ENHANCING DAIRY SAFETY: RAPID & VALIDATED MULTIPLEX PCR FOR *LISTERIA* AND *L. MONOCYTOGENES* DETECTION

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INTRODUCTION

Contamination of food with *Listeria monocytogenes* is a significant public health concern. Effective monitoring should focus on the Listeria "sensu stricto" group and L. monocytogenes, necessitating a rapid, dual-target method for robust and efficient risk management.

The **Hygiena® foodproof® *Listeria* plus *L. monocytogenes* Detection LyoKit** is a multiplex PCR assay designed to meet this need, offering simultaneous detection of all relevant *Listeria* targets and protocols optimized for throughput and sensitivity. This poster summarizes the validation study conducted by ADRIA Développement, France.

METHOD

The performance of the foodproof *Listeria* plus *L. monocytogenes* Detection LyoKit was compared against the EN ISO 11290-1:2017 reference method, following NordVal and ISO 16140-2 protocols.

Validated Matrices and samples: Samples (404 total) were analyzed across various categories, including **milk and dairy** products and **environmental samples**; 60% of the samples were naturally contaminated.

Three protocols were evaluated, differing in enrichment media and time, and DNA extraction procedure:

- **Protocol A – Optimized for high throughput and shortest incubation**: 22 h enrichment with ‚Actero™ *Listeria'*, followed by DNA extraction using the foodproof StarPrep® Two 8-Strip Kit.
- **Protocol B & C**: Enrichment with Half Fraser (ISO) for 25 hours (48 hours for **C**), followed by DNA extraction with the foodproof StarPrep Two Kit.

Interlaboratory Study: Blind-coded cheese samples inoculated with *Listeria monocytogenes* ranging from 0 CFU/25 g to 6 CFU/25 g were sent to laboratories for a side-by-side comparison of both methods.

RESULTS - SELECTIVITY, SENSITIVITY, RELATIVE LEVEL OF DETECTION (RLOD) AND LOD_{50}

The study critically assessed Method Performance Characteristics. **All Acceptability limits were met for each individual and combined categories for all three protocols.** Here are the results in detail:

Selectivity (Inclusivity + Exclusivity): The most challenging protocol A, with the shortest incubation time, was tested and demonstrated perfect specificity. It correctly identified 100% of the 50 target *Listeria* strains (inclusivity) and correctly excluded 100% of 30 other, non-target bacterial strains (exclusivity).

Excellent sensitivity (LOD_{50} & RLOD): The **LOD_{50} per test portion** delivered similar results for the reference method (0.3 to 1.1 CFU) and Hygiena's alternative method (Protocol A: 0.4 to 1.0 CFU; Protocols B and C: 0.3 to 1.1 CFU). **RLOD**: Both targets of the foodproof *Listeria* plus *L. monocytogenes* Detection LyoKit meet the acceptability limit of 2.5 for unpaired studies (Protocol A) and 1.5 for paired studies (Protocols B and C) for all matrix/strain pairs tested.

Sensitivity study (Correspondence): Especially for dairy matrices, the relative trueness RT (%) showed very high correspondence with both methods, ranging from 95.2 – 100% and from 83,7 – 100% for environmental samples (for all protocols and for *Listeria* spp. and *L. monocytogenes*). Observed values for the deviating results (ND-PD) and (ND+PD) met the acceptability limits for each individual category and for all the combined categories for all three protocols.

Interlaboratory study: Valid results for the **inoculated cheese samples** from 10 collaborators were analyzed and resulted in **comparable sensitivity rates** and the **acceptability limit** criteria (AL), according to ISO 16140-2, have been met.

Access the full dataset and the NordVal International Certificate (No 054) here:

CONCLUSIONS

These studies have shown that the alternative method fulfills the requirements of the NordVal International Protocol No. 1 / ISO 16140-2 and guarantees equivalent results to the reference method.

Consistent and reliable performance was proven across a wide range of challenging food types, including dairy, high-fat meats, seafood, ready-to-eat meals, as well as environmental samples. By combining the screening for *Listeria* species and the specific identification of *L. monocytogenes* into a single workflow, the kit saves significant hands-on lab time and allows for faster safety decisions.

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