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Food testing

Thermo Scientific SureTect Salmonella Infantis Assay method: Verification study report

Introduction

The prevalence of *Salmonella* in poultry in the United States has led the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) to set a Key Performance Indicator (KPI) which aims to reduce the proportion of poultry samples with *Salmonella* serotypes commonly associated with human illness. Data from both the Food Safety and Inspection Service and the Centers for Disease Control and Prevention (CDC) has determined that *Salmonella* Infantis, Enteritidis and Typhimurium should be the addressed serotypes for this measure[1]. This produces a need for a rapid and reliable typing method for all three *Salmonella* serotypes.

The Thermo Scientific™ SureTect™ Salmonella Infantis PCR Assay workflow (Figure 1) follows the same basic steps as other SureTect PCR Systems Assays and can be run in parallel with the Thermo Scientific™ SureTect™ Salmonella Species, Typhimurium and Enteritidis Multiplex PCR Assay to enable detection of these three serotypes in poultry meat.

These studies evaluated the performance of the SureTect Salmonella Infantis PCR Assay workflow for the detection of *Salmonella* Infantis from food samples.

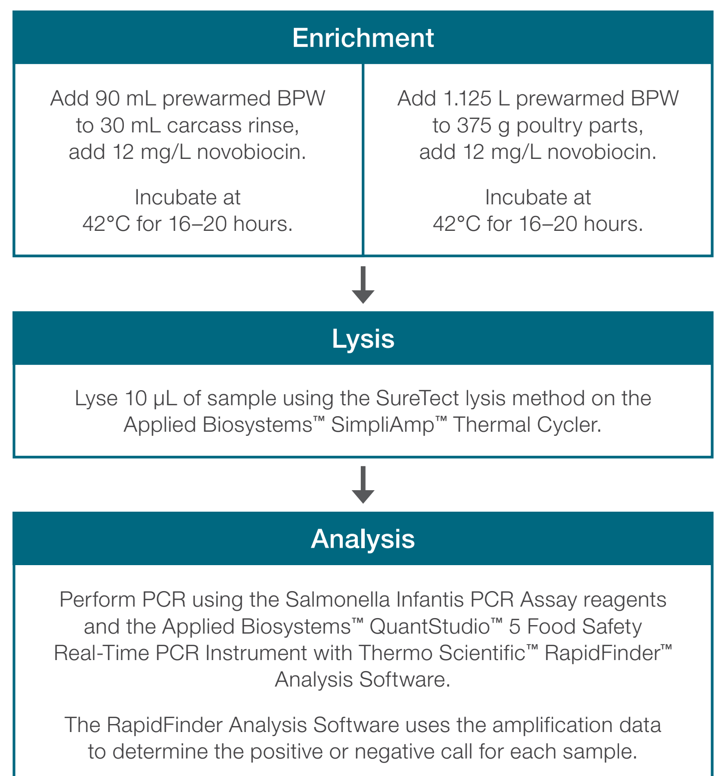

Figure 1: SureTect Salmonella Infantis PCR Assay workflow

Inclusivity and Exclusivity

The inclusivity and exclusivity of the method was assessed both in silico and in vitro.

In silico

Computational methods were used to screen the SureTect Salmonella Infantis PCR Assay against reference genome assemblies in the National Center for Biotechnology Information (NCBI) GenBank Bacteria database (updated Sept 22, 2021, with the addition of *Salmonella* Infantis genomes submitted after this date). A total of 980,831 genomes, including 21,707 for *Salmonella* Infantis, were screened using internal bash script, serotype annotations were obtained with the *Salmonella* In Silico Typing Resource (SISTR) command line tool.

In vitro

DNA extracted from a total of 52 *Salmonella* Infantis and 141 non-target isolates were analyzed in-vitro, including 96 non-Infantis *Salmonella* at a concentration of 2 x 10^5 copies per test. Isolates were sourced from the Thermo Fisher Scientific Culture Collection and global culture collections including CCUG[2], NCTC[3], ATCC[4], CIP[5], MIRRI[6], NCIMB[7], UKHSA[8] and APHA[9].

Testing condition	Inclusivity	Exclusivity
In silico	99.88% (21,681/21,707)	>99.99% (33/959,124)
In vitro	98.07% (51/52)	100.00% (0/141)

Results

In silico analysis resulted in >99.8% inclusivity and >99.9% exclusivity. The non-target hits represent small portion of genomes of multiple serovars.

In vitro testing showed no false identifications of the 141 non-target isolates. Fifty-one out of 52 inclusivity isolates tested positive with the PCR assay resulting in >98% inclusivity. The single isolate that was not detected was genetically distinct from other *S*. Infantis strains analyzed and is likely to be rarely found in food matrices.

Sensitivity

An unpaired study was conducted to compare the sensitivity of the SureTect Salmonella Infantis PCR Assay (alternative method) to the USDA FSIS MLG 4.14 method for the isolation and identification of *Salmonella*[10] (reference method). A total of 30 samples of two matrices were artificially contaminated with a total of 15 different *Salmonella* Infantis strains at low levels (raw poultry parts ~4.5 CFU / test portion and carcass rinses ~2.5 CFU / test portion) then stored at 2-8°C for 2-3 days before testing with both methods. Three negative samples were also tested in this way with a further 42 negative samples tested using only the alternative method. Results were confirmed via direct inoculation of enriched samples on Thermo Scientific™ Oxoid™ *Brilliance*™ Salmonella Agar before applying the Thermo Scientific™ Oxoid™ Salmonella Latex Test Kit for confirmation of *Salmonella* spp. For both methods, the presence of *Salmonella* Infantis was confirmed with relevant *Salmonella* agglutinating antisera.

Results

Between the two methods there were 26 positive agreements, one negative deviation and three positive deviations. All other negative samples tested and confirmed as negative via culture confirmation. Salmonella was not recovered from sub-cultures of the negatively deviating sample. This confirms that this deviation was caused by the unpaired study design, and not a false negative PCR result. As there were more positive deviations than negative deviations, the sensitivity of the alternative method (96.60%) was greater than the reference method (90.00%).

	USDA FSIS MLG 4.14 method positive (R+)	USDA FSIS MLG 4.14 method negative (R-)	Sensitivity reference	Sensitivity alternative
SureTect™ Salmonella Infantis PCR Assay method positive (A+)	26 (PA)	3 (PD)	90.00%	96.60%
SureTect™ Salmonella Infantis PCR Assay method negative (A-)	1 (ND)	3 (NA)*		

*42 additional samples were tested with the alternative method only. All results were negative and were culture confirmed as negative.

PA= positive agreement PD= positive deviation ND = negative deviation NA = negative agreement

Relative Level of Detection (RLOD) and Probability of Detection (POD)

An unpaired study was conducted to determine the relative limit of detection (RLOD) and probability of detection (POD) of the SureTect Salmonella Infantis PCR Assay (alternative method) when compared to the USDA FSIS MLG 4.14 method for the isolation and identification of *Salmonella*[10] (reference method). For each matrix type, 14 samples were tested with both methods; two without contamination, ten at a low contamination level (1-3 CFU per sample), and two at a high contamination level (3-5 CFU per sample). Results were confirmed via direct inoculation of enriched samples on *Brilliance* Salmonella Agar before applying the Salmonella Latex Test Kit for confirmation of *Salmonella* spp. For both methods, the presence of *Salmonella* Infantis was confirmed with relevant *Salmonella* agglutinating antisera.

Results

There were no differences in the number of positive / negative results during the testing of chicken carcass rinses. For poultry parts there were differences in the number of positive results at both the low and high contamination levels, however RLOD values were >1 and below the acceptability limit of 2.5 for an unpaired study. All of the dPOD 95% upper and lower confidence interval values contained a zero, indicating no significant difference in performance between methods.

Matrix	Contamination level / test portion (CFU)	N	SureTect Salmonella Infantis PCR Assay			USDA FSIS MLG 4.14			dPOD (C*,R)	95% LCL	95% UCL	RLOD
			X	POD_C	LOD_{50}	X	POD_R	LOD_{50}				
Poultry Parts	0	2	0	0.00		0	0.00		0.00	-0.66	0.66	
	1.5	10	7	0.70	1.24	6	0.60	1.02	0.10	-0.28	0.45	1.22
	4.0	2	1	0.50		2	1.00		-0.50	-1.00	0.33	
Chicken Carcass Rinse	0	2	0	0.00		0	0.00		0.00	-0.66	0.66	
	1	10	5	0.50	0.96	5	0.50	0.96	0.00	-0.37	0.37	1.00
	5	2	2	1.00		2	1.00		0.00	-0.66	0.66	

*Alternative method is referred to as candidate method for the purpose of POD calculations

N = number of samples tested. X= number of positive samples

Method modification for complex matrices

A paired study was conducted to determine if enrichment conditions established for chicken carcass rinses could be directly applied to rinses taken during pre-chill stages of production.

Twenty 30 mL aliquots of rinse samples from the hot rehang stage of production were artificially contaminated, with <5 CFU of a total of 10 different *Salmonella* Infantis strains, and tested using the enrichment protocol designed for chicken carcass rinses (Figure 1). After 16 hours incubation, samples were tested using the alternative method. Samples were then returned to the incubator for an additional 8 hours (24 hours in total) before subculturing into Rappaport-Vassiliadis Soya Peptone Broth and plating onto *Brilliance* Salmonella Agar (reference method).

Results

There were a total of three negative deviations between the methods, where samples returned a positive culture result at 24 hours but a negative PCR result at 16 hours.

Additional PCR testing was performed on the samples after 20 hours incubation, 1/3 of the samples returned a positive result at this time point. Further aliquots were taken from the rinses that gave deviating results. Testing was repeated on the same samples with the addition of 20 mg/L of novobiocin and yielded positive results for all samples at both 16 and 20 hours. It was concluded that high levels of non-target flora in these samples contributed to a delay in *Salmonella* growth, and that the increase in both enrichment time and novobiocin concentration overcame this challenge.

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Conclusions

The SureTect Salmonella Infantis PCR Assay method was able to identify *Salmonella* Infantis both in silico and in vitro with a high level of accuracy.

Low levels of *Salmonella* Infantis were readily detected from poultry matrices with a single 16 hour enrichment step with comparable performance to the reference method.

Modifications to the enrichment method to overcome high levels of background flora in complex matrices are recommended. This should be verified using methods outlined in the ISO 16140 series[11,12].

Combining the SureTect Salmonella Species, Typhimurium and Enteritidis Multiplex PCR Assay and the SureTect Salmonella Infantis PCR Assay enables simultaneous detection and differentiation of the three *Salmonella* serotypes covered by the USDA KPI within a single PCR run.

References

1. USDA FY 2022-2026 FOOD SAFETY KPI https://www.fsis.usda.gov/inspection/inspection-programs/inspection-poultry-products/reducing-salmonella-poultry/salmonella-0
2. Culture Collection University of Gothenburg, Sweden
3. National Collection of Type Cultures, USA
4. American Type Culture Collection, USA
5. Pasteur Institute, France
6. Microbial Resource Research Infrastructure, Portugal
7. NCIMB Ltd, UK
8. UK Health Security Agency, UK
9. American Public Health Association USA
10. USDA FSIS MLG 4.14 Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, and Siluriform (Fish) Products and Carcass and Environmental Sponges
11. EN ISO 16140-2:2016. Microbiology of food and animal feed - Method validation - Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method.
12. EN ISO 16140-3:2021. Microbiology of the food chain — Method validation — Part 3: Protocol for the verification of reference methods and validated alternative methods in a single laboratory.

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