

# Validation of the Thermo Scientific SureTect Real-Time PCR Method for Detection of *Salmonella* in Poultry meat

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## Overview

**Purpose:** To validate the Thermo Scientific™ SureTect™ Salmonella species assay according to AOAC Research Institute (RI) *Performance Tested Methods*<sup>SM</sup> validation criteria for raw poultry meat.

**Methods:** The SureTect method was compared to the reference method detailed in ISO 6579:2002.

**Results:** The SureTect Salmonella species assay reliably detected the presence of *Salmonella* in raw poultry meat and was equivalent to the ISO reference method.

## Introduction

The Thermo Scientific SureTect Salmonella species assay (PT0100A) is a new Real-Time PCR test for the detection of *Salmonella* from food, animal feeds and environmental samples, which combines pre-dispensed lysis reagent and lyophilised, tableted PCR reagents to simplify and improve assay handling, along with dedicated software to run the assays as well as interpret and display PCR results. This study was conducted using the AOAC RI *Performance Tested Methods*<sup>SM</sup> program<sup>1</sup> to validate the SureTect Salmonella species assay in comparison to the reference method detailed in ISO 6579:2002<sup>2</sup> with raw poultry meat.

## Methods

### Sample Preparation

A bulk sample of raw chicken breast meat was screened for natural contamination with *Salmonella* before splitting into three samples; unspiked (control), low spiked (0.2-2 CFU/25g) and high spiked (2-5 CFU/25g) samples. Once spiked, all samples were allowed to equilibrate as per AOAC instructions.

### SureTect Assay Method

25g samples of raw meat were added to 225ml of room temperature Buffered Peptone Water (BPW) (ISO) and incubated at 37°C for 20h at 37°C.

Following enrichment 10µl of each sample was added to the pre-filled SureTect Lysis Tubes (prepared by additionally adding Proteinase K Reagent) and the sample lysed according to the SureTect lysis protocol (37°C for 10 minutes followed by 95°C for 5 minutes).

Once lysed, 20µl of the lysate was added to the SureTect PCR Tubes, which contain lyophilised PCR reagents before running on the Thermo Scientific™ PikoReal™ Real-Time PCR instrument.

Assay results were automatically interpreted as “positive” or “negative” by the SureTect Software.

All SureTect results were confirmed culturally using the SureTect confirmation method of direct plating onto Thermo Scientific™ Oxoid™ *Brilliance*<sup>TM</sup> Salmonella Agar and confirming presumptive positive purple colonies with the Thermo Scientific™ Oxoid™ Salmonella Latex Kit (DR1108A) and additionally using the reference method confirmation protocol.

### ISO Reference Method

The reference method detailed in ISO 6579:2002 was followed, using *Brilliance* Salmonella Agar as the second plating medium. Confirmations were performed using the Thermo Scientific™ Remel™ microID™ kit or bioMérieux API™ 20E kit, Triple Sugar Iron (TSI) slants and poly-O and poly-H antisera.

### Inclusivity

One-hundred and seventeen *Salmonella* isolates covering a wide variety of O- serogroups and subspecies were cultured in BPW (ISO) and analysed at a level of approximately 10<sup>4</sup> CFU/ml using the SureTect assay protocol according to AOAC-RI PTM requirements.

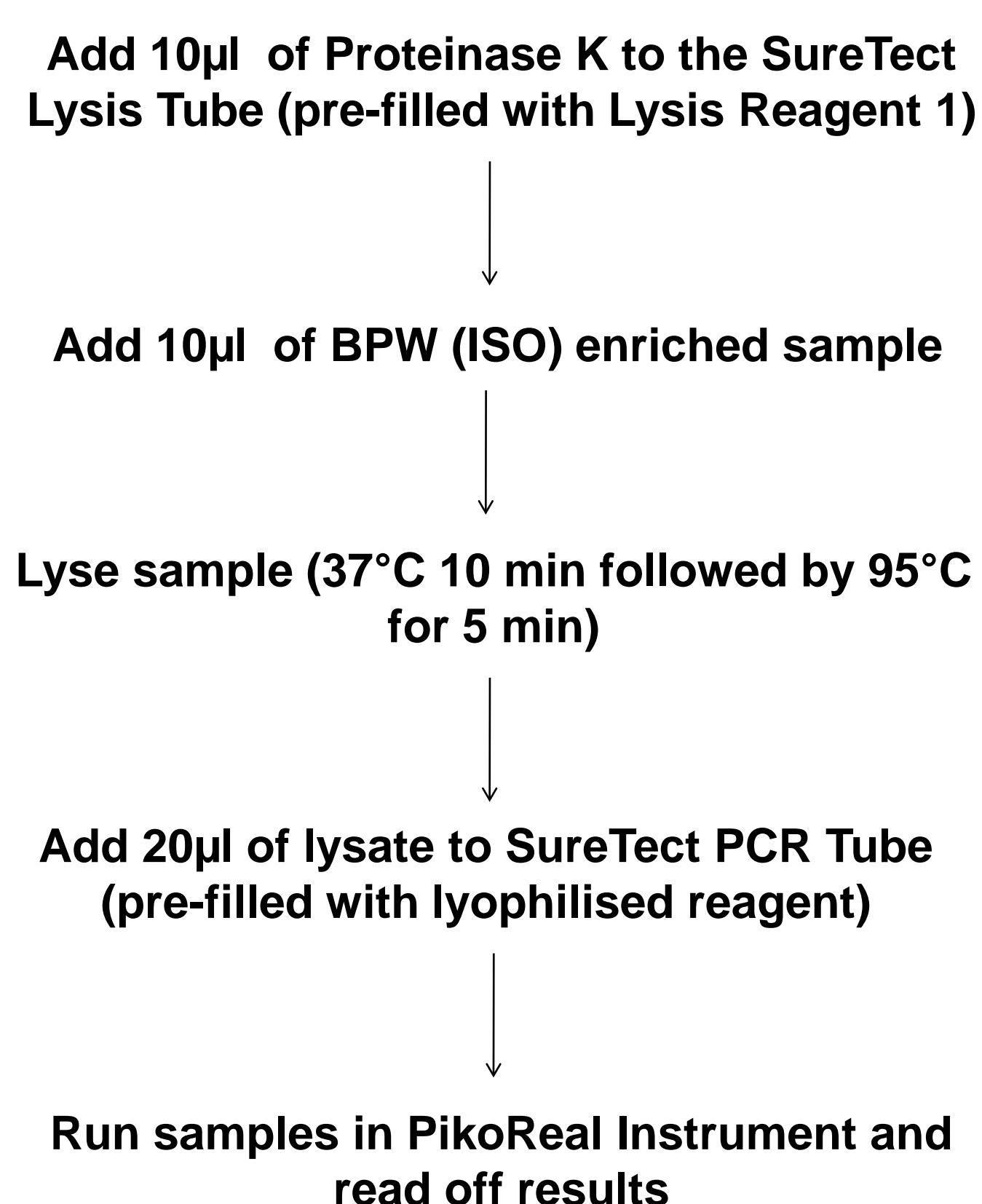
### Exclusivity

Thirty-six exclusivity isolates were cultured in TSB for 18-24 hours and analysed at a level of approximately 10<sup>8</sup> CFU/ml using the SureTect assay protocol according to AOAC-RI PTM requirements.

FIGURE 1. The Thermo Scientific SureTect System.



FIGURE 2. SureTect Assay Workflow.



## Results

### Inclusivity and exclusivity

All 117 *Salmonella* isolates were detected as positive by the SureTect Software. None of the 36 exclusivity isolates were detected by the SureTect Software.

FIGURE 3. Inclusivity of the SureTect Salmonella species assay.

Serotype	Number	% Positive
<i>Salmonella bongori</i>	4	100%
<i>Salmonella enterica</i> subsp. <i>salamae</i>	5	100%
<i>Salmonella enterica</i> subsp. <i>arizoniae</i>	3	100%
<i>Salmonella enterica</i> subsp. <i>diarizoniae</i>	4	100%
<i>Salmonella enterica</i> subsp. <i>indica</i>	3	100%
<i>Salmonella enterica</i> subsp. <i>houtenae</i>	4	100%
<i>Salmonella enterica</i> subsp. <i>enterica</i>	94	100%

### Food Matrix Analysis

No statistically significant difference, by probability of detection analysis (POD), was demonstrated during the validation study with raw chicken breast meat. The SureTect method was therefore shown to be identical in performance to the ISO reference method.

FIGURE 3. Method Developer Results for the ISO and SureTect Salmonella species Methods.

Matrix/Inoculating Organism	Level	MPN/25g	No. Test portions	ISO	SureTect	
					Presum* <sup>a</sup>	Con* <sup>a</sup>
Raw chicken breast <i>Salmonella</i> Indiana	Low	1.2	20	13	12	12
	High	2.3	5	5	5	5

\* Presumptive (Presum) and Confirmed (Con) SureTect results

## Conclusion

The SureTect Salmonella species assay was shown to be an accurate and user-friendly method, due to the use of pre-dispensed lysis reagent, tableted PCR reagents and automatic interpretation of results. Results from the analysis of raw chicken meat demonstrated the assay was able to reliably detect the presence of *Salmonella*.

## References

- AOAC International, Method Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces 2012.
- ISO, Microbiology of Food and Animal Feeding stuffs- Horizontal Method for the Detection of *Salmonella* spp. ISO 6579:2002.

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