



One Health Diagnostics™

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INTRODUCTION:

Fresh fruits and vegetables are a staple in healthy lifestyles; however, reports of food-borne disease outbreaks have been substantially increasing in recent years. Produce can become contaminated at any stage during its growth, harvesting, processing, distribution and final preparations (1). This makes the detection and identification of microbial hazards extremely important specifically because produce is sold and consumed raw and unprocessed.

PURPOSE:

This study was designed to evaluate the performance of the BAX® System method in comparison to the FDA BAM reference methods for the detection of *E. coli* O157:H7 and *Salmonella* in organic cut baby carrots.

REGISTERED TRADEMARKS:

BAX® is a registered trademark of Hygiena for its line of equipment, reagents and software used to analyze samples for microbial contamination.

Detection of *E. coli* O157:H7 and *Salmonella* in Baby Carrots using Hygiena’s BAX® System

BAX® System Q7

BAX® System X5

foodproof®

microproof®

METHODS:

Organic cut baby carrots (25 g) were co-inoculated with *E. coli* O157:H7 and *Salmonella* Typhimurium at a low fractional level expected to product 25-75% positives and a high level expected to produce 100% positives. Additional samples were left uninoculated for negative controls. After a 4 °C equilibration period, samples were divided for testing using each of the 3 methods and enriched accordingly.

For the test method; samples were enriched with BPW, incubated at 42 °C for 8-24 hours and tested by real-time PCR. Reference method samples were enriched and confirmed according to the procedures in the FDA BAM Chapter 4A: Diarrheagenic *Escherichia coli*, and FDA BAM Chapter 5: *Salmonella*.

Test Method

- 5 negatives, 20 low fractional samples, 5 high samples
- Enrichment: 1:10 BPW

E. coli O157:H7 Reference Method

- 5 negatives, 20 low fractional samples, 5 high samples
- Enrichment: 1:10 mBPWp + ACV supplements

Salmonella Reference Method

- 5 negatives, 20 low fractional samples, 5 high samples
- Enrichment: 1:10 UPB

RESULTS:

Test method samples returned positive results for *E. coli* O157:H7 in 10/20 low spiked samples and for *Salmonella* in 8/20 low spiked samples, satisfying fractional recovery. All 5 high spiked samples were positive for both organisms. All real-time PCR results were identical to culture at 8 hours with no false positives or false negatives (Table 1).

The corresponding reference methods returned culture positive results for *E. coli* O157:H7 in 11/20 low spiked samples and 5/5 high spiked samples, while *Salmonella* returned culture positive results for 13/20 low spiked samples and 5/5 high spiked samples.

Results between the test method and reference methods were compared using the difference in probability of detection (dPOD). There were no significant differences for either *E. coli* O157:H7 or *Salmonella* since the 95% confidence interval of the dPOD contains zero (Table 2).



DATA:

Table 1. BAX System Method Presumptive vs. Confirmed Results

Sample Type	Target Strain	CFU/Test Portion	MPN/Test Portion	N	8 h	10 h	24 h	Culture Confirmed
Baby Carrots (25 g)	<i>E. coli</i> O157:H7 DD13490	Control	0	5	0	0	0	0
		1.79	0.72	20	10	10	10	10
		17.9	7.2	5	5	5	5	5
Baby Carrots (25 g)	<i>S.</i> Typhimurium DD1467	Control	0	5	0	0	0	0
		1.51	0.75	20	8	8	8	8
		15.1	7.5	5	5	5	5	5

CFU/Test Portion = Actual inoculation level of samples prior to 48-72 hour equilibration
MPN/Test Portion = Most Probable Number is based on the POD of reference method test portions
N = Number of test portions

SIGNIFICANCE:

This study shows that the BAX System Real-Time PCR assays are indistinguishable from the FDA BAM reference methods and are therefore fit-for-use to rapidly screen organic cut baby carrots for the detection of *E. coli* O157:H7 and *Salmonella* using a single enrichment.

Table 2. BAX System Method vs. Reference Method Results

Sample Type	Target Strain	MPN/Test Portion	N	BAX System Method			Reference Method			dPOD _C	95% CI
				X	POD _C	95% CI	X	POD _R	95% CI		
Baby Carrots (25 g)	<i>E. coli</i> O157:H7 DD13490	0	5	0	0	0.00, 0.00	0	0.00	0.00, 0.00	0.00	0.00, 0.00
		0.72	20	10	0.5	0.30, 0.70	11	0.55	0.34, 0.74	-0.05	-0.32, 0.24
		7.2	5	5	1	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Baby Carrots (25 g)	<i>S.</i> Typhimurium DD1467	0	5	0	0	0.00, 0.00	0	0.00	0.00, 0.00	0.00	0.00, 0.00
		0.75	20	8	0.4	0.22, 0.61	13	0.65	0.43, 0.82	-0.25	-0.50, 0.05
		7.5	5	5	1	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

REFERENCES:

1. Iwu C. D, Okoh A. I. 2019. Preharvest Transmission Routes of Fresh Produce Associated Bacterial Pathogens with Outbreak Potentials: A Review. *Int J Environ Res Public Health*. 16(22):4407.