncontaminated levels were included as negative controls. All samples were equilibrated at 2 to 8 °C for 48-72 hours.

Test method samples (450 g) were enriched with BAX MP media and incubated at 42 °C for 10-24 hours. Aliquots were tested by real-time PCR and confirmed by culture.

Reference samples, one set for *E.coli* O157:H7 (200 g) and a second set for *Salmonella* (25 g), were enriched and confirmed according to the procedures in the FDA BAM Chapter 4A and Chapter 5, respectively.

RESULTS:

***E. coli* O157:H7**

- Test method samples (450 g): 14/20 low-inoculated positives and 5/5 high-inoculated positives showed consistent results for both real-time PCR and culture.
- USDA reference method samples (200 g): 16/20 low-inoculated positives and 5/5 high-inoculated positives confirmed.

Salmonella

- Test method samples (450 g): 18/20 low-inoculated positives and 5/5 high-inoculated positives showed consistent results for both real-time PCR and culture.
- USDA reference method samples (25 g): 14/20 low-inoculated positives and 4/5 high-inoculated positives confirmed.

When comparing methods using the difference in probability of detection (dPOD), the statistical results showed no significant differences between the test method and reference method for either *E.coli* O157:H7 or *Salmonella* (Table 1).

Table 1: Test method results vs. Reference method results

Sample Type	Target Strain	MPN/Test Portion	N	BAX System Method			Reference Method			dPOD	95% CI
				X	POD_C	95% CI	X	POD_R	95% CI		
Romaine (450 g)	*E. coli* O157:H7 DD1980	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	0.00, 0.00
		0.97	20	14	0.70	0.48, 0.85	16	0.80	0.58, 0.92	-0.10	-0.35, 0.16
		8.9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	Salmonella Saintpaul DD13596	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	0.00, 0.00
		1.2	20	18	0.90	0.70, 0.97	14	0.70	0.48, 0.85	0.20	-0.05, 0.43
		9.5	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

MPN/Test Portion = Most Probable Number is based on the POD of reference method test portions
N = Number of test portions
X = Number of positive test portions
POD_C = Confirmed BAX System method positive results divided by the total number of test portions
POD_R = Confirmed reference method positive results divided by the total number of test portions
$dPOD_C$ = Difference between the BAX System method and reference method POD values
95% CI = If the confidence interval of dPOD does not contain zero, then the difference is statistically significant at the 5% level

SIGNIFICANCE:

The results of this study demonstrate that the BAX® System Real-Time PCR assays for *E. coli* O157:H7 and *Salmonella* are sensitive and specific for the detection of the aforementioned target analytes in 450 g samples of romaine lettuce.

REFERENCES:

1. Alegbeleye, O.O., I. Singleton, and A.S. Sant'Ana. (2018). Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: A review. *Food Microbiol*. 73:177-208. doi: 10.1016/j.fm.2018.01.003

2. York, T. What's Behind LGMA's Preharvest Testing Requirement. California LGMA News. August 2, 2021. https://lgma.ca.gov/news/whats-behind-lgmas-preharvest-testing-requirement

3. California LGMA. August 2, 2021. *Commodity Specific Food Safety Guidelines for the Production and Harvest of Lettuce and Leafy Greens: Appendix*. https://lgma-assets.sfo2.digitaloceanspaces.com/downloads/August-2021-CA-LGMA-Metrics_FINAL-v20211208_A11Y.pdf