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Maisons-Alfort Laboratory for
Food Safety



EU-RL Lm

European Union Reference Laboratory for
Listeria monocytogenes

2014 Interlaboratory Proficiency Testing trial

Detection of *Listeria monocytogenes* in iceberg salad

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Unit SEL

9th Workshop of the EURL for *Listeria monocytogenes*

25th to 27th March 2015

French Agency for Food, Environmental and Occupational Health Safety – Maisons-Alfort Laboratory for Food Safety
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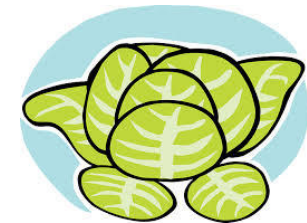
Presentation of the 2014 Proficiency Testing Trial

Proficiency of participants to use the Standard method:

EN ISO 11290-1: 1996/Amdt 1: 2004 “Microbiology of food and animal feeding stuffs – Horizontal method for detection and enumeration of *Listeria monocytogenes*, part 1. Detection method / Amendment 1: Modification of the isolation media and the haemolysis test, and inclusion of precision data”

11290-1 Standard method (Anonymous, 1996, 2004a)



- Food matrix : iceberg salad
 - Competitive mesophilic microflora (naturally contaminated): 5-6 log cfu.g⁻¹
 - 25g of food matrix per sample dispatched frozen at -18 °C
 - 7 samples/participant
 - 1 sample for negative control
 - 3 samples artificially contaminated with a low level of *L. monocytogenes* (15-30 cfu/sample)
 - 3 samples artificially contaminated with a high level of *L. monocytogenes* (150-300 cfu/sample)



Organisation of the 2014 Proficiency Testing Trial

- 34 laboratories registered to participate in the 2014 PT trial (deadline 15th August 2014)
- Period of analysis: 13th October 2014 to 21st October 2014
- Deadline to send results: 7th November (sphinx)

- Preliminary report of results sent: 6th January
- Discrepancy recording form sent to participant on December 2014 and closed on January 2015.
- Final report of 2014 PT trial will be send to participants and to DG SANTE in the coming months.

	Title	Published on	Type	Date
 424 Ko	[EURL Lm] Circular E-mail N°2014/19 - Report of the 2013 PT trial on Lm enumeration	26/11/2014	Circular letter	26/11/2014
 1490 Ko	Report of the 2013 Proficiency Testing trial on the enumeration of <i>Listeria monocytogenes</i> in diced poultry	21/11/2014	Report	21/11/2014
 Ko →	Instructions to participants on the implementation of the PT (2014 Lm detection PT Trial)	10/10/2014	Interlaboratory trial	10/10/2014
 425 Ko →	Cover letter concerning the samples (2014 Lm detection PT trial)	10/10/2014	Interlaboratory trial	10/10/2014
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 66 Ko	[EURL Lm] Circular E-mail N°2014/14 - EFSA report on Lm baseline survey-part B	25/08/2014	Circular letter	19/08/2014
 428 Ko →	[EURL Lm] Circular E-mail N°2014/12 - 2014 Detection PT Trial Invitation	25/07/2014	Circular letter	25/07/2014
 197 Ko →	Information to Participants (2014 PT trial Lm detection)	25/07/2014	Proficiency test	25/07/2014

EURL used for the first time on-line survey software Sphinx: this tool allows sending electronic questionnaires, for different steps of the PT:

- 1. Registration of the PT (participation form)**
- 2. Acknowledgement of receipt of samples**
- 3. Test report**



Homogeneity & stability

Homogeneity & stability have been also studied: CEN mandate*

- **Homogeneity study :**

- Checked at all levels (negative control, low level and high level) of PT trial

- **Stability study :**

- Defined in preliminary study:

- ↳ At -18 °C about 26 days

- ↳ For abuse temperature: overnight at 4°C and 8 °C

- ↳ Checked at all levels during the PT trial

*Revision of Standards EN ISO 11290-1 and -2 and CEN Mandate M/381

Results: Enrichment steps

Participation of 34 NRLs

All laboratories declared performing analysis according Standard method

EN ISO 11290-1:1996 amended 2004

- **Samples preparation**

- All laboratories declared performing analysis according to the Standard method EN ISO 6887

- **Enrichment stage and plating**

- All laboratories used appropriate enrichment broth medium (half Fraser/Fraser).
- All laboratories used appropriate plating medium (**ALOA** for the 1st medium and see fig1 for the 2nd medium)
- Incubation temperature and time → OK (only some typing mistake in the test report)

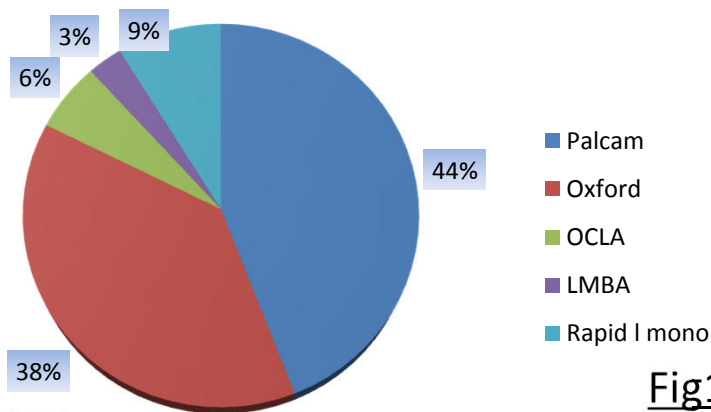


Fig1: 2nd plating medium

**Enrichment steps :
according standard:
satisfactory for all lab**

Standard method EN ISO 11290-1 : Confirmation stage

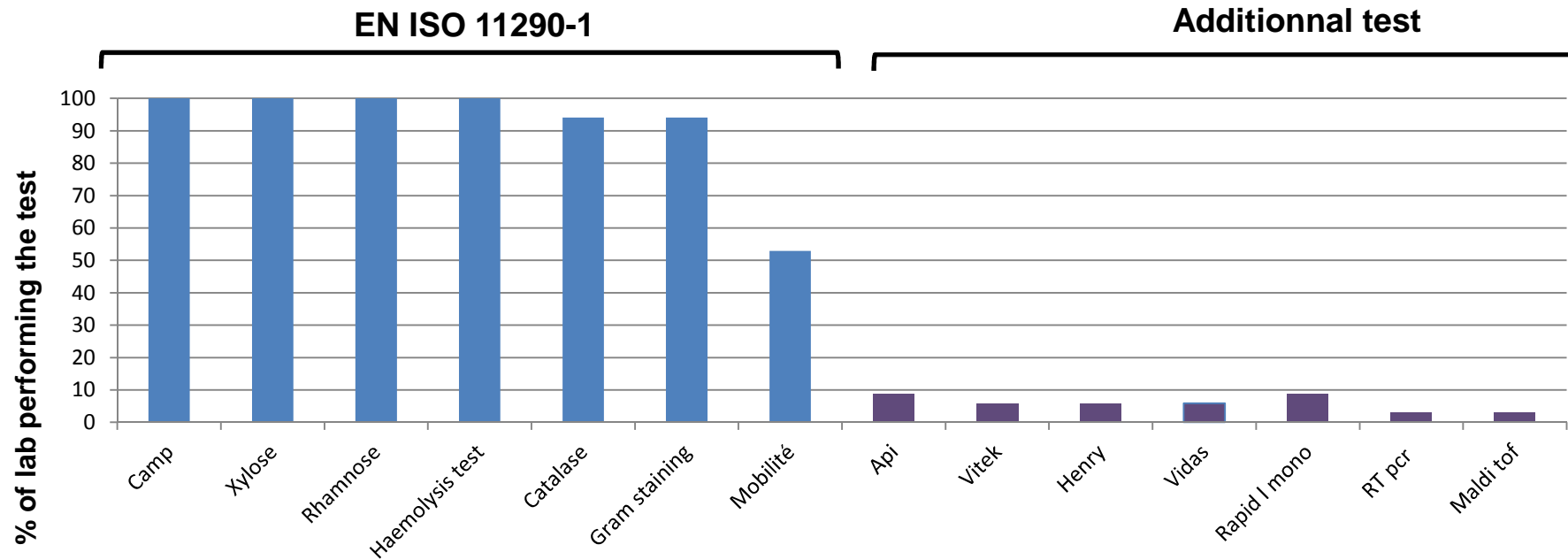


Fig2: Confirmation test performed

- Gram and Catalase not performed : **Minor deviation** (according to the revision of the Standard EN ISO 11290-1)

**Confirmation stage:
according standard:
satisfactory for all lab**

Standard method EN ISO 11290-1 : Expression of Results

- **EN ISO 11290-1 Amended: *L. monocytogenes* present or absent in X g**
- **34 NRLs have respected this point of standard -> you had to put a cross in the relevant box**

**Expression of results:
according standard:
satisfactory for all lab.**

**Results: implementation of Standard method by participants
All results included in the data processing**



Standard method EN ISO 11290-1 : Statistical analyses

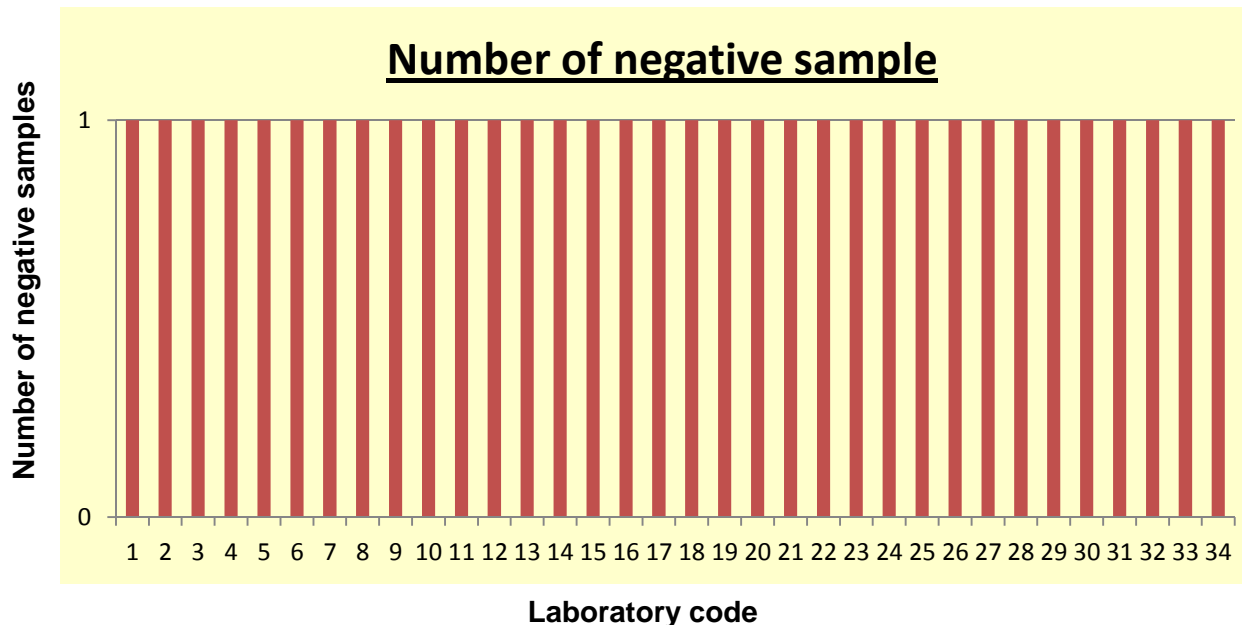
- **Negative control: One sample/Laboratory**

- **Individual performance:**

- ↳ Satisfactory criteria: 100% (if 0% ⇒ laboratory excluded of the following data processing)

- **NRL's Network performance:**

- ↳ $\frac{\text{Number of correct results by the network (Ncr)}}{\text{Number of expected results by the network (Nexr)}} \times 100 = \%$



Individual performance:

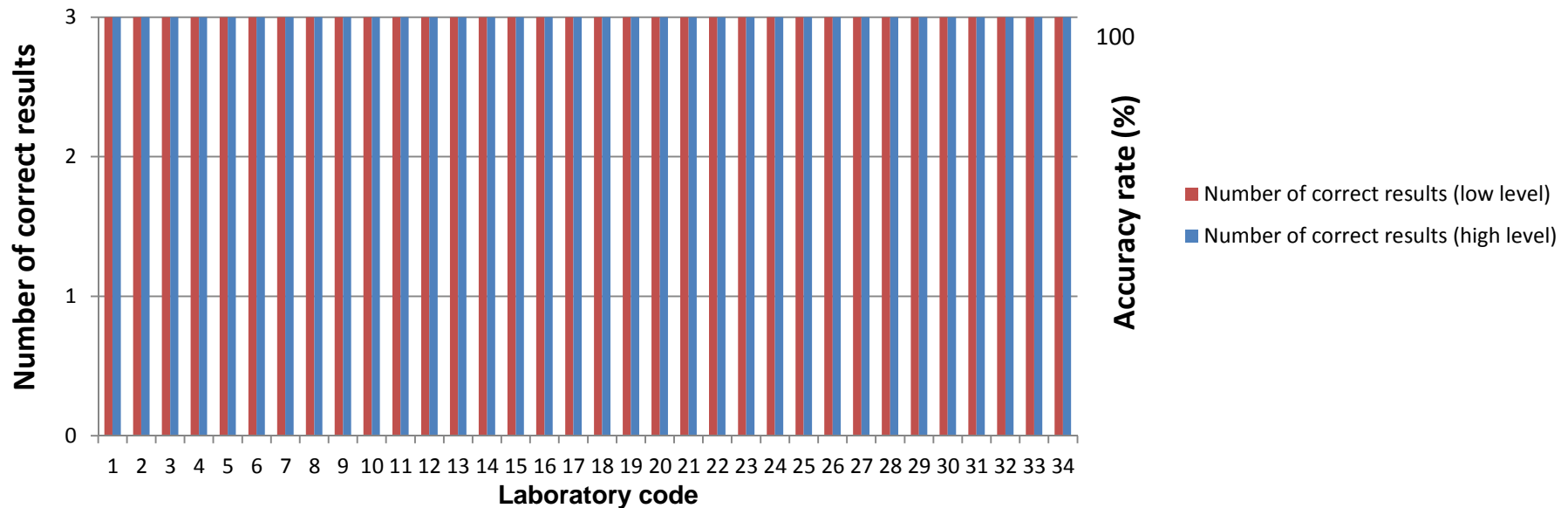
↳ 34 laboratories ⇒ 100% satisfactory

- **Network's performance:**

- ↳ 34/34 ⇒ 100% satisfactory

Standard method EN ISO 11290-1 : Statistical analyses

- **Low level of contamination:** 3 samples/Laboratory (artificially contaminated with *L. monocytogenes* 26 cfu/sample)
- **High level of contamination:** 3 samples/laboratory (artificially contaminated with *L. monocytogenes* 240 cfu/sample)



Individual performance:

$$\Rightarrow \frac{N_{cr}}{N_{exr}} \times 100 = \% \text{ accuracy rate}$$

⇒ Low level => All satisfactory

⇒ High level => All satisfactory

Network's performance:

$$\Rightarrow \frac{N_{cr}}{N_{exr}} \times 100 = \% \text{ accuracy rate}$$

⇒ Low level: $102/102 \times 100 = 100\%$

⇒ High level: $102/102 \times 100 = 100\%$



Standard method EN ISO 11290-1 : Statistical analyses

- Performance of Network of NRL to PT-trial 2014 : EN ISO 11290-1 Amd (Detection method)

↪ 34 Laboratories included in the data processing

↪ 7 samples



- Individual performance:

↪
$$\frac{N_{cr}}{N_{exr}(7)} \times 100 = \% \text{ accuracy rate}$$

⇒ All NRLs satisfactory

- Network's performance:

↪
$$\frac{N_{cr}}{N_{exr}} \times 100 = \% \text{ accuracy rate}$$

⇒ $238/238 \times 100 = 100\%$

⇒ Satisfactory



Conclusion

	Number of participants	34	100 %
	Provided results	34	100 %
Laboratory without deviation	EN ISO11290-1	34	100 %
	Sample preparation	34	100%
	Enrichment and plating stage	34	100 %
	Genus identification	34	100 %
	Species identification	34	100%
	Expression of results	34	100%
	Included in data processing	34	100 %
	Results satisfactory	34	100 %
	Fully satisfactory (Method + Results)	34	100%
	Number of minor deviation	3	9 %
	Number of major deviation	0	0%



Conclusion-perspectives

- **About the organisation**
 - ❖ Documents available on the website
 - ❖ Questionnaire with Sphinx
- **Next year : EURL will improve the questionnaire concerning the test report (software Sphinx)**

- **About the standard method (EN ISO 11290-1)**
 - ❖ No major deviation = 100% of NRLs included in the data processing
 - ❖ Compared to the last PT-trial 2012 ⇒ significant improvement

- **About results**
 - Satisfactory performance of the network of NRLs

Congratulations and THANK YOU



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Listeria monocytogenes



Questions?



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 197 Ko	Information to Participants (2014 PT trial Lm detection)	25/07/2014	Proficiency test	25/07/2014



Letter addressed to:

- *The National Reference Laboratories of the European Union for Listeria monocytogenes.*

Maisons-Alfort, 06/10/2014

Subject: Instructions for the Proficiency Testing trial on Listeria monocytogenes detection in iceberg salad

EURL Coordination:

- Bertrand LOMBARD, EURL Manager
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- Adrien ASSÉRE, Deputy EURL Manager
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File followed by:

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Sophie ROUSSEL
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Unit:

Salmonella E. coli Listeria Unit

Direct lines:

+33 149 7728 37
+33 149 7728 20

Dear Colleagues,

In order to carry out the proficiency testing trial on the detection of *Listeria monocytogenes* in iceberg salad according to the **EN ISO 11290-1:1996 Amd 1:2004**, we will send to you 7 samples of salad. You will receive the samples on the 14th, 15th or 16th October 2014. **You must start analyses before the 21th October 2014.**

Please find attached the document "Instructions to participants for carrying out the Interlaboratory test". This document describes in detail the procedure to be followed to perform the 2014 PT.

The samples will be kept in freezing condition (-18/-24°C) in the parcel. You will have to store samples at the same temperature before the analyses.

As soon as you receive the samples, please don't forget to fill out the "online acknowledgment of receipt" (link sent separately by email).

In the same time, you have to send back to us **the thermotrack in the envelope**



Maisons-Alfort Laboratory for
Food Safety



Listeria monocytogenes

Circular letter addressed to:

- the Heads of the National Reference Laboratories of the European Union for *Listeria monocytogenes*;

and for information to:

- Mr Martial PLANTADY and Mr Koen van DYCK (EC/DG SANCO Unit G4)

Maisons-Alfort, 22/07/2014

IRL Coordination:

Bertrand LOMBARD, EURL Manager
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Adrien ASSÉRE, Deputy EURL Manager
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Be followed by:
Anaïs BARRE

With the following title:
Salmonella, E.coli, Listeria

*Subject: Call for **participation to the** Proficiency Testing trial on Listeria monocytogenes detection in iceberg salad matrix*

Dear Colleagues,



We are organising an Inter-Laboratory Proficiency Testing Trial dedicated to *Listeria monocytogenes* detection, for the NRLs by reference method, Standard EN ISO 11290-part 1 (amended), prescribed by regulation EC2073/2005 for official controls.

Please, find a brief note "information about the Inter-Laboratory Trial" containing



INFORMATION FOR PARTICIPANTS ON THE INTERLABORATORY TEST PLAN

ILPT code	EILA/Anses/LSAI/SEL/2014/03
Version	01

Test			
Name of test	Interlaboratory Proficiency testing trial dedicated to detection of <i>Listeria monocytogenes</i> in iceberg salad according to EN ISO 11290-1:1996 Amd 1:2004		
Type	Inter-laboratory proficiency testing trial		
Purpose	Check the performance of the NRL Lm Network on the detection of Lm in iceberg salad matrix according the standard EN ISO 11290-1:1996 Amd 1:2004		
Participation fee	0 €		
Identification of the Organiser			
Name	Anses - Maisons-Alfort Laboratory for Food Safety		
Address	23 avenue du Général de Gaulle 94706 Maisons-Alfort France		
Identification of coordinator			
Name	BARRE Léna	Unit/team	SEL/ Listeria
	+33 1 49 77 28 37	Fax	+33 1 49 77 46 66
Email	lena.barre@anses.fr		
Identification of the national external contact person <i>(to be completed by the organiser)</i>			
Name			





INSTRUCTIONS TO PARTICIPANTS FOR CARRYING OUT THE INTERLABORATORY TEST

Code	EILA/Anses/LSAI/SEL/2014/03		
Version	01		
Description of the test	Interlaboratory Proficiency testing trial dedicated to detection of <i>Listeria monocytogenes</i> in iceberg salad according to ISO 11290-1:1996 Amd 1:2004		
Objective	Inter-laboratory proficiency testing trial		
Justification	Check the performance of the NRL Lm Network on the detection of Lm in iceberg salad matrix according to the standard ISO 11290-1:1996 Amd 1:2004		
Organization of the Organiser			
	Anses - Maisons-Alfort Laboratory for Food Safety		
	23, avenue du Général de Gaulle 94706 Maisons-Alfort Cedex		
Organization of the coordinator			
	BARRE Léna	Unit/team	SEL/ Listeria
+33 1 49 77 28 37	Fax	+33 1 49 77 46 66	Email lena.barre@anses.fr



PARTICIPATION FORM

Version: 01

**Commitment to return SIGNED before 18/08/2014
BY mail, fax or email to the coordinator of the PT trial.**

Name of trial: Interlaboratory Proficiency testing trial dedicated to detection of *Listeria monocytogenes* in iceberg salad according to EN ISO 11290-1:1996 Amd 1:2004

ILPT code: EILA/Anses/LSAI/SEL/LRUElm/2014/01

IDENTIFICATION OF THE PARTICIPATING LABORATORY

Name of participating laboratory

Address of the laboratory

Address 1
Address 2
Address 3
Zip code
City
Country

Delivery address for samples (if different)

Delivery address 1
Delivery address 2
Delivery address 3
delivery ZIP code
Delivery City
Delivery Country

Contact person during the trial

Phone: Fax: Email:

ANSES
LSAI/LSan

Fiche MQ E VII.03

Revision 04 project 00 - 17/07/2013

Detection of listeria

	<i>L. monocytogenes</i>	<i>Listeria spp.</i>
Agar Listeria according to Ottaviani and Agosti	Gram (optional) Catalase (optional) Mobility (optional) Haemolysis CAMP (optional) Rhamnose Xylose	Gram Voges Proskauer Catalase (optional) Mobility (optional) Other tests if speciation is required: see informative annex
Second medium if not specific for pathogenic <i>Listeria</i> species	Gram Haemolysis CAMP (optional) Rhamnose Xylose	Gram Voges Proskauer Catalase (optional) Mobility (optional) Other tests if speciation is required: see informative annex

Revision of EN ISO 11290-1 and -2

- **MAIN MODIFICATIONS OF DRAFT EN ISO 11290-1**
 - **Primary enrichment in half-Fraser broth:** incubation of 24 +/- 2 h but a note was added: **“A minimum of 24 h incubation is recommended, in particular in the case of stress or low contamination levels of *Listeria* spp. in the samples.”**
 - **Secondary enrichment in Fraser broth: incubation of 24 +/- 2 h** (according to EURL *L. monocytogenes* study) and addition of a note: **“In the case of *Listeria* spp. detection, an additional 24 h incubation may allow recovery of more species.”**
 - **Half-Fraser and Fraser broth may be refrigerated** before transfer or isolation on selective agar during a maximum of 72h, if a validation study of the commercially available broth is available or the laboratory has performed a verification study, according to ISO 16140-3 (note)
 - Storage of isolation plates: **incubated plates can be refrigerated for a maximum of 2 days before reading** (as for enumeration plates)
 - **If positive, only one colony per sample can be confirmed**



Revision of EN ISO 11290-1 and -2

- **MAIN MODIFICATIONS OF DRAFT EN ISO 11290-2**
 - Primary suspension: **half Fraser broth with or without supplements**
 - **Resuscitation step deleted**
 - **Maximum of 150 colonies by plate, but a note was added:** “In case of mixed cultures of blue-green colonies with or without opaque halo, or in case of blue-green colonies with large and overlapping opaque halos, it is preferable to count the colonies on each Petri dish containing **less than 100 characteristic colonies of *Listeria* spp....**”



Revision of EN ISO 11290-1 and -2

- **MAIN MODIFICATIONS OF DRAFTS EN ISO 11290-1 and -2: CONFIRMATION**
 - **Gram and catalase are optional** for *L. monocytogenes*
 - **Carbohydrates (L-rhamnose / D-xylose): incubation 24 to 48h**
 - **Camp-test is optional** for *L. monocytogenes*
 - Blood agar for haemolysis or Camp-test: defibrinated **sheep, calf or bovine blood** can be used
 - **Haemolysis test: blood agar is inoculated by stabbing**, or by streaking if positive at purification step
 - For **more clarity**, the definition and confirmation stage description were split in two: one part for *L. monocytogenes* and another part for *Listeria* spp.



Revision of EN ISO 11290-1 and -2

- **References**
- EN ISO 6887 series “Microbiology of food and animal feed — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination “

