**EU Reference Laboratory for *E. coli***

*Department of Veterinary Public Health and Food Safety*

*Unit of Foodborne Zoonoses*

**Istituto Superiore di Sanità**

# EU Reference Laboratory (EU-RL) for *Escherichia coli*, including Verotoxigenic *E. coli* (VTEC)

## Work Program

**1st January 2016 - 31st December 2017**

The Commission Work Programme on financing contribution to the EU-RLs adopted in July 2015 was established for a two-year period. Accordingly, the EU-RL for *E. coli* (EU-RL VTEC) has planned its work program for the years 2016 – 2017. The program will consist of the following activities, objectives and expected outputs, that have been defined taking into account the Commission Work Program and the responsibilities laid down by the Article 32 of Regulation (EC) No 882/2004.

The EU-RL VTEC activities planned for the period 2016 – 2017 are:

1. **Development and dissemination of high quality analytical methods to the NRLs and laboratories in third countries**
   - ***1.1. Evaluation of the ISO TS 13136:2012 for testing sprout irrigation water***
   - ***1.2. Revision of the standard ISO TS 13136:2012***
   - ***1.3. Development of a MLVA protocol for molecular typing of non-O157 VTEC***
   - ***1.4. Applied research and development activities to improve molecular methods for the detection and typing of VTEC***
   - ***1.5. Support to the NRLs for the accreditation of methods for the detection of pathogenic* E. coli**
   - ***1.6. Assistance to the NRLs: provision of reference materials* and advice on specific issues**
     - *1.6.1. Development and provision of certified reference materials*
     - *1.6.2. Preparation and provision of certified reference samples for quality control*
     - *1.6.3. Problem-solving plans related to analytical issues and provision of back-up test samples*
     - *1.6.4. Communications to the NRLs*
   - ***1.7. Collaboration with laboratories in third countries***
   - ***1.8.* Training for the benefit of staff from the NRLs and of experts from third countries**
     - *1.8.1. Annual Workshop with the NRLs*
     - *1.8.2. Training activities: Courses on the use of BioNumerics*
     - *1.8.3. 2nd Course on bioinformatics tools for NGS data analyses*
     - *1.8.4. Training activities: Short-term laboratory training visits to the EU-RL*

2. **Organization of comparative testing.**
   - ***2.1. Identification and typing of pathogenic E. coli (PT17)***
   - ***2.2. PFGE typing of E. coli strains (PT-PFGE5 and PT-PFGE6)***
   - ***2.3. Detection of VTEC in food (PT18 and PT20)***
   - ***2.4. Detection of VTEC in complex matrices (PT19)***
   - ***2.5. Addressing underperforming related issues***
3. **Provision of scientific and technical assistance to the Commission and other EU structures related with food safety**
   - **3.1.** ***Scientific and technical support to DG SANTE***
   - **3.2.** ***The European Food Safety Authority (EFSA)***
   - **3.3.** ***The European Committee for Standardization (CEN)***
   - **3.4.** ***The European Centre for Disease Prevention and Control (ECDC)***
   - **3.5.** ***Support to EFSA and the NRL network in the implementation of a database of molecular typing data for VTEC strains from animal and food sources***
4. **Consolidation of the EU-RL structures**
   - ***4.1. Staff***
   - ***4.2. Administration and Reporting***
   - ***4.3. Definition of ex-ante performance indicators and their ex-post evaluation***
   - ***4.4. Update of the infrastructure and equipment available for the EU-RL activities***
   - ***4.5. Maintaining and Implementing the EU-RL-VTEC web site***
5. **Missions**
6. **Other activities not co-financed under the EU-RL budget**

The objectives and the expected outputs of each action are indicated. Performance indicators (PI) are indicated where appropriate, making reference to the PI spreadsheet that is attached to this program.

## 1. Development and dissemination of high quality analytical methods to the NRLs and laboratories in third countries

### *1.1. Evaluation of the ISO TS 13136:2012 for testing sprout irrigation water*

Commission Regulation (EU) No 209/2013 of 11 March 2013, laying down microbiological criteria for sprouts, gives the food business operators producing sprouts the possibility to replace the sampling and testing of sprouts with the analysis of 5 samples of 200 ml of the water that was used for their irrigation. However, testing spent irrigation water for the presence of VTEC or other enteric pathogens may pose technical problems, due to some characteristics of this particular matrix. For instance, if concentration of VTEC is pursued by a filtration step, the high density of the irrigation water, due to substances released by some species of sprouts, can make such a filtration difficult. Based on the evaluation of the preliminary work done by the EU-RL and the results of the first inter-laboratory study that will be conducted in November 2015 on VTEC detection using spent irrigation water as complex matrix, the EU-RL will provide the NRLs with a SOP for the pre-treatment of spent irrigation water samples to be entered in the analytical flow of the ISO/TS 13136:2012 standard. The SOP will consider the treatment of spent irrigation water samples deriving from the production process of the most common sprout species. Given the high number of seed species used in the production of this food commodity and the high diversity in the composition and density of the spent irrigation water obtained, more seeds species will be considered over the next two years and the SOP will be updated regularly.

***Objectives:*** *to provide a suitable procedure to detect VTEC contamination in a matrix for which a microbiologic criterion has been included in the EU legislation.*

***Expected output:*** *a SOP to detect VTEC contamination in sprout irrigation water.*

***Performance indicators:*** *i) SOPs published on the EU-RL VTEC website; ii) the performance parameters of the ISO/TS 13136 method updated with the spent irrigation water. See also FF.PT.5 and FF.ANA.1 in the PI spreadsheet.*

***Duration****: 2016 - 2017*

### *1.2. Revision of the standard ISO TS 13136:2012*

After three years from its publication in November 2012, the standard ISO/TS 13136:2012 can undergo a revision. Such a revision has already been agreed during the 2015 CEN/TC/275/WG6 general meeting (Recommendation N. 393) and the EU-RL will coordinate the revision procedure (see also point 3.3). Based on the

experience done in the first three year of adoption, the revision will touch all the analytical steps of the method and will be focused on the possible improvement of enrichment and isolation media and on the evaluation of the appropriateness of the *stx* gene primers and probes. The revision process will start as soon as the CEN/TC/275/WG6 will launch a call for the experts to be enrolled in a STEC *ad hoc* group, which will discuss these issues under the chairmanship to the EU-RL VTEC.

***Objectives:*** *to coordinate the revision process of the standard ISO/TS 13136:2012.*

***Expected output:*** *a suitable revision of the standard.*

***Performance indicators:*** *a first draft of the revision submitted 12 months after the enrollment of the STEC ad hoc group. See also FF.ANA.1 and FF.CEN.2 in the PI spreadsheet.*

***Duration****: 2016 - 2017*

***1.3. Development of an MLVA protocol for molecular typing of non-O157 VTEC.***

Multi-locus variable number of tandem repeat analysis (MLVA) has been successfully used to further differentiate strains of VTEC O157. An MLVA scheme has been recently published for the typing of VTEC O26 strains (Løbersli *et al*, 2012). In 2015, a protocol has been developed in the framework of a collaborative study between the EU-RL and the Oklahoma City University, to characterize VTEC by MLVA regardless their serogroup. The protocol has been tested on selected VTEC strains to test its robustness and reproducibility. In 2016, the experimental protocol will be evaluated on a larger panel of strains and a SOP for MLVA typing of VTEC regardless their serogroup will be prepared. Additionally, a bioinformatics tool to extract the MLVA profiles from whole genome sequences of VTEC will be developed and made available on the Bioinformatics analytical framework ARIES (see point 1.4), already presented to the *E. coli* NRL during 2015. Both the MLVA method and the bioinformatics tools could allow a fast a reliable typing strategy for VTEC and could be particularly useful in view of the shift of the typing technology from restriction fragment length polymorphism (RFLP) analyses to whole genome sequencing.

***Objectives:*** *to provide an alternative procedure for molecular typing of VTEC non-O157.*

***Expected output:*** *availability of an additional typing method VTEC non-O157.*

***Performance indicators:*** *SOPs, to be partially evaluated by comparison with PFGE. See also FF.PT.5 in the PI spreadsheet.*

***Duration**: 2016 - 2017*

### *1.4. Applied research and development activities to improve molecular methods for the detection and typing of VTEC*

The rapid development of next generation sequencing (NGS) platforms and the parallel development of bioinformatics tools for NGS data management and analyses have made the genome sequence-based investigation a realistic alternative to conventional molecular typing of bacterial isolates. In particular, this approach promises to become a credible alternative to PFGE and its designated successor for molecular surveillance of VTEC infections. Discussion groups have been recently organized by the European Food Safety Authority (EFSA) and by the European Centre for Disease Control (ECDC) to define the most appropriate context for the introduction of NGS as the standard technology for molecular surveillance of foodborne infections (see also points 3.4 and 3.5). Therefore, the EU-RL VTEC will continue the research studies on the genomics of pathogenic *E. coli*, to better understand the epidemiology of the infections, to improve the detection of these pathogens in their animal reservoirs and food vehicles, and to increase the spectrum of molecular tools available for strain typing. The research activities will be particularly devoted to next generation sequencing (NGS) approaches for investigating VTEC biology and to the development of bioinformatics tools providing suitable strategies for the study of VTEC genomes. Additionally, efforts will be made to develop new tools for *E. coli* typing and to make them accessible to the NRLs for *E. coli*. In 2015, the EU-RL VTEC, in collaboration with the IT services of ISS, opened the web service ARIES. The portal is destined to the analysis of data intensive applications, such as those derived from the NGS technology. ARIES features all the tools needed for the basic analyses of bacterial genomes and in particular some tools for the characterization of pathogenic *E. coli* from whole genome sequences, including the analysis of virulence genes content (virulotyping), the identification of O and H antigens genes (serotyping), and the multi-locus-sequence typing (MLST). In the period 2016-2017, the range of tools dedicated to the analysis of *E. coli* will be incremented with new applications for molecular typing (*e.g.* SNP analyses) and NGS-based diagnostic applications. In 2016, the portal ARIES will be fully accessible to the NRLs network and will be used in the two-year period for practical training courses on the use of NGS for detection and typing of pathogenic *E. coli*. The ongoing collaborations with Public Health England (PHE),

London, UK, and the Oklahoma State University, Oklahoma City, OK, USA, will be maintained and strengthened with new research programs. Further collaborations will be established, *e.g.* with the US Food and Drug Administration (FDA) and other will be encouraged in order to set up a multi national group of scientists working on the development of innovative approaches and tools to investigate the biology and ecology of VTEC and other pathogenic *E. coli*, including their routes of transmission to humans.

***Objectives:*** *i) to improve the knowledge of the pathogenic mechanisms of VTEC and other pathogenic* E. coli *and of the biological bases of the emergence of new pathogenic clones; ii) to identify candidate molecular targets for the identification and typing of VTEC and other pathogenic* E. coli*; iii) to expand the detection capacity of the available methods to a wider range of pathogenic VTEC clones; iv) to provide the network of* E. coli *NRLs with new and efficient typing methods for VTEC and other pathogenic* E. coli*.*

***Expected output:*** *development of innovative flexible methodologies for detection and typing of pathogenic* E. coli*.*

***Performance indicators:*** *i) papers published in peer reviewed journals and pathogenic* E. coli *strains whole genomes determined and released; ii) development of procedures for whole genome-based typing and cluster detection. See also FF.ANA.2 and "Other Activities" in the PI spreadsheet.*

***Duration****: 2016 - 2017*

***1.5. Support to the NRLs for the accreditation of methods for the detection of pathogenic* E. coli**

The EU-RL will continue to support the NRLs in the process of setting up and accrediting the methods for the detection and typing of VTEC and other pathogenic *E. coli.*

As for the standard ISO 16654:2001 for the detection of *E. coli* O157 in food, after the full validation completed in 2014, a revision of the standard will be done, in order to include the performances obtained in the collaborative studies performed in 2012 on milk and in 2014 on sprouts, as well as those listed in the validation study performed by NMKL on 2002. An amendment to the ISO 16654:2001 containing the performance parameters of the methods with all the matrices will be produced in 2016 for the publication by ISO.

In order to ensure the possibility of a full compliance with Regulation (EU) 209/2013,

the performance of the method for the detection of *E. coli* genes specifying the serotype O104:H4, determined during an internal validation, will be updated with those derived from the PT program of the EU-RL VTEC and the consolidated results will be made available to the NRLs through the EU-RL website.

***Objective:*** *to coordinate the application of analytical methods.*

***Expected output:*** *more NRLs applying and accrediting the methods for the detection of VTEC in sprouts, according to Reg.EU 209/2013.*

***Performance indicators:*** *i) revision of the ISO 16654:2001 sent to CEN; ii) documents containing the performance parameters of the ISO TS 13136:2012 and the method for the detection of VTEC O104:H4 published on the EU-RL VTEC website. See also FF.PT.6 in the PI spreadsheet.*

***Duration****: 2016 - 2017*

### *1.6. Assistance to the NRLs: provision of reference materials* and advice on specific issues

The EU-RL-VTEC will continue to assist the NRLs in the field of pathogenic *E. coli* detection and typing, providing methods and standard operating procedures via the website, reference materials and advice on specific issues. The use by the NRLs of the methods developed and/or validated by the EU-RL VTEC, or prescribed for official controls by the EU legislation, will be monitored by dedicated surveys.

The EU-RL-VTEC will visit at least one NRL to strengthen the liaison with the NRL network and, if needed, to help in solving problems.

#### *1.6.1. Development and provision of certified reference materials*

The EU-RL VTEC will continue to collect, characterize, maintain and distribute *E. coli* reference strains to be used as controls in the assays for the detection and characterization of VTEC and other pathogenic *E. coli*.

Plasmids harboring the target genes of the method ISO/TS 13136:2012 for the detection of VTEC in food will be also distributed upon request. These plasmids can be used as positive control in the molecular detection assays aimed at the detection of genes *stx1a, stx2a, eae, rfbE*$_{O157}$*, wzx*$_{O26}$*, wzx*$_{O103}$*, wbdl*$_{O111}$*, ihp1*$_{O145}$.

#### *1.6.2. Preparation and provision of certified reference samples for quality control*

Upon request, the EU-RL VTEC will send to the NRLs or to qualified laboratories in third countries certified food samples artificially contaminated with VTEC or other pathogenic *E. coli*, to be used for quality control purposes.

*1.6.3 Problem-solving plans related to analytical issues and provision of back-up test samples*

Upon request, the EU-RL VTEC will continue to provide advice and problem-solving plans related to analytical issues in the field of pathogenic *E. coli* to the NRLs and other Official Laboratories. Back-up test samples will be provided to the NRLs that will obtain insufficient results in a PT, as a measure of management of the underperformance (see also point 2.5).

*1.6.4. Communication to the NRLs*

The EU-RL VTEC will continue to provide on a regular basis updates on methodologies, epidemiology, applied research, publications and events on VTEC infections, using the section named "*Focus on*" of the EU-RL website, where highlights on the *E. coli* subject are added regularly (see also point 4.5).

***Objectives*****:** *to provide updated diagnostic methods, reference materials and advice to the NRL Network and other laboratories.*

***Expected output*****:** *increased capability of the NRLs to detect and type VTEC.*

***Performance indicators:*** *see FF.NRL.4, FF.R&D.1 in the PI spreadsheet.*

***Duration:*** *2016 - 2017*

***1.7. Collaboration with laboratories in third countries***

The EU-RL VTEC will continue to collaborate with laboratories responsible for analyzing feed and food or carrying out investigations on *E. coli* in third countries. At present, collaborations are established with:

- *The Central Laboratory of Residue analysis of Pesticides and Heavy Metals in Food (QCAP) in Egypt.* The collaboration was established after the outbreak of VTEC O104:H4 infections in 2011, associated with sprouts produced with seeds imported from Egypt. It includes the provision of scientific and technical advice, reference materials, external quality assessment and possible on-site visits, in order to help the QCAP in the establishment and accreditation of the methods for testing food for the presence of pathogenic *E. coli.* There will be the possibility that QCAP scientists, on their own costs, undertake training visits to the EU-RL.
- *The National Food Safety and Quality Service (SENASA), in Argentina.* SENASA (*Servicio Nacional de Sanidad y Calidad Agroalimentaria*) is the agency of the Argentine government dealing with surveillance, regulation and certification of products of animal and plant origin and with the prevention, eradication and control of diseases and plagues that affect them. SENASA laboratories are

involved in the official controls of food in Argentina, including meat products intended for export. Since 2014, the EU-RL is interacting with SENASA and will continue to provide protocols, reference materials, technical advice, and the possibility to participate in its PTs schemes, to help them in the establishment and accreditation of the methods for testing food for the presence of pathogenic *E. coli.*

***Objectives*****:** *maintain relationships and provide support to competent laboratories in third countries.*

***Expected output*****:** *increased capability of the laboratories in third countries to detect and type VTEC.*

***Performance indicators:*** *see FF.CEN.1 in the PI spreadsheet.*

***Duration:*** *2016 - 2017*

**1.8. Training for the benefit of staff from the NRLs and of experts from third countries**

The EU-RL will continue to provide training opportunities to the staff from the NRLs and to experts from third countries. These will include the Annual Workshop, the organization of courses and the availability of standard programs for laboratory training on specific issues.

*1.8.1. Annual Workshop with the NRLs*

The 11$^{th}$ annual workshop will be held in the second half of 2016 and the 12$^{th}$ in the second half of 2017. Both workshops will be held in Rome or, in alternative, upon agreement with DG SANCO, one of the NRLs could host the workshop at its own Institute. The program of the workshops will include the presentation and discussion of the results of the inter-laboratory studies carried out in the respective years. The training programs for the benefit of NRLs will be discussed as well and plans for the following year will be established according to the NRLs needs. The program, developed considering also the inputs from the NRLs obtained through the replies to an *ad hoc* e-mail enquiry, will include updates on the surveillance and monitoring activities of VTEC infections carried out in the EU, information on new diagnostic tools, research results, recommendations and exchange of experiences, with presentations made from the NRL representatives. Representatives from the European Food Safety Authority (EFSA) and from the European Centre for Disease Control (ECDC) will be invited. The workshops will also represent an opportunity to evaluate the state of play of the initiative of the database of molecular typing data on

VTEC strains isolated from non-human sources, described at point 3.5. The level of satisfaction of the participants toward the workshop organization, the proposed topics and the quality of presentations will be evaluated by questionnaires. The results will be used for the continuous improvement of the organization.

***Objectives:*** *i) to provide updates on the different aspects of VTEC infections; ii) to plan the training programs according to the NRLs needs; iii) to strengthen the relationships with and among the NRLs.*

***Expected output:*** *consolidation of the NRL network.*

***Performance indicators:*** *see FF.NRL.1, FF.NRL.2, FF.NRL.3 and FF.NRL.QI in the PI spreadsheet.*

***Duration****: 2016 - 2017*

*1.8.2. Courses on the use of the software package BioNumerics for PFGE fingerprints analysis*

In 2013, EFSA received from the EC a mandate to set-up and manage a database for molecular typing data from food and animal isolates of VTEC, Listeria and Salmonella, in collaboration with the relevant EU-RLs (see also point 3.5). The data would be submitted to EFSA by the Member States' NRLs and the EU-RLs would act as curators of the data and would contribute to the data analyses. To contribute to the preparedness of the NRLs to submit PFGE profiles to the EFSA database, the EU-RL VTEC and the EU-RL Listeria organized laboratory training programs on PFGE. In particular, since 2012, scientists from 12 NRLs for *E. coli* visited the EU-RL VTEC to receive this specific laboratory training, and a basic course on the use of the software package BioNumerics for PFGE profile analysis was held in 2014, with the participation of 9 NRLs for *E. coli*. 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. It is the framework for the analyses of molecular profiles of foodborne pathogens selected for the collection of molecular data jointly coordinated by EFSA and ECDC.

In the years 2016 and 2017, the EU-RL VTEC will organize an advanced training course on the use of the software package BioNumerics 7.5 for PFGE fingerprints analysis together with the EU-RL Listeria and the EU-RL Salmonella. The course will be directed to the networks of the NRLs for *E. coli*, Listeria and Salmonella. A first edition will be held at ANSES in Maison Alfort (France) in June 2016. A second edition will be held at ISS in Rome (Italy) in June 2017. A third edition should be held

at RIVM in 2018.

For the first two courses in 2016 and 2017, the ANSES and ISS IT services will make available didactic rooms equipped with at least 12 computer workstations and each course will be attended by representatives of 4 NRLs for *E. coli*, 4 NRLs for Listeria and 4 NRLs for Salmonella. The courses will be managed by staff members of the three EU-RLs. The company owner of the software package BioNumerics (Applied Maths NV, Sint-Martens-Latem, Belgium) will be contacted for the release of the temporary licenses needed for the course. The courses will have a duration of 2 full days and will focus on the analysis of PFGE profiles and on the management of the metadata related with each isolate. The courses will have a hands-on approach, with demonstrations and exercises. At the end of the courses, the participants will be able to correctly perform band assignment and profile analyses and to identify the relatedness between PFGE profiles.

The travel and accommodation costs for 8 participants from the NRLs for *E. coli* will be covered by the EU-RL VTEC funds.

***Objectives*****:** *to provide specific molecular typing training to the staff of the NRLs.*

***Expected output*****:** *i) improved preparedness of the NRL network for providing molecular typing data to the database that is under construction at EFSA; ii) improved harmonization of the NRL networks.*

***Performance indicators:*** *see FF.NRL.3 FF.NRL.5, FF.NRL.6 and FF.NRL.QI in the PI spreadsheet.*

***Duration:*** *2016 - 2017.*

*1.8.3. 2nd Course on bioinformatics tools for NGS data mining: use of bioinformatics tools for typing pathogenic* E. coli

The EU-RL VTEC will organize in 2016 the second training course on the use of bioinformatics tools for NGS data mining. The course will be focused on the typing of pathogenic *E. coli* strains based on NGS. It will be held at ISS and the participants will learn how to come to a deep typing of *E. coli* isolates starting from whole genome sequence (WGS) data, using the tools available on the ARIES portal. ARIES is a webserver developed to be a shared workspace for intensive data analyses, particularly those deriving from NGS (see also point 1.4). The platform has been developed within a collaborative work among the EU-RL VTEC, the ISS IT service, and the *Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise*, one of the Italian Official Laboratories that is part of the national veterinary laboratory

network. ARIES allows the analysis of whole genome sequences for the identification and typing of pathogenic *E. coli* by using bioinformatics tools that operate on the ISS server, where it is installed. ARIES was presented to the NRLs of the *E. coli* Network during the *1st Course on bioinformatics tools,* held in Rome in June 2015 (course program and report available at the EU-RL website, Section *E. coli* genomics, www.iss.it/vtec).

***Objectives:*** *to provide advanced molecular typing training to the NRL staff.*

***Expected output:*** *improved preparedness of the NRL network for providing typing data based on NGS to the Competent Authorities.*

***Performance indicators:*** *see FF.NRL.3, FF.NRL.5, FF.NRL.6 and FF.NRL.QI in the PI spreadsheet.*

*1.8.4.Training activities: Short-term laboratory training visits to the EU-RL*

Upon request from NRLs within EU or from governmental institutions of third countries, the EU-RL will receive visits of scientists for individual laboratory training on specific topics related with detection and typing methods. The available standard programs for short-term training visits on techniques for VTEC detection, identification and typing will be updated, according to the needs of the NRLs and the evolution of the epidemiological picture of *E. coli* infections in the EU. A particular effort will be dedicated to provide training on:

- Molecular typing techniques (PFGE, MLVA), to increase the number of NRLs capable to submit profiles to the database of molecular typing data on VTEC strains isolated from non-human sources (see point 3.5).
- The organization of proficiency tests (PTs). This represents a duty also for the NRLs, which should organize PTs at the national level to the benefit of the laboratories involved in the official control of food. Conversely, a recent inventory showed that only a minor part of the NRLs organizes PTs on VTEC.

The standard training programs have a 5-days duration and the requested budget will allow the support with the EU-RL funds of at least 10 training visits from the NRLs in the two years. A call for applications will be sent to the NRLs at the beginning of each year. The level of satisfaction of the trainees toward the organization, the program, and the quality of the stage will be evaluated by a questionnaire. The results will be used for the continuous improvement of the training program. The travel and accommodation costs for at least six visits from NRLs will be covered by the EU-RL funds.

***Objectives*****:** *to provide specific training to the staff of the NRLs or other laboratories, with particular focus on molecular typing and PT organization.*

***Expected output*****:** *i) improved capability to detect and type VTEC in the laboratories receiving training; ii) preparedness of NRLs for providing molecular typing data to the database that is under construction at EFSA; iii) increase in the number of NRLs able to organize PTs on VTEC in their own country.*

***Performance indicators:*** *see FF.NRL.3, FF.NRL.5, FF.NRL.6 and FF.NRL.QI in the PI spreadsheet.*

***Duration:*** *2016- 2017*

## 2. Organization of comparative testing

The EU-RL will continue to organize proficiency tests (PTs) on the detection, identification and typing of VTEC and other pathogenic *E. coli.*

Three PT rounds will be organized in 2016 and other three in 2017. The PT program is summarized below:

- A study on the identification and typing of pathogenic *E. coli* strains: PT17 (April 2016).
- Two rounds of proficiency testing for PFGE typing: PT-PFGE5 and PT-PFGE6 (April 2016 and April 2017).
- Two studies on the detection of VTEC in food matrices, presumably bovine meat and sprouts: PT18 and PT20 (November 2016 and November 2017).
- A study on the detection of VTEC in a complex matrix, such as sprout spent irrigation water: PT19 (April 2017).

The choice of the matrices to be analyzed and the VTEC serotypes to be detected will depend on the evolution of the epidemiologic and regulatory frameworks concerning VTEC infections. Details on each PT round are given in the following paragraphs:

### *2.1. Identification and typing of pathogenic* E. coli *(PT17)*

A study on strain identification and typing will be organized to verify and improve the performance of the NRLs in the identification and typing of VTEC and other groups of pathogenic *E. coli*. The study will include the identification of VTEC strains, as well as the identification of strains belonging to other patho-groups that have recently been associated to foodborne outbreaks, such as entero-aggregative *E. coli* (EAggEC) and

entero-invasive *E. coli* (EIEC). The strains to be included will be chosen taking into account the data on human infections collected and published by the ECDC.

The study will consist of two parts:

1. The identification of *E. coli* patho-groups by Real Time PCR amplification of the following target virulence genes:

- *vtx1* group and *vtx2* group for VTEC
- *eae* for EPEC
- *aaiC* and *aggR* for EAggEC
- *ipaH* for EIEC

2. Serogrouping of the VTEC strains identified, using both conventional and molecular methods.

***Objectives:*** *to maintain and improve the capacity of the NRLs to identify and type VTEC and other pathogenic* E. coli *strains.*

***Expected output:*** *capacity to identify and type VTEC and other pathogenic* E. coli *strains.*

***Performance indicators:*** *see FF.PT.1, FF.PT.2, FF.PT.3 and FF.PT.4 in the PI spreadsheet.*

***Duration****: 2016*

***2.2. PFGE typing of* E. coli *strains (PT-PFGE5 and PT-PFGE6)***

As described at point 3.5, the EU-RL will continue to support EFSA and the NRL network in the implementation of a database of molecular typing data for VTEC strains from animal and food sources. Such data, mainly PFGE profiles, will be provided to EFSA by the NRLs, and the activities toward the creation of the database include the organization of an external quality assessment (EQA) scheme to assess the quality of the PFGE profiles produced by the NRLs. This activity is crucial to maintain a good level of performance of the NRLs that will contribute the molecular typing data on VTEC to EFSA. The EQA program was initiated in 2013 and 4 PT rounds on PFGE typing have already been carried out. To continue the EQA program, new PT rounds on PFGE typing (PT-PFGE5 and PT-PFGE6) will be conducted each year in the period 2016-2017. PFGE will be carried out using the standard operating procedures for the production of PFGE profiles of VTEC recently published by EFSA (http://www.efsa.europa.eu/en/supporting/doc/704e.pdf). The NRLs will submit the PFGE profiles both as TIFF files and XML export files,

uploading directly the files in the Restricted Area of the Proficiency Tests Section of the EU-RL web page. The NRLs that don't have the BioNumerics software will upload only the TIFF image of the gel. The NRLs that have the BioNumerics software will analyze the TIFF image by themselves, using an *ad hoc* XML file provided by the EU-RL. Then they will upload both the TIFF file and the XML file in the website.

The performance of each NRL will be evaluated by assigning a score based on the number of PFGE profiles provided that will be considered suitable for inclusion in the upcoming EFSA database, according to the standard operating procedures for PFGE profiles interpretation and curation recently published by EFSA (http://www.efsa.europa.eu/en/supporting/doc/704e.pdf) and already applied within PT-PFGE3 and PT-PFGE4. Each participant will receive its own individual report with the performance evaluation, the critical assessment of the gel image, and the BioNumerics analyses carried out, together with suggestions on how to improve the performance.

***Objectives:*** *i) further evaluation of the capability of the NRLs to produce PFGE profiles suitable for the inclusion in the upcoming EFSA database of molecular typing data; ii) first evaluation of the capability of the NRLs to assign the profile bands using the BioNumerics software.*

***Expected output:*** *capacity to produce and analyze high quality PFGE profiles of* E. coli *strains.*

***Performance indicators:*** *see FF.PT.1, FF.PT.2, FF.PT.3 and FF.PT.4 in the PI spreadsheet*

***Duration****: 2016-2017*

### *2.3. Detection of VTEC in food (PT18 and PT20)*

The PT scheme on the detection VTEC in food will be continued and implemented with new matrices, in order to ensure a proper analytical response to the management of the risks posed by VTEC infections and to comply with the existing regulations (Reg. (EU) 209/2013) concerning VTEC in sprouts and sprouted seeds. Even though the choice of the matrices could be modified according to the evolution of the epidemiologic and regulatory frameworks, the matrices selected for the two PTs that will be carried out are bovine meat and sprouts.

#### *2.3.1. PT18: detection of VTEC in bovine meat*

Bovine meat represents a food category historically associated with VTEC infections.

Moreover, since 2012, the number of tests for VTEC detection on beef lots imported into the EU Member States from third countries has increased, as well as the border rejections due to the presence of these pathogens. Testing meat samples represents therefore an important task for the laboratories involved in the official controls on food. The proposed study will consist on the examination of artificially contaminated beef samples for the presence of VTEC strains using the ISO/TS 13136:2012 standard.

*2.3.2. PT20: detection of VTEC in sprouts*

Reg. (EU) 209/2013, amending Regulation (EC) 2073/2005 as regards microbiological criteria for sprouts, established that VTEC belonging to serotypes O157, O26, O103, O111, O145 and O104:H4 must be absent in sprouts placed on the market. Testing sprout samples represents therefore an important challenge for food and public health laboratories. The proposed study will consist on the examination of artificially contaminated sprout samples for the presence of VTEC strains belonging to the serogroups indicated in the Reg. (EU) 209/2013.

***Objectives:*** *to build up the capacity of the NRLs to detect VTEC contamination in relevant food matrices.*

***Expected output:*** *capacity to identify meat and sprout samples contaminated with VTEC.*

***Performance indicators:*** *see FF.PT.1, FF.PT.2, FF.PT.3 and FF.PT.4 in the PI spreadsheet.*

***Duration****: 2016 - 2017*

***2.4. Detection of VTEC in complex matrices: sprout spent irrigation water (PT19)***

As stated at point 1.1, Regulation (EU) No 209/2013 allows the testing of spent irrigation water as an alternative to testing sprouts. Based on activities that are ongoing and will continue in 2016 (see point 1.1), a SOP for the pre-treatment of spent irrigation water samples to be entered into the analytical flow of the ISO/TS 13136:2012 standard will be released by the EU-RL within 2016. The objective of PT19 will be a first assessment of the capacity of the NRLs to apply the SOP for testing irrigation water samples from different sprout species and with different physical and chemical characteristics.

***Objectives:*** *to build up the capacity of the NRLs to detect VTEC contamination in a complex matrix.*

***Expected output:*** *possibility to control VTEC contamination in the sprout production process by testing an alternative matrix.*

***Performance indicators:*** *see FF.PT.1, FF.PT.2, FF.PT.3 and FF.PT.4 in the PI spreadsheet.*

***Duration****: 2017*

### *2.5. Addressing underperforming related issues*

The performance of the NRLs participating in the PTs will be evaluated by using a score-based system, based on the assignment of penalty points to the incorrect results reported. Such penalty points will be used to identify the aspects of the NRLs proficiency that need to be improved and will be assigned according to the criteria defined for each PT and communicated to the participants with the invitation. The total score obtained by summing up the penalty points will be used to evaluate the performance of each NRL. In particular, the score will place the underperformance into one of three major groups, providing an objective parameter used to define the management measures to be adopted by the EU-RL VTEC, according to the following scheme.

- If the score corresponds to a “light” under-performance, it will be managed by sending backup samples to the NRL (follow-up PT).
- If the score corresponds to a “medium” under-performance, it will be managed by interviewing the NRL staff, with the aim of highlighting the problems encountered and identifying an adequate training program that best fit the NRL training needs. The NRL staff will be invited to attend a training session at the EU-RL, which will include the analysis of backup samples.
- If the score corresponds to a “heavy” under-performance, an on-site visit to the NRL will be added to the previous measures. The NRL could be requested to perform the assay during the on-site visit, if this will be considered as necessary by the visit team. This action will be agreed with the laboratory and placed in the agenda before the visit is carried out.

***Objectives:*** *to improve the capacity of the NRLs to detect VTEC contamination in food.*

***Expected output:*** *improved capacity of the NRLs to detect VTEC contamination in food.*

***Performance indicators:*** *see FF.PT.3 in the PI spreadsheet.*

***Duration****: 2016 – 2017*

## 3. Provision of scientific and technical assistance to the European Commission (EC) and other EU structures related with food safety

The EU-RL will continue to provide scientific and technical assistance to the Commission and to cooperate with EC structures and initiatives in the field of human and animal health and food safety. The following liaisons will be maintained and implemented:

### *3.1. Scientific and technical assistance to DG SANTE*

Scientific and technical support will be provided to DG SANTE for all the food safety issues related with VTEC or other groups of pathogenic *E. coli*. In particular, the EU-RL scientists will be available to assist the EC in the elaboration of documents and in facing crisis situations, with competences including: microbiologic criteria, microbiologic and molecular detection methods, epidemiology and outbreak investigation. The EU-RL VTEC staff will be ready to carry out any type of laboratory work on site.

***Objectives:*** *to support DG SANTE in managing any food safety issues related with* E. coli*.*

***Expected output:*** *scientific and technical support to DG SANTE**.***

***Performance indicators:*** *see FF.COM.1 and FF.COM.2 in the PI spreadsheet.*

***Duration:*** *2016 -2017*

### *3.2. The European Food Safety Authority (EFSA)*

The EU-RL VTEC will provide scientific and technical support to EFSA in building up the database of molecular typing data on VTEC strains isolated from food and animals (see details at the specific point 3.5).

In addition, the EU-RL will continue to provide scientific and technical advice to any EFSA initiative in the field of *E. coli*, including the evaluation of specific issues or the implementation of monitoring programs by the EFSA Task Force on Zoonoses Data Collection, according to the document "*Technical specifications for the monitoring and reporting of VTEC on animals and food on request of EFSA*" (*EFSA Journal; 7(11): 1366*). The EU-RL scientists will be available to participate in EFSA working groups upon invitation. Upon request, the EU-RL will support EFSA in the analysis and interpretation of monitoring data on VTEC reported by the EU Member States in accordance with Directive 2003/99/EC and will support EFSA in the preparation of the relative chapter of the annual European Union summary report on zoonoses and food-borne outbreaks.

The expenses for participation in EFSA working groups and meetings are usually covered by EFSA and will not be included in the EU-RL budget.

***Objectives:*** *to provide scientific and technical support to EFSA on any food safety issues related with pathogenic* E. coli*.*

***Expected output:*** *scientific and technical support to EFSA.*

***Performance indicators:*** *see FF.COM.1 and FF.COM.2 in the PI spreadsheet.*

***Duration:*** *2016 -2017*

***3.3 The European Committee for Standardization (CEN), Technical Committee 275 – Food analysis – Horizontal methods, WG 6 – Microbial contamination.***

The EU-RL VTEC will continue to participate in the CEN/TC275/WG6, managing the current projects on *E. coli* (see the following points), and will be ready to assume the leadership of any new project dealing with pathogenic *E. coli*.

In particular, the EU-RL will coordinate the revision of the ISO/TS 13136:2012 after three years from its publication. Such a revision has already been agreed during the 2015 CEN/TC/275/WG6 general meeting (Recommendation N. 393). The CEN TC 275 WG6 will launch a call for experts and will request the formal revision of the technical specification that will be developed as a full standard (EN ISO standard) under the leadership of the EU RL VTEC. Additionally, after the revision it will be notified to ISO/TC147/SC4 “Water quality - Microbiological methods" that the scope would be broadened to include analysis of irrigation water for vegetables including sprouts.

The EU-RL VTEC will also prepare the Final Amendment to the ISO 16654:2001, which will contain the performance parameters of the method, obtained in the collaborative studies on milk and sprouts carried out in 2012 and 2014, respectively. The first draft of the amendment, prepared by the EU-RL, was adopted by CEN during the 2015 CEN/TC275/WG6 general meeting with a few comments. During 2016, the EU-RL will prepare the final version and will follow the publication process.

In 2016, the EU-RL will continue the work initiated in 2015 in collaboration with the *Netherlands Food and Consumer Product Safety Authority* to define the performance parameters of the DNA extraction procedures included in the ISO standards based on PCR, including the ISO/TS 13136:2012. The data produced will be included in a document on the standardization of the quality of the nucleic acid to be used in the following steps of PCR amplification, aimed at facilitating the accreditation of the PCR-based methods.

***Objectives:*** *i) to coordinate the CEN projects on methods dealing with pathogenic* E. coli*; ii) to coordinate the revision process of* the ISO/TS 13136:2012.

***Expected output:*** *i) revision of the ISO/TS 13136:2012 standard; ii) definition of the performance parameters of the ISO 16654:2001 for sprouts.*

***Performance indicators:*** *see FF.CEN.1 and FF.CEN.2 in the PI spreadsheet.*

***Duration:*** *2016 - 2017*

### *3.4. The European Centre for Disease Control (ECDC) Food- and Waterborne Diseases (FWD) Program*

The EU-RL will continue the liaison with the ECDC FWD Program, with the aim of ensuring connection and activity harmonization between this network and the network of Reference Laboratories in the veterinary and food safety fields (Regulation (EC) No. 882/2004). In particular, the forthcoming EFSA database of molecular typing data on VTEC strains isolated from non-human sources will be structured according to the procedures that ECDC has developed for its own repository of molecular typing data on strains from human infections (see point 3.5).

The EU-RL VTEC will also continue the liaison with the ECDC reference laboratory for VTEC infections (the WHO International *Escherichia* and *Klebsiella* Centre of the Statens Serum Institut, Copenhagen), which is in charge of the external quality assessment activities for the network. This will allow the harmonization of the identification and typing schemes, making the respective monitoring programs and databases compatible for comparison of human and non-human data.

Upon request, the staff of the EU-RL will continue to participate in the ECDC committees and working groups. In particular:

- Dr. Rosangela Tozzoli will participate in the *FWD Network Coordination Committee*. The Committee supports the development of the FWD Network by providing advise on all aspects of its work, including surveillance, prevention and control or any other technical, epidemiological or scientific aspects, thus enabling the network to improve its effectiveness and added value.
- Dr. Valeria Michelacci will participate in the "*Ad hoc Expert Group on the introduction of next generation typing methods for Food- and Waterborne Diseases (FWD NEXT)"*. The Group, which has the task to promote the smooth transition of genotyping competence in EU/EEA Member States from traditional typing methods to whole genome sequence-based methods. The Group will also evaluate the impact of non-culture methodologies on diagnostics and,

subsequently, on surveillance.

***Objectives:*** *i) to harmonize the identification and typing schemes for pathogenic* E. coli *used in the monitoring programs carried out by the medical and veterinary networks of NRLs; ii) to ensure the harmonization of the forthcoming database of molecular profiles of VTEC of human and non-human origin; iii) to support the ECDC coordination and working groups*.

***Expected outputs****: i) shared protocols for identification and typing of pathogenic* E. coli *of human and non-human origin; ii) scientific and technical support to ECDC.*

***Performance indicators****: see FF.COM.1 and FF.COM.2 in the PI spreadsheet****.***

***Duration:*** *2016 - 2017*

### *3.5. Support to EFSA and the NRL network in the implementation of a database of molecular typing data for VTEC strains from animal and food sources*

In 2012, DG SANCO decided to organize the collection of molecular typing data for isolates of VTEC, Listeria and Salmonella from food and animals, to improve the surveillance and trace-back of food-borne infections at the national, European and international level, as well as the preparedness to face foodborne outbreaks. The responsibility of the management of the related database was assigned to EFSA, with the scientific and technical support of the relevant EU-RLs. The EU-RL VTEC will therefore support EFSA with the following initiatives.

#### *3.5.1. Curation of the EFSA database of PFGE profiles of VTEC from food and animals*

According to the terms of reference of the mandate assigned to EFSA by DG SANCO, the NRLs will provide molecular typing data on VTEC isolates from food and animals to EFSA. The EU-RL shall take care of the curation of the database. The curation process will be accomplished according to the SOPs specifically developed by the EU-RL in conjunction with EFSA and will include:

- The assessment of the quality of any PFGE image provided and its acceptance for inclusion in the database.
- The assessment of the gel normalization and the correct band assignment
- The evaluation of variations in normalization and band assignment through the whole process.
- The process of nomenclature assignment by matching the profile to reference

types.

- The analyses for cluster detection.

In 2016, the EU-RL VTEC staff members involved in the curation will have a meeting with the staff members of the EU-RL Listeria and the EU-RL Salmonella who take care of the curation of the Listeria and Salmonella EFSA databases of molecular profiles, to better harmonize the application of the curation SOPs of the respective databases.

*3.5.2. PFGE Training*

To increase the number of NRLs capable to submit PFGE profiles and to improve the quality of the submitted profiles, the EU-RL will continue to offer the possibility of specific training to the NRLs, through short-term visits for individual training (see point 1.8.4).

*3.5.3. External quality assessment (EQA) for PFGE*

To verify the capability of the NRLs to perform PFGE and the quality of the profiles produced, the EQA program, initiated in 2013, will be continued. Two new PT rounds for PFGE typing (PT-PFGE5 and PT-PFGE6) will be conducted (see point 2.2). If possible, the PT will be carried out jointly with the ECDC-FWD network involved in the typing of VTEC strains from human infections.

***Objective:*** *i) to support EFSA in the management of the database of molecular profiles of VTEC of non-human origin*; *ii) to build up the capacity of the NRLs to produce PFGE profiles of* E. coli *suitable for inclusion in the database and comparison.*

***Expected outputs:*** *i) implementation of the PFGE database for VTEC strains from food and animals; ii) NRL capacity to produce high quality PFGE profiles of* E. coli *strains.*

***Performance indicators:*** *see FF.COM.1 and FF.COM.2 in the PI spreadsheet.*

***Duration:*** *2016 - 2017*

## 4. Consolidation of the EU-RL structures

The EU-RL VTEC will continue to carry out its tasks in the framework of its management system, which is constantly improved through the use of the quality policy, according to its accreditation EN/ISO IEC 17025:2005 (N. 0779) obtained in 2007 from the Italian accreditation body (ACCREDIA). Beside the management of the laboratory, the accreditation covers the methods for detection and typing of

VTEC related with EU-RL's tasks and activities. The possibility to submit additional methods for accreditation is continuously evaluated.

### *4.1. Staff*

The permanent staff of ISS will continue to devote significant working time to the EU-RL's activities. Five persons, already hired with EU-RL funds, will continue to work full time at the EU-RL-related activities with the status of "temporary staff employees". These will include a Post Doc scientist skilled in food microbiology and molecular detection methods, a Post Doc scientist skilled in phenotypic and genotypic bacterial typing methods, a Post Doc scientist skilled in molecular typing and bioinformatics, who will be in charge of the curation of the EFSA database of molecular profiles of VTEC from food and animals, a laboratory technician skilled in PT organization, quality assurance systems and equipment maintaining, and a technical management assistant fluent in English language and skilled in managing EC grants and scientific meeting organization. The qualification of the staff is continuously maintained and improved through specific procedures.

***Objective:*** *to appropriately cope with the EU-RL tasks.*

***Expected output:*** *involvement of experienced and skilled staff in EU-RL activities.*

***Performance indicators:*** *see FF.PT.QI, FF.ANA.QI, FF.NRL.QI, FF.COM.QI, FF.CEN.QI and FF.R&D.QI in the PI spreadsheet.*

***Duration:*** *2016 - 2017*

### *4.2. Administration and reporting*

The EU-RL will continue to manage the administration procedures related with the purchasing of materials, the shipment of reference materials and proficiency test samples, the missions of the staff, the reimbursement of the NRL representatives entitled to reimbursement for their participation in the annual workshop, courses and laboratory training periods. The general activity reports will be prepared, as well as those specific for the inter-laboratory studies and other actions reportable to both the EC and the NRLs network.

***Objective:*** *support to the EU-RL activities and communication of the results obtained.*

***Expected output:*** *efficient organization of the EU-RL activities and timely production of high quality reports.*

***Performance indicators:*** *no reports rejected by the EC or delivered beyond deadline (adequacy of the reports and timely delivery). Workshop, PT and courses*

*report published in the web site.*

***Duration:*** *2016 - 2017*

### *4.3. Definition of ex-ante performance indicators and their ex-post evaluation*

The EU-RL VTEC will continue to establish ex-ante indicators to measure the performance of its main activities. The performance indicators (PI) of each activity are defined according to the guidelines provided by DG SANTE and are attached to the work programs. The PI ex-post evaluation is carried out at the end of the year and the results are attached to the scientific and technical report sent to DG SANTE. However, partial ex-post evaluations are also carried out at the end of each activity (*e.g.* the annual workshop or a PT round), to adopt promptly possible adjustments of the activity organization.

***Objective:*** *evidence-based evaluation of the EU-RL activities*

***Expected output:*** *constant improvement of the EU-RL activities.*

***Performance indicators****: no ex-ante performance indicators list and ex-post evaluation rejected by the EC. See also FF.NRL.3.*

***Duration:*** *2016 - 2017*

### *4.4. Update of the infrastructure and equipment available for the EU-RL activities*

As stated at point 1.4, the EU-RL VTEC is doing efforts in developing NGS platforms and bioinformatics tools specifically dedicated to the genomics of pathogenic *E. coli,* and to make them available for the NRLs that begin to use these approaches. In this respect, the related technical infrastructure and equipment available at the EU-RL will be updated and potentiated through the acquisition of the following equipment.

- A blade server to be added to the ISS cluster of servers currently supporting the computational power of ARIES, the bioinformatics framework for the analyses of high intensity data from NGS, developed by the EU-RL with the collaboration of the IT services of ISS (see points 1.4 and 1.8.3). The additional server unit will increase the computational resources that will be made available to the NRLs for NGS-based identification and typing of pathogenic *E. coli*.
- A Network Attached Storage (NAS) unit to ensure the availability of a backup facility storage for the data uploaded by the NRLs on the bioinformatics framework ARIES. Currently, the data are stored on the ISS storage central units (50 Gb granted to each user) without security backup and the users from the NRLs are required to store the data on their own devices for security reasons.

The adoption of a backup unit based on the NAS technology will assure a safe storage of the NRL data.

- An automated workstation for the preparation of NGS libraries, to be used for the whole genome sequencing of pathogenic *E. coli*. This equipment will allow the optimization of the operational fluxes for this critical step of the NGS technology.

***Objective:*** *to appropriately cope with the EU-RL tasks.*

***Expected output:*** *use of state-of-art equipment for EU-RL activities.*

***Duration:*** *2016 - 2017*

### *4.5. Maintaining and implementing the EU-RL VTEC web site*

The website of the EU-RL VTEC (http://www.iss.it/vtec) will be maintained and updated on a regular basis with documents, methods, workshops and inter-laboratory studies reports, information on the NRLs and links. The “Restricted Area” will be used for the on-line submission of the results of the inter-laboratory studies.

The new section named "Focus on", where highlights on the *E. coli* subject are added regularly, will be further implemented.

***Objectives:*** *to continuously implement a tool for: i) rapid dissemination of the EU-RL activities* and *the communication on follow up of research on VTEC; ii) collection of proficiency testing results from the NRLs;*

***Expected output:*** *i) improved communication with the EC and the NRLs; ii) improved collection of data and requests from the NRLs.*

***Performance indicators:*** *evaluation of the number of contacts to the different sections. See also FF.R&D.1 in the PI spreadsheet.*

***Duration:*** *2016 - 2017*

## 5. Missions

The following missions may be needed in the period 2016 – 2017.

### *5.1. 2016 missions*

- Participation of a EU-RL representative in meetings of the Technical Working Group for Microbiological Criteria of the Standing Committee on the Food Chain and Animal Health, or other meetings, upon request of DG SANTE. Estimation: two meetings at the EC offices in Brussels.
- A visit to one NRL, upon agreement with the interested country.

- Participation of a EU-RL representative in the 22nd CEN/TC275 WG6 annual plenary meeting (Paris, June 2016).
- Mission of two EU-RL representative to the EU-RL Listeria, at the ANSES headquarter in Maison Alfort (Paris, France) to participate as teachers in the joint training course for the NRLs on the use of the software package BioNumerics (see point *1.8.2*). After the course, the two EU-RL representatives will take part in a half-day meeting with the colleagues of the EU-RL Listeria and the EU-RL Salmonella who take care of the curation of the Listeria and Salmonella EFSA databases of molecular profiles. The objective of the meeting will be a better harmonization of the application of the SOPs for the curation of the respective databases (see point *3.5.1*).

***5.2. 2017 missions***

- Participation of a EU-RL representative in meetings of the Technical Working Group for Microbiological Criteria of the Standing Committee on the Food Chain and Animal Health, or other meetings, upon request of DG SANTE: Estimation: a meeting at the EC offices in Brussels.
- A visit to one NRL, upon agreement with the interested country.
- Participation of a EU-RL representative in the 23rd CEN/TC275 WG6 annual plenary meeting (Place to be defined, June 2017).

***Objective:*** *to maintain and strengthen the institutional and scientific relationships of the EU-RL.*

***Expected output:*** *i) support to DG SANTE; ii) scientific networking; ii) visibility of the EU-RL activities.*

***Performance indicators:*** *see FF.COM.1, FF.COM.2, FF.CEN.1, FF.CEN.2 and FF.NRL.4 in the PI spreadsheet.*

***Duration****: 2016 - 2017*

## 6. Other activities not co-financed under the EU-RL budget

Upon request, the EU-RL VTEC will continue to be available to cooperate with initiatives undertaken by bodies and technical assistance and training instruments of the EC, such as the Technical Assistance and Information Exchange Instrument (TAIEX) and the Better Training for Safer Food (BTSF) initiative.

***Objectives:*** *to contribute specific competences to EC training programs*

***Output:*** *support to EC training programs with specific competences on VTEC and the organization of reference laboratories.*

***Performance indicators****: See FF.CEN.1 and FF.CEN.2 in the PI spreadsheet.*

***Duration****: 2016 - 2017*

**September 25, 2015**

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