

# AOAC-RI Performance Tested Method Validation Of The Thermo Scientific SureTect Listeria Species PCR Assay

J. Cloke<sup>1</sup>, C. Leon-Velarde<sup>2</sup>, N. Larson<sup>2</sup>, K. Dave<sup>2</sup>, H. Simpson<sup>1</sup>, C. Hopper<sup>1</sup>, K. Evans<sup>1</sup>, D. Crabtree<sup>1</sup>, A. Hughes<sup>1</sup>, M. Oleksiuk<sup>1</sup>, S. Withey<sup>1</sup>. <sup>1</sup>Thermo Fisher Scientific, Basingstoke, UK and <sup>2</sup>Agriculture and Food Laboratory, University of Guelph, Guelph, Ontario, Canada

## Overview

**Purpose:** To validate the Thermo Scientific™ SureTect™ Listeria species PCR Assay (PT0200A) according to AOAC Research Institute (RI) *Performance Tested Methods*<sup>SM</sup> validation criteria.

**Methods:** The SureTect method was compared to the reference method detailed in ISO 11290-1:1996 including Amendment 1:2004.

**Results:** The SureTect Listeria species PCR Assay reliably detected the presence of *Listeria* in a wide variety of food matrices and surface samples.

## Introduction

The SureTect Listeria species PCR Assay (PTM#071304) is a Real-Time PCR kit for the detection of *Listeria* species. This study was conducted using the AOAC-RI *Performance Tested Methods*<sup>SM</sup> program<sup>1</sup> to validate the assay in comparison to the ISO reference method detailed in ISO 11290-1:1996, including Amendment 1:2004.

## Methods

### SureTect Assay Method

The SureTect method was followed as detailed in the product instructions for use to analyse 25g samples of all food matrices and surface samples (figure 1). Regardless of SureTect method results, all enrichments were confirmed by both the SureTect & ISO reference method confirmation protocols.

### Reference Method

All matrices were validated in comparison to the reference method detailed in ISO 11290-1:1996 including Amendment 1:2004 which used Oxford Agar as the second plating medium. Confirmations (Gram stain, catalase, haemolysis, CAMP test and rhamnose and xylose utilisation) were performed as detailed by the reference method.

### Inclusivity And Exclusivity

Sixty-eight different isolates of *Listeria* species covering all known serotypes (except 4ab) of *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri* and *L. seeligeri* were analysed according to the SureTect kit protocol and AOAC-RI PTM study requirements at a level of ~10<sup>4</sup> CFU/ml. Thirty-three exclusivity isolates were also analysed as required by AOAC-RI PTM study requirements at a level of ~10<sup>8</sup> CFU/ml with the SureTect method.

### Independent Laboratory Study

As required for AOAC-RI PTM validation, an independent laboratory study was conducted on three of the matrices (figure 3) following the same methods as the method developer study.

## Results

### Inclusivity And Exclusivity

All 68 isolates of *L. species* were detected as positive and all 33 exclusivity isolates gave a negative result with the SureTect method.

### Food Matrix Analysis

There was a statistically significant difference, by probability of detection analysis at 95% confidence levels for low spiked samples of salami in favour of the SureTect method during the method developer study (figure 1). For all other matrices, there was no statistical difference between the two methods.

**FIGURE 1. Method Developer & Independent Laboratory Study Results For The SureTect and ISO Methods**

Matrix/Inoculating organism and serotype	Level	MPN/25g	No. Test portions	ISO	SureTect	
					Presum*	Con*
Cantaloupe melon <i>L. monocytogenes</i> 3c	Low	0.70	20	11	13	13
	High	3.00	5	5	5	5
Salami <i>L. monocytogenes</i> 1/2a	Low	0.29	20	4	10	11
	High	0.40	5	3	4	4
Smoked salmon <i>L. monocytogenes</i>	Low	0.60	20	8	7	8
	High	1.25	5	2	4	4
Spinach <i>L. innocua</i>	Low	1.38	20	15	11	11
	High	4.37	5	5	5	5
Cooked deli turkey <i>L. innocua</i>	Low	0.69	20	10	9	9
	High	1.02	5	2	5	4
Pork Frankfurters <i>L. innocua</i>	Low	0.74	20	10	7	7
	High	1.18	5	4	5	5
Cooked deli ham/ <i>L. welshimeri</i>	Low	1.02	20	15	13	14
	High	2.96	5	5	5	5
Cooked prawns <i>L. monocytogenes</i> 4b	Low	1.00	20	14	15	15
	High	1.88	5	4	4	4
Processed cheese <i>L. monocytogenes</i> 1/2a	Low	0.53	20	9	6	6
	High	1.48	5	4	4	4
Raw ground beef <i>L. monocytogenes</i> 1/2c	Low	1.13	20	14	13	13
	High	1.88	5	4	4	4
Stainless steel surface <i>L. monocytogenes</i> 3a	Low	N/A	20	12	11	11
	High	N/A	5	4	5	5
Plastic surface <i>L. monocytogenes</i> 3a	Low	N/A	20	15	16	16
	High	N/A	5	5	4	4
Pork Frankfurters <i>L. innocua</i> Independent lab study	Low	1.38	20	15	12	12
	High	2.97	5	5	5	5
Spinach <i>L. innocua</i> Independent lab study	Low	0.66	20	9	14	14
	High	2.97	5	5	5	5
Stainless steel surface <i>L. monocytogenes</i> 1/2a Independent lab study	Low	N/A	20	8	8	8
	High	N/A	5	4	5	5

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

## Conclusion

The SureTect Listeria species PCR Assay proved to be an accurate method for the detection of *Listeria* with a wide range of food matrices, when compared to the ISO reference method and is designed to provide fast results for product release when compared with other rapid methods.

## References

1. AOAC International, Method Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces 2012.
2. ISO, Microbiology of Food and Animal Feeding stuffs-Horizontal Method for the Detection of *Listeria monocytogenes*. ISO 11290-1:1996. Including Amendment 1:2004