



EURL Lm

European Union Reference Laboratory for
Listeria monocytogenes

Maisons-Alfort laboratory for
food safety

2012 Work Programme of the European Union Reference Laboratory for *Listeria monocytogenes*

Version 3 – 20 October 2011

INTRODUCTION

In May 2006, the Maisons-Alfort laboratory for food safety of Anses (French agency for food, environmental and occupational health safety) has been nominated European Union Reference Laboratory for *Listeria monocytogenes* (EURL Lm) (see Regulation 776/2006).

The EURL Lm foresees to undertake the following actions in 2012, according to the actions planned at the 5th Workshop of the National Reference Laboratories (NRLs) (10&11 March 2011).

Most of these activities aim at implementing, from an analytical point of view, the EC Regulation 2073/2005 on microbiological criteria for foodstuffs, which includes in particular 4 food safety criteria on *L. monocytogenes* (Annex I, Chapter 1):

- either qualitative criteria: absence of *L. monocytogenes* in 25 g, for
 - ready-to-eat foods intended for infants and for special medical purposes,
 - other ready-to-eat foods able to support the growth of *L. monocytogenes*, when leaving the producer;
- either quantitative criteria: a limit of 100 cfu/g, for
 - ready-to-eat foods able to support the growth of *L. monocytogenes*, placed on the market during their shelf-life,
 - ready-to-eat foods unable to support the growth of *L. monocytogenes*, placed on the market during their shelf-life.

NB: in brackets under each item, the scheduled duration of the action is indicated: either annual (limited to 2012), either multi-annual (on-going programme on several years).

0 GENERAL ASPECTS

0.1 GENERAL COORDINATION (EURL MANAGEMENT TEAM, PAFT DEPARTMENT) (MUTI-ANNUAL)

General coordination of the network of the NRLs (dispatch of circular letters and documents, coordination of the scientific and technical support to NRLs,...).

Relations with DG SANCO, coordination of the scientific and technical advice to DG SANCO, management of annual contract with DG SANCO (annual budgets and work programmes, annual technical and financial reports).

In-house follow-up of EURL activities, expenses, support to EURL units.

0.2 WORKSHOP OF THE NRLS (ANNUAL)

The EURL will organise the 6th Workshop of the NRLs in 2012, of general scope:

- to make a progress report on works undertaken by the EURL since the 2011 Workshop;
- to envisage the work programme for 2013 and further.

0.3 SCIENTIFIC MONITORING AND COMMUNICATION (MUTI-ANNUAL)

The EURL teams will conduct scientific monitoring in its area of competence, as well as communicate on the works conducted as EURL Lm, disseminate the outcome of works in the international scientific community (drafting of written publications, oral presentations and posters to international symposia).

1 DETECTION AND ENUMERATION OF *L. MONOCYTOGENES* IN FOOD

Frame: The Standard methods EN ISO 11290-parts 1&2 are cited as reference methods in the qualitative and quantitative criteria of EC Regulation 2073/2005 for *L. monocytogenes*.

1.1 INTER-LABORATORY PROFICIENCY TESTING

The inter-laboratory proficiency testing (PT) trials organised by the EURL for the NRLs aim at evaluating the ability of the NRLs to apply satisfactorily the methods for the analyses performed in the frame of controls prescribed by Regulation 2073/2005.

1.1.1 STUDY OF SAMPLE TYPES FOR INTER-LABORATORY TRIALS (ANNUAL)

The EURL (Unit EDB) will conduct a study to develop the sample types to be used for the PT trial to be organized in 2012, using as matrix diced poultry. In particular, the homogeneity and stability of this type of samples will be studied.

1.1.2 DETECTION OF *L. MONOCYTOGENES* (ANNUAL)

The EURL (Unit EDB) will organize in 2012 a PT trial for the NRLs on the detection of *L. monocytogenes* by the reference method EN ISO 11290-1, using diced poultry as matrix.

1.1.3 COMPARISON OF INOCULATION TECHNIQUES OF SOLID FOOD MATRICES (MULTI-ANNUAL)

Solid food matrices can be contaminated in depth or on surface, with various techniques, according to packaging in particular.

Different inoculation techniques of solid food matrices will be tested and compared so as to optimise the combination solid food matrix/inoculation technique.

This study would be used by the EURL for its future PT trials and could help NRLs for the organization of their interlaboratory PT trials at national level.

1.2 ANALYTICAL DEVELOPMENT (MULTI-ANNUAL)

1.2.1 ENUMERATION METHOD USING A MEMBRANE FILTRATION METHOD

Frame: The Standard horizontal method EN ISO 11290-2 for enumeration of *L. monocytogenes* in food is characterized by a theoretical limit of enumeration of 10-100 cfu/g or ml. Meanwhile, it has been shown that the precision of this Standard method is quite poor, especially at low levels. Even if the Standard has been amended with a more precise method (an enumeration agar, ALOA, now more specific to *L. monocytogenes*), the method still lacks of enough sensitivity to control precisely a limit at 100 cfu/g or ml or lower.

The EURL (Unit EDB) has developed and validated a more sensitive enumeration method, including a concentration step based on membrane filtration followed by transfer of the filter to a selective medium, for the enumeration of *L. monocytogenes* at low levels. Since 2008, the EURL has tested the applicability of this membrane filtration method to various foods.

As agreed at the 2011 workshop, the method will be further studied in 2012 for some food categories (vegetables, seafood products...). Moreover, studies will be conducted on filtration membrane in collaboration with one of the Cyprus NRL (SGL, George PAPAGEORGIOU), which has investigated how to improve the method.

In the purpose of developing/evaluating enumeration methods at low levels of contamination, it was agreed that the EURL Lm would investigate the interest of MPN methods as alternatives to membrane filtration method, in the cases where the latter would not be applicable: a bibliographic review will be conducted at first in 2012.

1.2.2 REDUCTION OF THE SECOND ENRICHMENT STEP IN FRASER BROTH FROM 48 TO 24H

Frame: Several NRLs have pointed out the length of the Standard reference method for the detection of *L. monocytogenes* in food, EN ISO 11290-1, based on conventional microbiology and requiring in particular for this bacterium two successive enrichment steps (3 days are needed to get a negative result and 4-7 days are required for a positive result). It makes the method not optimal for obtaining results shortly in the frame of own checks or official controls.

The two enrichment steps of the Standard method EN ISO 11290-1 are the following: first enrichment in Half Fraser broth, incubated for 24 hours at 30°C and second enrichment in Fraser selective broth, incubated for 48 h at 37°C.

The purpose of this study is to investigate the possibility to reduce the duration of the second enrichment step from 48 h to 24 h, which would enable to shorten the total duration of the Standard detection method by 24h, representing a significant improvement in its practicability.

The EURL already conducted a study on the enrichment phases of the detection method, suggesting that a 24h-incubation in Fraser broth could be sufficient to reach the maximum population, instead of the current practice of 48h. However, to substantiate the reduction of the 2nd enrichment step requires the analysis of additional naturally contaminated samples from various origins, comparing the modified method with the current Standard method. In case of positive outcome of the study, the EURL would transmit it to CEN/TC 275/WG 6 and ISO/TC 34/SC 9, the structures in charge of EN ISO 11290-1, with a recommendation to reduce the 2nd enrichment phase of this Standard detection method.

In 2012, the EURL will go on this study on naturally contaminated samples. To conduct this stage of the study, there is a need to receive from NRLs naturally contaminated samples from various origins. A call for samples has been launched at the 2011 workshop. The transportation of samples from NRLs to the EURL will be sub-contracted.

1.2.3 ENVIRONMENTAL SAMPLING TECHNIQUES

After having reviewed the ISO 18593 Standard, the EURL conducted a bibliographic study in order to check whether the ISO Standard fully covers the case of *L. monocytogenes* control in the environment of food production and food handling, or whether there would be a need to add to the ISO Standard specific guidance on sampling techniques for *L. monocytogenes* control. Given the outcome of this bibliographic review, it was agreed that the EURL, in association with a working group of volunteering NRLs and other FBOs, would draft a technical guidance document (TGD) on environmental sampling techniques specific to *Listeria monocytogenes*. The EURL has conducted in 2010 a large enquiry to NRLs, competent authorities and food business operators to survey the different practices in terms of environmental sampling techniques. On the basis of the bibliographic study and the outcome of the survey, the EURL Lm has been drafting with the WG the TGD in 2011.

By the end of 2012, the EURL Lm will finalize the version 1 of the EURL Lm Technical Guidance Document on environmental sampling techniques specific to *Listeria monocytogenes*.

In addition, the EURL Lm will evaluate the suitability of the EN ISO 11290-1 method to analyse environmental samples: the second part of the survey will be analysed, and if necessary experimental studies would be proposed at the 2012 workshop. Such studies may deal with the choice of diluent and if necessary neutralizer to avoid loss of culturability.

1.2.4 MEASUREMENT UNCERTAINTY

Context: To conduct analyses for own checks and official controls related to the quantitative criteria on *L. monocytogenes* in ready-to-eat food defined in EC Regulation 2073/2005 modified (criteria 1.2 & 1.3 in Annex I, Chapter 1), it is important to know and to control the measurement uncertainty associated to the analytical results. For example, the result found may comply with the limit settled in the microbiological criterion (here 100 cfu/g) whereas the true result (lying in the uncertainty range) may not comply: in that case, a wrong interpretation of the result may be taken if ignoring the measurement uncertainty. A correct interpretation of analytical results, in terms of conformity with regulatory limits, thus requires the knowledge of measurement uncertainty associated to these results as well as the limitation of this uncertainty as far possible.

In the series of Standards EN ISO 6887-2 to 5 on the preparation of test samples for microbiological analyses, it is not specified how to sub-sample the test portion in the laboratory sample (sample that is sent to the laboratory), depending on the different types of food matrices to be submitted to microbiological analyses. This stage is however recognized as a major source of measurement uncertainty, in particular for solid matrices characterized by heterogeneous bacterial contaminations, such as matured cheeses, smoked fishes or meat products.

The EURL Lm has launched a study to assess the impact of test portion sub-sampling on measurement uncertainty, in order to evaluate heterogeneity of contamination, to harmonize how to sub-sample test portions and to reduce measurement uncertainty. The purpose of this study is to harmonize the procedure of sub-sampling the test sample in solid matrices, thus (i) reducing the overall measurement uncertainty, and (ii) better ensuring that the contamination of a sample is correctly reflected in the test portion taken and analyzed.

This study will be continued in 2012, and it requires the analysis of naturally contaminated samples from various origins. This study could be conducted in collaboration with some NRLs. The transportation of samples from NRLs to the EURL will be sub-contracted.

1.2.5 ALTERNATIVES FOR CONFIRMATION STAGE.

Frame: Several NRLs have underlined that the confirmation stage of the Standard methods for detection and enumeration of *L. monocytogenes* (EN ISO 11290-1&2) is laborious and long to implement in routine use, for large scale own checks or official controls. This confirmation is based on biochemical tests, haemolysis reaction and CAMP test (inoculation of test and reference cultures), which take 48 h to perform. The NRLs wished to be able to use, for confirmation purposes, alternative methods, in particular PCR methods.

Further to the 2011 workshop, the EURL *Lm* will undertake a bibliographic review on alternatives (PCR techniques) to confirmation tests for *Lm*, included in EN ISO 11290, and will review in particular their validation status.

1.3 TRAINING OF THE NRLS

Upon request, the EURL could receive NRLs for individual training.

2 PREDICTIVE MICROBIOLOGY

Frame: The EC Regulation 2073/2005 on microbiological criteria defines a quantitative limit for *L. monocytogenes* of 100 cfu/g, which is applicable to certain categories of products placed on the market during their shelf-life. The manufacturer needs to be able to demonstrate that the product will not exceed the limit of 100 cfu/g throughout its shelf-life. For that purpose, Annex II of the regulation lists the different types of data and studies that can be used.

2.1 STRAIN COLLECTION FOR CHALLENGE TESTS (MULTI-ANNUAL)

According to the current (2nd) version of the “Technical Guidance Document (TGD) on shelf-life studies for *L. monocytogenes* in ready-to-eat foods” (see 2.2), the inoculation of the samples intended to the evaluation of the growth potential when performing challenge tests is made with a mixture of at least 3 strains: a reference strain and strains isolated from the same or a similar strain matrix.

The EURL Lm is constituting a collection of strains from various origin (meat, dairy products, fish,...) and various serotypes (4b, 1/2a, 1/2b, ...). Strains will be selected for their ability to grow rapidly and to grow in harsh conditions of temperature, pH and a_w .

Once established, the strain collection will be made available for each NRL.

2.2 REVISION OF TECHNICAL GUIDANCE DOCUMENT ON SHELF-LIFE STUDIES (MULTI-ANNUAL)

The current (2nd) version of the “Technical Guidance Document (TGD) on shelf-life studies for *L. monocytogenes* in ready-to-eat foods”¹ was prepared by the EURL Lm (Unit MOB) in 2008 and describes the microbiological procedures for determining growth of *L. monocytogenes* using durability studies and challenge tests, which are quoted in Annex II of EC Regulation 2073/2005 modified as studies which can be used by manufacturers to demonstrate that their products will not exceed the limit of 100 cfu/g throughout their shelf-life.

The purpose of the revision of the TGD to be launched is to take into account (i) the articles recently published in scientific journals and (ii) the experience of the laboratories implementing the tests. The EURL Lm will conduct the revision with a working group of volunteering NRLs.

¹ http://ec.europa.eu/food/food/biosafety/salmonella/docs/shelflife_listeria_monocytogenes_en.pdf

2.3 NRL TRAINING (ANNUAL)

In 2012, the EURL Lm (Unit MOB) will propose to the NRLs not yet trained a 3-day training session of the theoretical and practical training on:

- durability studies;
- challenge tests;
- predictive microbiology softwares;
- shelf-life of foods related to *L. monocytogenes*.

3 CHARACTERIZATION AND TYPING OF STRAINS, EPIDEMIOLOGICAL SURVEILLANCE

Frame: In the DG SANCO support document to the call for the selection and designation of the new CRLs (SANCO/2214/2005), the Annex 1 describes the specific functions of the EURL *L. monocytogenes*, which includes to keep abreast of developments in *Listeria* epidemiology and to cooperate, as appropriate, with the Community structures involved into surveillance of *Listeria*.

3.1 SETTING UP A CENTRAL EUROPEAN MOLECULAR DATABASE (MULTI-ANNUAL)

The EURL *Lm* data base testing version was launched by the EURL *Lm* (Unit CEB) together with a steering committee in 2011 and was named EURL *Lm* DB. Eight NRLs participated to the first steering committee (SCOM) meeting (8-9 March), as well as a representative from ECDC. After this meeting, a memorandum of understanding of the EURL *Lm* DB, a standard operating procedure for profile interpretation and an epidemiological scheme were written and dispatched to the NRLs and DG-SANCO by circular letter of 14/06/2011. In particular, it has been agreed that the data of this data base would be made fully available to EFSA for risk assessment and surveillance, as well as to ECDC for comparison with profiles of human strains and investigation of listeriosis outbreaks.

In 2012 the EURL will go on:

- 1) Its administrator and curator activities;
- 2) To qualify and train new NRLs willing to join the EURL *Lm* DB;
- 3) To improve and test BioNumerics plug-in improvement, using the outcome of the submission test organized in 2011 with SCOM members;
- 4) To analyze PFGE profiles submitted by NRLs to the EURL *Lm* DB database (in particular of strains isolated in the European baseline survey and submitted by SCOM members).
- 5) To organize SCOM /working group meetings.

In the frame of the EURL *Lm*-DB, the EURL will strengthen its relationships with PulseNet USA and PulseNet Canada.

The operation of PFGE profile software will require subcontracting.

3.2 TECHNICAL & SCIENTIFIC ASSISTANCE TO NRLS, DISPATCH OF STRAINS (MULTI-ANNUAL)

Upon request of the NRLs, the EURL *Lm* (Unit CEB) would provide technical and scientific assistance (in particular to perform PFGE and PCR), and would send them *Lm* field strains from its collection, as well as the control strain *Salmonella* Branderup H9812.

3.3 INVESTIGATION OF RECENT MOLECULAR TYPING TECHNIQUES (MULTI-ANNUAL)

The EURL *Lm* (Unit CEB) will go on in 2012 the development of the Multi Locus Variable number tandem repeat Analysis (MLVA) technique, coupled with capillary electrophoresis system.

In 2011, 8 VNTR loci, detected by conventional electrophoresis (agarose gel), were demonstrated as useful and suitable for the sub-typing of *L. monocytogenes*. In 2012, the detection of small and polymorphic loci will be tested on capillary electrophoresis. The EURL *Lm* will use this MLVA sub-typing method in addition to PFGE for the investigations of certain listeriosis cases.

In addition, molecular serotyping using a real-time PCR method (hydrolysis probes) will be explored and compared to the conventional PCR one.

The implementation of these recent techniques will require subcontracting for gene sequencing of *Lm* strains.

3.4 EUROPEAN LM BASELINE STUDY IN RTE FOOD PRODUCTS: SUB-TYPING OF FOOD ISOLATES

The European monitoring programme on the prevalence of *Listeria monocytogenes* in certain ready-to-eat foods (see EC Decision 2010-678 of 5 November 2010) will be completed by the MSs by the end of 2011.

For undertaking the sub-typing of *Lm* strains isolated in the frame of this baseline survey, the EURL *Lm* (Unit CEB) will coordinate the NRL network as a consortium of laboratories:

- The NRLs having successfully participated to the 2010 PT trial organized by the EURL *Lm* will sub-type the strains: PFGE analysis and PCR serotyping analysis. SCOM members will submit their data online.
- The NRLs, which don't have the capacity/assessed proficiency to perform PFGE or PCR serotyping or both, will send their strains to the EURL *Lm* which will sub-type these strains.

The subtyping data will be centralised and verified by the EURL *Lm*. The molecular profiles would be entered and saved into the EURL *Lm* DB. Data exchange with the laboratory which will be entrusted by ECDC of human strain typing would be undertaken, in close collaboration with ECDC, in the frame of the ELITE project.

4. TECHNICAL AND SCIENTIFIC ASSISTANCE TO THE EUROPEAN COMMISSION

4.1 DG SANCO ACTIVITIES (MULTI-ANNUAL)

Upon request of the services of DG SANCO in charge of food hygiene:

- participation of the EURL manager, for the analytical aspects, to the update of Regulation 2073/2005 on microbiological criteria related to *Lm* (in particular to the on-going addition to the existing criteria on *Lm*);
- technical and scientific assistance of the Unit MOB for the implementation of the Annex II on studies to verify compliance with the 100 cfu/g-ml limit at the end of shelf-life;
- and any new question which may arise during the year.

4.2 PARTICIPATION TO CEN/ISO STANDARDIZATION ACTIVITIES (MULTI-ANNUAL)

On behalf of the EURL *Lm* and as EC representative, participation to the activities and to the joint plenary meeting of ISO/TC 34/SC 9² & CEN/TC 275/WG 6³ (scheduled in Brussels, BE, June 2012), in particular for aspects related to the standardization of reference methods for *L. monocytogenes*.

² Sub-Committee 9 « Microbiology » of Technical Committee 34 « Food products »

³ Working Group 6 « Microbial Contaminants » of Technical Committee 275 « Food analysis – Horizontal methods »