Second Joint Training Course on the Use of BioNumerics Software to analyse PFGE data of Shiga-toxin producing *Escherichia coli*, *Salmonella* and *Listeria monocytogenes*

3-4 July 2017

Istituto Superiore di Sanità, SIDBAE Training Room

Content

- **Scientific Report**
- **List of participants (Annex 1)**
- **Course agenda (Annex 2)**

Scientific Report

In 2012, the EC DG SANTE decided to organize the collection of molecular typing data for isolates of *Listeria monocytogenes*, *Salmonella* and Shiga-toxin producing *Escherichia coli* (STEC) from food animal feed and samples from the primary production stage, to improve the surveillance and trace-back of food-borne infections as well as the preparedness to face foodborne outbreaks at the European level. The strategy of this molecular surveillance system is described in the DG SANTE document "Vision paper on the development of data bases for molecular testing of foodborne pathogens in view of outbreak preparedness", available at the url: http://ec.europa.eu/food/food/biosafety/salmonella/docs/vision-paper_en.pdf.

According to the DG SANTE mandate, the responsibility of data collection was assigned to the European Food Safety Authority (EFSA), with the scientific and technical support of the relevant European Union Reference Laboratories (EURLs), which have been assigned the role of curators of the submitted data. The data collection has started during 2017.

The bulk of molecular typing data on food/animal isolates is primarily produced by the networks of National Reference Laboratories (NRLs) operating with the relevant EURLs. Therefore, among the initiatives to provide training to the respective NRL networks, the EURLs for *Listeria monocytogenes*, *Salmonella* and STEC decided to organize joint training courses on the use of the software BioNumerics, which offers an integrated platform for the analysis of PFGE fingerprints and allows the storage of gel images and epidemiological metadata in a single database.

The first edition of the training course took place in July 2016 at the laboratory for food safety located at Maisons-Alfort of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES), located at Maisons-Alfort (Paris, France), hosting the EURL for *Listeria monocytogenes*.

The second edition was held on 3-4 July 2017 at the IT-training room of the Istituto Superiore di Sanità in Rome, Italy, hosting the EURL-VTEC.

The objective of the course was to make the participants able:
- to visually evaluate the quality of PFGE gel images;
- to know the technical flaws resulting in poor-quality and not-analyzable pictures;
- to use the BioNumerics software package for PFGE profile analysis, through hands-on exercises on the acquisition and normalization of .tiff files, band assignment;
- to build up a database of PFGE profiles;
- to perform band assignment and profile analysis;
- to perform cluster analysis, in order to identify the level of similarity between the profiles.

Representatives of the three EURLs gave presentations and guided the trainees through the application of the standard operating procedures (SOPs) for PFGE analysis published by EFSA in 2014:

- Listeria SOP: http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2014.EN-702/pdf
- Salmonella SOP: http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2014.EN-703/pdf
- VTEC SOP: http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2014.EN-704/pdf

In the last training section, hosted at EURL-VTEC in the 3-4 July 2017, a total of twelve scientists participated in the event, representative of four National Reference Laboratories for each of the three EURLs networks. The participants were divided in three groups according to their preparedness in PFGE images analysis, assigned computers organized in as many rows and assigned two trainers per group among the representatives from the three EURLs.

The course consisted in interactive oral presentations, followed by extended hands-on training sessions regarding the subjects discussed.

Each participant was placed in front of a workstation equipped with the software BioNumerics version 7.5 for the practical sessions. The BioNumerics temporary licenses were provided by Applied Maths NV (Sint-Martens-Latem, Belgium).

The presentations and the exercises were managed by staff from the three EURLs. An invited talk was given by Dr. Valentina Rizzi from EFSA, who described the state of play of the data collection in the EFSA-ECDC joint database for the molecular-typing based surveillance of foodborne infections.

The Course was opened by **Dr. Rosangela Tozzoli**, researcher at the EURL-VTEC and involved in the Steering Committee for the Molecular Typing joint ECDC-EFSA data collection program, who briefly introduced its aims and program.

Dr. Valeria Michelacci, from EURL-VTEC, presented three examples of case studies demonstrating the relevance of PFGE analysis for the molecular typing of STEC. In detail, she showed how PFGE analysis can be crucial to identify the sources of infection and recall the concerned products from the market, implement hygiene measures when animal contact is involved or rule out the involvement of suspected food products differentiating isolates which could be undistinguishable when typed with other molecular methods.

Dr. Antonella Maugliani, from EURL-VTEC, then gave a presentation illustrating all the phases of PFGE typing, from DNA preparation to image acquisition, with particular attention to the possible problems encountered, giving a sort of 'trouble shooting' and introducing the criteria to be adopted to perform a preliminary visual evaluation of the gel image before importing it in BioNumerics software for the analysis.

Dr. Wilma Jacobs, from EURL-Salmonella, and **Dr. Maugliani** jointly presented the steps to perform when starting using BioNumerics software for PFGE interpretation, starting from databases creation and import of digital images of the gel in TIFF format.

In the following presentation, **Dr. Ludivine Bonanno,** from EURL-Listeria, and **Dr. Maugliani** carefully explained the process of PGFE images analysis, describing how to set a reference system, how to normalize the gel and to assign bands for producing the final fingerprints.

This presentation was followed by a practical session managed by **Dr. Maugliani** and **Dr. Valeria Michelacci**. The session consisted of examples of visual evaluation of PFGE images and exercises on the same topic carried out by the participants.

A practical session followed the lunch break, where all participants experienced the use of BioNumerics for database creation, experiments set up and PFGE image import and analysis.

The second day of the training was opened by **Dr. Benjamin Felix** from EURL-Listeria, who presented introduced the cluster analysis, with particular focus on the purposes, the algorithms and the parameters to be applied for such analysis. He finally gave an overview of the different protocols which are available for the exchange of files across databases.

The last presentation was given by **Dr. Valentina Rizzi** from EFSA, who described the state of play of the EFSA-ECDC joint database for molecular surveillance of foodborne pathogens.

The rest of the morning was completely devoted to a practical session mainly focused on cluster analysis, during which the participants had the possibility to go in depth in profiles analysis and comparison with the concrete help of the trainers from the three EURLs to answer all the technical questions arising explaining in depth the functionalities of the BioNumerics software to serve the purpose of PFGE molecular typing of foodborne pathogens.

The training was concluded by **Dr. Tozzoli,** who presented a wrap up of the topics discussed during the course.

EURL Lm
European Union Reference Laboratory for
Listeria monocytogenes
http://eurl-listeria.anses.fr

EURL Salmonella

EU-RL
VTEC

Second Joint Training Course on the Use of BioNumerics Software to analyse PFGE data of Shiga-toxin producing *Escherichia coli*, *Salmonella* and *Listeria monocytogenes*

A total of 12 participants were reimbursed by the three EURLs and are listed below.

Name	Affiliation	Funding EURL
Ana AMARO	National Institute for Agrarian and Veterinary Research, Portugal	EURL-VTEC
Laetitia BONIFAIT	ANSES – Ploufragan, France	EURL-Salmonella
Paola CHIANI	Istituto Superiore di Sanità, Italy	EURL-VTEC
Lucia DINCAKOVA	Public Health Authority, Slovak Republic	EURL-Listeria
Maria EMMANUEL	Veterinary Services, Cyprus	EURL-Listeria
Linda GRESOVA	Veterinary and Food Institute, Slovak Republic	EURL-Salmonella
Juris KIBILDS	Institute of Food Safety, Animal Health and Environment "BIOR", Latvia	EURL-Salmonella
Monika KURPAS	National Veterinary Research Institute, Poland	EURL-Listeria
Justina PETRASKAITE	National Food and Veterinary Risk Assessment Institute, Lithuania	EURL-VTEC
Monika STASIAK	National Institute of Public Health, Poland	EURL-VTEC
Monica VANGHELE	Institute for Diagnosis and Animal Health, Romania	EURL-Salmonella
Bavo VERHAEGEN	Scientific Institute of Public Health, Belgium	EURL-Listeria

The following table lists the speakers of the course.

Name	Affiliation
Ludivine BONANNO	EURL for L. monocytogenes, ANSES-Laboratory for Food Safety, France
El BOUW	EURL for Salmonella, RIVM, The Netherlands
Benjamin FELIX	EURL for L. monocytogenes, ANSES-Laboratory for Food Safety, France
Wilma JACOBS	EURL for Salmonella, RIVM, The Netherlands
Antonella MAUGLIANI	EURL-VTEC, Istituto Superiore di Sanità, Italy
Valeria MICHELACCI	EURL-VTEC, Istituto Superiore di Sanità, Italy
Valentina RIZZI	EFSA, Italy
Rosangela TOZZOLI	EURL-VTEC, Istituto Superiore di Sanità, Italy

EURL Lm
European Union Reference Laboratory for
Listeria monocytogenes
http://eurl-listeria.anses.fr

EURL Salmonella

EU-RL VTEC

Second Joint Training Course on the
Use of BioNumerics Software to analyse PFGE data of
Shiga-toxin producing *Escherichia coli*, *Salmonella* and
Listeria monocytogenes

3-4 July 2017

SIDBAE Training Room
(*Building 1, Floor B*)
Istituto Superiore di Sanità
Viale Regina Elena, 299 – Rome, Italy

Organized for the 3 networks of National Reference Laboratories for STEC, Listeria monocytogenes & Salmonella by:

− **The EU Reference Laboratory for STEC**
  (**ISS**: Antonella MAUGLIANI, Valeria MICHELACCI, Rosangela TOZZOLI)
− **The EU Reference Laboratory for *L. monocytogenes***
  (**ANSES-Laboratory for Food Safety**: Benjamin FELIX, Ludivine BONANNO**)**
− **The EU Reference Laboratory for *Salmonella***
  (**RIVM**: El BOUW, Wilma JACOBS**)**

Funded by the European Commission – DG SANTE

DIRECTOR OF THE COURSE

Stefano MORABITO
EU Reference Laboratory for *E. coli*
Dipartimento di Sanità Pubblica Veterinaria e Sicurezza Alimentare
Istituto Superiore di Sanità

TECHNICAL SECRETARIAT

Susan BABSA, Clarissa FERRERI

EU Reference Laboratory for *E. coli*
Department of Veterinary Public Health and Food Safety
Istituto Superiore di Sanità
Rome, Italy

Corrado Di Benedetto, Arnold KNIJN

IT Services
Istituto Superiore di Sanità
Rome, Italy

GENERAL INFORMATION

Venue: Istituto Superiore di Sanità, SIDBAE Training Room (Building 1, Floor B)
Viale Regina Elena 299, 00161 Rome

This event is part of the scientific and tutorial activities of the EU-RL VTEC, EU-RL Listeria and EU-RL Salmonella, funded by the European Commission – DG SANTE

For any information regarding the event, please send an email to crl.vtec@iss.it

PROGRAM

Monday, 3 July

9.00   **Registration**

9.15   Welcome, housekeeping and general overview on the training course (S. Morabito)

9.30   Case studies on PFGE typing: Listeria; Salmonella, STEC (The three EURLs)

10.30   Molecular typing by PFGE: gel production and staining, image acquisition, analysis and self-evaluation (A. Maugliani)

11.00   **Coffee break**

11.30   The BioNumerics Software: database creation, import of TIFF files, setting up experiments and image analyses (A. Maugliani/W. Jacobs)

12.00   Profiles analysis and interpretation (L. Bonanno, A. Maugliani)

12:30   **HANDS-ON EXERCISES:** Visual evaluation of PFGE gel images fingerprint data analysis (all)

13.00   **LUNCH**

14.00   **PRACTICAL SESSION**
Creation of the groups based on specific needs
Description of the dataset
Start of exercises

17.00   End of the first day

Tuesday, 4 July

9.00   Introduction to cluster analysis: purpose and parameters and Global Database Management (*e.g.* re-normalisation, re-mapping) (B. Felix)

9.30   Molecular surveillance of foodborne infections in the EU: EFSA-ECDC Joint Database. State of play (V. Rizzi - EFSA)

10.00   **Coffee break**

10.30   **PRACTICAL SESSION**

13.00   Concluding remarks