ISO 16140 VALIDATION STUDY OF THE ALOA®ONE DAY METHOD FOR LISTERIA SPECIES DETECTION IN FOODSTUFFS AND ENVIRONMENTAL SAMPLES



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ABSTRACT

Introduction:

ALOA®One Day is a certified method for *Listeria* monocytogenes detection. It has recently been granted validation for the detection of Listeria species in foodstuffs and environmental samples. The proposed protocol is simpler, shorter and cheaper than the ISO mandatory reference method.

Purpose:

The aim of this study was to compare the ALOA® One Day alternative Method to the NF EN ISO 11290-1 standard according to the ISO 16140: 2003 requirements. Relative specificity, relative accuracy and limit of detection were tested for both methods.

Methods:

The specificity of both Methods (ALOA® One Day and NF EN ISO11290-1) were compared by testing 63 Listeria species (Inclusivity) and 32 non-*Listeria* species (Exclusivity).

379 samples including 123 naturally contaminated were tested by the reference and the alternative Method. During these tests relative accuracy, sensitivity and specificity were calculated. ALOA® One Day is a 2-step Method :an enrichment step (24h at 30°C in half-fraser), a detection step (subculture of 0.1 ml on one ALOA® plate and incubate 24h at 37°C). The positive samples must be confirmed by a 5h procedure using one typical colony and a lateral flow test.

Results:

Exclusivity and inclusivity data have shown a perfect recovery of all the Listeria species and a perfect discrimination or inhibition of the 32 Non-Listeria species strains.

The statistical analysis performed on the 379 food samples tested according to ALOA®One day and ISO 11290-1 has proved that the two methods were equivalent: Relative accuracy (AC) = 99.4%, Relative specificity (SP) = 99.45% and relative sensibility (SE)=99.49%.

Significance:

ALOA® One Day is a suitable method to detect Listeria species in foodstuffs, animal feeding and environmental samples. This method showed equivalent specificity and sensitivity to the ISO reference method and enables laboratories to save time and money.

Introduction

ALOA® One Day is a well established method for the detection of Listeria monocytogenes in foods and environmental samples in Europe. This method was the first method based on the Agosti and Ottaviani chromogenic agar to be certified by NF Validation for the detection of Listeria monocytogenes more than ten years ago. The validation of the method was then extended according to the ISO16140 standard in 2006 and it has recently been granted validation for the detection of Listeria species in foodstuffs and Environmental samples. In comparison with the ISO11290-1 reference method, the ALOA® One Day method shortens the batch release delay and is an economical alternative method for the labs.

The aim of this study was to evaluate the performances of the ALOA® One Day against the ISO11290-1 reference method according the ISO16140 method comparison standard. The different points described in the ISO16140 guideline have been tested including accuracy, sensitivity, specificity, but also detection limit and practicability.

Material and Method

Method Comparison

D + 1:

D + 2 : Results

Absence of *Listeria* spp

A comparison between the ALOA® One Day alternative method (Fig1) and the ISO11290-1 reference method (Fig 2) has been carried out on 379 food and environmental samples including 123 naturally contaminated samples. The different categories tested during this study are described in table 1.

Catégories	Positive samples including (naturally contaminated)	Négatif Samples	Total
Meat and cooked pork meat	58 <mark>(58)</mark>	40	98
Fruits and vegetables	31 (13)	32	63
Fish and custaceans	42 (11)	38	80
Dairy products	31 <mark>(12)</mark>	34	65
Environnemental samples	35 (29)	38	73
Total	197 (123)	182	379

Table 1: Samples categories tested for the method comparison

This method comparison allowed calculating the relative accuracy (AC), the relative sensitivity (SE) and the relative specificity (SP) of the alternative method according to the ISO16140 protocol.

Detection of *Listeria monocytogenes* and other *Listeria* spp

in foodstuffs and environmental samples

ALOA[®] One Day Method

Incubation : 24 ± 2 hours at 30 ± 1 °C

Spreading or isolation of 0.1 ml on ALOA®

Incubation : 24 hours at 37 \pm 1 $^{\circ}$

It is possible to prolong the incubation up to 48 hours.

Absence of typical colonies of *Listeria*

Inclusivity and exclusivity

Sixty three strains of *Listeria* species were tested by the ALOA® One Day method to evaluate inclusivity and 32 non-Listeria species strains for exclusivity. The inclusivity test was performed following the entire ALOA® One Day protocol (including the half-Fraser enrichment step). The exclusivity test was performed by streaking a non selective enrichment broth of tested micro-organism on to an ALOA® plate .the target strains. ALOA® plates were then incubated at 30°C and read after 24 and 48h.

Relative Level of detection

In order to determine the minimal detection level of Listeria spp in a contaminated sample by both Alternative Method and Reference Method, several artificially contaminated samples were tested. The assay was carried out by using 4 target contamination levels (0; 0.25; , 0.5 and 1 cells/25 g) in each 2 couples of sample/ Listeria strain. Six replicates per couple were tested.

Practicability

Thirteen criteria described in the ISO16140 standard have been evaluated for the ALOA® One Day alternative method, including the Time To Result in comparison with the ISO11290-1 Method.

Results

Inclusivity and Exclusivity

Listeria species	Number of tested strains	ALOA results	Listeria species confirmation strip	Palcam results
Listeria monocytogenes 20		Typical: Blue green colonies with opaque halo	to + +	
Listeria grayi	4	Typical: Blue green colonies	+	+
Listeria ivanovii	9	Typical: Blue green colonies with small opaque halo	+	+
Listeria innocua	11	Typical: Blue green colonies	+	+
Listeria seeligeri	10	Typical: Blue green colonies	+	+
Listeria welshimeri	9	Typical: Blue green colonies	+	+

Table 2: Inclusivity test

OPTION 1:

PROTOCOL 24 HOURS

From 1 colony isolated on ALOA

noculate by prick a PALCAM ag

(up to 12 pricks per 90 mm pla

ncubation 24 hours at 37 ± 1

= Listeria spp

aesculin -

Sample dilution of Xg (or ml) in 9X ml (ou g) of **Half Fraser broth** Confirmation of the presence of *Listeria* spp **ALOA[®] One Day Method** Incubation for (24±2) hours at (30±1) $\mathcal C$ **SPECIFIC CASE OF CONFIRMATION** 'solation of the previous culture onto ALOA® agar Secondary enrichment of the medium b culture in 10ml of Fraser broth to the first agar, for example: Incubation for (48±2) hours at (37±1)% **PROTOCOL 5 HOURS** From 1 colony isolated on ALOA inoculate 1ml of **BRAIN HEAF** Incubation for (24±3)h at (37±1) \mathfrak{C} **INFUSION** broth Incubation for further (24±3)h if necessary. **AEB110101 - Pack of 20 tube** ncubation : 5 hours at 30 ± 1% and onto a complementary second agar to the first agar, for example Possibility to prolong incubati Select 5 presumptive colonies (on ALOA®: blue-green colonies with a or Palcam agar frank halo) and streak onto a TSYE agar Dip a strip *Listeria* species onfirmation Strip AEB40005 Incubation for 18 to 24 hours Incubation for (24±3)h at (37±1) \circ at (35 ± 1) ℃ or at (37 ± 1) ℃ Incubation for further (24±3)h if necessary directly in the tube. Reading after 10 to 20 minutes of contact. 1 - Catalase reaction Select 5 presumptive colonies 2 – **Gram** staining (on **ALOA**®: blue-green colonies with 3 – **Motility** test *(optional test)* frank halo) and streak onto a TSYE agar 4 – Haemolysis test (AEB522865- 20 plates Ø90 mm) 5 - Carbohydrates utilisation 6 – **CAMP** test Incubation for 18 to 24 hours at (35 ± 1) ℃ or at (37 ± 1) ℃ Positive test 2 – **Gram** staining - Motility test (optional test) 1 line = absence of *Listeria* spp 4 – Haemolysis test 2 lines = presence of *Listeria* spr 5 – **Carbohydrates** utilization 6 – **CAMP** test

Species	Strain origin	ALOA results
Bacillus cereus	Sprouts	Non typical
Bacillus cereus	Tabbouleh	Non typical
Bacillus cereus	wheat	_
Bacillus circulans	Lactic ferment	_
Bacillus megaterium	Salad	Non typical
Bacillus mycoïdes	Radish	Non typical
Bacillus subtilis	Collection ATCC 6633	_
Candida albicans	Orange juice	_
Corynebacteriaceae sp	Poultry offal	_
Enterococcus faecalis	Collection ATCC 29212	_
Enterococcus faecalis	Cow Milk	-
Enterococcus faecium	cut duck	_
Enterococcus faecium	Collection CIP 5855	Non typical
Enterococcus hirae	Water	-
Escherichia coli	beef raw meat	-
Escherichia coli	goat Milk	-
Lactobacillus acidophilus	Milk product	_
Lactobacillus casei	Milk product	_
Lactobacillus casei	Milk powder	_
Pediococcus pentosaceus	Lactic ferment	_
Pseudomonas aeruginosa	veal liver	Non typical
Pseudomonas aeruginosa	ATCC 27853	Non typical
Rhodococcus equi	Meat product	_
Saccharomyces cerevisiae	Collection ATCC 9763	Non typical
Staphylococcus aureus	Cow Milk	_
Staphylococcus aureus	Cow Milk	_
Staphylococcus intermedius	Food industry	_
Streptococcus agalactiae	Cow Milk	_
Streptococcus dysgalactiae	Cow Milk	_
Streptococcus uberis	Cow Milk	_
Micrococcus luteus	ATCC 9341	_
Micrococcus luteus	CIP 5345	_

Table 3: Exclusivity test

These results show a perfect ability of the ALOA® One Day method to recover the Listeria species and a perfect specificity because non-Listeria strains are either inhibited or grow as non typical (colour and colony morphology). This selectivity cuts out on costly and time consuming confirmation tests.

Method Comparison

	ISO 11290 Positive (R+)	ISO 11290 Negative (R-)	Total
ALOA one Day Positive (A+)	PA = 191	PD = 1	192
ALOA One Day Negative (A-)	ND = 5	NA = 182	187
Total	196	183	379

Table 4: Method comparison results

	PA	NA	ND	PD	SUM N	Relative Accuracy AC (%) [100x(PA+NA)]/N	N+ PA + ND	Relative sensitivity SE (%) [100xPA]/N+	N- NA + PD	Relative specificity SP (%) [100xNA]/N-
TOTAL	55	116	0	3	174	98.2	55	100	119	97,4

Table 5: Relative Accuracy, sensitivity, specificity calculation

These results show very close performances between ALOA® One Day method and ISO11290-1 reference Method for the detection of Listeria species in food and environmental sample. The value of the relative Accuracy (AC), relative sensitivity (Se) and relative specificity (Sp) and the low number of discordant result allow concluding to equivalence between both methods according to the ISO16140 standard.

Detection level

Couples (strains, matrix)	Relative Detection level (CFU / 25 g or 25 ml) according to the Spearman-Kärber* test			
	Reference Method	Alternative Method		
Rillettes, Listeria welshimeri	0.5 [0.3-0.7]	0.5 [0.3-0.7]		
Process water, Listeria innocua	0.4 [0.3-0.6]	0.4 [0.3-0.6]		

* «Hitchins A. Proposed Use of a 50 % Limit of Detection Value in Defining Uncertainty Limits in the Validation of Presence-Absence Microbial Detection Methods, Draft 10th December, 2003».

Table 6: ALOA® One Day and ISO11290-1 Listeria species detection level

The relative detection level for the ALOA® One Day Method is between 0.3 and 0.7 CFU/25g. It is similar from the ISO11290-1 Reference method.

Practicability

ANALYTICAL STEP	TTR Reference Method NF EN ISO 11290-1	TTR Alternative Method « ALOA® One DAY »	
Half Fraser Enrichment	Day o	Day o	
Subculture in Fraser Broth	Day 1	-	
Streaking on Selective Agar Spreading or streaking on ALOA	Day 1 & Day 3 -	- Day 1	
Negative results (absence of typical colonies)	Day 5	Day 2	
Positive results (typical colonies) or negative after the confirmation step			
Genus confirmation			
- Standardized test	Day 6 to Day 7	Day 3 to Day 4	
- Immunochromatographic Test	_	Day 2	
- Palcam Spot	_	Day 3	
Species confirmation			
- Standardized test	Day 8 to Day 12	Day 4 to Day 9	
- ALOA ConfirmationTM	-	Day 3	

Table 7: Time To Results Comparison between ALOA® One Day and ISO11290-1 Method.

These results put forward the ability of the ALOA® One Day protocol to answer in 2 or 3 days when the reference method needs 4 to 12 days.

Conclusion

The results presented in this study show that the ALOA® One Day is reliable for the screening of Listeria monocytogenes and other Listeria species contamination in food and environmental samples. ALOA® One Day yields results as good or better than the corresponding reference method, but in a shorter time to obtain final result especially for Listeria monocytogenes and /or Listeria species free samples food and environmental samples.

