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Harmonized PCR Workflows for *Salmonella* and STEC Detection

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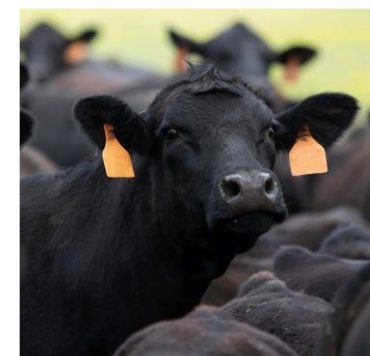
Introduction

Salmonella species and Shiga-toxin producing *Escherichia coli* (STEC) are significant food pathogens in raw beef[1] and leafy produce[2] (Figure 1). A harmonized PCR workflow using the Thermo Scientific™ SureTect™ Salmonella species PCR Assay and the Thermo Scientific™ SureTect™ STEC Screening and Identification workflow has been developed to enable detection of both pathogens from one short enrichment protocol using a large 375 g sample of either raw beef or leafy green produce (Figure 1). This provides end users with a reliable, fast and efficient method.

Figure 1. Raw beef and spinach

Study Design

The harmonized workflow was evaluated according to AOAC Appendix J[3] requirements and included the following studies:

Probability of Detection (POD) Study – 375 g Raw Beef Trim

Thirty samples at three contamination levels were analyzed for all PCR assays following a 1-in-5 dilution of pre-warmed buffered peptone water (BPW) enrichment incubated at 41.5°C for 8 and 24 hours

POD Study – 375 g Leafy Produce

Thirty samples were analyzed for all PCR assays following a 1-in-10 pre-warmed BPW enrichment incubated at 41.5°C for 10 and 24 hours
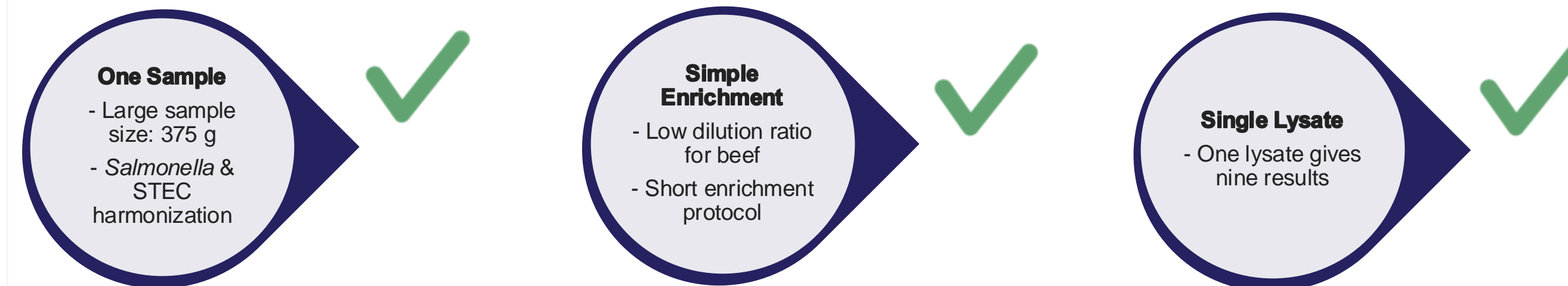
Reference Methods

The SureTect Salmonella PCR Assay was compared against the ISO 6579-1:2017[4] reference methods.

The SureTect STEC PCR Screening and ID Assays were compared against the ISO/TS 13136:2012[5] and FSIS MLG Ch.5C.00[6] (for raw beef only) reference methods.

Key Method Benefits

Figure 2: Key benefits of the harmonized SureTect Salmonella PCR Assay and SureTect STEC PCR Screening and ID Assay workflows

One Sample
- Large sample size: 375 g
- *Salmonella* & STEC harmonization

Simple Enrichment
- Low dilution ratio for beef
- Short enrichment protocol

Single Lysate
- One lysate gives nine results

Results

The SureTect PCR Assay Methods showed superior or comparable performance to the corresponding reference methods with no statistically significant differences (Table 1) at either timepoint. The 375 g raw beef trim had a difference in probability of detection value between the SureTect and reference method (dPOD) of 0.05 for the Salmonella method and 0.10 for the STEC method at the fractional level. The 375 g leafy produce had a dPOD of -0.05 for the Salmonella PCR Assay method and 0.10 for the STEC PCR Assay method at the fractional level. Both reference methods for the SureTect STEC PCR Assay method returned the same results.
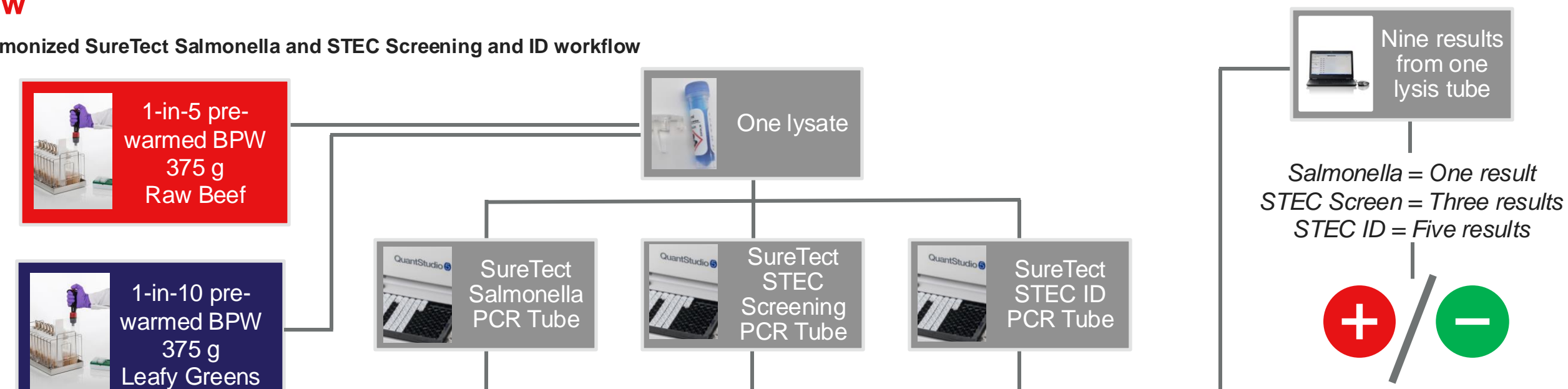
Table 1. POD study results for SureTect Salmonella vs. ISO 6579 and STEC Screening and STEC ID vs. ISO/TS 13136:2012 and FSIS MLG Ch.5C.00

	Matrix	Timepoint	No. samples	Difference in POD	95% POD Confidence Interval	Acceptance
SureTect Salmonella	375 g Raw Beef Trim	8 & 24 h**	5	0.00	-0.43, 0.43	
			20	0.05	-0.24, 0.33	
			5	0.00	-0.43, 0.43	
	375 g Leafy Produce	10 & 24 h**	5	0.00	-0.43, 0.43	
			20	-0.05	-0.32, 0.23	**PASS**
			5	0.00	-0.43, 0.43	
SureTect STEC Screening and ID*	375 g Raw Beef Trim	8 & 24 h**	5	0.00	-0.43, 0.43	
			20	0.10	-0.19, 0.37	
			5	0.00	-0.43, 0.43	
	375 g Leafy Produce	10 & 24 h**	5	0.00	-0.43, 0.43	
			20	0.00	-0.19, 0.37	
			5	0.00	-0.43, 0.43	

*Both reference methods for SureTect STEC returned the same results. **Both timepoints had identical results.

Workflow

Figure 3. Harmonized SureTect Salmonella and STEC Screening and ID workflow

1-in-5 pre-warmed BPW 375 g Raw Beef

1-in-10 pre-warmed BPW 375 g Leafy Greens

One lysate

SureTect Salmonella PCR Tube

SureTect STEC Screening PCR Tube

SureTect STEC ID PCR Tube

Nine results from one lysis tube

Salmonella = One result
STEC Screen = Three results
STEC ID = Five results

Conclusions

EFFICIENT

One enrichment, one DNA extraction, and three PCR reactions leading to nine results increasing throughput and laboratory efficiency.

RELIABLE

Equivalent or superior performance to gold standard FDA and ISO reference methods.

CERTIFIED

Workflows rigorously tested and granted AOAC PTM certification.

References

1. FSIS Compliance Guideline for Minimizing the Risk of Shiga Toxin-Producing *Escherichia coli* (STEC) and *Salmonella* in Beef (including Veal) Slaughter Operations, https://www.fsis.usda.gov/sites/default/files/import/Compliance-Guideline-STEC-Salmonella-Beef-Slaughter.pdf (accessed May 2024)

2. Elias, S. O., *et al. Salmonella* spp. and *Escherichia coli* O157:H7 prevalence and levels on lettuce: A systematic review and meta-analysis, (2019), *Food Microbiology*, Vol. 84

3. Official Methods of Analysis of AOAC INTERNATIONAL (2019) 21st Ed., Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, AOAC INTERNATIONAL, Gaithersburg, MD, http://www.eoma.aoac.org/app_j.pdf (accessed May 2024)

4. ISO 6579-1:2017: Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp., https://www.iso.org/standard/56712.html (accessed May 2024)

5. ISO/TS 13136:2012: Microbiology of food and animal feed - Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens - Horizontal method for the detection of Shiga toxin-producing Escherichia coli (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups, https://www.iso.org/standard/53328.html (accessed May 2024)

6. FSIS MLG Chapter 5C.00 - Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing Escherichia coli (STECs) from Meat Products and Carcass and Environmental Sponges, https://www.fsis.usda.gov/sites/default/files/media_file/2021-03/mlg-5.pdf (accessed May 2024)

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