



# Matrix Verification of 25 g of Soppressata for the Detection of Shiga Toxin-Producing *E. coli* (STEC) using Hygiena's BAX<sup>®</sup> System Real-Time PCR Assay

Christine Chapman<sup>1</sup>, Julie Weller<sup>1</sup>, and Glen Feller<sup>2</sup>  
 1. Hygiena<sup>®</sup>, 2 Boulden Circle, New Castle, DE 19720  
 2. Kansas State University, Manhattan, KS

BAX<sup>®</sup> System Q7

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## INTRODUCTION:

Ready-to-eat (RTE) meat and poultry products such as hot dogs and cold cuts are in an edible form that does not require any additional preparation by the consumer. These products are processed by the manufacturer by cooking, smoking, fermenting, or other lethality treatment to ensure product safety.

Despite these processing techniques, RTE foods are still at risk for contamination and are often implicated in product recalls.

## PURPOSE:

This study was designed to verify the use of a real-time PCR assay for the detection of non-O157 STEC organisms compared to the USDA FSIS reference methods in unpaired samples of soppressata.

## REGISTERED TRADEMARKS

The BAX<sup>®</sup> System is a registered trademark of Hygiena<sup>®</sup> for its line of equipment, reagents and software used to analyze samples for microbial contamination. Hygiena<sup>®</sup> is a registered trademark of Hygiena<sup>®</sup>.

## METHOD:

Two unpaired matrix studies for soppressata were performed to compare a commercial real-time PCR assay to the USDA reference method for the detection of Shiga Toxin-Producing *E. coli*.

Twenty-five-gram (25 g) test portions for each method were inoculated with either *E. coli* O121 or *E. coli* O103 to create 6 replicate samples at a low level, expected to yield fractional positive (25 to 75%) results. An additional 6 replicate samples per method were left uninoculated as negative controls. All samples were equilibrated at 4 °C for 48-72 hours prior to enrichment and testing.

Test method samples (25 g, n=12) were enriched in BAX<sup>®</sup> MP media and incubated for 16-24 hours before being tested by real-time PCR and culture-confirmed.

Reference method samples were enriched according to the USDA FSIS STEC reference method (1).

## RESULTS:

### *E. coli* O121

- Test method: 5/6 low-level positives, consistent between real-time PCR and culture.
- USDA reference results: 6/6 low-level positives confirmed.
- For both methods, all uninoculated samples returned negative results.

### *E. coli* O103

- Test method results: 4/6 low-level positives, consistent between real-time PCR and culture.
- USDA reference results: 3/6 low level positives confirmed.
- For both methods, all uninoculated samples returned negative results.

When compared to the reference methods, the difference in probability of detection (dPOD) indicated no significant difference for either organism (Table 1) when using the BAX real-time PCR assay.

## SIGNIFICANCE:

The results of this study demonstrate that the BAX System Real-Time STEC Suite is accurate and reliable for the detection of Shiga Toxin-Producing *E. coli* in 25 g samples of soppressata, statistically equivalent to culture.



TABLE 1. Test Method Results vs. Reference Method Results

Target Strain	MPN/Test Portion	N	BAX System Method			Reference Method			dPOD <sub>C</sub>	95% CI
			X	POD <sub>C</sub>	95% CI	X	POD <sub>R</sub>	95% CI		
<i>E. coli</i> O121 DD13362	Control	6	0	0.00	0.00, 0.41	0	0.00	0.00, 0.41	0.00	0.00, 0.00
	0.65	6	5	0.83	0.44, 0.97	6	1.00	0.61, 1.00	-0.17	-0.56, 0.25
<i>E. coli</i> O103 DD13372	Control	6	0	0.00	0.00, 0.41	0	0.00	0.00, 0.41	0.00	0.00, 0.00
	0.70	6	4	0.67	0.30, 0.90	3	0.50	0.19, 0.81	-0.17	-0.31, 0.56

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<sub>C</sub> = Confirmed BAX System method positive results divided by the total number of test portions, POD<sub>R</sub> = Confirmed reference method positive results divided by the total number of test portions, dPOD<sub>C</sub> = 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. United States Department of Agriculture, Food Safety and Inspection Service. MLG 5C.03 Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing *Escherichia coli* (STEC) from Meat Products, Carcass, and Environmental Sponges. Effective February 01, 2023.