Food testing

Application note: Quantitation, detection, and differentiation of Salmonella from poultry

Importance of *Salmonella* testing in poultry

Salmonella is a bacterial pathogen that poses significant risks to public health, particularly with respect to foodborne illnesses. In the United States, poultry products have been identified as one of the primary sources of *Salmonella* contamination. Consequently, rigorous testing and monitoring of poultry for *Salmonella* have become crucial steps in ensuring food safety and preventing outbreaks.

The US Department of Agriculture's Food Safety and Inspection Service (FSIS) plays a pivotal role in overseeing *Salmonella* testing in poultry. The agency has established a comprehensive testing program, known as the *Salmonella* Verification Testing Program, which is designed to assess the prevalence and control measures of *Salmonella* in poultry establishments across the country. As part of these performance standards, poultry establishments must perform Process Control Testing to evaluate the effectiveness of the preventative measures, Category 1 Testing to detect the presence of *Salmonella* from poultry rinses, and Category 2 Testing to serotype the *Salmonella* strains detected from Category 1 Testing.

Quantification of *Salmonella* can help poultry establishments evaluate the effectiveness of their process control measures, as the level of *Salmonella* can be quantified at the beginning and the at the end of the production process. Detection and differentiation of *Salmonella* as part of Category 1 and 2 testing demonstrates the establishments compliance to the performance standards and allows them to take appropriate action to prevent *Salmonella* contamination, protecting public health.

To ensure poultry establishments meet regulatory requirements and perform their testing accurately and reliably, Thermo Fisher Scientific offers a range of PCR assay workflows that can quantify, detect, and differentiate *Salmonella* from the same test portion for relevant poultry samples. Table 1 provides an overview of the available assays and their capabilities. The Thermo Scientific™ SureCount™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit method, which has been evaluated according to AOAC Appendix J guidelines, allows for simultaneous differentiation and accurate estimated quantitation within a single shift. To further aid method users, the same test portion used for quantification can also be used for detection and further differentiation by re-incubating the test portion. The Thermo Scientific™ SureTect™ Salmonella species PCR Assay, Thermo Scientific™ RapidFinder™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit, and Thermo Scientific™ SureTect™ Salmonella Infantis PCR Assay allow for detection and differentiation of *Salmonella* from the same test portion used in the quantitation method.

Table 1. Thermo Scientific PCR Assay for poultry testing

Assay name	Detection	Quantification	Serotyping
SureCount Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit		*Salmonella* species *S.* Enteritidis *S.* Typhimurium	
SureTect Salmonella species PCR Assay	*Salmonella* species		
RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit	*Salmonella* species *S.* Enteritidis *S.* Typhimurium		*S.* Enteritidis *S.* Typhimurium
SureTect Salmonella Infantis PCR Assay	*S.* Infantis		*S.* Infantis

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Figure 1A. Harmonised workflows for *Salmonella* quantitation, detection, and differentiation from poultry

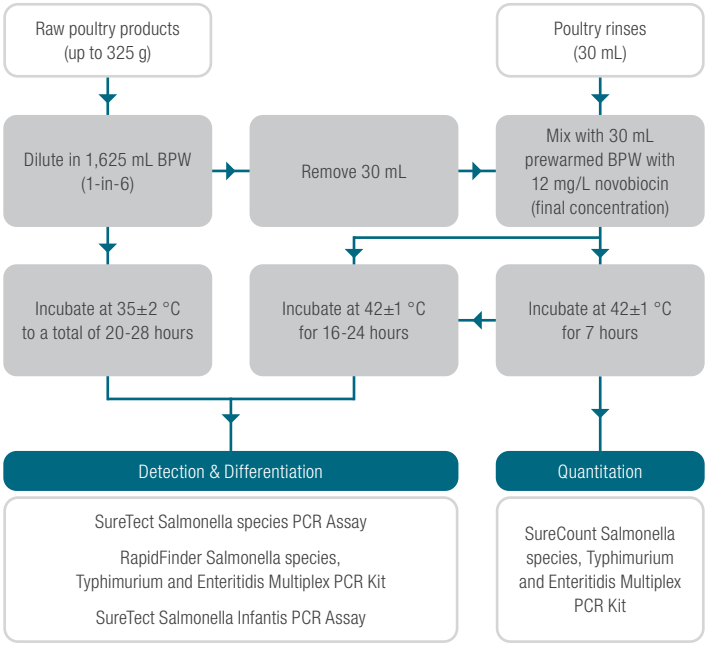
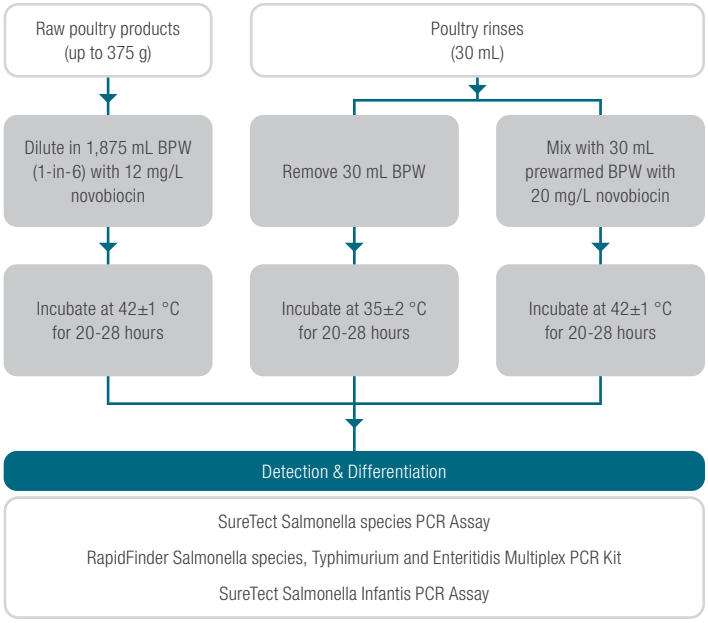


Figure 1B. Alternative workflows for *Salmonella* detection and differentiation from poultry



Thermo Scientific end-to-end PCR workflows for *Salmonella* testing in poultry

The Thermo Scientific PCR assays offer a reliable and accurate solution for poultry testing. These assays are designed to simplify the testing process while ensuring the highest level of performance. With verified and certified protocols available for different poultry matrices, users can have confidence in the results obtained. Figure 1 A and B give an overview of the workflows.

These workflows are designed to be user-friendly and efficient, allowing for streamlined testing processes. For both poultry rinses and raw poultry products, enrichment protocols are harmonized allowing one test portion to be tested with any of the assays in Table 1. For certain poultry matrices, more than one verified protocol exists, meaning end users can select the protocol which fits their needs best. Table 2 summarizes the verified/certified protocols available for poultry testing.

Table 2. Poultry matrices and enrichment protocols

Matrix category	Matrix	Enrichment	SureCount Salmonella	SureTect Salmonella	RapidFinder Salmonella	SureTect Infantis
Poultry rinse	Pre-chill rinses: 30 mL	Mix with 30 mL pre-warmed BPW + 20 mg/L novobiocin, at 42±1 °C	Custom verification available	20-28 hours[a]	20-28 hours	20-28 hours
	Post-chill rinses: 30 mL	Mix with 30 mL pre-warmed BPW + 12 mg/L novobiocin (final concentration), at 42±1 °C	7 hours[a]	16-24 hours[a]	16-24 hours	16-24 hours
		Mix with 30 mL BPW at 35±2°C.	Not applicable	20-28 hours[a]	20-28 hours	20-28 hours
Raw poultry products	Ground turkey: up to 325 g	Mix in 1,625 mL (1-in-6) BPW at 35±2 °C SureCount: Take 30 mL and mix with 30 mL pre-warmed BPW + 12 mg/L novobiocin (final concentration), at 42±1 °C	30 mL aliquot - 7 hours[a]	20-28 hours[a]	20-28 hours	20-28 hours
	Ground turkey: up to 375 g	Mix in 1,875 mL (1-in-6) BPW + 12 mg/L novobiocin at 42±1 °C[b]	30 mL aliquot - 7 hours[a, b]	20-28 hours[a]	20-28 hours	20-28 hours

a. AOAC certified protocol.
b. Aliquot should be removed from enrichment before novobiocin is added.

Methods of Verification

Qualitative Method Assessment

Probability of detection (POD) studies were conducted to compare performance of SureTect and RapidFinder workflows to the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) MLG 4.14 – Isolation and Identification of *Salmonella* from meat, poultry, pasteurized egg, Siluriformes (fish) products and carcass and environmental sponges reference method with poultry matrices.

For each matrix, 30 samples were tested: 20 at a low inoculation level (0.2-2 CFU/test portion), five at a high inoculation level (>2 CFU/test portion) and five left uninoculated. Samples were allowed to equilibrate at 2-8 °C for 48-72 hours.

Samples were enriched and tested following the SureTect Salmonella or the RapidFinder Salmonella Multiplex workflows on the Applied Biosystems™QuantStudio™ 5 Food Safety Real-Time PCR System and the Applied Biosystems™ 7500 Fast Real-Time PCR System; all samples were culture confirmed (Figure 1). Each poultry matrix was tested alongside the method outlined in USDA FSIS MLG Chapter 4 in an unpaired study design.

Quantitative Method Assessment

Quantitative analysis studies were conducted to compare performance of the SureCount workflow to the USDA FSIS MLG Chapter 4 and MLG Appendix 2.05 reference method to quantify *Salmonella* from poultry matrices.

For each matrix, five non-inoculated portions (0 CFU/test portion), five low level inoculated portions (1–10 CFU/test portion), five medium level inoculated portions (10–100 CFU/test portion), and five high level inoculated portions (100–1000 CFU/test portion) were tested using the SureCount method and the reference method.

Matrix Study Results

The matrix study results for each combination can be seen in Tables 3 and 4.

Table 3. SureCount™ Salmonella species, Typhimurium and Enteritidis PCR Kit Results

Matrix	Innoculating strain(s)	Cont. level[a]	DOM[b]	95% CI[c]		Results
				Lower	Upper	
Fresh Ground Turkey 325 g	N/A[d]	Non	N/A	N/A	N/A	Pass
	S. Enteritidis	Low	-0.241	-0.816	0.334	
	S. Enteritidis & *S.* Typhimurium	Med	0.093	-0.106	0.291	
	S. Enteritidis	Med	-0.040	-0.245	0.166	
	S. Typhimurium	Med	0.821	0.616	1.027	
	S. Enteritidis	High	0.225	-0.095	0.545	
Chicken Carcass Rinse	N/A	Non	N/A	N/A	N/A	Pass
	S. Typhimurium	Low	0.492	0.228	0.757	
	S. Typhimurium	Med	0.197	-0.174	0.568	
	S. Typhimurium & *S.* Hadar	High	-0.356	-0.842	0.130	
	S. Typhimurium	High	-0.477	-0.808	-0.145	
	S. Hadar	High	0.543	-0.864	1.949	
Fresh Ground Turkey 325 g (Independent Laboratory)	N/A	Non	N/A	N/A	N/A	Pass
	S. Enteritidis	Low	-0.011	-0.488	0.466	
	S. Enteritidis & *S.* Typhimurium	Med	0.085	-0.468	0.639	
	S. Enteritidis	Med	0.012	-0.193	0.218	
	S. Typhimurium	Med	0.366	0.160	0.571	
	S. Enteritidis	High	0.223	-0.078	0.524	

a. All matrixes were artificially contaminated. Non=non-inoculated.
b. Difference of means between the candidate and reference methods, analyzed using an unpaired statistical analysis.
c. Confidence interval (95%).
d. Not applicable.

Table 4. Detection and Differentiation Thermo Scientific PCR Assay POD Results at Fractional Level for Poultry Matrices

Matrix	Enrichment	SureTect Salmonella spp. PCR Assay		RapidFinder Salmonella Multiplex Kit						SureTect Salmonella Infantis PCR Assay		Result
		Salmonella spp. target		Salmonella Typhimurium target		Salmonella Enteritidis target		Salmonella spp. target		Salmonella Infantis target		
		$dPOD_c$[a]	CI[b]	$dPOD_c$	CI	$dPOD_c$	CI	$dPOD_c$	CI	$dPOD_c$	CI	
Whole bird and parts rinse (30 mL)	30 mL pw[c]-BPW + 12 mg/L novobiocin	-0.15	-0.41, 0.15			-0.15	-0.40, 0.13	-0.15	-0.41, 0.15	-0.05	-0.31, 0.22	Pass
	30 mL pw-BPW + 20 mg/L novobiocin	-0.10	-0.37, 0.19			-0.10	-0.36, 0.18	-0.10	-0.37, 0.19	0.05	-0.23, 0.32	Pass
	30 mL BPW	0.00	-0.13, 0.13			0.00	-0.28, 0.28	0.00	-0.13, 0.13	0.00	-0.27, 0.27	Pass
Raw Poultry products (325 g ground turkey)	1,625 mL BPW	0.00	-0.13, 0.13	0.00	-0.27, 0.27			0.00	-0.13, 0.13	0.00	-0.26, 0.26	Pass
Raw Poultry products (375 g ground turkey)	1,875 mL BPW + 12 mg/L novobiocin	0.05	-0.24, 0.33	0.00	-0.27, 0.27			0.05	-0.24, 0.33	0.10	-0.18, 0.36	Pass

a. $dPOD_c$: Difference between the confirmed candidate method result and reference method confirmed result POD values. Reference method used was USDA FSIS MLG 4.
b. CI (95%): If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
c. Pre-warmed.
d. Reference method used in testing was ISO 6579-1:2017.

For all detection/serotyping PCR assay-matrix combinations, the Thermo Scientific PCR Assays met AOAC performance requirements. There were no statistically significant differences observed in the dPOD results between the methods.

For the quantitative PCR assay, results met AOAC performance requirements.

Conclusion

The Thermo Scientific PCR assays provide a reliable and accurate solution for *Salmonella* testing in poultry. These assays offer the following benefits:

- They enable the quantitation, detection, and differentiation of *Salmonella* (up to three serotypes) from a single enrichment, reducing laboratory resource requirements.
- Quantitation can take place in a single shift, streamlining the testing process.
- Improve accuracy and measurability of Process Control Measures.
- The workflows provided include automated data analysis, which supports real-time decision making.
- The poultry testing PCR workflows have been certified by AOAC or meet AOAC testing requirements, ensuring their quality and adherence to industry standards.

Overall, the Thermo Scientific PCR assays offer an efficient and verified solution for *Salmonella* testing in poultry, providing reliable results and supporting timely decision making.

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