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Maisons-Alfort laboratory for food safety

EURL Lm
European Union Reference Laboratory for *Listeria monocytogenes*

Report of the 5th Workshop of the National Reference Laboratories for *Listeria monocytogenes*

10 & 11 March 2011, Maisons-Alfort

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1 OPENING : THURSDAY 10TH MARCH, 9.15 AM

Laurent LALOUX, Director of the European Union Reference Laboratory for *Listeria monocytogenes* (EURL *Lm*) at the ANSES Maisons-Alfort Laboratory for Food Safety, opened the meeting and welcomed the participants (55).

L. LALOUX gave some news (see his slides) about the changes that took place in the laboratory: the new agency ANSES (French Agency for Food, Environmental and Occupational Health Safety), which resulted of the merging between Afssa and Afsset. Bertrand LOMBARD, EURL *Lm* Manager, introduced the meeting. He was glad that at least one NRL representative from all EU Member States (MSs), except Bulgaria, took part to the workshop.

Roll-call of delegates

Each delegate introduced itself (see the list of attendance, appended). 29 NRLs from 25 EU Members States (MSs) and from Norway were represented, as well as the Czech National Institute of Public Health Laboratory, associated NRL to the Czech NRL. Leena RÄSÄNEN represented EC/ DG SANCO Health & Consumer Protection. Excuses were received from Svetlana FLOROVA (BG), Jens Kirk ANDERSEN (DK), Elisabeth BAGGE (SE), Zuzana SIROTNÁ (SK).

Elena MAZZOLINI, EFSA (European Food Safety Authority), Andreas JANSEN, ECDC (European Center for Disease Prevention and Control), and Marc LECUIT, Pasteur Institute were also invited in order to present the epidemiological context of *Listeria* and listeriosis. Eva Møller NIELSEN, SSI (Statens Serum Institute, DK) was invited to present a project conducted on typing, in collaboration with the EURL *Lm*, as well as to give the input from a human reference centre on the topic of strain typing, epidemiosurveillance and the setting-up of the European database.

All additional documents (i.e. agenda and presentations) are available on the EURL website:

http://www.afssapro.fr/eurl-listeria/

2 GENERAL TOPICS

2.1 DG-SANCO UPDATE

L. RÄSÄNEN presented this topic (see her slides).

2.1.1 EVALUATION OF THE EURLS

L. RÄSÄNEN presented the process of evaluation of the EURLs by Civic Consulting and the result for the EURL *Lm* which was very satisfactory (one of the 5 EURLs with A ranking), giving also some recommendations for improvement. The evaluation report would be soon published.

B. Lombard thanked the NRL network for the quality of the cooperation and for their support.

2.1.2 UPDATE ON THE REVISION OF MICROBIOLOGICAL CRITERIA ON *LM* IN REGULATION EC 2073/2005

The Regulation EC 2073/2005 on microbiological criteria has been amended on 28 April 2010 (EC Regulation 335/2010), by including food grade salt RTE food in the list of products having derogation from regular *Lm* testing in normal circumstances (footnote 4 in Annex I, Chapter 1).

L. RÄSÄNEN informed that DG SANCO had not yet launched the amendment to Regulation 2073/2005 to clarify the *Lm* criterion, for RTE foods able to support the *Lm* growth when the food business operator is not able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 cfu/g throughout the shelf-life.

2.1.3 VALIDATION REQUIREMENTS FOR ALTERNATIVE METHODS – USE FOR OFFICIAL CONTROLS

Regarding the use of alternative methods for official controls, if respecting the conditions of Article 5 of Regulation 2073/2005, L. RÄSÄNEN recalled that Competent Authorities (CAs) can allow their use at national level but EC legal services had not yet provided their opinion on the possibility to allow this use at European level. An opportunity to clarify the situation may also come with the revision of Regulation 882/2004 on official controls.

2.2 PROFICIENCY TESTING TRIAL ORGANISATION

Soraya AMAROUCHE, for a personal reason, could not give her presentation.

2.2.1 ISO TS 22117 ON GENERAL REQUIREMENTS FOR PROFICIENCY TESTING IN FOOD MICROBIOLOGY

Bertrand LOMBARD introduced CEN ISO TS 22117, published in 2010, giving guidance on proficiency testing (PT) trials in food microbiology (see his slides).

This is a very useful tool for the organisation and exploitation of (PT) trials in addition to EN ISO 17043 and ISO 13528.

➔ NRLs were asked to use CEN ISO 22117 to organize their own PT trials for the national networks they coordinate.

2.2.2 OUTCOME ON THE SURVEY ON THE PT ORGANISED BY NRLS LM

Adrien ASSÉRÉ, EURL *Lm* deputy manager, presented the typology of the PT trials organised by the NRLs where around half of the NRLs had organised PT trials dedicated to *Lm* detection since 2006 (see his slides). Moreover 3 NRLs are even accredited for PT organisation.

2.2.3 EXPERIENCE OF 5 NRLS

Vincenza PRENCIPE (IT), Enne de BOER (NL), Katarzyna DMOWSKA (PL), Susanne THISTED LAMBERTZ (SE) and Irena ZDOVC (SI) presented their activity as PT organiser for the official control laboratories.

Concerning the Italian NRL, the presentation of Vincenza PRENCIPE gave a focus on the program aiming to guarantee the equivalence between the US and Italian official control systems.

Enne de BOER presented CHEK, an independent branch of the Dutch Food and Consumer Product Safety Authority (VWA). CHEK has specialised in quality assurance for laboratory testing where the main activities are dedicated to the production of reference materials and the organisation of microbiological and chemical proficiency studies.

Katarzyna DMOWSKA presented the whole process of organisation of PT trials at PIWet (Pulawy), as well as the participation and global results of the 33 participants.

Susanne THISTED LAMBERTZ presented the PT activity at NFA which is accredited for PT organisation since 2004. PT samples are freeze-dried mixtures, not in a food matrix.

Irena ZDOVC presented the PT organisation in her institute. 8 laboratories participate to these PT on brain tissue, minced meat and milk matrices.

2.3 EPIDEMIOLOGY

2.3.1 EUROPEAN LISTERIA MONOCYTOGENES BASELINE SURVEY

Leena RÄSÄNEN gave an update on the *Listeria* baseline survey which was finally adopted on 5 November 2010 (EC Decision 2010/678). Sampling started in 2010 for a one-year period.

Analysis (*Lm* detection/enumeration) should be conducted by NRLs or by laboratories designated by CAs. 19 NRLs indicated that they would perform the analysis themselves (totally or partly).

The results of the 12080 collected samples would be processed and published by EFSA in 2012.

2.3.2 UPDATE ON EFSA ACTIVITIES AND *L. MONOCYTOGENES* REPORTING IN ANIMAL AND FOOD

Elena MAZZOLINI gave a short introduction to EFSA and to the main activities of the EFSA Zoonoses Unit on data collection and presented the results from the 2009 European Union Summary Report for *L. monocytogenes* (Listeriosis in humans and animals and from foodborne outbreaks).

In 2009 the number of notified listeriosis cases in humans increased by 19% compared to 2008, with 1645 confirmed cases recorded in 2009 having conducted to 270 human deaths. 6 MSs (AT, DK, ES, HU, IT, SE) are facing with a significant increase of listeriosis cases.

In 2009 only three verified and four possible food-borne outbreaks –FBOs- (7/5550) were reported to be due to *Listeria* with 15 fatal cases, representing the 1/3 of the number of deaths reported for FBOs in the EU (46 fatal cases).The *Listeria monocytogenes* outbreaks had the highest case fatality rate (22%) out of all the agents associated with FBOs in 2009.

The main sources of contamination were cheese and pig meat. The proportion of samples exceeding the legal safety limit of 100 cfu/g was low as it had been the case in earlier years. RTE fishery products, mainly smoked fish, represented the highest proportion of units over the 100 cfu/g limit (0.6 %).

2.3.3 ECDC ACTIVITIES RELATED TO *LM* AND ELITE PROJECT

Andreas JENSEN presented the role the European Centre for Disease Prevention and Control (ECDC). ECDC was established in Stockholm in 2004 with the support of EC. Among other activities this agency prepares the Annual epidemiological report and also launched the European Listeria Typing Exercise (ELiTE). One of the objectives of ELiTE project is the study of molecular epidemiology of human listeriosis, comparing human typing data to the food data from the European *Listeria* baseline survey and assessing the importance of different RTE foodstuffs as sources of infection.

2.3.4 RECENT TRENDS IN HUMAN LISTERIOSIS

Marc LECUIT, Director of the French Human Reference Center and WHO Collaborative Center for Listeria (Institut Pasteur, Paris, France), together with Sylvain BRISSE, Head of the technological platform Genotyping of pathogens and Public Health (Institut Pasteur, Paris, France) presented an update on epidemiological and microbiological investigations, as well as a R&D project, involving also the EURL/French NRL laboratory, on clonal diversity and evolution of Lm (see their slides).

A proposal was to combine MLST and PFGE to characterize the pathogenicity of the Lm strains.

3 MAIN WORKING AREAS

3.1 DETECTION / ENUMERATION

3.1.1 INTRODUCTION

Nathalie GNANOU-BESSE, Unit EDB (EURL *Lm*), introduced the 2010 and 2011 work programmes, which was detailed afterwards. See her slides.

3.1.2 2010 INTER-LABORATORY TRIAL ON LM DETECTION

Anne-Laure LARDEUX, Unit EDB (EURL *Lm*), presented the results of the 2010 PT trial dedicated to *Lm* detection in powdered infant food formula.

Only 60% of the NRLs applied entirely the official reference method (EN ISO 11290-1), the deviation came mainly from the identification step. Apart from this fact, the results of the participants were satisfactory.

➔ NRLs were once more requested to use entirely the official reference methods in the frame of PT trials organized by the EURL.

3.1.3 MEASUREMENT UNCERTAINTY

Bertrand LOMBARD presented this part. See his slides.

3.1.3.1 ENQUIRY ON THE REVISION OF THE EURL GUIDE

Among the 8 replies collected during the enquiry, limited suggestions were proposed to revise the guide and only 2 NRLs volunteered to participate to a working group dedicated to the revision. In addition, B. Lombard informed that ISO/TC 34/SC 9 had recently launched the revision of ISO/TS 19036 on the estimation of measurement uncertainty (MU) in food microbiology.

➔ It was agreed that there was no need to revise now the EURL Guide on MU estimation for *Lm*. But the EURL would contribute to the revision of ISO/TS 19036 and NRLs were strongly encouraged to participate also to this revision through their respective national standardization bodies.

3.1.3.2 TRIALS ON THE INFLUENCE OF SUB-SAMPLING TEST PORTION

Bertrand LOMBARD presented the design of the study to be launched by the EURL and pointed out the need of naturally contaminated samples.

3.1.4 SURVEY ON SURFACE SAMPLING METHODS TO DETECT L. MONOCYTOGENES

Brigitte CARPENTIER, Unit MAHY (EURL *Lm*), presented the outcome of the survey with 137 replies from 15 MSs. Several inconsistencies in replies and wrong practices (sampling after C&D, sampling of too small areas) were found. Most repliers (76) declared they don't apply recommendations from the ISO 18593 standard. The main proposed improvements are to increase the sampling area (10 replies) and to change the scrubbing device (6 replies). The survey shows that European guidelines are needed.

The next steps are the following:

- First draft guidelines written by the EURL *Lm* to be reviewed by June.
- September: teleconference of WG of NRLs and samplers to deal with comments received.
- First version of the guidelines finalized by February 2012.
- The other part of the work will be dedicated to the sample analysis. Consequently, the second part of the survey will be analysed, and if necessary experimental studies will be proposed at the next workshop. Such studies would also concern the choice of diluent and if necessary neutralizer to avoid loss of culturability.

3.1.5 ENUMERATION METHOD USING FILTRATION MEMBRANE

Emilie de COURSEULLES, Unit EDB (EURL *Lm*), presented the progress of the project. It showed that this method gives better sensitivity than the EN ISO 11290-2 method (0,2 CFU/g), good trueness and precision but it is hardly applicable to some food products (such as dairy products), because of interference due to background microflora and filterability problems. However good results were obtained for some matrices (raw meat, some delicatessen, fish products…). More results are needed for some food categories such as vegetables.

George PAPAGEORGIOU (CY) proposed to collaborate with the EURL, this proposal was much welcomed. This work concerns the improvement of the method for some difficult products such as cheeses.

Some delegates suggested to investigate also MPN and PCR methods.

➔ It was agreed that the EURL Lm would investigate the interest of MPN method as an alternative to membrane filtration method where the latter would not be applicable (bibliographic review first).

3.1.6 UPDATE ON CEN/ISO STANDARDISATION

Enne de BOER (NL-NRL) presented the revision of the Standard ISO 11290 and its modifications (see his slides). The revision was stopped, waiting for the CEN Mandate to be launched to validate the Standard methods.

Nathalie GNANOU-BESSE was the project leader for the validation by inter-laboratory trials of the revised EN ISO 11290-1&2 methods, in the frame of the CEN Mandate.

➔ The EURL strongly encouraged the NRLS to take part to these validation inter-laboratory studies in the CEN Mandate.

3.1.7 PROPOSAL OF WORK PROGRAMME FOR 2012

Based on suggestions from Nathalie GNANOU-BESSE, the following items were retained for the 2012 tentative work programme (see her slides), to be confirmed after agreement of DG SANCO on the programme and associated budget:

1. Inter-laboratory PT trial on *Lm* detection in diced poultry samples.
2. Type of samples for PT trials: comparison of various inoculation techniques of solid food matrices.
3. PCR review and validation status for *Lm* alternative confirmation stage in EN ISO 11290.
4. Development/evaluation of enumeration methods at low levels of contamination.
    a. Further studies with filtration membrane in collaboration with George PAPAGEORGIOU (CY)
    b. Bibliographic review on MPN methods
5. Environmental (surface) sampling
    a. Choice of diluents/neutraliser.
    b. Finalization of version 1 of the EURL *Lm* Technical Guidance Document on environmental sampling techniques specific to *Listeria monocytogenes*
    c. Suitability of the EN ISO 11290-1 method to analyse environmental samples
6. Measurement uncertainty: study on impact of test portion sub-sampling on MU values (continuation).
7. Reduction of the 2nd enrichment step in the EN ISO 11290-1 detection method (continuation).
8. Survey of NRLs on details concerning the methods for *Lm* detection and enumeration in the European baseline survey.

3.2 STRAIN TYPING / EPIDEMIOLOGY

3.2.1 INTRODUCTION

Anne BRISABOIS, Head of Unit CEB (EURL *Lm*), introduced the 2010 and 2011 work programmes, which was detailed afterwards. See her slides.

3.2.2 EUROPEAN DATA BASE

Benjamin FELIX, Unit CEB (EURL *Lm*), presented the project of the European Union Reference Laboratory for *Listeria monocytogenes* Data Base (EURL *Lm* DB) dedicated to *Lm* typing (see his slides). A working group of 8 NRLs representatives, Eva Møller NIELSEN (SSI, DK, as former PulseNet Europe Coordinator), Ivo van WALLE (ECDC, Stockholm) and the EURL *Lm* has been settled (WG EU-DB). The minutes of the first meeting will be dispatched soon to the NRLs.

The property of data was still to be defined; a proposal would be prepared and submitted to the Competent Authorities (through the Standing Committee of Food Chain). The advice of EFSA was needed on food classification with regards to *Lm* risk.

This project would allow the collection of the profiles of the strains isolated in particular during the European *Listeria* baseline survey. Close cooperation with ECDC was wished in order to allow compatibility of the food data base and the future human data base, to be developed in the ELiTE project of EFSA. Further comparison of profiles of food and human strains would thus be made possible.

The next WG EU-DB meeting was scheduled at the end of 2011.

3.2.3 TYPING OF STRAINS FROM THE EUROPEAN BASELINE SURVEY

Benjamin FELIX presented this topic. See his slides.

In the frame of the European baseline survey (BLS), at least one confirmed *Listeria monocytogenes* strain per positive sample shall be stored for possible further typing studies.

The EURL *Lm* proposal, in agreement with DG SANCO, was that the molecular sub-typing would be undertaken by a consortium of EURL and certain NRLs. The method selected for sub-typing would be PFGE, in connection with ECDC (see 3.2.2).

➔ It was agreed that:

- A consortium of certain NRLs coordinated by EURL would undertake the typing of strains isolated in their country in the frame of the BLS. These NRLs should have obtained satisfactory results in EURL PT trials. Serogroups, PFGE profiles and epidemiological data would be submitted to EURL either through the EURL *Lm* DB or as raw data.

- NRLs not involved in the consortium would send strains to the EURL which would undertake the typing itself for the countries concerned, covered by the EURL budget.
- A call for NRL participation to typing or to send strains to the EURL would be launched.
- L. RÄSÄNEN would ask the approval of MSs in Standing Committee of the Food Chain for this typing exercise as a follow-up of the baseline survey.

3.2.4 DEVELOPMENT OF MOLECULAR SUBTYPING METHODS

3.2.4.1 FAFLP VS PFGE

Sophie ROUSSEL, Unit CEB (EURL *Lm*), presented a project of comparison between the PFGE and fAFLP techniques, conducted in association with HPA (UK-NRL) (see her slides).

The conclusions are promising because fAFLP has a slightly more discriminatory power than PFGE, is more rapid (2 days), less costly and less labour intensive than PFGE, but laboratories need to have capillary electrophoresis. It could be envisaged to perform a validation study of fAFLP with different laboratories.

3.2.4.2 MLVA ON AGAROSE GEL

Sophie ROUSSEL presented the development of a *Listeria monocytogenes* sub-typing method using Multiple Locus Variable Analysis (MLVA) (see her slides).

The conclusion of the study was that on agarose gel, the discriminatory power is slightly lower than PFGE and MLVA on agarose gel cannot be recommended.

3.2.4.3 MLVA WITH SEQUENCING

Eva Moller NILSEN presented the work done at SSI (DK) on MLVA with a sequencer for the surveillance of human cases.

This method is promising, being faster and easier to perform than PFGE, with a comparable discriminatory power.

3.2.5 PROPOSAL OF WORK PROGRAMME FOR 2012

Based on suggestions from Sophie ROUSSEL, the following items were retained for the 2012 tentative work programme (see her slides), to be confirmed after agreement of DG SANCO on the programme and associated budget:

1. European molecular PFGE database management (phase 2)
2. PT trial
    a. PFGE-typing method
    b. Gel analysis and submission to EU-DB
3. EC *Lm* baseline survey in RTE food products: coordination of the EURL/NRLs consortium and typing of strains from certain MSs

4. Characterization of genetic *Lm* diversity based on molecular methods

3.3 MICROBIOLOGICAL SHELF-LIFE STUDIES AND PREDICTIVE MICROBIOLOGY

3.3.1 INTRODUCTION

Annie BEAUFORT, Head of Unit MOB (EURL *Lm*), introduced the 2010 and 2011 work programmes, which was detailed afterwards. See her slides.

3.3.2 SUPPORTING DOCUMENT TO EVALUATE THE IMPLEMENTATION OF CHALLENGE TESTS ASSESSING THE GROWTH POTENTIAL OF *L. MONOCYTOGENES*

Annie BEAUFORT presented this document intended to the auditors who would assess the competence of laboratories implementing challenge tests. This document is being prepared by the EURL in collaboration with a working group of NRLs. This group had already held 2 meetings. A final version would be dispatched by the end of 2011.

It was noticed that such studies are conducted by/at the request of food business operators and are thus not part of official control: the assessment of competence to conduct these studies may not be under the responsibility of NRLs.

> ➔ The way to perform the assessment of the laboratories conducting shelf-life studies was to be clarified by L. RÄSÄNEN within DG-SANCO.

3.3.3 CONSTITUTION OF LISTERIA STRAINS COLLECTION

Laurent GUILLIER, Unit MOB (EURL *Lm*), presented this project. A collection would be constituted in 2011. The growth parameter of these strains would be assessed by 2012. This collection would be made available for NRLs once finalised.

3.3.4 EFFECT OF ORGANIC ACID ON LM GROWTH

Laurent GUILLIER and Anne-Laure LARDEUX, Unit MOB (EURL *Lm*), presented the study of *Lm* growth, in the case of lactate in custard (see her slides). The addition of potassium lactate in a food matrix slightly reduced the growth of *Lm.* A report of this study would be soon dispatched.

3.3.5 EFFECT OF THE SIZE OF THE *INOCULUM*

Anne-Laure LARDEUX presented this project conducted by the EDB Unit (EURL *Lm*). A significant reduction of *Lm* growth (3 to 8 $\log_{10}$) has been observed in the presence of background flora, with a reduction much intensified with modified atmosphere. The effect of the inoculum size on *Lm* growth was confirmed: the final level of contamination reached by *Lm* depends on the initial bacterial concentration through bacterial competition with the background flora, with no impact on the growth potential.

3.3.6 ABILITY OF L. MONOCYTOGENES TO GROW IN BUTTER

Hélène BERGIS, Unit MOB (EURL *Lm*), presented the experimental design of this study to be launched soon. See her slides.

3.3.7 PROPOSAL OF WORK PROGRAMME FOR 2012

Based on suggestions from Annie BEAUFORT, the following items were retained for the 2012 tentative work programme (see her slides), to be confirmed after agreement of DG SANCO on the programme and associated budget:

1. Study on the *Lm* ability to grow in fats from animal and vegetal origins,
2. Constitution of *Lm* strains collection (continuation),
3. Launching of revision of the EURL Technical Guidance Document,
4. Study the effect of terpenes on the evolution of *Lm* in food.

4 CONCLUSION

B. Lombard thanked all the attendees for their participation to that 5th workshop, the speakers and organizers, hoping that this workshop met the expectations of the participants.

The progressive departure of several participants during Friday afternoon would require to modify the organization of the workshops, to be launched on Wednesday noon and to be ended on Friday noon.