Food Testing

Rapid Detection Method to Identify *Salmonella* Infantis in Food Samples

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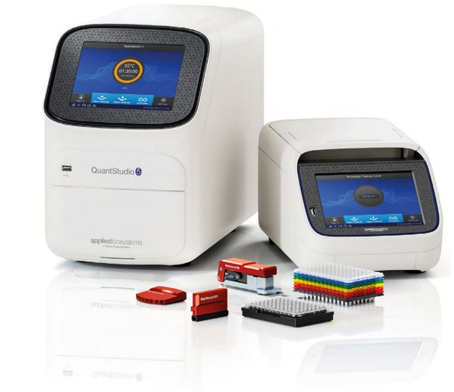
Introduction

The prevalence of *Salmonella* in poultry in the United States has led the USDA Food Safety and Inspection Service to set a KPI of reducing the proportion of poultry samples with *Salmonella* serotypes commonly associated with human illness. Data from both FSIS and the CDC has determined that Infantis, Enteritidis and Typhimurium should be the addressed serotypes for this measure. This produces a need for rapid and reliable identification method for all three *Salmonella* serotypes.

A detection method for *Salmonella* Infantis in poultry meats was developed to complement the RapidFinder™ Salmonella Species, Typhimurium and Enteritidis Multiplex Assay (RapidFinder Salmonella Multiplex assay) in order to detect these three serotypes. The method utilizes the established Thermo Scientific™ SureTect™ Food Safety PCR System (Figure 1).

The purpose of this study was to evaluate the performance of a *Salmonella* Infantis PCR assay (PCR assay) for the detection of *Salmonella* Infantis from enriched food samples and to assess accuracy by conducting inclusivity and exclusivity testing.

Figure 1. Thermo Scientific SureTect Food Safety PCR System



Methods

Inclusivity and Exclusivity

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

Computational methods were used to screen the assay against reference genome assemblies in the 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.

DNA extracted from a total of 52 *Salmonella* Infantis and 94 non-target strains were analyzed in-vitro, including 75 non-Infantis *Salmonella* at a concentration of 2 x 10^5 copies / test. Strains 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].

Methods Continued

Sensitivity

An unpaired study was conducted to compare the performance of the PCR assay method (Figure 2) with the USDA FSIS MLG 4.14 method for the isolation and identification of *Salmonella*[9] (reference method). At least 22 replicates 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) to achieve a fractionally positive dataset. Replicates were divided evenly across each method for testing. Results were confirmed via direct inoculation of enriched samples on Thermo Scientific™ Oxoid™ *Brilliance*™ Salmonella Agar before confirmation with Thermo Scientific™ Oxoid™ Salmonella Latex Test Kit. For both methods, the presence of *Salmonella* Infantis was confirmed with relevant *Salmonella* agglutinating antisera.

Relative level of detection (RLOD) and probability of detection (POD)

An unpaired study was conducted with raw poultry parts and carcass rinses to establish the relative level of detection of the PCR assay method. For both matrices bulk contaminated and uncontaminated matrix were combined to achieve two high-level (4-5 CFU), ten low level (1 – 1.5 CFU) and two negative test portions before analysis using the PCR assay workflow (Figure 2) and the reference method.

Figure 2. PCR Assay workflow

Sample preparation

Artificially contaminate 375 g poultry parts and store at +2-8°C for 2-3 days.	Artificially contaminate each chicken carcass and store at +2-8°C for 2-3 days. Rinse with 400 mL BPW for 1 min.

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Enrichment

Matrix specific enrichment detailed in Figure 3.

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Lysis

Lyse 10 µL of sample using the SureTect lysis method on the Applied Biosystems™ SimpliAmp™ Thermal Cycler.

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Analysis

Perform PCR using the *Salmonella* Infantis PCR assay reagents and the Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR Instrument with Thermo Scientific™ RapidFinder™ Analysis Software.

The RapidFinder Analysis Software uses the amplification data to determine the positive or negative call for each sample.

Figure 3. Matrix-specific enrichment procedures for the PCR assay

Add 90 mL prewarmed BPW to 30 mL carcass rinse

Add 12 mg/L novobiocin

Incubate at 42°C for 16-20 hours

Add 1.125 L prewarmed BPW to 375 g poultry parts

Add 12 mg/L novobiocin

Incubate at 42°C for 16-24 hours

Results

Inclusivity and exclusivity

The in silico analysis resulted in 99.88% inclusivity and >99.99% exclusivity with only 33 non-target genomes out of 959,124 genomes expected to produce false positive PCR amplification. Fifty one out of 52 inclusivity strains tested positive on the PCR assay resulting in >98 % inclusivity. The negative strain was genetically distinct from other *S.* Infantis strains tested and is likely to be rarely found in food matrices. All of the exclusivity strains tested negative with the PCR assay method.

Table 1. In silico inclusivity and exclusivity results

Genome type	N	Expected positives	Expected negatives	Inclusivity	Exclusivity
Salmonella Infantis	21,707	21,681	26	99.88%	-
Non-target	959,124	33	959,091	-	>99.99%

Sensitivity, RLOD and POD

Results from the sensitivity study indicated that the two methods were equivalent. All positive results from both methods were confirmed via culture and relevant agglutinating sera (Table 2). The RLOD for both matrices was below the acceptability limit of 2.5 for an unpaired study, the results were not statistically different (Table 3).

Table 2. Sensitivity results

Matrix	Number of replicates tested (per method)	PCR Assay positive results	Culture confirmed	Reference method Positives results
Carcass rinse	18	17	17	15
Poultry parts	12	12	12	12

Table 3. RLOD and POD results

Matrix	Contamination level / Test portion	N	PCR assay		Reference		dPOD	95% Ci	RLOD
			N positive	POD	N positive	POD			
Poultry Carcass Rinse	N/A[i]	2	0	0.00	0	0.00	0.00	-0.66, 0.66	1.00
	1.0	10	5	0.50	5	0.50	0.00	-0.37, 037	
	5.0	2	2	1.00	2	1.00	0.00	-0.66, 0.66	
375 g Poultry Parts	N/A	2	0	0.00	0	0.00	0.00	-0.66, 0.66	1.03
	1.0	10	7	0.70	6	0.60	0.10	-0.28, 0.45	
	3.0	2	1	0.50	2	1.00	-0.50	-1.00, 0.33	

Conclusions

The *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.

Combining the RapidFinder Salmonella Multiplex Assay and the *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
web address 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 [7] UK Health Security Agency, UK
8. American Public Health Association USA
9. USDA FSIS MLG 4.14 Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, and Siluriform (Fish) Products and Carcass and Environmental Sponges

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