Technical Bulletin: Validation of the BAX® System for the Detection of *E. coli* O157:H7 and *Salmonella* from Microtally™ Swabs in 8 Hours

A validation study was performed on the BAX® System PCR assays for the detection of *E. coli* O157:H7 and *Salmonella* from MicroTally™ manual sampling swabs. After sampling raw beef trim, swabs were co-inoculated at a low level expected to produce fractional positive results and a high level expected to produce all positive results. After a 12-hour equilibration at 4°C, samples were enriched, tested and culture confirmed according to the U.S. Department of Agriculture's Food Safety and Inspection Service (USDA FSIS) reference methods. The results were analyzed using the probability of detection (POD) demonstrating equivalent performance between the BAX® System method and the reference methods.

Introduction

The USDA FSIS collects and analyzes raw beef products for both Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella*. Typically, a single sample collected by N60 exicison or N60 Plus is co-analyzed for the presence of both pathogens. However, these methods are labor intensive and result in product loss. A novel sampling methodology that utilizes a manual sampling device (MSD) composed of cloth rather than enriching actual portions of meat to be tested, has the potential to produce rapid cost-efficient results when screened with PCR along with other production benefits (1).

Sample Preparation and Enrichment

Pure cultures of *E. coli* O157:H7 DD13078 and *Salmonella* Typhimurium DD586 were grown overnight in BHI broth, serially diluted and enumerated for use in this study. Thirty MicroTally™ manual sampling swabs were used to firmly swab 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 manufacturer's instructions.

Swabs were co-inoculated with aliquots of each diluted culture to create 20 low-level (0.2-2 CFU/test portion) and 5 high-level (5-10 CFU/test portion) samples. Five swabs were left uninoculated to serve as negative controls. All swabs were held at 4°C for approximately 12 hours to equilibrate the target organism in the matrix.

Swabs were homogenized with 400 mL of pre-warmed (45°C) BAX® System MP media and incubated at 42°C for 8-15 hours. Sample aliquots were removed at 8, 9, 10, 12 and15 hours and tested by the BAX® System method described below. All samples were culture confirmed at 15 hours.

Method

BAX® System Method – For each sample, two separate lysates were made to accommodate both *E. coli* O157:H7 and *Salmonella* lysis procedures. Twenty microliters or 5 µL of enrichment was added to 200 µL prepared lysis reagent (150 µL of protease to one 12 mL bottle of lysis buffer) in cluster tubes for *E. coli* O157:H7 and *Salmonella* respectively. Lysis was performed by heating cluster tubes at 37°C for 20 minutes and 95°C for 10 minutes, and then cooling tubes at 4°C.

For *E. coli* O157:H7 testing, Real-Time *E. coli* O157:H7 (KIT2000), STEC Screening

(KIT2021) and *E. coli* O157:H7 Exact (KIT2039) PCR tubes were hydrated with 30 µL of lysate.

For *Salmonella* testing, Real-Time *Salmonella* (KIT2006) PCR tubes were hydrated were 30 µL of lysate and held on a chilled (4°C) PCR cooling block for 10 minutes and *Salmonella* 2 (KIT2011) PCR tubes were hydrated with 50 µL of lysate. All PCR tubes were capped securely and loaded into the BAX® System Q7 instrument. A full process was run according to the procedures described in the BAX® System User Guide.

Reference Method – Each sample was confirmed by culture regardless of BAX® System results following the USDA FSIS MLG Method 5C.00 for the Top Seven Shiga Toxin-Producing *Escherichia coli* (STECs) and the USDA FSIS MLG Method 4.10 for *Salmonella*.

Results and Discussion

For *E. coli* O157:H7, all PCR assays returned positive results for 17/20 low spiked samples and 5/5 high spiked samples at each timepoint (8, 9, 10, 12 and 15 hours). All results were identical to culture (Table 1).

For *Salmonella*, all PCR assays returned positive results for 12/20 low spiked samples and 5/5 high spiked samples at each timepoint. All results were identical to culture (Table 1).

For the method comparison, the results of matched samples from the low-level inoculation tested by both the BAX® System method and reference culture method were analyzed using the probability of detection (POD). No significant difference between the methods were observed for either *E. coli* O157:H7 or *Salmonella* at any timepoint (Table 2).

Table 1. BAX® System Presumptive vs. Confirmed Results

Sample Type	Target Organism	CFU/Test Portion	N	BAX® System Presumptive Positive					Culture Positive
				8 h	9 h	10 h	12 h	15 h	
MicroTally™ Swabs	*E. coli* O157:H7	Control	5	0	0	0	0	0	0
		1.27	20	17	17	17	17	17	17
		12.7	5	5	5	5	5	5	5
	Salmonella Typhimurium	Control	5	0	0	0	0	0	0
		1.16	20	12	12	12	12	12	12
		11.6	5	5	5	5	5	5	5

Table 2. BAX® System Method Paired Study Results										
Target Organism	**CFU/Test Portion**	**N**	**FN**	**FP**	**Method Agreement**	**$\sum d_i$**	**dPOD**	**s_d**	**SE dPOD**	**95% CI**
E. coli O157:H7	1.27	20	0	0	20	0.00	0.00	0.00	0.00	-0.14, 0.14
Salmonella Typhimurium	1.16	20	0	0	20	0.00	0.00	0.00	0.00	-0.14, 0.14

N = Number of test portions
FN = Number of false negatives
FP = Number of false positives
$\sum d_i$ = The difference of the replicates tested by the BAX® System method and reference culture method
dPOD = Difference between the mean of differences
s_d = The standard deviation of the differences
SE dPOD = The standard error of the dPOD
95% CI = If the confidence interval of dPOD does not contain zero, then the difference is statistically significant at the 5% level

Conclusions

The results of this study demonstrate the ability of the BAX® System to accurately detect *E. coli* O157:H7 and *Salmonella* from a single enriched MicroTally™ manual sampling swab, equivalent to the reference culture methods, using the following enrichment protocol:

- Homogenize 1 MicroTally™ swab with 400 mL pre-warmed (45°C) BAX® System MP Media and incubate at 42°C for 8-15 hours.

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.