Detection Of Listeria Monocytogenes In Cheese And Samples Of The Production Environment: Comparison Of The One-Step Enrichment Broth "OneBroth™" With Reference Method ISO 11290-1

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For *Listeria monocytogenes* (LMO), two-step enrichment procedures gave the most satisfiying recovery rates in all relevant food matrices so far, but they are still time-consuming. Recently, a procedure called "One Broth (TM)" containing a single enrichment step with 24 hour incubation was successfully validated against the reference method ISO 11290-1/Amd:2004. However, before implementing an alternative method, one has to verify the performance, especially on matrices which are often used or known to be problematic for LMO recovery . Results of our verification procedure are reported here.

Methods*: „One Broth™"

ENRICHMENT:

1) Mix 10 g sample & 90 ml „One Broth™" (Oxoid)

2) Incubation at 30°C for 22 -26 h

ISO 11290-1/Amd:2004

1st ENRICHMENT:

1) Mix 10 g sample & 90 ml ½ Fraser Broth

2) Incubation at 30°C for 22 -26 h

2nd ENRICHMENT:

Fraser broth, 37°C, 46-50 h

PLATING OUT AND CONFIRMATION:

1) ALOA (Biolife), 37°C, 24-48 h; 2) confirm 1-5 blue colonies with halo per plate with gene probe (Accuprobe, Biomerieux)

Design of spiking experiments:

- smear water, brine (20% NaCl), red smear soft cheese, hard cheese smear analysed after scheme above
- 3 levels of contamination (low, medium, high)
- 3 strains, isol. from cheese (LMO 1/2a, LMO 4b & *L. innocua*)
- 15 samples per contamination level and strain (e.g. in Tab. 1)
- 5 blanks/food type

And 14 naturally smear water contaminated samples

		cfu/sample	OneBroth +ve of 15	ISO 11290-1 +ve of 15
Brine Samples Spiked with *L. monocytogenes* & *L. innocua*	LMO 1/2a	7	7	14 (1)*
		35	14	14 (8)*
		485	15	15 (14)*
	LMO 4b	3	6	15 (3)*
		28	15	15 (10)*
		402	15	14 (14)*
	LMO 4 b & LIN	3 LMO + 7 LIN	8	15 (5)*
		28 LMO + 82 LIN	15	15 (8)*
		402 LMO +680 LIN	15	15 (15)*

Tab. 1: Design of spiking experiments and results (brine samples)
* in (): samples positive from plating out after first enrichment

ISO 11290		+	-	Σ
One Broth ™	+	500	11	511
	-	31	32	63
	Σ	531	43	574 = n

Tab. 2: Summary of method comparison
Clear concordance between both methods (kappa=0.57) , calculated after Swiss SAS guideline No. 328

Results:

- For brine samples spiked with low levels, only 21/45 positive with „One Broth™" in contrast to reference (44/45 positive) (Tab.1)
- Second enrichment of ISO 11290 strongly needed for LMO recovery from brine samples (Tab. 1)
- Clear concordance between both methods (kappa=0.57, Tab 2); strong concordance (kappa=0.71), if brine sampels excluded
- Successful recovery of LMO at low numbers in brine, if „OneBroth™" is incubated for 48 h (data not shown)

* deviations from AFNOR validation study or ISO 11290 method: 10g sample; ALOA agar for all experiments ; no second agar; Accuprobe is a validated alternative confirmation test

The one-step enrichment procedure „One broth™" is suitable for the fast and successful recovery of *Listeria monocytogenes* in cheese and samples from the production environment, but may need modification in case of certain matrices (e.g. 48 h incubation of brine samples). These results are showing the need of a carefully performed verification procedure, even when already validated alternative methods are applied.

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