Rapid And Simple Molecular Workflow For The Detection Of *Listeria* In Food And Environmental Samples

Jani Holopainen[1], Laura Vaahtoranta[1], Jukka-Pekka Palomäki[1], Feng Huang[1], James Stringer[2], Katharine Evans [2]

[1]Thermo Fisher Scientific, Vantaa, Finland, [2]Thermo Fisher Scientific, Basingstoke, Hampshire, UK, RG24 8PW

Overview

Purpose: To verify the performance of the Thermo Scientific™ SureTect™ Listeria PCR assays on the Applied Biosystems™ 7500 Fast PCR platform with pasteurized whole milk, cold smoked salmon, roast beef and stainless steel surface.

Methods: SureTect Listeria monocytogenes and Listeria species PCR assays were compared to a slightly amended version of the ISO 11290-1:1996[1] including Amendment 1:2004[2].

Results: The SureTect Listeria PCR assays were comparable to the amended ISO reference method for the four matrices analysed following an enrichment time of 22 hours.

Introduction

SureTect assays are PCR based tests for the detection of pathogens in food, animal feed and environmental samples. SureTect Listeria PCR assays and Applied Biosystems 7500 Fast Real-Time PCR instrument were combined to bring the SureTect System´s streamlined workflow on to a high throughput 96-well PCR platform.

Methods

Sample Preparation

Pasteurized whole milk, cold smoked salmon, roast beef and stainless steel were spiked with *L.monocytogenes* isolates. In addition, pasteurized whole milk was spiked with *L.innocua* isolate. The spiking level was between 0.5 – 0.83 CFU/25g food. Surface samples of stainless steel were spiked with a 24 CFU/plate suspension of *L.monocytogenes* to account for the Listeria die off on the surface. Isolates for roast beef and whole milk were heat stressed prior to spiking. Ten replicate bags and two unspiked samples were set up for each matrix. Once spiked, all samples were allowed to equilibrate as per AOAC instructions.

SureTect Assay Method

Twenty five grams or millilitres of foods or surface sponges were added to 225 ml of room temperature Thermo Scientific™ Oxoid ™ 24 Listeria Enrichment Broth (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 followed by 10µl of Proteinase K Reagent and Lysis Reagent 2, and 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 lyophilized PCR reagents before running the Applied Biosystems 7500 Fast PCR platform using SDS software version 1.4.2.1. SureTect cycling protocol was applied and the ROX™ reference dye was turned off. Assay results were interpreted as “positive”, “negative” and “indeterminate” based on Cq values and cut off of 50 for the targets and 40 for the internal amplification control. Samples with indeterminate call (IAC and target above the cut off) were rerun from lysates.

All results were confirmed by the SureTect confirmation protocol (direct plating onto Thermo Scientific™ Oxoid™ *Brilliance*™ Listeria Agar and Thermo Scientific™ Microbact™ Listeria 12L biochemical identification kit).

Amended ISO Reference Method

SureTect *Listeria monocytogenes* and *Listeria* species PCR assays were compared to a slightly amended version of the ISO 11290-1:1996 reference method. The reference method´s primary enrichment was conducted in 24 LEB instead of Half Fraser broth followed by secondary enrichment in Fraser Broth. Thermo Scientific™ Oxoid ™ Chromogenic Listeria Agar (OCLA) (ISO) and Oxford Agar were used as the second plating media. Confirmations (Gram stain, oxidase, catalase, CAMP and sugar tests) were performed according to the reference method.

Results

A total of 48 and 60 samples were tested with the SureTect Listeria monocytogenes and Listeria species PCR Assays on the Applied Biosystems 7500 Fast PCR platform resulting in 23 (47.9%) and 28 (47.5%) positive calls with positive agreement to the reference method, respectively. Twenty two of the *L. monocytogenes* positive samples were also confirmed by the SureTect confirmation protocol. The high *Listeria* spp. background in the cold smoked salmon sample overgrew the spiked *L. monocytogenes,* and thus could not be confirmed.

The results for the three food matrices and the stainless steel surface did not show statistically significant difference between the SureTect Listeria PCR methods and the amended reference method.

FIGURE 1. Results for the amended reference and SureTect Listeria hybrid methods.

Matrix / Inoculating organism	Amended ISO	SureTect *L.monocytogenes*	
		Presum *	Con *
Roast beef *L.monocytogenes*	6	6	6
Cold smoked salmon *L.monocytogenes*	7	7	6
Pasteurized whole milk *L.monocytogenes*	2	2	2
Stainless steel *L.monocytogenes*	8	8	8

Matrix / Inoculating organism	Amended ISO	SureTect *Listeria* spp.	
		Presum *	Con *
Roast beef *L.monocytogenes*	6	6	6
Cold smoked salmon *L.monocytogenes*	10	10	10
Pasteurized whole milk *L.monocytogenes,* *L.innocua*	2 2	2 2	2 2
Stainless steel *L.monocytogenes*	8	8	8

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

Conclusion

The SureTect Listeria PCR methods were comparable to the amended reference method for the four matrices following an enrichment time of 22 hours.

The study provided the evidence that the high throughput SureTect methods offer reliable workflow for the detection of Listeria in food and environmental samples.

References

1. ISO, Microbiology of Food and Animal Feeding stuffs – Horizontal Method for the Detection of Listeria monocytogenes. ISO 11290-1:1996.
2. ISO, Microbiology of Food and Animal Feeding stuffs — Horizontal Method for the Detection and Enumeration of Listeria monocytogenes — Part 2: Enumeration Method AMENDMENT 1: Modification of the Enumeration Medium 11290-2:1998/Amd 1:2004

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