Validation of the Thermo Scientific SureTect Real-Time PCR Method for Detection of *Listeria monocytogenes* in Food and Environmental Samples

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Overview

Purpose: To validate the Thermo Scientific™ SureTect™ Listeria monocytogenes Assay according to AOAC Research Institute (RI) *Performance Tested Methods*SM validation criteria.

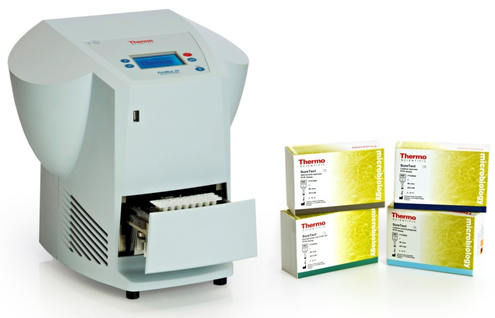
Methods: The SureTect method was compared to the reference method detailed in ISO 11290-1:1996 including Amendment 1:2004.

Results: The SureTect Listeria Monocytogenes Assay reliably detected the presence of *L. monocytogenes* in a wide variety of matrices.

Introduction

The Thermo Scientific SureTect Listeria monocytogenes Assay (PT0300A) is a new Real-Time PCR test for the detection of *Listeria monocytogenes* from food 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*SM program[1] to validate this SureTect assay in comparison to the ISO 11290-1 reference method.

FIGURE 1. The Thermo Scientific SureTect System.



Methods

Sample Preparation

Bulk samples foods were screened for natural contamination with *Listeria* spp. 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. Surface samples of stainless steel and plastic were spiked with a suspension of *L. monocytogenes.*

SureTect Assay Method

25g samples of foods and surface sponges were added to 225ml of room temperature Oxoid™ 24 LEB supplemented with both 24 LEB Buffer and Selective supplements. All samples were incubated at 37°C for 22h. Following enrichment 10µl of each sample was added to the prefilled SureTect Lysis Tubes (prepared by additionally adding Proteinase K Reagent and Lysis Reagent 2) 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, (containing 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 results were confirmed by the SureTect confirmation protocol (direct plating onto Oxoid™ *Brilliance*™ Listeria Agar and Oxoid™ Microbact™ 12L biochemical identification) as well as by the confirmation procedure in the ISO reference method.

ISO Reference Method

The reference method detailed in ISO 11290-1:1996 including Amendment 1:2004, was followed, using Oxford Agar as the second plating medium. Confirmations (Gram stain, catalase, haemolysis, CAMP test and rhamnose and xylose) were performed according to the reference method.

Inclusivity

Fifty-three different isolates of *L. monocytogenes* covering all known serotypes (except 4ab) were cultured in 24 LEB supplemented with 24 LEB Buffer and Selective supplements and analysed at a level of approximately 10^4 CFU/ml using the SureTect assay protocol according to AOAC-RI PTM requirements.

Exclusivity

Thirty-eight exclusivity isolates were cultured in Tryptone Soya Broth and analysed at a level of approximately 10^8CFU/ml using the SureTect assay protocol according to AOAC-RI PTM requirements.

FIGURE 2. SureTect Assay Workflow.

Add 10µl of Proteinase K and 10µl of SureTect Lysis Reagent 2 to the SureTect Lysis Tube (pre-filled with Lysis Reagent 1)

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Add 10µl of 24 LEB enriched sample

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Lyse sample (37°C 10 min followed by 95°C for 5 min)

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Add 20µl of lysate to SureTect PCR Tube (pre-filled with lyophilised reagent)

↓

Run samples in PikoReal Instrument and read off results

Results

Inclusivity

All 53 isolates of *L. monocytogenes* from a wide range of serotypes were detected as positive by the SureTect Software

Exclusivity

None of the 38 exclusivity isolates, including isolates of non-*monocytogenes Listeria,* were detected by the SureTect Software.

Food Matrix Analysis

There was a statistically significant difference, by probability of detection analysis (POD), for salami, smoked salmon, spinach, cooked sliced turkey, pork Frankfurters, ice-cream, cooked prawns and stainless in favour of the SureTect method during the method developer study, and with pork Frankfurters and lettuce during the independent laboratory study demonstrating that the SureTect Listeria monocytogenes Assay is more reliable than the ISO reference method in detecting *L. monocytogenes* contamination of foods and surface samples. The results for the remaining matrices showed no statistically significant different by POD analysis between the SureTect and ISO methods.

FIGURE 3. Method Developer Results for the ISO and SureTect Listeria monocytogenes Methods.

Matrix/Inoculating organism and serotype	Level	MPN/ 25g	No. Test portions	ISO	SureTect	
					Presum*	Con*
Cantaloupe melon *L. monocytogenes* 3c	Low	0.70	20	11	13	13
	High	3.00	5	5	5	5
Salami *L. monocytogenes* 1/2a	Low	0.29	20	4	10	11
	High	0.40	5	3	4	4
Smoked salmon *L. monocytogenes*	Low	0.60	20	8	7	8
	High	1.25	5	2	4	4
Fresh spinach *L. monocytogenes* 3b	Low	0.38	20	6	8	8
	High	0.39	5	5	5	5
Cooked deli turkey *L. monocytogenes* 4b	Low	1.06	20	12	19	19
	High	4.37	5	5	4	4
Pork Frankfurters *L. monocytogenes* 4e	Low	0.72	20	9	14	14
	High	1.00	5	5	5	5
Ice-Cream *L. monocytogenes* 1/2b	Low	0.24	20	1	12	12
	High	0.64	5	0	4	4
Cooked prawns *L. monocytogenes* 4b	Low	1.00	20	14	15	15
	High	1.88	5	4	4	4
Processed cheese *L. monocytogenes* 1/2a	Low	0.53	20	9	6	6
	High	1.48	5	4	4	4
Raw ground beef *L. monocytogenes* 1/2c	Low	1.13	20	14	13	13
	High	1.88	5	4	4	4
Stainless steel surface *L. monocytogenes* 3a	Low	N/A	20	12	11	11
	High	N/A	5	4	5	5
Plastic surface *L. monocytogenes* 3a	Low	N/A	20	15	16	16
	High	N/A	5	5	4	4

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

FIGURE 4. Independent Laboratory Results for the ISO and SureTect Listeria monocytogenes Methods.

Matrix/Inoculating Organism	Level	MPN/ 25g	No. Test portions	ISO	SureTect	
					Presum*	Con*
Pork Frankfurters *L. monocytogenes* 4b	Low	0.59	20	8	12	12
	High	2.97	5	5	5	5
Fresh lettuce *L. monocytogenes* 4b	Low	0.37	20	5	10	10
	High	2.19	5	5	5	5
Stainless steel surface *L. monocytogenes* 1/2a	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 monocytogenes 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 a wide range of foods, including matrices deemed as “challenging” such as salami and smoked salmon, demonstrated the assay was able to reliably detect the presence of *L. monocytogenes* from foods and surface samples.

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.

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