Matrix Validation of 30 g Test Portions of Soil for the Detection of *E. coli* O157:H7, *Salmonella* and *Listeria* Using Hygiena's BAX® System

Christine Chapman[1], Julie Weller[1], and Ryan Morrow[2]
1. Hygiena®, 2 Boulden Circle, New Castle, DE 19720
2. PrimusLabs™, 2810 Industrial Pkwy, Santa Maria, CA, 93455

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INTRODUCTION

Good soil quality provides essential elements for crop growth and yield as well as a diverse microbial community that aids in regulating deleterious organisms. Shifts in weather and climate factors can expose agroecosystems to harmful pathogens and put food crops at high risk.

Agriculture management practices can sustain and increase the resiliency of agroecosystems to prevent harmful organisms from becoming established in the soil, but those systems, when left exposed, can lead to foodborne outbreaks and recalls. In addition, natural sources that expose soil to pathogens, such as weather and climate factors are more difficult to prepare for and put food crops at high risk.

PURPOSE

This study was designed to validate soil impacted by heavy storms for the detection of *E. coli* O157:H7, *Salmonella* and *Listeria* using a rapid, real-time PCR-based method.

METHOD

Two unpaired matrix validations for agricultural soil were performed following the technical guidelines in Appendix J of the AOAC INTERNATIONAL Official Methods of Analysis to compare three commercial real-time PCR assays to the EPA reference methods for the detection of *E. coli* O157:H7, *Salmonella,* and *Listeria.*

E. coli* O157:H7 and *Salmonella
Thirty-gram (30 g) test portions for each method were co-inoculated with *E. coli* O157:H7 and *Salmonella* at low levels (0.2-2 CFU/test portion) and high levels (≥ 5 CFU/test portion). Additional samples were reserved for use as negative controls. All samples were equilibrated at 4 °C for 48-72 hours before enrichment and testing.

Test method samples (30 g, n=30) were enriched in MP media and incubated for 18-24 hours before being tested by real-time PCR and culture confirmed. Reference method samples for *E. coli* O157:H7 (30 g, n=30) and *Salmonella* (30 g, n=30) were enriched and confirmed according to their respective procedures in the EPA guidance documents.

Listeria
Thirty-gram (30 g) test portions for each method were inoculated with *Listeria monocytogenes* at a low level (0.2-2 CFU/test portion) and a high level (≥ 5 CFU/test portion). Additional samples were reserved for use as negative controls. All samples were equilibrated at 4 °C for 48-72 hours before enrichment and testing.

Test method samples (30 g, n=30) were enriched in 24 LEB Complete media and incubated for 48 hours before being tested by real-time PCR and culture confirmed. Reference method samples for *Listeria* (30 g, n=30) were enriched and confirmed according to their respective procedures in the FDA guidance documents.

RESULTS

***E. coli* O157:H7**
- Test method results: 6/20 low-level positives, 5/5 high-level positives with consistent results between real-time PCR and culture.
- EPA reference results: 4/20 low level positives, 5/5 high level positives confirmed.

Salmonella
- Test method results: 7/20 low-level positives, 5/5 high-level positives with consistent results between real-time PCR and culture.
- EPA reference results: 6/20 low-level positives, 5/5 high-level positives confirmed.

Listeria
- Test method results: 10/20 low-level positives, 5/5 high-level positives consistent between real-time PCR and culture.
- FDA reference results: 13/20 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 any organism (Table 1).

TABLE 1. Test Method Results vs. Reference Method Results

Target Strain	CFU/Test Portion	N	BAX System Method			Reference Method			$dPOD_c$	95% CI
			X	POD_c	95% CI	X	POD_R	95% CI		
***E. coli* O157:H7 DD642**	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	0.00, 0.00
	0.91	20	6	0.30	0.15, 0.52	4	0.20	0.08, 0.42	0.10	-0.17, 0.35
	0.81	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
***Salmonella* Typhimurium DD13752**	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	0.00, 0.00
	0.53	20	13	0.65	0.43, 0.82	6	0.30	0.15, 0.52	0.35	0.04, 0.58
	3.98	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
***L. mono* D13781**	Control	5	0	0.00	0.00, 0.00	0	0.00	0.00, 0.00	0.00	0.00, 0.00
	1.05	20	10	0.50	0.30, 0.70	13	0.65	0.43, 0.82	-0.15	-0.41, 0.14
	21.6	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

This study shows that the BAX® System Real-Time PCR assays are specific, sensitive and accurate for the detection of *E. coli* O157:H7, *Salmonella* and *Listeria* in 30 g samples of soil.

REFERENCES

1. Samaddar, S., D. S. Karp, R. Schmidt, N. Devarajan, J. A. McGarvey, A. F. A. Pires, and K. Scow. 2021. Role of soil in the regulation of human and plant pathogens: soils' contributions to people. *Phil. Trans. R. Soc. B.* 376: 20200179.