Basic Course on the use of BioNumerics Software for the analysis of Pulsed Field Gel Electrophoresis-generated profiles of *E. coli*

12-13 June, 2014

Istituto Superiore di Sanità, SIDBAE Training Room

Content

Scientific Report

In January 2013, the European Food Safety Authority (EFSA) received a mandate from the European Commission to provide technical support for the collection of molecular typing results of food/animal isolates of Salmonella, *Listeria monocytogenes* and Shiga toxin-producing *Escherichia coli*. According to this mandate, EFSA was requested to set-up and manage a database for molecular typing data from food and animal isolates of food-borne pathogens in collaboration with the relevant European Union Reference Laboratories (EU-RLs). The data would be submitted to EFSA by the Member States' National Reference Laboratories (NRLs) and the EURLs would act as curators of the data and would contribute to the data analyses.

To contribute to the preparedness of the NRLs for *E. coli* to submit VTEC PFGE profiles to the EFSA database, the EU-RL VTEC organized a training program on PFGE. Between 2012 and 2013, scientists from 11 NRLs visited the EU-RL to receive this specific laboratory training. The next step in the training program was the organization of a basic course on the use of the software package BioNumerics for PFGE profile analysis. The software package BioNumerics 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 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 understand the negative effects of such flaws on the analysis of profiles, after their import in the BioNumerics software;
- to use the BioNumerics software package for of 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 profile cluster analysis.

The course was held on 12-13 June 2014, at the IT-training room of the *Istituto Superiore di Sanità* (ISS). Eleven scientists from 10 NRLs participated in the event. Two scientists visiting the EU-RL VTEC in that period also took part in the course as observers. The course was organized in sessions, each composed of interactive oral presentations, followed by hands-on training regarding the subject discussed.

Each participant was placed in front of a workstation equipped with the software BioNumerics version 6.5 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 EU-RL and ISS staff. An invited talk was given by Damien Michelon from the EURL *Listeria monocytogenes*, who described the BioNumerics-based molecular data collection system in place at ANSES.

The Course was opened by **Dr. Alfredo Caprioli**, Director of the EU-RL VTEC, who briefly introduced its aims and program. Dr. Caprioli also introduced the state of play of molecular typing-based surveillance of foodborne pathogens in the EU.

Dr. Rosangela Tozzoli showed a brief outline of the project of setting up an EFSA-managed database for molecular typing of foodborne pathogens isolated from non-human sources (food, feed, animals and the related environment). Dr. Tozzoli then introduced the PFGE typing of VTEC strains, considering all the phases of this technique, from DNA preparation to image acquisition, with particular attention to the possible problems encountered, and giving a sort of ‘trouble shooting’.

This presentation was followed by a practical session managed by **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.

Dr. Antonella Maugliani introduced the use of the BioNumerics software to the participants with presentations on the creation of databases and experiments and import and analysis of PFGE images. A practical session came after these presentations, 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. Damien Michelon** from EU-RL Listeria, who presented the functionalities of the molecular typing database for Listeria monocytogenes managed by the EU-RL. The database is named "EURL *Lm* DB" and comprises data on strains isolated from food, feed, animals and the environment, submitted by 10 NRLs of the EU-RL Listeria network.

Dr. Stefano Morabito introduced the cluster analysis, with particular focus on the purposes and the parameters available for such analysis. The presentation was followed by a hands-on session, aiming at letting all the trainees experience this kind of analysis, using the data introduced during the previous practical sessions in the newly created BioNumerics database.

Dr. Arnold Knijn, from ISS, showed the significance and the syntax of the 'eXtensible Markup Language' (XML), which allows data sharing between databases and, after his presentation, an exercise was carried out aiming at producing XML packages exporting sample data, image analysis and parameter settings.

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

Evaluation of the satisfaction level towards the Course

A questionnaire was administered to the NRL representatives and observers attending the Course to collect their evaluation of the following aspects:

- **Organization** (Availability of multimedia equipment, venue, duration and content, social dinner).
- **Presentations** (Clarity of presentations, completeness of presentations, appropriateness of question time).
- **Topics** (Relevance, Completeness of elaborations, compliance with the expectations transfer of knowledge).

The evaluation was provided according to the following score**:**

1: Very poor; 2: Poor; 3; Good; 4; Very good; 5: Excellent.

Questionnaires were returned by 12 participants and the results were the following.

Organization:

The median score obtained was 5 (excellent) for the availability of multimedia equipment, the venue, the duration and content of the Course.

Presentations:

The median score obtained was 5 (excellent) for the clarity and completeness of presentations and for appropriateness of question time.

Topics:

The median score obtained was 5 (excellent) for the relevance of the topics, the completeness of elaborations, the transfer of knowledge and the compliance with the expectations.

The details of the evaluation scores are provided in **Figures 1 – 3**.

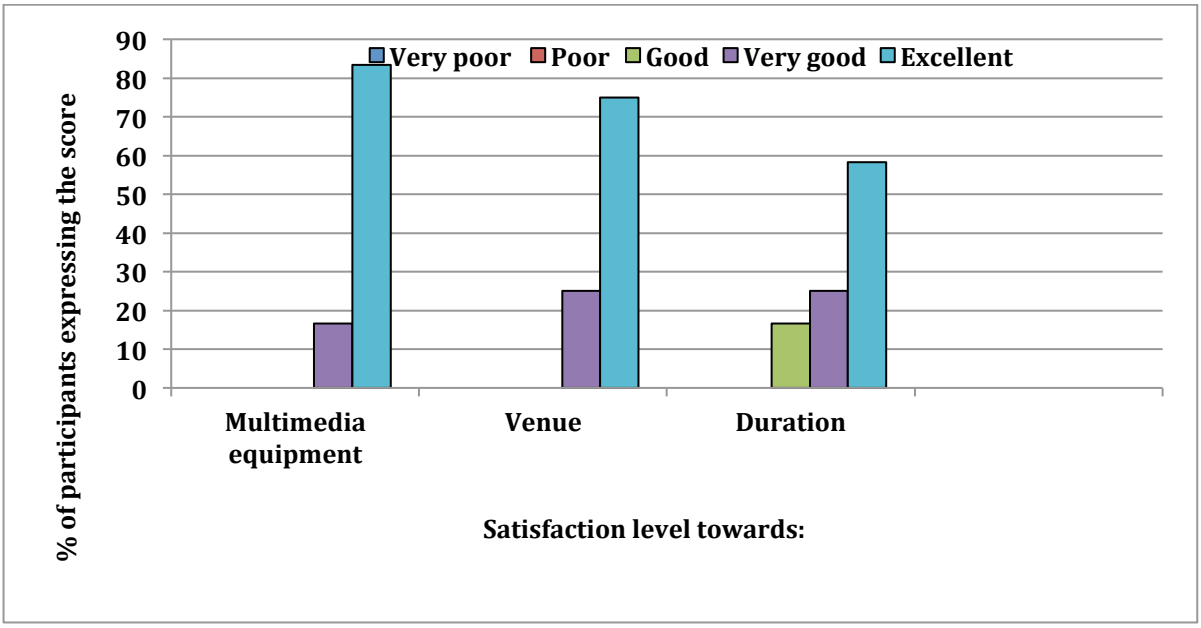

Figure 1. Evaluation of the satisfaction level toward the Course organization.

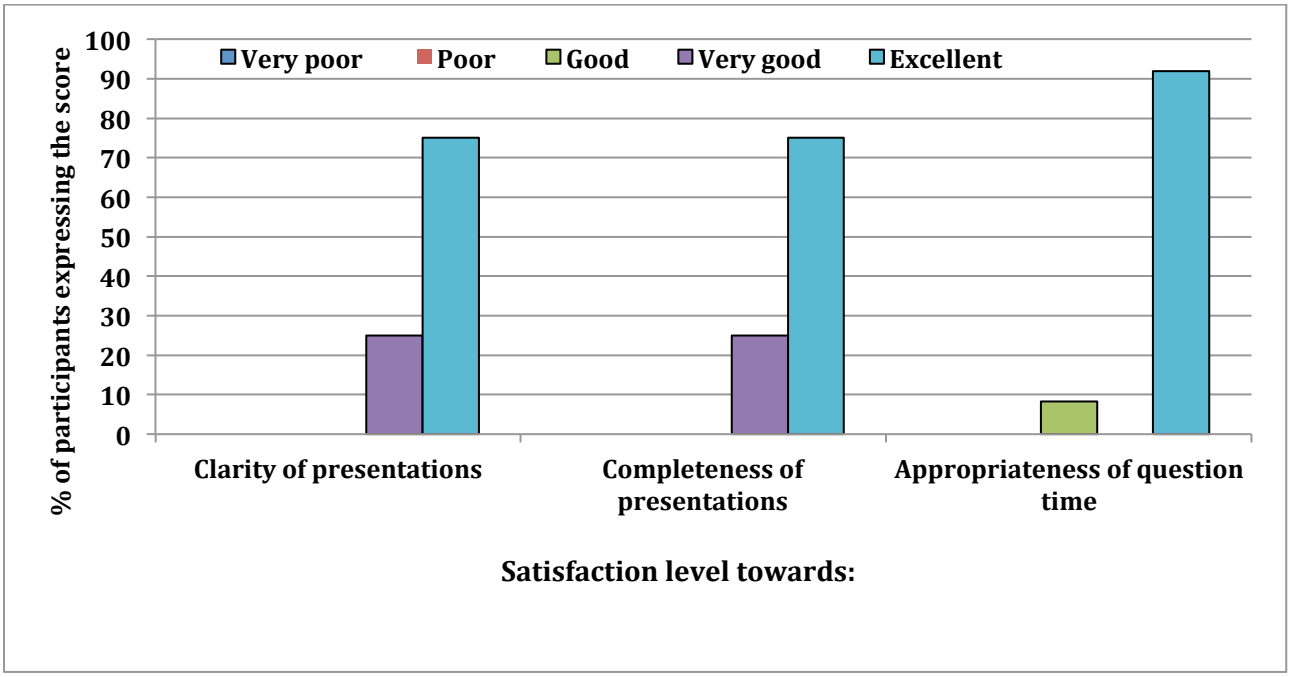

Figure 2. Evaluation of the satisfaction level towards the Course presentations

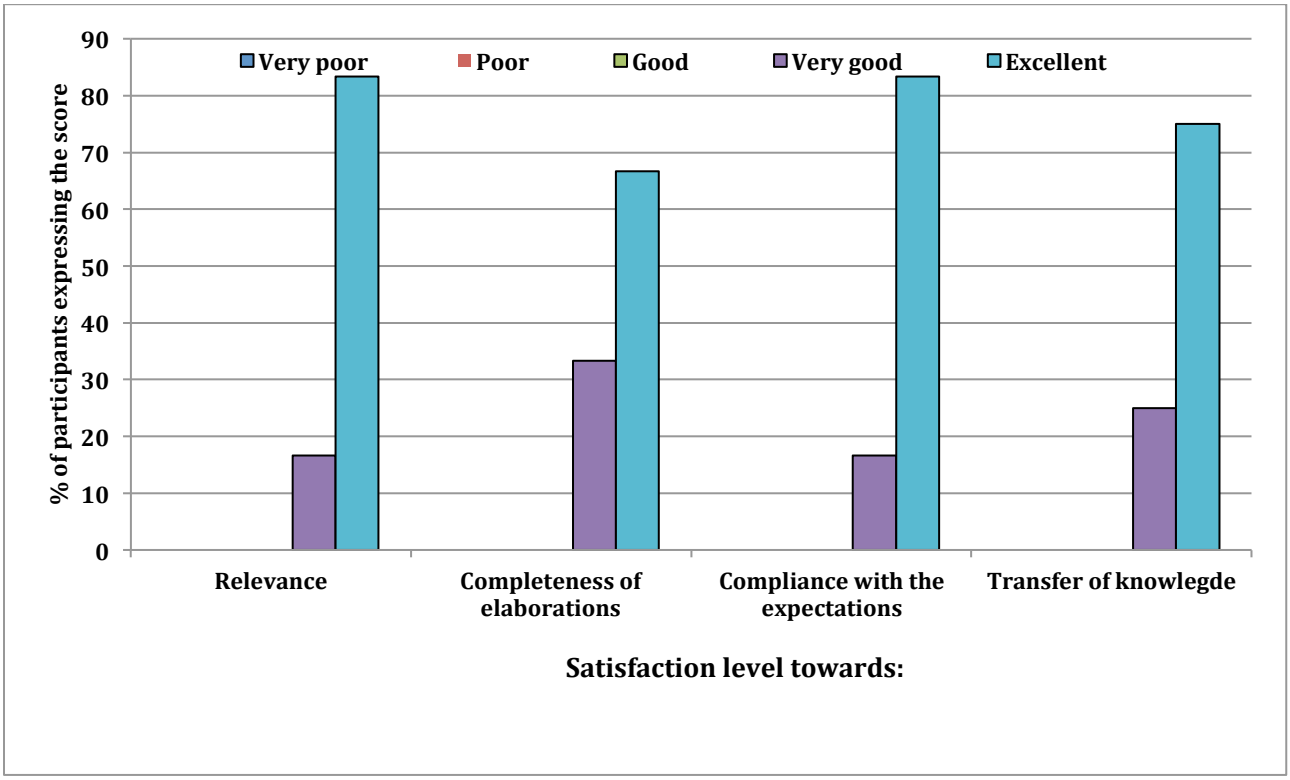

Figure 3. Evaluation of the satisfaction level towards the topics treated in the Course.

Annex 1

EU Reference Laboratory for *E. coli*
Department of Veterinary Public Health and Food Safety
Unit of Foodborne Zoonoses
Istituto Superiore di Sanità

Basic Course on the use of BioNumerics Software for the analysis of Pulsed Field Gel Electrophoresis-generated profiles of *E. coli*

Istituto Superiore di Sanità, Rome, 12-13 June 2014

List of Participants

Representatives of National Reference Laboratories (NRLs) for *E. coli*

1. Constantinos ARSENOGLOU, NRL Cyprus
2. Laurentiu CIUPESCU, NRL Romania
3. Francisco Javier GARCÍA PEÑA, NRL Spain
4. Anna GIČOVÁ, NRL Slovakia
5. Laura GRANDE, NRL Italy
6. Žygimantas JANELIŪNAS, NRL Lithuania
7. Elżbieta MAĆKIW, NRL Poland
8. Isabela NICORESCU, NRL Romania
9. Mateja PATE, NRL Slovenia
10. Ariane PIETZKA, NRL Austria
11. Kinga WIECZOREK, NRL Poland

Observers

Kabiru LAWAL, University Ahmadu Bello, Zaria, Nigeria

Chris TIMMONS, National Institute for Food and Agricultural Biosecurity, Oklahoma, USA

Speakers

Alfredo CAPRIOLI, EU-RL VTEC

Arnold KNIJN, SIDBAE, ISS

Antonella MAUGLIANI, EU-RL VTEC

Valeria MICHELACCI, EU-RL VTEC

Damien MICHELON, EU-RL Listeria, Maisons Alfort, France

Stefano MORABITO, EU-RL VTEC

Rosangela TOZZOLI, EU-RL VTEC

Annex 2

EU Reference Laboratory for *E. coli*
Department of Veterinary Public Health and Food Safety
Unit of Foodborne Zoonoses
Istituto Superiore di Sanità

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12-13 June, 2014

SIDBAE Training Room

(*Building 1, Floor B*)

Istituto Superiore di Sanità

Viale Regina Elena, 299 – Rome, Italy

Program

Organized by:

- **The EU Reference Laboratory for *E. coli***
- **The ISS IT Service (SIDBAE)**

Funded by the European Commission – DG SANCO

DIRECTOR OF THE COURSE

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

SPEAKERS

EU Reference Laboratory for *E. coli*, Rome, Italy

Alfredo CAPRIOLI
Antonella MAUGLIANI
Valeria MICHELACCI
Stefano MORABITO
Rosangela TOZZOLI

EU Reference Laboratory for Listeria, Maison Alfort, ANSES, France

Damien MICHELON

TECHNICAL SECRETARIAT

Susan BABSA, Clarissa FERRERI

EU Reference Laboratory for *E. coli*
Dipartimento di Sanità Pubblica Veterinaria e Sicurezza Alimentare
Istituto Superiore di Sanità
Rome, Italy

Fabio GALATI, Arnold KNIJN

Servizio Informatico, documentazione, biblioteca ed attività editoriali (SIDBAE)
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, funded by the European Commission – DG SANCO

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

PROGRAM

Thursday 12 June		
9.00	**Registration**	
9.15	Welcome, housekeeping, and general overview on the training course	**Alfredo Caprioli** **Stefano Morabito (EU-RL VTEC)** **Arnold Knijn (SIDBAE)**
	Session 1	
9.45	Molecular surveillance of foodborne infections in the EU	**Alfredo Caprioli**
10.00	Molecular typing of VTEC by PFGE: gel production and staining, image acquisition and self-evaluation	**Antonella Maugliani** **Rosangela Tozzoli**
11.00	**Coffee break**	
11.30	Exercises: visual evaluation of PFGE gel images	**Antonella Maugliani** **Valeria Michelacci** **Rosangela Tozzoli**
12.00	The BioNumerics Software: database creation, import of TIFF files, and setting up experiments	**Rosangela Tozzoli** **Antonella Maugliani** **Valeria Michelacci**
13.00	**Lunch**	
14.00	Image analysis: profiles analysis and interpretation	**Stefano Morabito** **Antonella Maugliani**
15.00	Hands-on exercises: fingerprint data analysis	**Antonella Maugliani** **Valeria Michelacci** **Rosangela Tozzoli**
16.30	Discussion and concluding remarks on the first session	

Friday 13 June

Session 2

9.30	Molecular typing data collection: the experience of EU-RL Listeria	**Damien Michelon**
10.15	Introduction to cluster analysis: purpose and parameters	**Stefano Morabito**
11.00	**Coffee break**	
11.30	Practical Session on cluster analysis	**Stefano Morabito** **Antonella Maugliani**
12.15	Sharing data in the network: The markup language XML	**Arnold Knijn**
12.45	Concluding remarks	**Stefano Morabito**
13.00	**Farewell lunch**	

Annex 3

Basic Course on the use of BioNumerics Software for the analysis of Pulsed Field Gel Electrophoresis-generated profiles of *E. coli*

Participants reimbursed by the EU-RL

A total of 10 participants were reimbursed by the EU-RL. Those included the representatives of nine NRLs and one invited speaker, Damien Michelon, who presented the experience of EU-RL *Listeria monocytogenes* on the application of molecular typing for surveillance.

The participants reimbursed by the EU-RL are listed below.

1. Constantinos ARSENOGLOU, NRL Cyprus
2. Francisco Javier GARCÍA PEÑA, NRL Spain
3. Anna GIČOVÁ, NRL Slovakia
4. Žygimantas JANELIŪNAS, NRL Lithuania
5. Elżbieta MAĆKIW, NRL Poland (NIPH)
6. Isabela NICORESCU, NRL Romania
7. Mateja PATE, NRL Slovenia
8. Ariane PIETZKA, NRL Austria
9. Kinga WIECZOREK, NRL Poland (NVRI)
10. Damien MICHELON, EU-RL *Listeria monocytogenes*, Maisons Alfort, France, invited speaker