Validation of the BAX® System Real-Time PCR Assays for *Salmonella*, STEC Suite and *E.coli* O157:H7 Exact for the Detection of *Salmonella* and Shiga-toxin producing *Escherichia coli* (STEC; *stx*1 and/or *stx*2 positive) in Beef Trim Sampling Cloths

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INTRODUCTION:

Salmonella has been recognized as a primary cause of foodborne illness worldwide. *Salmonella* can contaminate a wide range of foods including poultry, meat, eggs, dairy, fruit, and vegetables as well as pet food.

Escherichia coli is a bacterium that is commonly found in the human gastrointestinal tract and can be pathogenic. Shiga toxin-producing *E. coli* (STEC) are *E. coli* that produce Shiga toxins encoded by *stx* genes. The main modes of transmission of STEC infections to humans are in the consumption of contaminated food such as meat products, milk and dairy products made with pasteurized or unpasteurized cow's milk or goat's milk, consumption of raw vegetables contaminated by animal feces, and the ingestion of contaminated water and contact with animals, particularly bovines (1).

The beef industry needs a reliable, easy-to-use, non-destructive beef sampling device that allows the accurate detection of STEC and *Salmonella* in a shorter time period. Hygiena's BAX® System and Fremonta's MicroTally® Manual Sampling Device provide the industry with a combined method that provides reliable results in a shortened period of time.

PURPOSE:

The objective of this study was to evaluate the below assays for the detection of *Salmonella* and STEC species from sampling cloths swabbed on 375 g beef trim test portions.

1. BAX System Real-Time PCR Assays for *Salmonella*
2. BAX System Real-Time PCR Assays for STEC Suite
   A. Screening Assay for *stx* and *eae*
   B. Panel 1 Assay for *E. coli* O26, O111, O121
   C. Panel 2 Assay for *E. coli* O45, O103, O145
3. BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact

REGISTERED TRADEMARKS/CERTIFICATIONS:

METHODOLOGY:

Forty sampling cloths used to swab 375 g beef trim test portions were fractionally inoculated with either *E. coli* O157:H7 (N=20), STEC (*E. coli* O26:H11) (N=20) or *Salmonella* Typhimurium (N=20) and held at 4 °C for 48 hours. Sampling clothes were then either enriched with 200 mL of pre-warmed (42 °C) MP media or mTSB+caa. All samples were incubated at 42 °C for 8-24 hours and tested using real-time PCR. Results were confirmed using the appropriate USDA MLG confirmation method.

RESULTS:

In samples enriched with MP media , 10 of 10 (*E. coli* O157:H7) were detected at 10 and 24 hours; 9 of 9 (STEC) at 8, 10 and 24 hours; and 10 of 10 (*Salmonella*) at 10 and 24 hours. Using mTSB+caa, 8 of 8 (*E. coli* O157:H7) at 8 and 24 hours; 13 of 13 (STEC) at 8 and 24 hours; and 10 of 10 (*Salmonella*) positives were detected at 8, 10 and 24 hours. All positives were confirmed by the appropriate reference confirmation method.

Table 1. Matrix study: BAX Real-Time PCR Assay for STEC Suite Presumptive vs. Confirmed Results in Beef Trim (375 g) Sampling Cloths

Matrix and Inoculum	CFU[a]/ Test Portion	N[b]	x[c]	Presumptive		x	Confirmed		dPOD$_{cp}$[f]	95% CI[g]
				POD$_{cp}$[d]	95% CI		POD$_{cc}$[e]	95% CI		
Beef trim Sampling cloth (E. coli O26:H11 DD 9703[h]) 8 h, MP media	NA[i]	5	0	0.00	0.00, 0.00	0	0.00	0.00, 0.00	0.00	(-0.47, 0.47)
	0.64	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	(-0.13, 0.13)
	4.76	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Beef trim Sampling cloth (E. coli O26:H11 DD 9703[h]) 10 h, MP media	NA	5	0	0.00	0.00, 0.00	0	0.00	0.00, 0.00	0.00	(-0.47, 0.47)
	0.64	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	(-0.13, 0.13)
	4.76	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Beef trim Sampling cloth (E. coli O26:H11 DD 9703[h]) 24 h, MP media	NA	5	0	0.00	0.00, 0.00	0	0.00	0.00, 0.00	0.00	(-0.47, 0.47)
	0.64	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	(-0.13, 0.13)
	4.76	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)

[a]CFU/test portion = Inoculating strain was grown overnight, then serially diluted and plated in triplicate to determine appropriate concentration for inoculation.
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]POD$_{cp}$ = Candidate method presumptive positive outcomes divided by the total number of trials.
[e]POD$_{cc}$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[f]dPOD$_{cp}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]Hygiena Culture Collection, New Castle, DE.
[i]Not applicable.

Table 2. Matrix study: BAX Real-Time PCR Assay for *E. coli* O157:H7 Exact Presumptive vs. Confirmed Results in Beef Trim (375 g) Sampling Cloths

Matrix and Inoculum	CFU[a]/ Test Portion	N[b]	x[c]	Presumptive		x	Confirmed		dPOD$_{cp}$[f]	95% CI[g]
				POD$_{cp}$[d]	95% CI		POD$_{cc}$[e]	95% CI		
Beef trim Sampling cloth (E. coli O157:H7 DD1980[h]) 8 h, MP media	NA[i]	5	0	0.00	0.00, 0.00	0	0.00	0.00, 0.00	0.00	(-0.47, 0.47)
	0.63	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	(-0.13, 0.13)
	5.42	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Beef trim Sampling cloth (E. coli O157:H7 DD1980) 10 h, MP media	NA	5	0	0.00	0.00, 0.00	0	0.00	0.00, 0.00	0.00	(-0.47, 0.47)
	0.63	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	(-0.13, 0.13)
	5.42	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Beef trim Sampling cloth (E. coli O157:H7 DD1980) 24 h, MP media	NA	5	0	0.00	0.00, 0.00	0	0.00	0.00, 0.00	0.00	(-0.47, 0.47)
	0.63	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	(-0.13, 0.13)
	5.42	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)

[a]CFU/test portion = Inoculating strain was grown overnight, then serially diluted and plated in triplicate to determine appropriate concentration for inoculation.
[b]N = Number of test potions.
[c]x = Number of positive test portions.
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[f]dPOD$_{cp}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]Hygiena Culture Collection, New Castle, DE.
[i]Not applicable.

Table 3. Matrix study: BAX Real-Time PCR Assay for *Salmonella* Presumptive vs. Confirmed Results in Beef Trim (375 g) Sampling Cloths

Matrix and Inoculum	CFU[a]/ Test Portion	N[b]	x[c]	Presumptive		x	Confirmed		dPOD$_{cp}$[f]	95% CI[g]
				POD$_{cp}$[d]	95% CI		POD$_{cc}$[e]	95% CI		
Beef trim Sampling cloth (S. Typhimurium DD 13557[h]) 10 h, MP media	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
	0.57	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	(-0.13, 0.13)
	4.68	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Beef trim Sampling cloth (S. Typhimurium DD 13557[h]) 24 h, MP media	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
	0.57	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	(-0.13, 0.13)
	4.68	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)

[a]cfu/test portion = Inoculating strain was grown overnight, then serially diluted and plated in triplicate to determine appropriate concentration for inoculation.
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]POD$_{cp}$ = Candidate method presumptive positive outcomes divided by the total number of trials.
[e]POD$_{cc}$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[f]dPOD$_{cp}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]Hygiena Culture Collection, New Castle, DE.
[i]Not applicable.

SIGNIFICANCE:

On February 1, 2023, the USDA FSIS changed its sampling method for beef trim from N60 excision to manual cloth sampling, a non-destructive sampling method (2). In order to most efficiently use this new sampling device, single enrichments in either BAX MP media or mTSB+caa were tested for the recovery of *Salmonella*, *E. coli* O157:H7 and STEC by using the BAX System portfolio of kits.

The real-time PCR assays evaluated allowed users to obtain presumptive positive results for *Salmonella,* STEC, and *E. coli* O157:H7 from one 8-10 hour enrichment after processing and PCR analysis. This quick turnaround time allows beef manufacturers to be able to test for multiple targets from one enrichment resulting in time and cost savings.

REFERENCES:

1. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED). Accessed August 2021. https://www.cdc.gov/ecoli/general/index.html
2. United States Department of Agriculture, Food Safety and Inspection Services, Microbiology Laboratory Guidebook, Chapter 5C.03. Accessed May 2024. https://www.fsis.usda.gov/sites/default/files/media_file/documents/MLG-5C.03.pdf