Food testing

Method modification of the Listeria detection and enumeration methods in accordance with ISO 16140-2:2016

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Introduction

The ISO 16140-2:2016[1] validated Thermo Scientific™ Listeria Precis™ detection and enumeration methods (UNI 03/14-06/22, UNI 03/04-04/05, UNI 03/14-06/22, UNI 03/15-12/22)[2] was extended to offer an improved time to result and flexibility for the detection and enumeration of *Listeria* species and *L. monocytogenes.*

Study objectives:

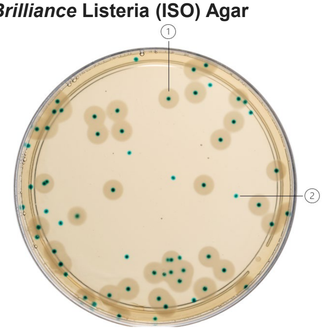
- Modify **enrichment** protocol (detection) and **dilution** protocol (enumeration).
- Introduce the **new** and **enhanced** Thermo Scientific™ Oxoid™ *Brilliance*™ Listeria (ISO) Agar.
- Offer a wider range of **confirmation tests**, including the rapid Thermo Scientific™ PrecisCheck™ Listeria species and Thermo Scientific™ PrecisCheck™ L. monocytogenes lateral flow tests.

Methods

The method modifications were validated against the ISO 11290-1:2017[3] and ISO 11290-2:2017[4] reference methods in an unpaired study design. For detection, tests portions were enriched in 24 LEB for a minimum of 20 hours, followed by streaking **10 µL** with a loop on *Brilliance* Listeria Agar (ISO) (Figure 1). The enumeration method consisted of a dilution in buffered 24 LEB (without selective supplement) as well as the diluents prescribed ISO 6887[5] series and ISO 11290-2 standard, followed by plating procedures on the new *Brilliance* Listeria Agar (ISO), including both surface and pour plating protocols.

Presumptive *Listeria* colonies were confirmed using the appropriate tests for the colony characteristic. This included the PrecisCheck Listeria species or PrecisCheck L. monocytogenes lateral flow tests that give results in 20 minutes or less. In addition, the incubation temperature for the 24 LEB (detection) and *Brilliance* Listeria Agar (ISO) is 37˚C, eliminating the need for multiple incubators set at different temperature.

Figure 1. *Brilliance* Listeria (ISO) Agar



Examples results – mixed culture
1. Listeria monocytogenes colony
2. Listeria species (non-*L. monocytogenes*) colony

Results

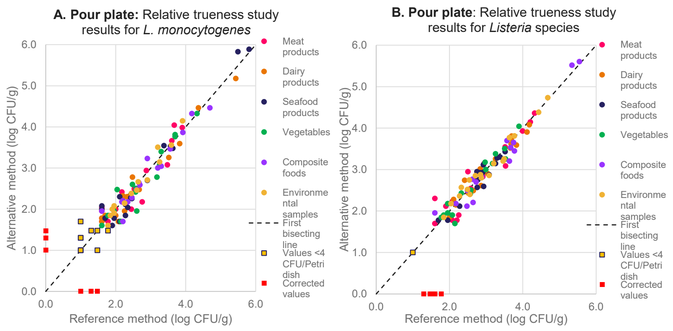
For the detection methods, n=395 samples tested for the *L. monocytogenes* target, with n=197 positive. For *Listeria* species, n=364 samples tested, with n=182 positive. The sensitivity and RLOD results can be seen in Figure 2. These studies showed that the modified Listeria Precis detection methods were statistically comparable or superior to the ISO 11290-1:2017 reference method. For the enumeration methods, the average difference in the relative trueness studies for *L. monocytogenes* were -0.02 log cfu/g with the pour plate protocol and 0.02 log cfu/g with the surface plate protocol. For *Listeria* species, the average difference was -0.03 log cfu/g with the pour plate protocol and 0.00 log CFU/g with the surface plate protocol. The relative trueness study results can be seen in Figure 3. The Listeria Precis enumeration methods were statistically comparable to the ISO 11290-2:2017 reference method.

Figure 2. Summary of ISO 16140-2:2016 Results for the Listeria Precis Detection methods

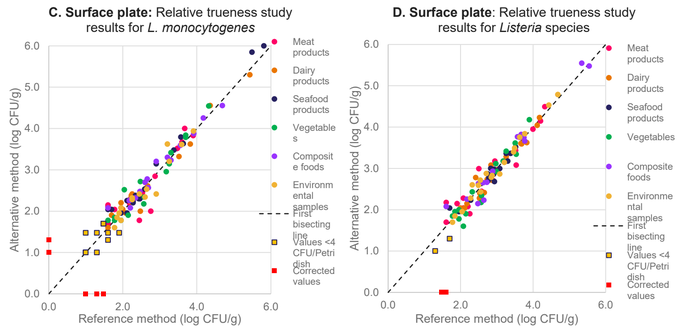
	Listeria monocytogenes	*Listeria* species
Sensitivity of Alternative method	90.4%	91.6%
Sensitivity of Reference method	89.3%	87.4%
Relative Trueness	89.9%	89.4%
False Positive Ratio	0%	1.6%
Relative Level of Detection	0.930	0.848

Summary of Listeria Precis (detection *Listeria monocytogenes* and detection *Listeria* species) results for all categories.

Figure 3. Summary of ISO 16140-2:2016 Results for the Listeria Precis Enumeration methods



Two-dimensional plots of all categories for the *L. monocytogenes* pour plate protocol.



Two-dimensional plots of all categories for the *L. monocytogenes* surface plate protocol.

Conclusions

The Listeria Precis methods were comparable to the relevant reference method throughout the studies. They provide a simple, fast, accurate and reliable culture-based method for the detection and enumeration of *Listeria* from a broad range of foods and environmental surfaces.

- 20-hour enrichment (detection)
- Pour plate option (enumeration)
- 20-minute confirmation

- New *Brilliance* Listeria Agar (ISO)
- Clearly visible halos in 24 hours
- 10 µL streak (detection)

- Equivalent or better than reference methods
- Simple user-friendly workflow

References

1. ISO 16140-2:2016: Microbiology of the food chain — Method validation — Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method
2. NF Validation - https://nf-validation.afnor.org/en/food-industry/
3. ISO 11290-1:2017 - Microbiology of the food chain — Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. — Part 1: Detection method.
4. ISO 11290-2:2017 - Microbiology of the food chain — Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. — Part 2: Enumeration method
5. ISO 6887-1 to 6:2017 - Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination

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