

REDUCTION OF ENVIRONMENTAL FOOTPRINT AND TIME TO RESULT FOR *LISTERIA MONOCYTOGENES* DETECTION BY CULTURAL ISO 16140-2 VALIDATED METHOD

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INTRODUCTION

Detection of *Listeria monocytogenes* in food and environmental samples following ISO 11290-1:2017 includes sample preparation step using 1/10 dilution followed by secondary enrichment and isolation, requiring four days to release negative results. The new cultural method developed allows significant reduction on the volume of enrichment broth with shorter time to result (less than 48 h). The ISO 16140-2 validation study for the new ALOA® ONE DAY SHORT protocol led to conform results allowing a new management method of time and waste in laboratories.

METHOD

Alternative ALOA® ONE DAY SHORT protocol (Figure 1) from bioMérieux includes sample dilution 1/6 (e.g. 25 g diluted in 125 mL) in proprietary **Listeria Boost Broth (LBB) (18 h at 30°C without prewarming)**, followed by isolation on ALOA® agar (22 h at 37°C) and confirmation of typical colonies by rapid or reference tests.

ISO 16140-2 validation was performed on **445 food items** covering five food categories and environmental samples, including spiked and natural contaminated samples. Sensitivity and Relative Limit Of Detection (RLOD₅₀) studies were performed for all categories and compared (unpaired study) with the reference ISO method.

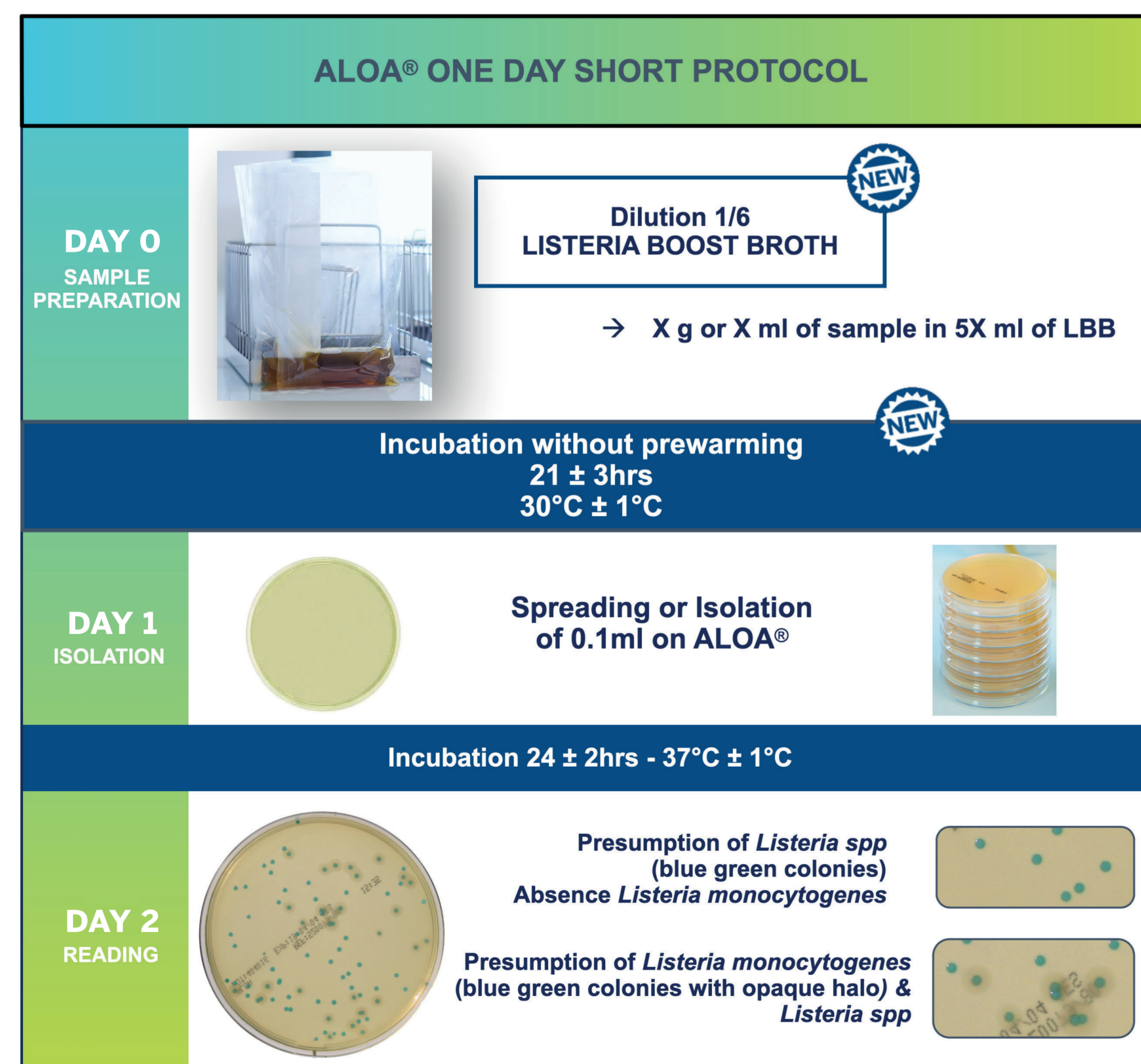


Figure 1: New ALOA® ONE DAY short protocol workflow allowing broth reduction with fast results.

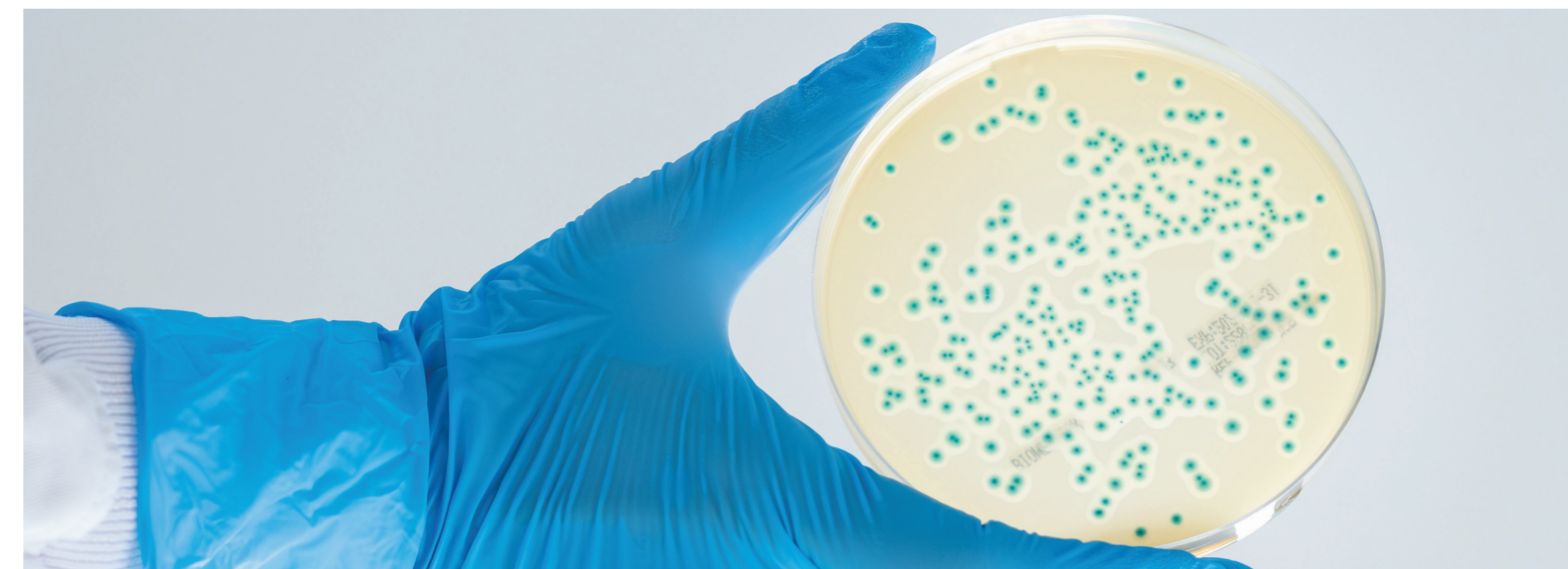


Figure 2: Illustration for results obtained on ALOA® agar medium

RESULTS

New sample preparation protocol allowed enrichment broth **reduction by 40%** with impact on contaminated waste treatment, incubation volume, media handling/storage, reducing time to result to **less than 48 hours**.

Category	PA	NA	PD	ND	Total
Meat Products	31	48	3	3	85
Dairy Products	30	36	2	1	69
Seafood Products	21	33	6	3	63
Vegetables	29	37	4	2	78
Composite Products	33	38	2	5	78
Environmental Samples	26	45	2	5	78
Total	170	237	19	19	445

Table 1: Summarizing results obtained on 6 food categories for the global ALOA® ONE Day short protocol performances

Among the 445 samples evaluated during this study, deviations for all categories (**ND-PD = 0**) were below the acceptability limits (AL=6).

Sensitivity for alternative and reference method was equal (90,9%). **Relative trueness** was **91,5%** and **False Positive Ratio** was **0%**.

RLOD50 combined values **0,908** and individual values (between 0,733 and 1,148) were below acceptability limits (AL=2,5).

	Formula EN ISO 16140-2	Reading 22h and 48h
Sensitivity Alternative Method (SE _{alt})	$SE_{alt} = \frac{(PA + PD)}{(PA + ND + PD)} \times 100\%$	90,9%
Sensitivity Reference Method (SE _{ref})	$SE_{ref} = \frac{(PA + PD)}{(PA + ND + PD)} \times 100\%$	90,9%
Relative Trueness (RT)	$RT = \frac{(PA + NA)}{N} \times 100\%$	91,5%
RLOD	$RLOD = \frac{LOD_{alt}}{LOD_{ref}}$	0,908
False Positive Ratio (FPR)	$FPR = \frac{FP}{NA} \times 100\%$	0,0%

Table 2: Summarizing results obtained for sensitivity, Relative Trueness, RLOD and FPR.

The new ALOA® ONE DAY short protocol allows significant reduction of volume needed for routine analysis and then significant save of cost dedicated to waste management. Table 3 is an example of saving for a 50 000 tests a year scenario.

Method	ALO A® ONE DAY	ALO A® ONE DAY short protocol	SAVING
Enrichment volume	225 ml	125 ml	100 ml
Dilution	1/10	1/6	
Minimum incubation time	21h	18 h	3 h
Nb of test/DILUBAG® 5L	22	40	Handling
Nbr of test/year	50 000	50 000	-
Total liter/year	11 250	6 250	40%
Total DILUBAG® 5L/year	2 250	1 250	Storage
Cost of waste treatment/tons (€)	1300	1 300	-
Nbr of tons/year	11.25	6.25	40%
Cost of waste treatment (€)/year	14 625 €	8 125 €	6 500 €

Table 3: Estimated cost savings in terms of volume thanks to the new ALOA® ONE DAY short protocol for 50,000 tests/year.



Figure 3: Illustration of the 40% volume saving with new ALOA® ONE DAY short protocol (on the left).

CONCLUSION

New ALOA® ONE DAY short protocol method for *Listeria monocytogenes* detection in broad range of foods and environmental samples showed equivalent results with :

- A shorter broth incubation time (18h to 24h) allowing faster time to result compared to reference and ALOA® ONE DAY methods
 - 40% reduction of culture media broth volume needed
 - 40 % decrease of waste treatment for laboratories
 - An optimization of sample preparation and laboratory workflows
 - Less handling & storage - More samples in 1 Dilubag (40 samples instead of 22)
- > ISO 16140-2: Extension validation with same full scope than ALOA® ONE DAY