TECHNICAL SHEET

Microlab Listeria monocytogenes

PRINCIPLE AND SCOPE OF THE TEST

Microlab Listeria monocytogenes is a qualitative test for the detection of *Listeria monocytogenes* in food and environmental samples.

The method consists of two stages: an enrichment phase and a detection phase. During the enrichment phase, the sample is incubated in a specific culture medium for 48 hours at 37°C, allowing the bacteria to multiply to detectable levels. Subsequently, these bacteria are detected using a lateral flow immunochromatographic assay (LFIA) based on a specific antigen-antibody reaction.

Microlab can be presented in two different formats: the Microlab device and the Microlab Basic:

- Microlab Listeria monocytogenes: This presentation integrates all phases into a ready-to-use device, eliminating the transfer of the enriched medium between containers and therefore the risk of cross-contamination. This format can be used in the food industry itself or in a laboratory and requires no qualified personnel or specific microbiology equipment.

- Microlab Basic Listeria monocytogenes: In this format, the culture medium for enrichment and the immunochromatographic test are presented separately and require prior preparation.

VALIDATION

Microlab Listeria monocytogenes has been approved by the AOAC Research Institute under the Performance Tested Methods program (license number PTM 062502). Validation was performed according to AOAC guidelines[1]. The food matrices included in the AOAC validation are listed in Table 1.

Table 1. Matrices validated with the Microlab Listeria monocytogenes method.

Category	Matrix analyzed
Ready-to-eat fish products	Smoked salmon
Ready-to-eat poultry products	Deli turkey
Milk and treated products thermally	Cheddar cheese
Ready-to-eat meat products (excluding poultry)	Deli ham
Milk and treated products thermally	Ice-cream

A different strain of *Listeria monocytogenes* was used for each validated matrix, and the results were compared with the ISO 11290-1:2017 reference method. All presumptive positive results were confirmed according to ISO standards. The tests performed on food matrices were:

- 5 negative controls (not inoculated).
- 5 positive controls (100% positive with reference method, POD=1).
- 20 samples with an inoculation level such that it resulted in 25-75% positives for the reference method (POD=0.25-0.75).

On the other hand, AOAC validation requires, in addition to matrix testing, the following studies:

- **Inclusivity:** The analysis of 50 different strains of *Listeria monocytogenes* was carried out in order to verify the ability of the method to reliably detect the different strains of the target microorganism.

- **Exclusivity:** A total of 32 bacterial species other than *Listeria monocytogenes* were analyzed. In these assays using high inoculation concentrations, *Listeria innocua* and *Listeria marthii* yielded positive results. Subsequent studies conducted within the validation framework demonstrated that the detection capability of the Microlab method for these bacteria is 100 times lower than for *Listeria monocytogenes*. Therefore, positive results will only be obtained in samples with high contamination levels of these microorganisms.

- **Sturdiness:** Controlled variations were introduced into the assay protocol (incubation temperature, enrichment time, and detection time) to evaluate

the ability of the Microlab system to maintain adequate performance under modified conditions.

- **Batch-to-batch consistency:** The functionality of three different batches of Microlab kits was verified, ensuring the reproducibility and uniformity of results between productions.
- **Stability:** Tests were carried out with kits at different points in their life cycle period, with the aim of ensuring the correct functioning of the test throughout its useful life.

VERIFICATION

Due to the large number of different food and environmental samples, in addition to the matrices included in the AOAC certification, performance verification studies of the Microlab Listeria monocytogenes method have been conducted with new matrices. The verified matrices are described in Table 2.

Number of tests per food matrix:

- 1 negative control (uninoculated).
- 1 sample with an inoculation level 10 times higher than the detection limit.
- 3 samples with an inoculation level between 2 and 5 times higher than the detection limit.

Table 2. Matrices verified with the Microlab Listeria monocytogenes method.

Category	Matrix analyzed
Meat and meat products ready to cook (excluding poultry)	Fresh meat, minced meat, burger meat, carpaccio
Ready-to-eat meat products (excluding poultry)	Vienna sausage, fuet, mortadella, cooked ham, smoked bacon, cured ham, pâté
Ready-to-cook poultry products	Fresh meat, minced meat, burger meat, raw chicken breast
Ready-to-eat poultry products	Cooked turkey, Cooked chicken, Vienna sausage
Raw milk and raw milk products	Raw cow's milk
Milk and heat-treated products	UHT and pasteurized milk, pasteurized milk cheese, cheddar cheese, feta cheese, mozzarella, yogurt, cream ice cream, powdered milk, grated cheese
Eggs and egg products	Powdered egg
Fruits and vegetables	Guacamole
Raw fish and seafood, ready to cook	Raw tuna
Fishmonger products ready to eat or reheat	Smoked salmon, cooked prawns
Environmental samples	Wash water, polycarbonate, stainless steel

BIBLIOGRAPHY

1. Appendix J, AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces (2023) in Official Methods of Analysis of AOAC INTERNATIONAL, 22nd Edition(New York, AOAC Publications, 4 Jan. 2023), https://doi.org/10.1093/9780197610145.005.010 (accessed January 11, 2024).