Testing beef trim sampling cloths for *Escherichia coli* O157:H7, STEC, and *Salmonella* species with harmonized enrichments

Introduction

Ensuring the safety of beef trim is paramount to public health and industry compliance. United States Federal Regulations mandate rigorous testing procedures to detect potential contaminants in beef products, prominently focusing on pathogens such as *Escherichia coli* O157:H7 and non-O157 Shiga toxin-producing *E. coli* (STEC) serotypes, along with *Salmonella* species. This includes a zero-tolerance policy for the presence of STEC, including *E. coli* O157:H7 in beef trim. Although presence of *Salmonella* in beef trim is permitted, beef product manufacturers are required to monitor and control the presence of *Salmonella* at their sites and in their products. These stringent regulations aim to prevent foodborne illnesses and uphold the quality standards expected by consumers.

To meet these critical requirements, we offer a comprehensive suite of solutions designed for the rapid and accurate detection of these pathogens from beef trim sampling cloths in as little as ten hours (Figure 1).

The performance of these assays has been verified according to AOAC Appendix J method validation guidelines. The assays are designed to offer high specificity and sensitivity, ensuring dependable results in a timely manner. By leveraging our advanced PCR technology, food manufacturers and relevant laboratories can enhance their testing protocols, protect public health, and maintain compliance with regulatory standards.

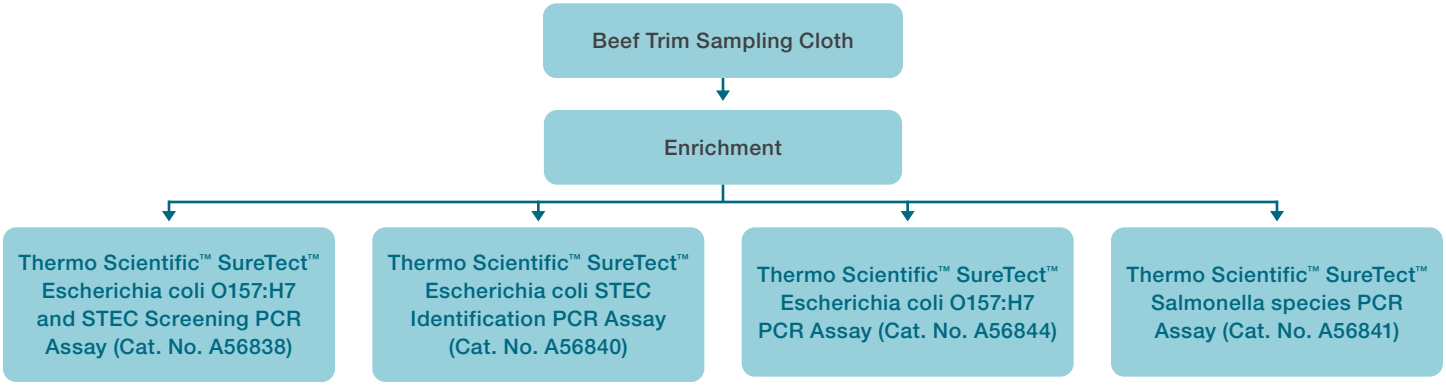

Figure 1. Overview of beef trim sampling cloth testing with SureTect PCR Assays.

Methods of verification

The study was conducted according to AOAC Appendix J method validation guidelines. The SureTect PCR Assays related to STEC and *E. coli* O157:H7 were compared to the USDA FSIS MLG 5C.03 reference method. The SureTect Salmonella species PCR Assay was compared to the USDA FSIS MLG 4.14 reference method.

To provide flexibility, beef trim sampling cloths were tested both with and without neutralizing Buffered Peptone Water (nBPW). The enrichment steps for all four SureTect PCR Assays were harmonized to offer end users streamlined protocols to meet their testing requirements (Table 1).

In addition, the sampling cloths were artificially contaminated with both *Salmonella* and STEC, simulating a worst-case real-world scenario. This methodological approach provided a robust evaluation of the harmonized enrichment performance, ensuring detection of both pathogens is possible, even in a co-infection scenario.

Study results

The PCR assay workflows met AOAC performance requirements and there were no statistically significant differences observed when comparing to the gold standard reference methods (Table 2).

Table 1. Choice of harmonized protocols for beef trim sampling cloth enrichment

Matrices	Enrichment protocols
	SureTect STEC Screening and Identification, SureTect E. coli O157:H7, SureTect Salmonella species
Beef trim sampling cloth without nBPW[a]	1x cloth and 225 mL pre-warmed BPW[b], 8–24 h at 41.5 ± 1°C
Beef trim sampling cloth with nBPW (25 mL)	1x cloth in 25 mL nBPW and 200 mL pre-warmed BPW, 8–24 h at 41.5 ± 1°C
	1x cloth in 25 mL nBPW and 200 mL mTSB[c], 15–23 h at 42 ± 1°C[d]

a. nBPW = neutralizing Buffered Peptone Water; b. BPW = Buffered Peptone Water; c. mTSB = modified Tryptone Soya Broth; d. USDA MLG enrichment protocols.

Table 2. POD results at fractional level in beef trim sampling cloth testing

Matrices	Enrichment	Timepoint	SureTect STEC Screening and Identification[a]		SureTect E. coli O157:H7		SureTect Salmonella species[b]		Result
			dPOD[c]	CI[d]	dPOD	CI	dPOD	CI	
Beef trim sampling cloth without nBPW[e]	225 mL pre-warmed BPW[f], 41.5 ± 1°C	8 h and 24 h	0.00	-0.28, 0.28	0.00	-0.28, 0.28	-0.10	-0.37, 0.19	**Pass**
Beef trim sampling cloth with nBPW (25 mL)	200 mL pre-warmed BPW, 41.5 ± 1°C	8 h and 24 h	0.05	-0.24, 0.3	0.05	-0.24, 0.33	-0.05	-0.33, 0.24	**Pass**
	200 mL mTSB[g], 42 ± 1°C[h]	15 h	0.00	-0.13, 0.13	0.00	-0.13, 0.13	0.00	-0.13, 0.13	**Pass**

a. Enrichment AOAC *Performance Tested Method*℠ Certified; b. Enrichment AOAC *Official Method of Analysis*℠ approved; c. dPOD = Difference in Probability of Detection; d. CI = Confidence Intervals (95%); e. nBPW = neutralising Buffered Peptone Water; f. BPW = Buffered Peptone Water; g. mTSB = modified Tryptone Soya Broth; h. Paired with USDA FSIS MLG 5C.03 and MLG 4.14.

Conclusions and significance

Ensuring the safety of beef trim through rigorous testing is crucial for public health and industry compliance. Our comprehensive suite of solutions offer a robust and efficient workflow for detecting *E. coli* O157:H7, non-O157 STEC serotypes, and *Salmonella* species from beef trim sampling cloths.

A single sample enrichment for all four PCR assays streamlines the testing process, enhancing efficiency. Additionally, the co-inoculation of sampling cloths with both *Salmonella* and STEC simulates the worst-case real-world scenario, proving method performance and ensuring reliable detection.

The methods meet AOAC acceptability requirements by demonstrating no statistically significant differences in performance compared to the relevant reference method, showcasing their high specificity and sensitivity. The SureTect Escherichia coli O157:H7 and STEC Screening PCR Assay and SureTect Escherichia coli STEC Identification PCR Assay were certified by AOAC *Performance Tested Methods*℠ for the enrichments described in Table 2, while the SureTect Salmonella species PCR Assay has been approved by AOAC *Official Methods of Analysis*℠ for the same enrichments. Furthermore, the simplicity and ease of use of these assays make them ideal for food safety professionals seeking dependable and timely results.

Through advanced PCR technology, Thermo Fisher Scientific aids in safeguarding public health and maintaining compliance with stringent regulatory standards.

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