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

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Introduction

Listeria monocytogenes is a major global foodborne pathogens with a severe impact on public health[1]. The ISO 16140-2:2016[2] validated Thermo Scientific™ Listeria Precis™ detection methods (UNI 03/14-06/22 & UNI 03/04-04/05) have been extended to offer an improved time to result and flexibility for the detection of *Listeria* species and *Listeria monocytogenes*.

Study objectives:
Validate the Precis methods with Thermo Scientific™ Oxoid™ 24 Listeria Enrichment Broth (24 LEB) with a **20-hour** minimum enrichment time.
- 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™ lateral flow tests.

Methods

The method modifications were validated against the ISO 11290-1:2017[3] reference method in an unpaired study design. The method consisted of enrichment in 24 LEB for a minimum of 20 hours, followed by streaking **10 µL** with a loop on *Brilliance* Listeria Agar (ISO) (Figure 1). 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 and *Brilliance* Listeria Agar (ISO) is 37˚C, eliminating the need for multiple incubators set at different temperature.

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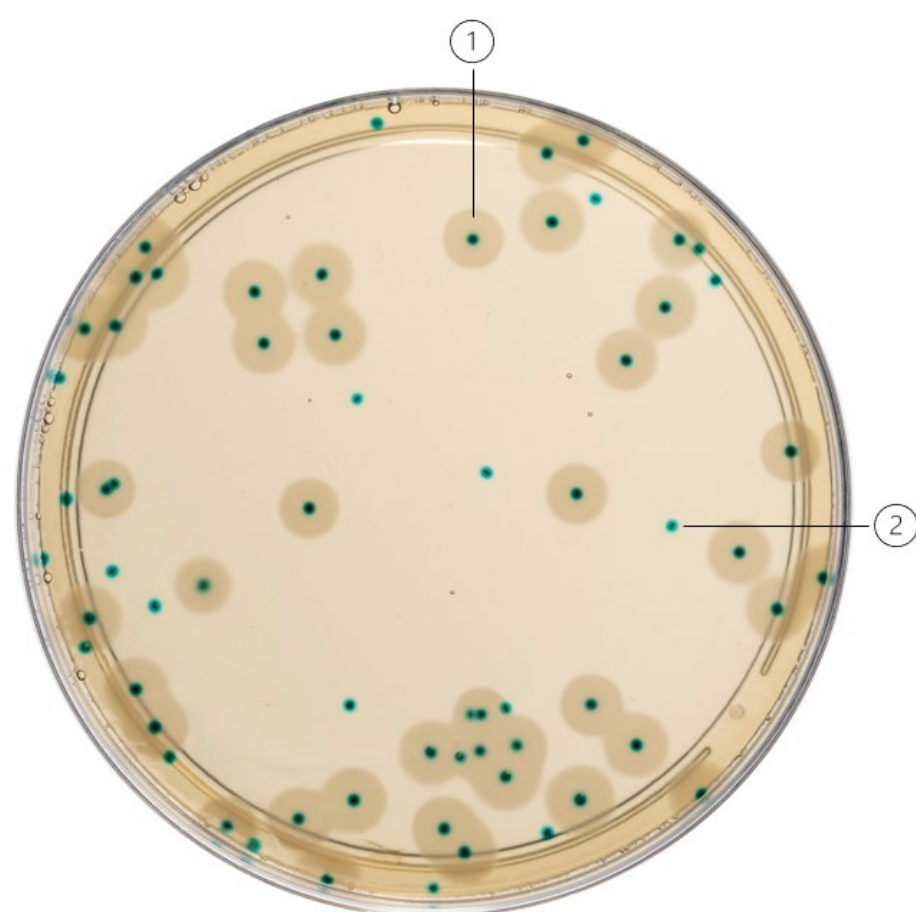
Examples results – mixed culture
① Listeria monocytogenes colony
② Listeria species (non-*L. monocytogenes*) colony

Results

A total of 395 samples were tested for the *L. monocytogenes* target, with 197 being positive. For *Listeria* species, a total of 364 samples were test and 182 were 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.

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

A. Summary of Listeria Precis (detection *Listeria monocytogenes* and detection *Listeria* species) results from each category.

| Listeria Precis method	Composite foods	Meat products	Dairy products	Seafood	Vegetables	Environmental	Total
Detection *Listeria monocytogenes* (ND-PD[a])	-2	-2	-1	2	-3	3	-3
Detection *Listeria* species (ND-PD)	-2	-2	3	-2	-3	-1	-7
Acceptability Limit	3						6

tability limits per category. [a]Positive deviations (PD) subtracted from Negative deviations (ND).

Conclusions

The two Listeria Precis detection methods for *Listeria* species and *L. monocytogenes* provide a simple, fast, accurate and reliable culture-based method for the detection of *Listeria* in a broad range of foods and environmental surfaces. Results achieved in two days, which is up to three days faster than traditional testing workflows and a day sooner than other 'rapid' workflows. Studies have shown that resources are saved by a 33% reduction in plate inoculation time compared to other rapid culture media methods

Enrichment:
20 hours
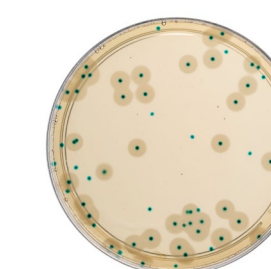
Detection:
22 hours

Confirmation:
20 minutes

References

1. Centers for Disease Control and Prevention, Listeria https://www.cdc.gov/Listeria/index.html.
2. 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
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.

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