Matrix Validation of 375 mL Spent Sprout Irrigation Water for *E. coli* O157:H7 and *Salmonella* Using Hygiena's BAX® System

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INTRODUCTION:

Pathogen contamination of sprouts has been a recurring public health challenge. Three critical control points of sprout production have been identified: the seeds, spent irrigation water and the sprouts (finished product). Growers can use approved seed treatments to reduce pathogens prior to sprouting, but this does not guarantee complete pathogen elimination (1). Therefore, it is equally important to perform microbiological testing of the spent irrigation water.

PURPOSE:

This study was designed to validate spent sprout irrigation water for the detection of *E. coli* O157:H7 and *Salmonella* using a rapid, real-time, PCR-based method.

METHOD:

An unpaired matrix validation for spent sprout irrigation water was performed following the technical guidelines in Appendix J of the AOAC INTERNATIONAL Official Methods of Analysis to compare two commercial real-time PCR assays to the FDA reference methods for the detection of *E. coli* O157:H7 and *Salmonella*.

Samples were co-inoculated with *E. coli* O157:H7 and *Salmonella* at a low level (0.2 – 2 CFU/test portion) and a high level (≥5 CFU/test portion). Additional samples were reserved for negative controls. All samples were equilibrated at 4 °C for 48 – 72 hours before enrichment and testing.

Test method samples (375 mL, n = 30) were enriched in BPW and incubated for 12 – 24 hours before being tested by real-time PCR and culture-confirmed. Reference method samples for *E. coli* O157:H7 (100 mL, n = 30) and *Salmonella* (375 mL, n = 30) were enriched and confirmed according to their respective procedures in the FDA guidance documents.

RESULTS:

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

- Test method results: 8/20 low-level positives, 5/5 high-level positives consistent between real-time PCR and culture.
- FDA reference results: 9/20 low-level positives, 5/5 high-level positives confirmed.

Salmonella

- Test method results: 5/20 low-level positives, 5/5 high-level positives consistent between real-time PCR and culture.
- FDA reference results: 4/20 low-level positives, 5/5 high-level positives confirmed.

When compared to the reference methods, the difference in probability of detection (dPOD) indicated no significant difference for either organism (Table 1).

SIGNIFICANCE:

The results of this validation demonstrated a sensitivity rate of 100% and a specificity rate of 100% for the detection of *E. coli* O157:H7 and *Salmonella* using real-time PCR. This study shows that the BAX® System assay is indistinguishable from the FDA reference method.

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)	
Spent Sprout Irrigation Water (375 mL)	*E. coli* O157:H7 DD12880	Control	5	0	0.00 (0.00, 0.45)	0	0.00 (0.00, 0.45)	0.00 (0.00, 0.00)
		0.45	20	8	0.40 (0.22, 0.61)	9	0.45 (0.26, 0.66)	-0.05 (-0.32, 0.24)
		3.65	5	5	1.00 (0.57, 1.00)	5	1.00 (0.57, 1.00)	0.00 (-0.43, 0.43)
	Salmonella Enteritidis DD13759	Control	5	0	0.00 (0.00, 0.45)	0	0.00 (0.00, 0.45)	0.00 (0.00, 0.00)
		0.33	20	5	0.25 (0.11, 0.47)	4	0.20 (0.08, 0.42)	0.05 (-0.21, 0.30)
		2.68	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** = 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. Center for Food Safety and Applied Nutrition, U.S. Food & Drug Administration, Office of Compliance. 2017. FY 2014 – 2016 Microbiological Sampling Assignment Summary Report: Sprouts. https://www.fda.gov/files/food/published/FY-2014-%E2%80%93-2016-Microbiological-Sampling-Assignment-Summary-Report-Sprouts-PDF.pdf

REGISTERED TRADEMARKS: