

# Thermo Scientific SureTect Salmonella species PCR Assay: NF Validation Using the Applied Biosystems 7500 Fast PCR Instrument

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## ABSTRACT

### Introduction

The Thermo Scientific™ SureTect™ Salmonella species PCR Assay is a real-time PCR assay intended for the detection of *Salmonella* spp. from food and production environment samples, which has previously gained NF VALIDATION™ by AFNOR Certification using the Thermo Scientific™ SureTect™ PikoReal™ PCR instrument and Thermo Scientific™ SureTect™ Software version 1.2.

### Purpose

The purpose of this study was to conduct an NF VALIDATION by AFNOR Certification extension study to validate use of the SureTect Salmonella species PCR Assay on the Applied Biosystems™ 7500 Fast PCR Instrument with Applied Biosystems™ RapidFinder™ Express v2.0 Software (the alternative method) for meat products, dairy products, infant formula, seafood and vegetables and production environment samples.

### Methods

A method comparison study and relative limit of detection (RLOD) study was conducted. For the alternative method, all samples underwent an enrichment step followed by direct lysis. Following direct lysis, PCR was run and results were automatically interpreted by the software. The reference method was conducted according to ISO 6579:2002.

### Results

Food samples were tested using the alternative and reference methods. For the RLOD study, five *Salmonella* isolates were selected from the culture collection at ADRIA Développement and were spiked into representative food and production environmental categories, as per the alternative method protocol and the reference method. The alternative method demonstrated equivalent performance to the reference method. The alternative method RLOD met the acceptability limits, according to the ISO 16140-2:2016 (LOD for the alternative method shall not be higher than 1.5 time (paired study) or 2.5 time (unpaired study) the LOD of the reference method for a paired study).

### Significance

The alternative method proved to be a suitable substitute to reference method for *Salmonella* spp. from food and environmental samples.

## INTRODUCTION

The SureTect Salmonella species PCR Assay is a real-time PCR kit for the detection of *Salmonella* spp. meat products, dairy products, infant formula, seafood and vegetables and production environment samples. The kit combines pre-dispensed lysis reagents and lyophilised and tableted PCR reagents to simplify and improve assay handling, along with software to automatically interpret and display results. This NF VALIDATION by AFNOR Certification ISO 16140 extension study was conducted to extend the use of the SureTect Salmonella species PCR Assay to a second PCR instrument, the Applied Biosystems 7500 Fast 96-well PCR Instrument with RapidFinder Express 2.0 Software using SureTect kit files for meat products, dairy products, infant formula, seafood and vegetables and production environment samples.

## MATERIALS AND METHODS

### Method comparison study

A total of 391 samples were analyzed as part of the expert laboratory study, which was designed to validate the performance of the alternative method for meat products, dairy products, infant formula, seafood and vegetables and production environment samples on the Applied Biosystems 7500 Fast system. One hundred and sixty-one samples were artificially contaminated (25 samples were artificially contaminated by a spiking protocol and 136 by a seeding protocol).

### Relative limit of detection (RLOD) study

For this study, five *Salmonella* isolates were selected from the culture collection at ADRIA Développement and were spiked into representative matrices from the food categories analyzed during the NF VALIDATION ISO 16140 method comparison part of this validation study (raw chicken meat, raw milk, infant formula with probiotics, raw ground beef, process water and frozen spinach).

Samples were prepared to give three batches of the matrices which consisted of five samples at 0 CFU/25 g, 20 samples at 0.5-1 CFU/25 g (to achieve fractional positive results) and 5 samples at 2 CFU/25 g. The samples were analyzed using the reference method detailed in ISO 6579:2002 prior to spiking in order to verify the absence of *Salmonella* spp. After inoculation, samples were tested using the ISO reference method and the alternative method.

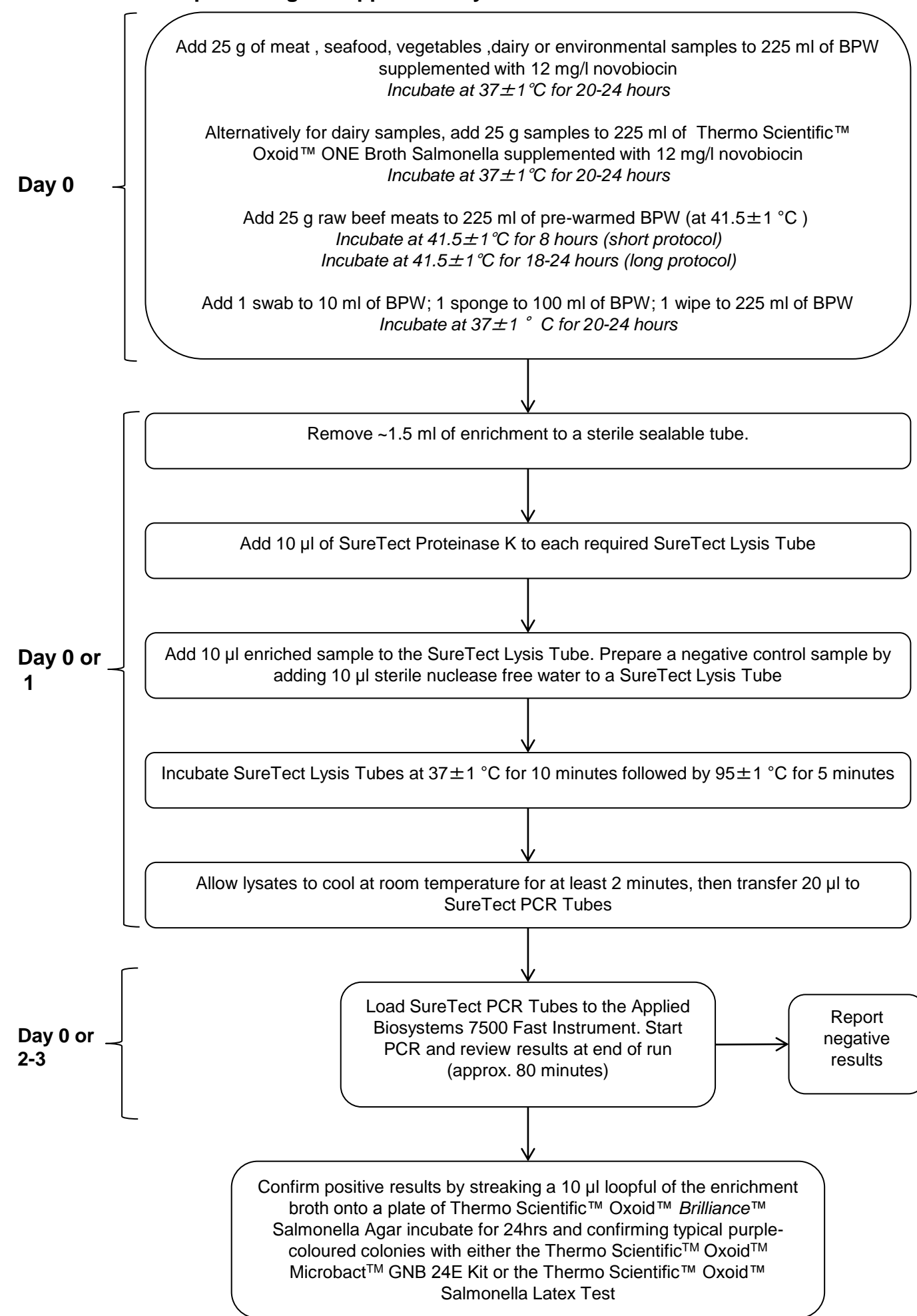
### SureTect Salmonella species PCR Assay method

When following the alternative method, food and environmental samples were enriched following the workflow detailed in Figure 1.

### ISO reference method

Twenty-five gram samples were analyzed according to ISO 6579:2002.

**Figure 1. SureTect Salmonella species PCR Assay protocol used for food and production environment samples using the Applied Biosystems 7500 Fast Instrument**



## RESULTS

### Method comparison study

The SureTect Salmonella PCR Assay results versus the ISO reference method results are listed in Table 1.

**Table 1. SureTect Salmonella species PCR Assay versus the ISO reference method**

Protocols	Methods	ISO method positive results	ISO method negative results
All protocols and Dairy products BPW + novobiocin & Raw beef 9 hour incubation	Alternative method positive results	154	20
	Alternative method negative results	20	197
All protocols and Dairy products BPW + novobiocin & Raw beef 24 hour incubation	Alternative method positive results	155	19
	Alternative method negative results	20	197
All protocols and Dairy products ONE Broth + novobiocin & Raw beef 9 hour incubation	Alternative method positive results	154	20
	Alternative method negative results	21	196
All protocols and Dairy products ONE Broth + novobiocin & Raw beef 24 hour incubation	Alternative method positive results	155	19
	Alternative method negative results	21	196

Thirty-one negative deviation results were recorded during the expert laboratory study. The presence of *Salmonella* was detected in 9 of these samples (by subculture of the BPW enrichment into RVS Broth before streaking onto *Brilliance* Salmonella Agar). The remaining 22 negative and the 33 positive deviation results were most likely due to the unpaired study design and the related sampling heterogeneity as *Salmonella* could not be isolated from the samples by the culture confirmation method meaning that it is likely that no target cells were present in the portion of matrix used for the alternative method. The relative trueness, sensitivity and false positive ratios of the SureTect Salmonella species PCR Assay method are listed in Table 2.

**Table 2. Relative trueness, sensitivity and false positive ratios of the SureTect Salmonella species PCR Assay**

	SureTect Assay methods comparative study			
	Dairy products BPW + novobiocin & Raw beef 9 hour incubation	Dairy products BPW + novobiocin & Raw beef 24 hour incubation	Dairy products ONE Broth + novobiocin & Raw beef 9 hour incubation	Dairy products ONE Broth + novobiocin & Raw beef 24 hour incubation
Relative Trueness	89.8%	90.0%	89.5%	89.8%
Relative Sensitivity for the reference method	89.7%	89.2%	89.7%	89.2%
Relative Sensitivity for the alternative method	89.7%	90.2%	89.7%	90.3%
False positive ratio	0.5%	0.5%	0.0%	0.0%

### Relative limit of detection (RLOD) study

The level of detection for the alternative method and the ISO reference method were determined according to the ISO 16140-2:2016 standard (Table 3). The aim was to determine the relative level of detection for all matrices analyzed during the NF VALIDATION by AFNOR Certification extension study.

**Table 3: Relative detection level results for the SureTect Salmonella species PCR Assay according to ISO 16140-2:2016**

Matrix/Strain pairs	Relative level of detection (CFU/25 g)
Raw chicken meat / <i>Salmonella</i> Bredeney 975	1.629 [0.696-3.814]
Raw milk / <i>Salmonella</i> Ohio Ad1482	BPW + novobiocin: 1.000 [0.447-2.240]
	ONE Broth <i>Salmonella</i> + novobiocin: 1.000 [0.420-2.383]
Infant formula with probiotics / <i>Salmonella</i> Anatum Ad298	1.000 [0.473-2.113]
Ground beef / <i>Salmonella</i> Typhimurium A00C060	9 h: 0.554 [0.239-1.285]
	24 h: 0.554 [0.239-1.285]
Process water / <i>Salmonella</i> Livingstone A00L058	1.170 [0.437-3.132]
Frozen spinach / <i>Salmonella</i> Virchow Ad1721	1 [0.385-2.599]

## CONCLUSIONS

The method comparison study conducted as part of this NF VALIDATION extension study demonstrated that the alternative method is equivalent in performance for the food and production environment samples analyzed to the ISO reference method detailed in ISO 6579:2002, when using the Applied Biosystems 7500 Fast System and RapidFinder Express v2.0 Software. The relative level of detection study conducted as part of the NF VALIDATION extension study demonstrated that the level of detection of the SureTect Salmonella species PCR Assay workflow met the acceptability limits as detailed in the ISO 16140-2:2016, using the Applied Biosystems 7500 Fast System with the RapidFinder Express v2.0 Software

## REFERENCES

- ISO 6579:2002 Microbiology of food and animal feeding stuff -- Horizontal method for the detection of *Salmonella* spp.
- ISO 16140-2:2016. Microbiology of the food chain -- Method validation -- Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method

## TRADEMARKS/LICENSING

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