

# Thermo Scientific SureTect Salmonella species PCR Assay Workflow NF VALIDATION; ISO 16140-2:2016

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## Summary

The Thermo Scientific™ SureTect™ Salmonella species PCR Assay workflow (PT0100A) (alternative method) has been certified by NF VALIDATION (UNI 03/07-11/13) for the detection of *Salmonella* species from meat, dairy, seafood, vegetables, raw beef meats with and without aromatics, production environment samples and powdered infant formula (PIF) 10 g and 375 g samples. The following report gives a summary of the validation studies performed to gain NF VALIDATION.

## Methodology

Study	Reference method
<b>Initial validation</b> PCR analysis was conducted using the Thermo Scientific™ SureTect™ PikoReal™ Real-Time PCR Instrument	<b>ISO 6579:2002</b> 'Microbiology of the food chain—Horizontal method for the detection of <i>Salmonella</i> species.'
<b>Extension study</b> To incorporate the Applied Biosystems™ 7500 Fast Food Safety Real-Time PCR System (7500 Fast Real-Time PCR Instrument and Applied Biosystems™ RapidFinder™ Express Software (version 2.0 or higher))	<b>ISO/TS 22964:2006</b> 'Microbiology of the food chain—Horizontal method for the detection of <i>Salmonella</i> species.'
<b>Extension study</b> To incorporate the following; <ul style="list-style-type: none"><li>Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR System (Applied Biosystems QuantStudio 5 Real-Time PCR Instrument and RapidFinder™ Analysis Software version 1.0)</li><li>Powdered milk products (375 g)</li></ul>	<b>ISO 6579-1:2017</b> 'Microbiology of the food chain—Horizontal method for the detection, enumeration and serotyping of <i>Salmonella</i> – Part 1: Detection of <i>Salmonella</i> species.'

The initial certification study and all subsequent extension studies were conducted by ADRIA Développement, Quimper, France.

The protocols for the alternative method and the reference method are summarized in Appendix 1 and 2, respectively.

**Inclusivity & exclusivity study**

Fifty-five inclusivity isolates were cultured in Thermo Scientific™ Brain Heart Infusion (BHI) Broth (incubated for 24 hours at 37±1°C). The BHI culture was inoculated into Thermo Scientific™ Buffered Peptone Water (BPW) (ISO) at a level of 10 CFU/225 mL (incubated for 8 hours at 41.5±°C).

Thirty exclusivity isolates were cultured in BHI Broth (incubated for 24 hours at 37±1°C). The BHI culture was inoculated into BPW (ISO) to obtain approximately 10<sup>5</sup> CFU/mL (incubated for 24 hours at 37±1°C).

Following incubation, the enrichment samples were analyzed following the alternative method protocol (Appendix 1).

**Inclusivity & exclusivity results**

The alternative method successfully identified all 55 inclusivity isolates and correctly gave a negative result for all 30 exclusivity isolates. The results show the alternative method is sensitive and specific with equivalent performance to the ISO reference method.

**Inter-laboratory study**

An inter-laboratory study was performed as part of the initial validation. A raw ground beef matrix was prepared and spiked with *Salmonella* Typhimurium and sent to all participating laboratories. Samples were analyzed following both the alternative method and the ISO reference method (Appendix 1 and 2). Of all the samples tested, one third were unspiked, one third were spiked with a low level inoculum (4.1 CFU/25 g) and the remaining samples were spiked with a high level inoculum (19.2 CFU/25 g).

**Inter-laboratory study results**

**Table 1. Inter-laboratory study results summary**

Relative accuracy	99.6%
Relative sensitivity	100.0%
Relative specificity	100.0%

The results displayed in Table 1 demonstrate that the alternative method is a reliable method for the detection of *Salmonella* species.

**Method comparison study**

A total of 456 samples including meat products, milk and dairy products, seafood and fishery products, vegetables, raw beef meats, environmental samples and PIF (10 g) categories were analyzed using the alternative method with the Applied Biosystems 7500 Fast Food Safety System and the Applied Biosystems QuantStudio 5 Food Safety System.

The current extension study aimed to extend the claims of the NF VALIDATION to include milk powders (375 g), and to incorporate the use of the Applied Biosystems QuantStudio 5 Food Safety System.

For the milk and dairy category and the raw beef meats category, two different enrichment protocols were analyzed as part of the method comparison study on the Applied Biosystems 7500 Fast Food Safety System and the Applied Biosystems QuantStudio 5 Food Safety System. Table 3 summarizes the categories and enrichment protocols.

**Table 3: Enrichment protocol summary**

Category or type		Enrichment broth	Incubation
1	Meat products	BPW (ISO) + 12 mg/L Novobiocin	20–24 h at 37±1°C
2	Dairy products	BPW (ISO) + 12 mg/L Novobiocin	20–24 h at 37±1°C
		Thermo Scientific™ ONE Broth-Salmonella (OBS) with 12 mg/L Novobiocin	20–24 h at 37±1°C
3	Infant formula	BPW (ISO)	16–20 h at 37±1°C
4	Vegetables	BPW (ISO) with 12 mg/L Novobiocin	20–24 h at 37±1°C
5	Seafood	BPW (ISO) with 12 mg/L Novobiocin	20–24 h at 37±1°C
6	Raw beef meats with and without aromatics	Pre-warmed BPW (ISO)	9 h at 41.5±1°C
		Pre-warmed BPW (ISO)	24 h at 41.5±1°C
7	Environmental samples	BPW (ISO)	20–24 h at 37±1°C
8	Powdered milk products	BPW (ISO) with 6 mg/L Vancomycin	18–22 h at 37±1°C

PA = Positive agreement    PD = Positive deviation    PP = Positive deviation positive presumptive non-confirmed samples    NA = Negative agreement  
 ND = Negative deviation    PPND = Predictive positive negative deviation    PPNA = Predictive positive negative agreement

The results for the method comparison study using the Applied Biosystems 7500 Fast and the Applied Biosystems QuantStudio 5 Food Safety Systems are detailed in Tables 4 and 5, respectively.

## Method comparison study results

**Table 4: Method comparison study results using the Applied Biosystems 7500 Fast Food Safety System**

Category		PA	NA	PD	ND	PPND	PPNA	Total
1	Meat products	26	35	7	5	0	0	73
2	Milk and dairy products (BPW + Novobiocin)	21	32	4	4	1	0	62
	Milk and dairy products ((One-Broth Salmonella) (OBS) + Novobiocin)	21	31	5	5	0	0	62
3	Infant formula	33	30	0	1	0	0	64
4	Vegetables	22	33	5	3	1	0	64
5	Seafood products	22	33	3	4	1	0	63
6	Raw beef meats 9 h	20	31	5	5	0	0	61
	Raw beef meats 24 h	21	31	5	4	0	0	61
7	Environmental samples	27	39	0	3	0	0	69
8	Powdered milk products (375 g)	21	30	6	6	0	0	63
All categories + Dairy enriched with BPW + Raw Beef incubated for 9 hours		192	263	30	31	3	0	519
All categories + Dairy enriched with OBS + Raw Beef incubated for 9 hours		192	263	31	32	2	0	519
All products + Dairy enriched with BPW + Raw Beef incubated for 24 hours		193	263	30	30	3	0	519
All products + Dairy enriched with OBS + Raw Beef incubated for 24 hours		193	262	31	31	2	0	519

PA = Positive agreement    PD = Positive deviation    PP = Positive deviation positive presumptive non-confirmed samples    NA = Negative agreement  
 ND = Negative deviation    PPND = Predictive positive negative deviation    PPNA = Predictive positive negative agreement

**Table 5: Method comparison study results using the Applied Biosystems QuantStudio 5 Food Safety System**

Category		PA	NA	PD	ND	PPND	PPNA	Total
1	Meat products	26	35	7	5	0	0	73
2	Milk and dairy products (BPW + Novobiocin)	21	32	4	4	1	0	62
	Milk and dairy products (OBS + Novobiocin)	21	29	6	3	2	1	62
3	Infant formula	33	29	0	1	0	1	64
4	Vegetables	22	31	6	3	1	1	64
5	Seafood products	22	32	4	4	1	0	63
6	Raw beef meats 9 h	21	32	4	3	1	0	61
	Raw beef meats 24 h	21	31	5	4	0	0	61
7	Environmental samples	28	39	0	2	0	0	69
8	Powdered milk products (375 g)	20	29	6	7	0	1	63
All categories + Dairy enriched with BPW + Raw Beef incubated for 9 hours		193	259	31	29	4	3	519
All categories + Dairy enriched with OBS + Raw Beef incubated for 9 hours		193	256	33	28	5	4	519
All products + Dairy enriched with BPW + Raw Beef incubated for 24 hours		193	258	32	30	3	3	519
All products + Dairy enriched with OBS + Raw Beef incubated for 24 hours		193	255	34	29	4	4	519

PA = Positive agreement    PD = Positive deviation    PP = Positive deviation positive presumptive non-confirmed samples    NA = Negative agreement  
 ND = Negative deviation    PPND = Predictive positive negative deviation    PPNA = Predictive positive negative agreement

During the current extension study, six negative deviations were observed for the Applied Biosystems 7500 Fast Food Safety System and seven negative deviations were observed for the Applied Biosystems QuantStudio 5 Food Safety System.

Among the samples in negative deviation, the presence of *Salmonella* was confirmed for three samples. For one sample, one of the three PCR replicates tested gave a negative result on both Food Safety Systems. For the remaining two samples, three PCR replicates were analyzed and all gave negative results. The contamination in the enrichment broths for these samples was likely below the limit of detection of the alternative method.

During the current extension study, six positive deviations were recorded when using both Food Safety Systems. The positive deviations were detected as positive using the alternative method but failed to be detected with the reference method. All six positive deviations were confirmed as true positives.

The results from all validation studies has been combined and is presented in Table 6.

Table 6: Sensitivity, relative trueness and false positive ratio summary

	Applied Biosystems 7500 Fast				Applied Biosystems QuantStudio 5			
	A	B	C	D	A	B	C	D
Sensitivity of the alternative method	88.7%	86.8%	87.1%	87.2%	87.2%	87.3%	87.2%	87.3%
Sensitivity of the reference method	88.3%	87.9%	88.3%	87.9%	87.9%	87.3%	87.6%	86.9%
Relative trueness	87.7%	87.5%	87.9%	87.7%	87.7%	87.3%	87.5%	87.1%
False positive ratio	1.1%	0.8%	1.1%	0.8%	2.7%	3.5%	2.3%	3.1%

A = All categories + Dairy enriched with BPW + Raw Beef incubated for 9 hours  
B = All categories + Dairy enriched with OBS + Raw Beef incubated for 9 hours

C = All products + Dairy enriched with BPW + Raw Beef incubated for 24 hours  
D = All products + Dairy enriched with OBS + Raw Beef incubated for 24 hours

The method comparison study results demonstrate that the alternative method was shown to be a reliable alternative to the ISO reference method for the detection of *Salmonella* spp. from the meat products, milk and dairy products, seafood and fishery product, vegetables, raw beef meats, environmental samples, powdered infant formula and powdered milk products categories.

Relative level of detection study

For the relative level of detection (RLOD) studies, eight *Salmonella* species isolates were spiked into eight matrices (see Table 7 for matrix/strain pairs) and analyzed using the Applied Biosystems 7500 Fast and the Applied Biosystems QuantStudio 5 Food Safety Systems.

Table 7: Defined matrix/strain pairs for the RLOD determination.

Category	Matrix	Inoculated strain
1 Meat products	Raw chicken meat	<i>Salmonella</i> Bredeney 975
2 Milk and dairy products	Raw milk	<i>Salmonella</i> Ohio Ad1482
3 Powdered infant formula (10 g)	Powdered infant formula with probiotics	<i>Salmonella</i> Anatum Ad298
4 Vegetables	Frozen spinach	<i>Salmonella</i> Virchow Ad1721
5 Seafood	Fish terrine	<i>Salmonella</i> Derby Ad1093
6 Raw beef meats	Ground beef	<i>Salmonella</i> Typhimurium A00C060
7 Production environmental samples	Process water	<i>Salmonella</i> Livingstone A00L058
8 Milk powder, powdered infant formula and infant cereals with and without probiotics including ingredients (375 g) <sup>a</sup>	Powdered infant formula with probiotics	<i>Salmonella</i> Mbandaka Ad1810

<sup>a</sup> Categories analyzed in current extension study

The samples were analyzed using the reference method detailed in ISO 6579-1:2017 prior to inoculation in order to verify the absence of *Salmonella* spp. After inoculation, samples were tested using the ISO reference method and the alternative method.

**Table 8: Relative level of detection results**

Matrix	Strain	QuantStudio 5 RLOD	7500 Fast RLOD	Acceptability level ( $\leq$ )
Raw chicken meat	<i>Salmonella</i> Bredeney 975	1.629	1.629	2.5
Raw milk (BPW + Novobiocin)	<i>Salmonella</i> Ohio Ad1482	0.761	1.000	2.5
Raw milk (OBS + Novobiocin)	<i>Salmonella</i> Ohio Ad1482	1.336	1.336	2.5
Powdered infant formula with probiotics (10 g) <sup>a</sup>	<i>Salmonella</i> Anatum Ad298	1.000	1.000	1.5
Frozen spinach	<i>Salmonella</i> Virchow Ad1721	1.000	1.000	2.5
Fish terrine	<i>Salmonella</i> Derby Ad1093	1.000	1.000	2.5
Ground beef (9 h and 24 h)	<i>Salmonella</i> Typhimurium A00C060	0.554	0.554	2.5
Process water	<i>Salmonella</i> Livingstone A00L058	1.000	1.170	2.5
Powdered infant formula with probiotics (375 g) <sup>b</sup>	<i>Salmonella</i> Mbandaka Ad1810	0.195	0.195	2.5
<b>Combined RLOD</b>		<b>0.822</b>	<b>0.852</b>	<b>/</b>

<sup>a</sup> Paired study design

<sup>b</sup> Categories analyzed in current extension study

As shown in Table 8, the alternative method gave a RLOD within the acceptability limit of  $\leq 1.5$  for the paired study and  $\leq 2.5$  for the unpaired study designs, when used with the Applied Biosystems QuantStudio 5 Food Safety System and the Applied Biosystems 7500 Fast Food Safety System.

## Conclusion

The NF VALIDATION studies demonstrate that the SureTect *Salmonella* species PCR Assay workflow gives equivalent or improved performance compared to the ISO reference method detailed in ISO 6579-1:2017 for the detection of *Salmonella* spp. from a broad range of foods and environmental samples when using the Applied Biosystems 7500 Fast or the Applied Biosystems QuantStudio 5 Food Safety Systems. The NF VALIDATION certificate and a summary of the validation report for this study are available from [www.thermofisher.com/foodsafety](http://www.thermofisher.com/foodsafety).

[www.thermofisher.com](http://www.thermofisher.com)

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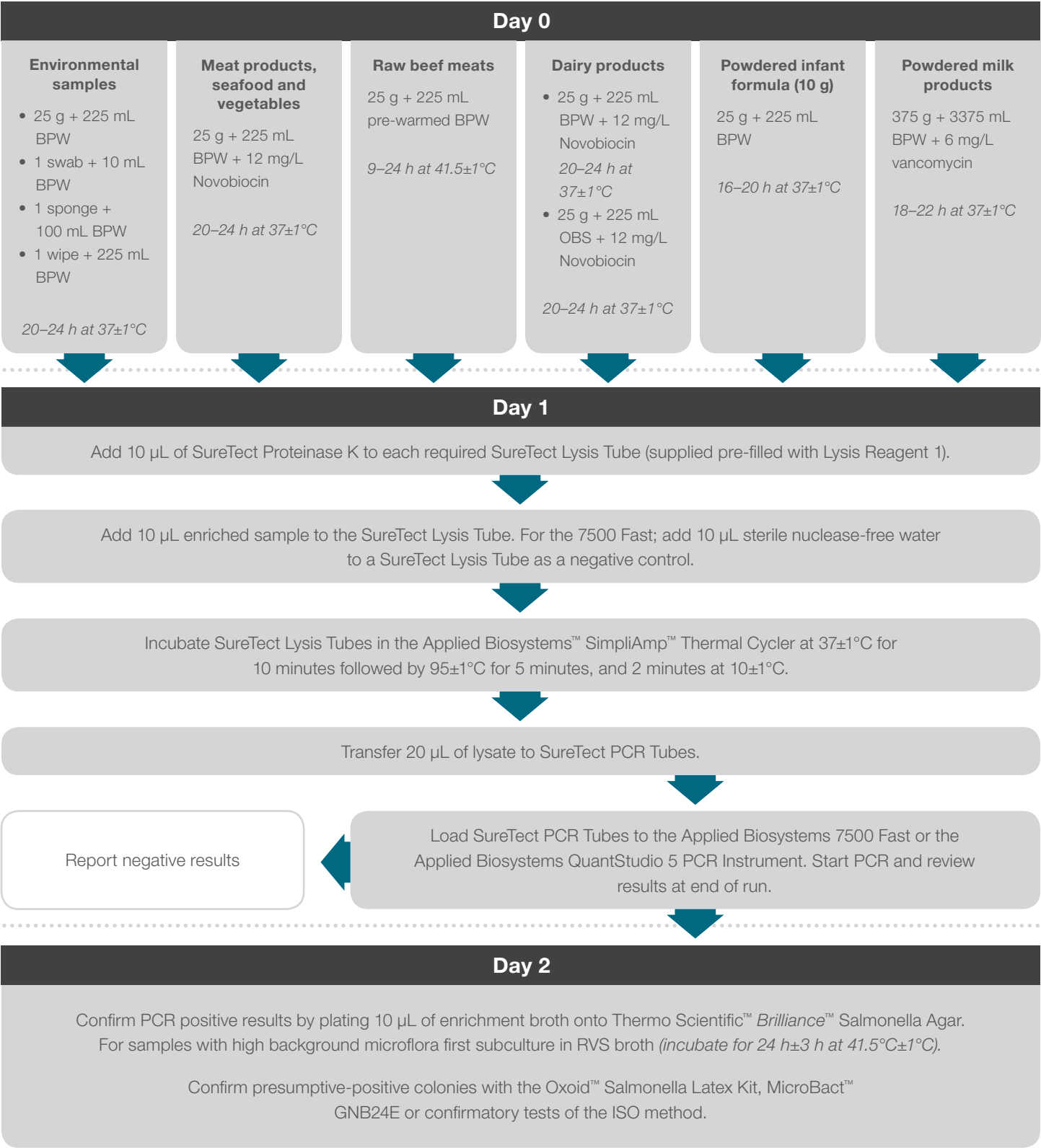
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Appendix 1: Workflow for alternative method—SureTest Salmonella Species PCR Assay for use on the QuantStudio 5 Food Safety System



Appendix 2: Protocol for the reference method: ISO 6579-1

