Evaluating Alternative Methods for the Detection of Listeria monocytogenes from Medical Nutrition Samples

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ABSTRACT

Purpose
This study assessed the suitability of the Thermo Scientific™ SureTect™ Listeria monocytogenes PCR Assay and a rapid culture method for use with a range of medical nutrition products while maintaining a shortened time to result.

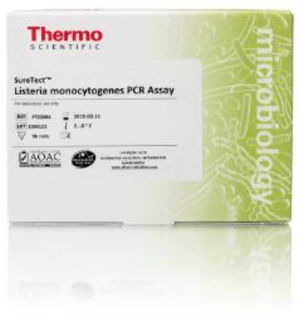
Methods
Medical nutrition samples sourced from Nutricia Advanced Medical Nutrition were artificially contaminated with *Listeria monocytogenes* then enriched and tested using the SureTect PCR Assay protocol and compared against the ISO 11290-1:1996 reference method[1]. Samples found to be inhibitory to growth of *L. monocytogenes* were tested with two alternative methods: a modified PCR method that included a non-selective regrowth following primary enrichment; and a rapid culture method.

Results
The SureTect PCR Assay and ISO reference method successfully detected *L. monocytogenes* from seven of nine samples tested. The remaining two samples were found to be inhibitory to growth of *L. monocytogenes* along with a further four additional samples independently identified by Nutricia Advanced Medical Nutrition. The modified PCR method detected *L. monocytogenes* from four of six inhibitory samples tested. All inhibitory samples were tested with the rapid culture method, achieving 100% sensitivity.

INTRODUCTION

Nutricia Advanced Medical Nutrition is a manufacturer of medical nutrition products, including matrices inhibitory to bacterial growth, which are routinely tested for the presence of *Listeria monocytogenes*. Reducing the time to result for detection of this pathogen from these matrices was desirable; the ISO reference method (ISO 11290-1:1996) provides a negative result in five days. The Thermo Scientific SureTect Listeria monocytogenes PCR Assay is validated for the detection of *L. monocytogenes* from all food types and production environment samples via AOAC® *Performance Tested Methods*℠ (PTM) and NF VALIDATION™ by AFNOR Certification (Image 1), giving a time to negative result in 24-28 hours. Thermo Scientific™ Oxoid™ 24 LEB is a proprietary medium for the selective growth of *Listeria* from food enrichments. To prepare the complete broth for use with the SureTect PCR Assay, 10 ml of 24 LEB Buffer Supplement must be added to 225 ml 24 LEB before incubation.

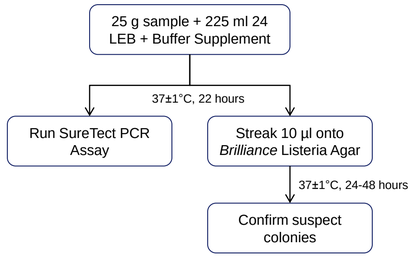
Image 1: Thermo Scientific SureTect Listeria monocytogenes PCR Assay



METHODS

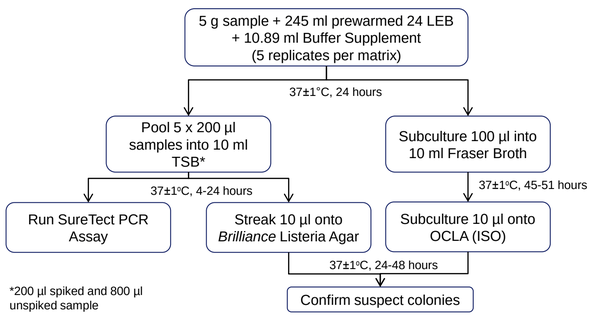
Part 1 – SureTect PCR Method
Nine medical nutrition samples were weighed into duplicate 25 g portions for each method tested. The first portion of each sample was diluted in 225 ml 24 LEB plus 10 ml 24 LEB Buffer Supplement and inoculated with 4.3 to 5.0 CFU *L. monocytogenes.* The second portion was tested as an unspiked sample using the same enrichment method. All samples were incubated at 37±1ºC for 22 hours. Post enrichment, samples were tested using the SureTect Listeria monocytogenes PCR Assay and results confirmed using a culture method using Thermo Scientific™ Oxoid™ *Brilliance*™ Listeria Agar (Figure 1). A replicate set of inoculated and uninoculated samples was tested against the ISO reference method[1].

Figure 1. Workflow for SureTect PCR Method



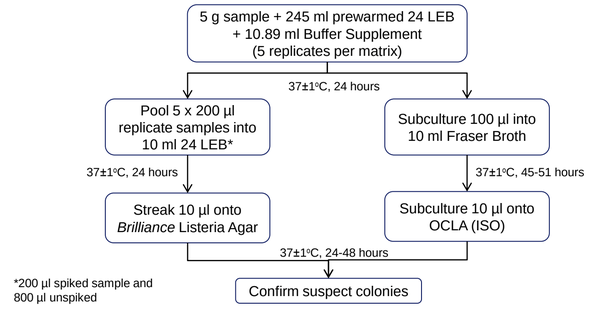
Part 2 – Modified SureTect PCR Method
Matrices identified to cause growth inhibition of *L. monocytogenes* were weighed into 5 g portions and diluted in 24 LEB plus 24 LEB Buffer Supplement at an increased ratio of 1/50, diluting the inhibiting compounds to a level that would no longer impact growth of *Listeria*. Samples were inoculated with 4.4 CFU *L. monocytogenes* and incubated at 37±1ºC for 4-24 hours. To ensure that 25 g total mass was tested per matrix, a pooling method was used by combining 200 µl from a spiked sample with 4 x 200 µl from an unspiked sample. This generated a worst case scenario for one positive sample out of five pooled samples (5 replicates of 5 g) to challenge the alternative method. The prepared pooled samples were inoculated into both 10 ml Tryptone Soya Broth (TSB) and 10 ml Fraser Broth and incubated at 37±1ºC for 24 hours. TSB regrowth samples were tested with the SureTect Listeria monocytogenes PCR Assay and both secondary enrichments were streaked onto plating media for culture confirmation (Figure 2).

Figure 2. Workflow for Modified SureTect PCR Method



Part 3 – Rapid Culture Method
The alternative rapid culture method used a 1/50 dilution ratio for the primary enrichment of inhibitory samples then inoculated and incubated in the same manner as Part 2. Samples were also similarly prepared as a composite post-primary enrichment to ensure the testing of a total mass of 25 g per matrix. One hundred microliters of each prepared pooled sample was inoculated into 10 ml complete 24 LEB and 10 ml Fraser Broth and incubated at 37±1ºC for 24 and 48 hours respectively. Secondary enrichments were then streaked onto plating media for culture confirmation (Figure 3).

Figure 3. Workflow for Rapid Culture Method



RESULTS

Table 1. SureTect method vs. the ISO reference method (Part 1)

	SureTect PCR	Brilliance Listeria	ISO 11290-1:1996
True Positive	7	7	7
False Negative	2	2	2
True Negative	9	9	9
False Positive	0	0	0

n=18; 9 spiked and 9 unspiked samples for both alternative and reference methods

Table 2. Alternative SureTect method vs. Fraser Broth confirmation (Part 2)

	4 hours		24 hours		Fraser Broth OCLA ISO
	SureTect PCR	BL	SureTect PCR	BL	
True Positive	4	4	4	4	4
False Negative	2	2	2	2	2
True Negative	6	6	6	6	6
False Positive	0	0	0	0	0

n=12; 6 spiked and 6 unspiked for both alternative and ISO confirmation method
BL: *Brilliance* Listeria Agar

Table 3. Alternative rapid culture method vs. ISO Fraser Broth confirmation (Part 3)

	Alternative Method	ISO Confirmation
Positive	6	4
False Negative	0	2
True Negative	6	6
False Positive	0	0

n=12; 6 spiked and 6 unspiked for both alternative and ISO confirmation method

Both the SureTect PCR assay and ISO reference method successfully detected *L. monocytogenes* from seven of nine matrices inoculated with 4.3 to 5.0 CFU; two samples were found to be inhibitory to growth of *L. monocytogenes* (Table 1). An increased dilution ratio in the primary enrichment of 1/50, tested with a sample pooling method, showed improved PCR and culture confirmation results with 4 out of 6 inhibitory matrices tested (Table 2). The alternative rapid culture method, using complete 24 LEB for both primary and secondary enrichments, showed 100% sensitivity while a Fraser Broth confirmation from the same samples failed to recover 2/6 samples tested, indicating reduced sensitivity of the ISO reference method from the secondary enrichment step (Table 3).

CONCLUSIONS

Detection of *L. monocytogenes* was achieved for all medical nutrition samples tested in a shorter time to result than the traditional ISO reference method. The SureTect PCR method achieved a reliable time to result of 24-28 hours for samples that were not inhibitory to growth of *Listeria*. Where inhibition was encountered, an alternative rapid 4-day culture method was identified that achieved 100% sensitivity compared to 67% for the Fraser Broth confirmation method, indicating superior performance to the 5-day ISO reference method. Where performance issues are encountered with a broad range of sample types, Thermo Fisher Scientific SureTect PCR method demonstrates the ability to enhance and optimise workflows that achieve faster, more reliable results for a variety of foodborne pathogens.

REFERENCES

1. ISO 11290-1:1996. Microbiology of food and animal feeding stuffs -- Horizontal method for the detection and enumeration of Listeria monocytogenes -- Part 1: Detection method

ACKNOWLEDGEMENTS

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TRADEMARKS/LICENSING

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