



Summary of 2014-2015 activities for the EURL *Lm* database

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Set up of EURL Lm DB - publication

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Building a molecular *Listeria monocytogenes* database to centralize and share PFGE typing data from food, environmental and animal strains throughout Europe



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ABSTRACT

The European Union Reference Laboratory (EURL) for *Listeria monocytogenes* (*Lm*) collaborates with a network of 35 National Reference Laboratories (NRLs) throughout Europe. Most of these NRLs are in charge of detecting and typing *Lm* strains from food, environment and animals, which are isolated nationally. The past few years EURL activities have enabled NRLs to reinforce typing capabilities according to standardised protocols. Consequently the need to exchange typing data within the NRL network has emerged. That is why the EURL has recently set up a EURL *Lm* Database (EURL *Lm* DB). Each NRL contributes data, which is then shared within the network. Data include strain-typing-results (PFGE and serotyping) and epidemiological information on the strains. This article describes (1) the EURL typing activities that led to the creation of the EURL *Lm* DB, (2) the different steps involved in developing the EURL *Lm* DB, and (3) the usefulness of this database for public health. The combined use of this database, with databases on human strains, is being integrated into the European surveillance system of *Lm* strains circulating throughout Europe. It should improve the detection of this pathogen and provide support for outbreak investigations.

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Validation of PFGE protocols

- Validation of PFGE protocol variations (comparison EURL protocol and PulseNet USA protocols 2009 and 2013) (Michelon et al. 2015 FPD head of print)

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Original Article

PFGE Standard Operating Procedures for *Listeria monocytogenes*: Harmonizing the Typing of Food and Clinical Strains in Europe

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Abstract

Listeria monocytogenes is a foodborne pathogen responsible for a severe disease known as listeriosis. The European Centre for Disease Prevention and Control (ECDC) coordinates a network of national public health laboratories (NPHLs) in charge of typing clinical strains. In food, it is the European Union Reference Laboratory for *L. monocytogenes* (EURL *Lm*), which manages a network of National Reference Laboratories (NRLs). A pulsed-field gel electrophoresis (PFGE) standard operating procedure (EURL SOP) has been used routinely at the EURL *Lm* since 2007. The EURL *Lm* has recommended that NRLs use the EURL SOP, whereas the Statens Serum Institut (SSI), under contract for ECDC, requested that NPHLs use Halpins' SOP (HSOP) published in 2010 for the PulseNet USA network. An update of Halpins' SOP (uHSOP) was published in 2013. To facilitate the exchange of profiles among human and food European reference laboratories, it is crucial to ensure that the PFGE profiles obtained with these different SOPs are comparable. The aim here was to compare the EURL SOP with HSOP and uHSOP. The panel comprised 114 well-characterized SSI/EURL strains. All

EFSA Standard Operating Procedures

<http://www.efsa.europa.eu/en/supporting/doc/702e.pdf>

anses
French agency for food, environmental
and occupational health safety



EURL for *Lm*

European Union Reference
Laboratory for
Listeria monocytogenes

EFSA supporting publication 2014:EN-702

EXTERNAL SCIENTIFIC REPORT

Molecular typing of *Listeria monocytogenes* strains isolated from food, feed and animals: state of play and standard operating procedures for pulsed field gel electrophoresis (PFGE) typing, profile interpretation and curation¹

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ABSTRACT

EFSA has contracted ANSES (Food Safety Laboratory, Maisons-Alfort, France) to provide assistance related to establishing molecular typing data collection for *Listeria monocytogenes* from food, feed and animals. This document is the final report scheduled in the contract; it comprises three parts. The first part contains a

SOP for PFGE (page 41)



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Appendix C. Standard Operating Procedures for PFGE

1. Scope

This standard operating procedure (SOP) describes the molecular subtyping of *Listeria monocytogenes* by Pulsed-Field Gel Electrophoresis (PFGE) in order to generate comparable results between laboratories.

SOP acquisition and quality assessment of image (page 59)

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Appendix D. Standard Operating Procedure for acquisition and quality assessment of images

1. Scope

Prior to any analysis of Pulsed Field Gel Electrophoresis (PFGE) profiles of *Listeria monocytogenes* (*L. monocytogenes*), the quality of the gel should be visually checked. This Standard Operating Procedure (SOP) details the basic steps required for visual assessment of the overall quality of the gel.

Visual validation does not mean that a gel will be accepted through the curation SOP. It is then highly recommended to perform an extended validation of the profiles by using the curator SOP. This SOP detail further validation step using the BioNumerics software.

SOP for curation (page 64)



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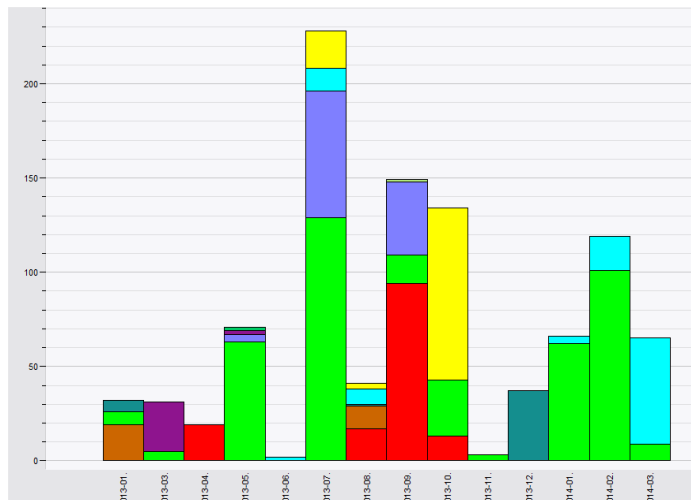
Appendix E. Standard Operating Procedure for curation

1. Scope

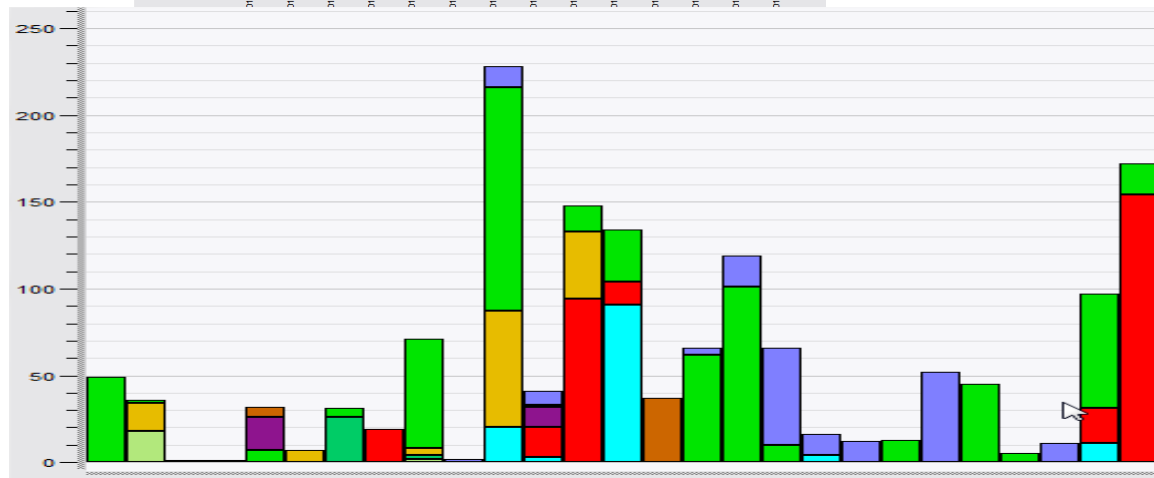
The present Standard Operating Procedure (SOP) describes the process of the curation of the *Listeria monocytogenes* (*L. monocytogenes*) Pulsed Field Gel Electrophoresis (PFGE) profiles uploaded by the database users.

The curation process implies the analysis of the PFGE profiles submitted by the database users, the assessment of their suitability for cluster analyses and the assignment of the profiles' nomenclature, which are the steps propaedeutic to every further analysis and comparison.

Submissions 2013-2014, beginning 2015



Total = 982



Total = 1674

Data transmission excluding BLS framework

EURL Lm DB evolution

EURL Lm DB developments currently in stand-by

Exclude the entry of the requestor from fast match (lines 44-45)

Pulsotype traceability functionality (lines 46-68)

Report MLST data for French NRL strains in EURL database specific fields (Lines 69-75)

Annual report format (Lines 91-93)

Implementation of the profile at fast band matching level (Lines 94-97)

Curation report available for users (Lines 99–114)

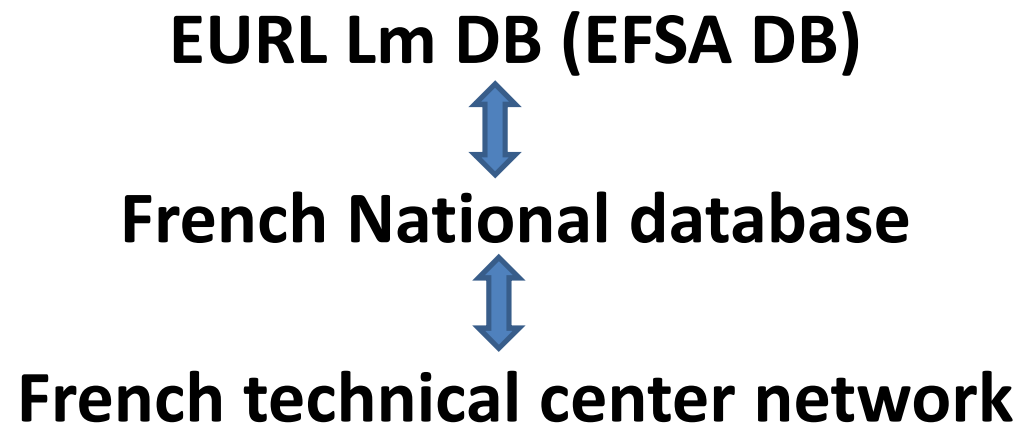
Multi selection for sector category 4 (line 254)

New EUListNet plug compatible BN66 & BN7

Administration tool improved

Batch submission of profiles

Database cascading system



MLST data

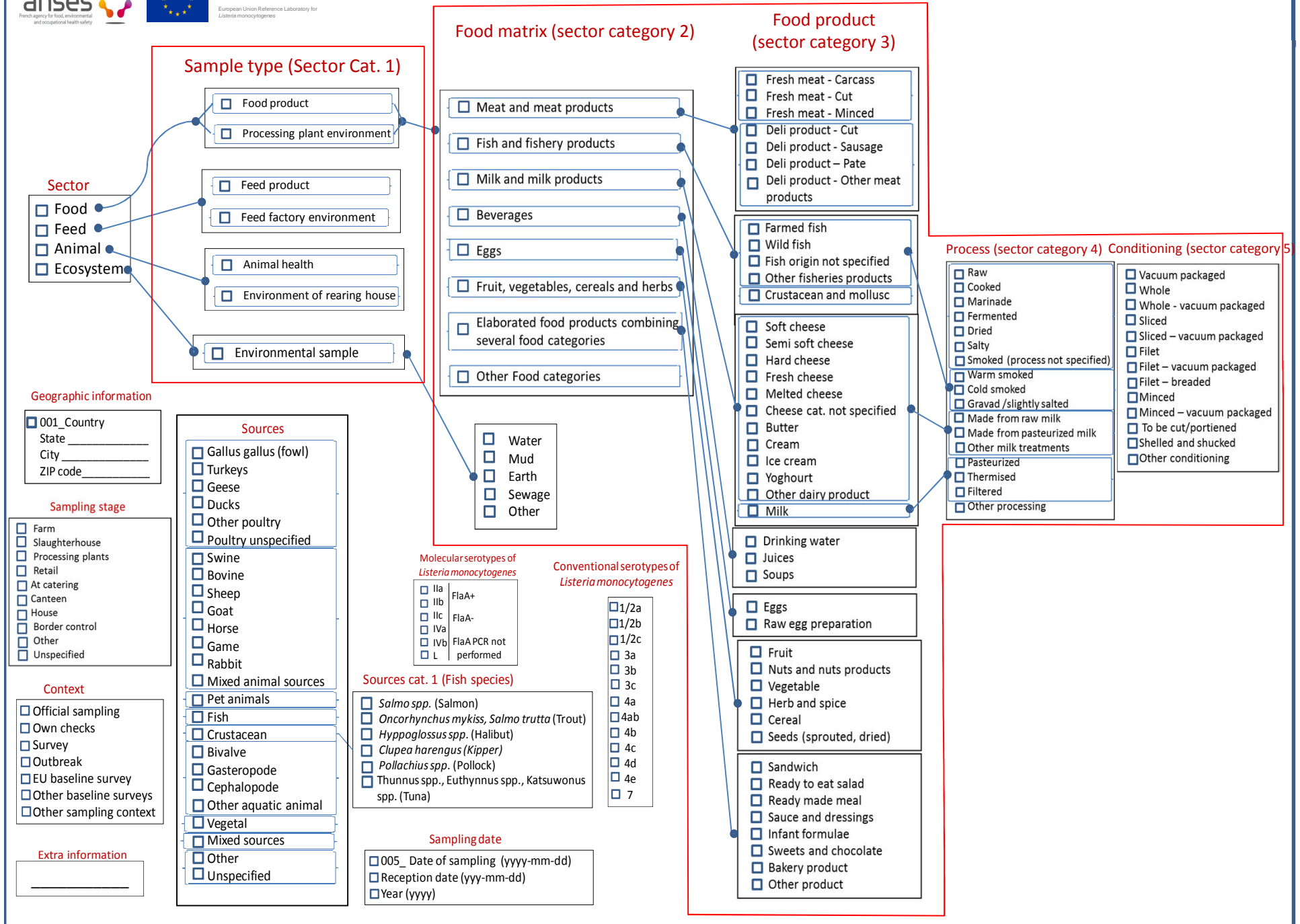
- Submission of ST for approx. 250 French strains
- MLST assigned according to Ragon MLST scheme :
<http://www.pasteur.fr/recherche/genopole/PF8/mlst/Lmono.html>
- Clonal complexes provided according to CC ST correspondence

Who can provide MLST on submitted strains ?

EFSA DB transition step

- 1- Feed back of profiles from EURL DB**
- 2- Connection to EFSA epidemiological scheme**
- 3- Submission to EFSA database**
- 4- Both script are compatible in the same DB**

Epidemiological scheme version 2015



Sample type (Sector Cat. 1)

Sector

- Food
- Feed
- Animal
- Ecosystem

- Food product
- Processing plant environment
- Feed product
- Feed factory environment
- Animal health
- Environment of rearing house
- Environmental sample

Geographic information

001_Country
State _____
City _____
ZIP code _____

Sampling stage

- Farm
- Slaughterhouse
- Processing plants
- Retail
- At catering
- Canteen
- House
- Border control
- Other
- Unspecified

Context

- Official sampling
- Own checks
- Survey
- Outbreak
- EU baseline survey
- Other baseline surveys
- Other sampling context

Extra information

Food matrix (sector category 2)

- Meat and meat products
- Fish and fishery products
- Milk and milk products
- Beverages
- Eggs
- Fruit, vegetables, cereals and herbs
- Elaborated food products combining several food categories
- Other Food categories

- Water
- Mud
- Earth
- Sewage
- Other

Molecular serotypes of *Listeria monocytogenes*

- IIa FlaA+
- IIb FlaA+
- IIc FlaA-
- IVa FlaA PCR not performed
- IVb FlaA PCR not performed
- L

Conventional serotypes of *Listeria monocytogenes*

- 1/2a
- 1/2b
- 1/2c
- 3a
- 3b
- 3c
- 4a
- 4ab
- 4b
- 4c
- 4d
- 4e
- 7

Sources cat. 1 (Fish species)

- Salmo* spp. (Salmon)
- Oncorhynchus mykiss*, *Salmo trutta* (Trout)
- Hyppoglossus* spp. (Halibut)
- Clupea harengus* (*Kipper*)
- Pollachius* spp. (Pollock)
- Thunnus* spp., *Euthynnus* spp., *Katsuwonus* spp. (Tuna)

Sampling date

- 005_ Date of sampling (yyyy-mm-dd)
- Reception date (yyy-mm-dd)
- Year (yyyy)

Food product (sector category 3)

- Fresh meat - Carcass
- Fresh meat - Cut
- Fresh meat - Minced
- Deli product - Cut
- Deli product - Sausage
- Deli product - Pate
- Deli product - Other meat products
- Farmed fish
- Wild fish
- Fish origin not specified
- Other fisheries products
- Crustacean and mollusc
- Soft cheese
- Semi soft cheese
- Hard cheese
- Fresh cheese
- Melted cheese
- Cheese cat. not specified
- Butter
- Cream
- Ice cream
- Yoghourt
- Other dairy product
- Milk
- Drinking water
- Juices
- Soups
- Eggs
- Raw egg preparation
- Fruit
- Nuts and nuts products
- Vegetable
- Herb and spice
- Cereal
- Seeds (sprouted, dried)
- Sandwich
- Ready to eat salad
- Ready made meal
- Sauce and dressings
- Infant formulae
- Sweets and chocolate
- Bakery product
- Other product

Process (sector category 4) Conditioning (sector category 5)

- Raw
- Cooked
- Marinade
- Fermented
- Dried
- Salty
- Smoked (process not specified)
- Warm smoked
- Cold smoked
- Gravad /slightly salted
- Made from raw milk
- Made from pasteurized milk
- Other milk treatments
- Pasteurized
- Thermised
- Filtered
- Other processing
- Vacuum packaged
- Whole
- Whole - vacuum packaged
- Sliced
- Sliced - vacuum packaged
- Filet
- Filet - vacuum packaged
- Filet - breaded
- Minced
- Minced - vacuum packaged
- To be cut/portioned
- Shelled and shucked
- Other conditioning

Forthcoming evolutions 2015 - 2016

- 1- NRLs Slovenia and Denmark will use the EURL Lm DB and integrate SCOM of EURL Lm DB**
- 2- Why no submission from DE, NL, IT, BE ?**
- 3- Transfer of EURL Lm DB to EFSA-ECDC DB during pilot phase of EFSA-ECDC DB, at the latest at the end of pilot phase**
- 4- Do you wish to use the EURL epi scheme for linking with EFSA DB ?**
- 5- EURL Lm DB used as research tool for the NRL network?**
- 6- What are your suggestions ?**