

# Recovery of *Escherichia coli* O157:H7 by the BAX<sup>®</sup> System in Beef Trim using Surface Sampling Swabs

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## INTRODUCTION:

The beef industry currently uses sampling techniques such as N60 or N60 Plus to detect *E. coli* O157:H7 contamination in beef trim. A novel sampling methodology has recently been developed that utilizes a manual sampling device (MSD) swab rather than taking actual portions of meat to be tested (1). This swab provides a simplified sampling approach for beef trim that is nondestructive and provides labor savings. In combination with post-enrichment pooling, which is commonly practiced, surface sampling has the potential to produce rapid cost-efficient results when screened with PCR and to reduce product waste.

## PURPOSE:

The purpose of this study was to evaluate the performance of the BAX<sup>®</sup> System Real-Time PCR assay for *E. coli* O157:H7 in comparison to the USDA FSIS reference culture method to detect *E. coli* O157:H7 from individual and pooled enriched MicroTally<sup>™</sup> sampling swabs used to sample raw beef trim.

## METHODS:

Thirty MicroTally<sup>™</sup> swabs were removed from their respective sample bags, unfolded and used following USDA developed manual sampling protocols to swab 50% lean raw beef trim. Once both sides of the cloth were used, swabs were folded back to the original dimensions with an additional horizontal fold per the manufacturers instructions and placed into the original bag (2). Using an enumerated *E. coli* O157:H7 culture, swabs were inoculated with the appropriate volume and dilution to create 20 low-level and 5 high-level samples. Five additional swabs were left uninoculated to serve as negative controls. All swabs were held at 4°C for 30 hours to cold stress the target organism before analysis.

Swabs were homogenized with 200 mL of pre-warmed (42°C) mTSB and incubated at 42°C for 8-15 hours. Following enrichment, samples were analyzed as a single test portion (n=30) and a 5-sample enrichment pool (n=10) by PCR at 8 and 10 hours and confirmed according to the USDA FSIS reference culture method at 15 hours.

## RESULTS:

The real-time PCR assay for *E. coli* O157:H7 returned positive results for 12/20 low spiked and 5/5 high spiked samples after both 8 and 10 hours of enrichment.

The proportion of positive results from the low-level sample set (POD 0.60) meets the validation criteria POD range of 0.25-0.75, indicating the expected true absence of cells in some samples. All samples were identical to culture (Table 1).

When a sub-set of 10 inoculated samples were pooled post-enrichment, real-time PCR detected 5/8 low spiked samples and 2/2 high spiked samples after both 8 and 10 hours of enrichment. These results were identical to its individual test result with no false negatives or false positives (Table 2).

Table 1. BAX <sup>®</sup> System Results vs. Reference Culture Results										
Sample Type	CFU/Test Portion	N	BAX <sup>®</sup> System Method			Reference Method			dPOD <sub>C</sub>	95% CI
			X	POD <sub>C</sub>	95% CI	X	POD <sub>R</sub>	95% CI		
MSD swab	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.43, 0.43
	0.94	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28
	5.64	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

**Table 1.** N = Number of test portions, X = Number of positive test portions, POD<sub>C</sub> = Confirmed BAX<sup>®</sup> 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<sup>®</sup> method and reference method POD values, 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 2. Individual vs. Pooled Results						
Sample Type	CFU/Test Portion	N	8 hours		10 hours	
			Individual	Pooled	Individual	Pooled
MSD swab	0.94	8	5	5	5	5
	5.64	2	2	2	2	2

**Table 2.** N = Number of test portions, Individual = Number of BAX<sup>®</sup> method positive results from individually enriched test portions, Pooled = Number of BAX<sup>®</sup> method positive results from combined aliquots of 5 enriched test portions

## SIGNIFICANCE:

This study demonstrates that beef producers can reliably use the BAX<sup>®</sup> System to test for *E. coli* O157:H7 contamination from mTSB enriched surface swabs for beef trimmings at 8-10 hours (individual and pooled samples) with equivalent sensitivity to the USDA FSIS culture methods.

**References:** 1. Wheeler, T.L., Arthur, T.M., 2018. Novel Continuous and Manual Sampling Methods for Beef Trim Microbiological Testing. Journal of Food Protection. 81(10), 1605-1613.; 2. FREMONTA Corporation. 2017. Microtally Instruction Insert. [https://docs.wixstatic.com/ugd/e42455\\_d80bf8f93335471dba0051b82e29d3a1.pdf](https://docs.wixstatic.com/ugd/e42455_d80bf8f93335471dba0051b82e29d3a1.pdf)

