



Integrating Pathogen Detection and Process Monitoring with the BAX® System Real-Time PCR Assay for *E. coli* O157:H7 Exact +S

Introduction

Escherichia coli O157:H7 is a significant foodborne pathogen in beef production. Effective sanitary dressing practices and process controls are critical for minimizing carcass contamination and reducing the risk of contaminated product entering commerce.

The BAX® System Real-Time PCR Assay for *E. coli* O157:H7 Exact +S (KIT2043) provides two distinct results from a single assay: specific detection of *E. coli* O157:H7 and detection of a proprietary S-target. The assay uses the same *E. coli* O157:H7 target as the AOAC-certified BAX® System Real-Time PCR Assay for *E. coli* O157:H7 Exact (KIT2039).

What is the S-Target?

The S-target is a proprietary genetic marker that is commonly present in a broader group of *E. coli* strains, including non-pathogenic strains that grow in the same environment as *E. coli* O157:H7. As a result, the S-target may be detected in samples that do not contain *E. coli* O157:H7. The assay reports the S-target independently of the *E. coli* O157:H7 result; therefore, a positive S-target result does not indicate the presence of *E. coli* O157:H7. The value of the S-target is derived from patterns observed across multiple samples and over time. Analysis of S-target detection patterns has demonstrated that changes in S-target frequency can provide an early indication of periods when *E. coli* O157:H7 positives may be more likely to occur within a facility.

When incorporated into a statistical process control program, S-target data can serve as a leading process indicator, helping beef processors monitor sanitary dressing performance and identify potential changes before they result in an *E. coli* O157:H7 finding. S-target data can help processors:

- Establish a facility-specific baseline.
- Monitor changes in S-target detection frequency.
- Identify shifts or trends that may indicate declining sanitary dressing performance.
- Investigate process changes before an O157:H7 result occurs.
- Evaluate corrective and preventive actions by monitoring whether the process returns to its expected operating range.

The S-target should not be interpreted from a single result in isolation. Its predictive value is derived from trending results over time and evaluating them in the context of the facility's historical performance, sampling plan, production conditions, and other process-control data.



How Can the Beef Industry Use the S-Target?

Statistical Process Monitoring

S-target results can be incorporated into a Sanitary Dressing Index or other statistical process control program. Establishing a facility-specific baseline and tracking and trending the percentage of S-target positive samples over time can help processors identify changes in sanitary dressing performance before those changes are reflected in an *E. coli* O157:H7 positive result.

Results can be compared across production dates, lines, shifts, sampling locations, product types, or cattle-source groups. An increase in S-target detection or a sustained shift from the established baseline may indicate that the process warrants further investigation. S-target results can be trended over time in Hygiena's SureTrend® software.

Preventive Process Improvement

Changes in S-target trends can help focus investigations on potential contributing factors, such as carcass dressing and hide-removal practices, equipment sanitation, intervention performance, line speed, employee practices, sampling consistency, or incoming cattle conditions.

Identifying these changes early allows processors to take targeted preventive or corrective action before an O157:H7 positive result occurs.

Verification and Decision Support

Following corrective action, S-target results can be monitored to determine whether the process returns to its established baseline. This provides a data-driven way to evaluate whether the action produced the intended improvement.

Used together, the O157:H7 and S-target results provide both pathogen detection and process performance information. The O157:H7 result supports product-testing decisions, while S-target trends help facilities prioritize investigations, additional sampling, and preventive action. Product-disposition decisions should continue to follow the facility's validated food safety program and applicable regulatory requirements.

Validation Data

The BAX System Real-Time PCR assay for *E. coli* O157:H7 Exact + S has been independently reviewed and certified by the AOAC Performance Tested MethodsSM (PTM) Program under PTM 032602 (Tables 1 and 2). The method was validated for the detection and colony confirmation of the *E. coli* O157:H7 target only in sampling cloths (such as MicroTally®) from 375 g beef trim, raw beef trim (375 g and 325 g), raw ground beef (375 g and 325 g) and spinach (375 g) (Table 3). The method performance for these validated matrices and sample sizes was determined to be equivalent to that of the applicable recognized reference methods (USDA MLG and FDA BAM).

**Table 1. Method Performance Claims**

Matrix	Test Portion	Enrichment Conditions				Reference Method ^b	Claim ^c
		Broth ^a	Volume	Temperature	Time		
Sampling cloth from raw beef trim	1 cloth	pw BAX MP	200 mL	42 ± 1 °C	8-24 h	MLG 5C.04	NSDD
	1 cloth ^d	mTSB	200 mL	42 ± 1 °C	8-24 h	MLG 5C.04	Eq
Raw beef trim	375 g	pw-BAX MP	1500 mL	42 ± 1 °C	8-24 h	MLG 5C.04	NSDD
	325 g	mTSB	975 mL	42 ± 1 °C	15-24 h	MLG 5C.04	Eq
Raw ground beef	375 g	pw-BAX MP	1500 mL	42 ± 1 °C	8-24 h	MLG 5C.04	NSDD
	325 g	mTSB	975 mL	42 ± 1 °C	15-24 h	MLG 5C.04	Eq
Spinach	375 g	pw-BAX MP	1500 mL	42 ± 1 °C	8-24 h	BAM Ch. 4A (2020)	NSDD

^a pw-BAX MP = BAX MP media pre-warmed to 42 ± 1 °C before use for sampling cloths and spinach and pre-warmed to 45 ± 1 °C for raw beef trim and raw ground beef; mTSB = Modified Tryptone Soya Broth with casamino acids.

^b MLG = Microbiology Laboratory Guidebook; BAM = Bacteriological Analytical Manual

^c NSDD = No statistical difference detected using SLV study design from OMA Appendix J (2012). The SLV qualitative method comparison study design from OMA Appendix J (2012) is not intended to demonstrate statistical equivalence in unpaired studies. Expert opinion is that the method is appropriate for its intended use. Eq = The study data indicate with 95% confidence that the candidate presumptive results are equivalent to the candidate confirmation results. Equivalency criteria are dependent on the observed POD value and are determined according to TR364 (Least Cost Formulations, Virginia Beach, VA).

^d Add 25 mL neutralizing buffered peptone water (2-8 °C) to the cloth after sampling.

Table 2. Method Selectivity

Claim	Media ^a	Temperature	Inclusivity Strains		Exclusivity Strains ^b		Claim ^c
			No. Tested	No. Positive	No. Tested	No. Positive	
Detection	BAX MP media	42 ± 1 °C	54	54	32	0	MAL
Confirmation	CT-SMAC	37 ± 1 °C	25	25	110	0	MAL
	RBA	37 ± 1 °C	25	25	110	0	MAL
	mRBA	37 ± 1 °C	25	25	110	0	MAL
	TSA	37 ± 1 °C	25	25	110	0	MAL

^a BAX MP media; CT-SMAC = MacConkey Agar with Sorbitol, Cefixime and Tellurite; RBA = Rainbow Agar; mRBA = modified Rainbow Agar; TSA = Tryptic Soy Agar

^b Exclusivity organisms for detection were comprised of 17 non-O157:H7 *E. coli* strains and 15 non-*E. coli* species. Exclusivity organisms for confirmation were comprised of 70 non-O157:H7 *E. coli* strains and 13 non-*E. coli* species.

^c MAL = Met Acceptability Limit for method comparison study outlined in ISO 16140-6:2019

**Table 3. Matrix study: BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact +S vs. Reference Method (unpaired test portions) – POD results**

Matrix ^a	Inoculating Organism	CFU/test portion	N	BAX System			Reference Method		
				X	POD	95% CI	X	POD	95% CI
Meat Sampling Cloth (375 g Beef Trim) – 8 and 24 h	<i>E. coli</i> O157:H7 ATCC 43894	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43
		0.66	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89
		1.18	5	5	1.00	0.57, 1.00	4	0.80	0.38, 1.00
Raw Beef Trim (375 g) – 8 and 24 h	<i>E. coli</i> O157:H7 ATCC 43894	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43
		0.44	20	8	0.40	0.22, 0.61	6	0.30	0.14, 0.52
		1.78	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00
Raw Beef Trim (325 g) – 15 and 24 h	<i>E. coli</i> O157:H7 ATCC 43894	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43
		0.44	20	6	0.30	0.14, 0.52	6	0.30	0.14, 0.52
		1.78	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00
Raw Ground Beef (375 g) – 8 and 24 h	<i>E. coli</i> O157:H7 QL ^b 2-710	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43
		0.75	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66
		2.23	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00
Raw Ground Beef (325 g) – 15 and 24 h	<i>E. coli</i> O157:H7 QL ^b 2-710	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43
		0.75	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66
		2.23	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00
Spinach (375 g) – 8 and 24 h	<i>E. coli</i> O157:H7 QL ^b 2-705	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43
		0.87	20	10	0.50	0.30, 0.70	12	0.60	0.39, 0.78
		2.23	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00

^a Matrices were incubated in the following media: Meat Sampling Cloth (Microtally) in pw-BAX MP media; 375 g samples in pw-BAX MP media; 325 g samples in mTSB.

^b Q Laboratory strain

One Assay. Two Levels of Information

The BAX System Real-Time PCR assay for *E. coli* O157:H7 Exact + S enables laboratories to obtain complementary pieces of information from a single test. This combination can help transform rapid molecular testing from a single pathogen detection event into a broader process-monitoring tool.

Result	What it tells you	Application
<i>E. coli</i> O157:H7 Target	Specific molecular detection of <i>E. coli</i> O157:H7	Pathogen testing and confirmation
S-Target	Indicator of conditions associated with <i>E. coli</i> O157:H7 contamination	Process monitoring and preventative control
IPC	Confirms assay performance	Validity of PCR result

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