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

Work Programme

1st January - 31st December, 2015

The work programme of the EU-RL for *E. coli* (EU-RL VTEC) for the year 2015 will consist of the following activities, listed according to the responsibilities laid down by the Article 32 of Regulation (EC) No 882/2004.

1. **Provision of analytical methods, including reference methods, to the NRLs.**
   - ***1.1. Evaluation of the ISO TS 13136:2012 for testing sprout irrigation water***
   - ***1.2. Development of a MLVA protocol for molecular typing of non-O157 VTEC***
   - ***1.3. Applied research and development activities to improve molecular methods for the detection and typing of VTEC***
2. **Coordination of the application of analytical methods by the NRLs and organization of comparative testing.**
   - ***2.1. Support to the NRL for the accreditation of methods for pathogenic* E. coli**
   - ***2.2. Proficiency testing***
     - 2.2.1. *Detection of VTEC in food samples*
     - 2.2.2. *Identification and typing of pathogenic* E. coli *strains*
     - 2.2.3. *PFGE typing of* E. coli *strains*
3. **Training for the benefit of staff from the NRLs and of experts from developing countries**
   - ***3.1. Annual Workshop with the NRLs***
   - ***3.2. Training***
   - ***3.3. Assistance to NRLs***
4. **Provision of scientific and technical assistance to the Commission and other EU structures related with food safety**
   - **4.1. *Scientific and technical support to DG SANCO***
   - **4.2. *The European Food Safety Authority (EFSA)***
   - **4.3. *The European Committee for Standardization (CEN)***
   - **4.4. *The European Centre for Disease Prevention and Control (ECDC)-Food- and water-borne diseases (FWD) program***
5. **Collaboration with laboratories responsible for analyzing feed and food in third countries.**
   - ***5.1. Central Laboratory for Food Analysis (QCAP), Egypt***
   - ***5.2. The National Food Safety and Quality Service (SENASA), Argentina***

The objectives, and the expected outputs of each action are indicated, as well as its duration, which will be either limited to 2015 or multi-annual (ongoing programme). Performance indicators are indicated where appropriate, making reference to the PI spreadsheet that is attached to this programme.

1. Provision of analytical methods, including reference methods, to the NRLs.

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 the irrigation of the sprouts. 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 the sprouts, can make such a filtration difficult. The EU-RL will evaluate different techniques for the manipulation of spent irrigation water, in order to find the most appropriate for the detection of VTEC using the ISO TS 13136:2012 standard. The methods for treatment of spent water samples will range from a simple centrifugation to the filtration using filter aids. Once a suitable procedure will be defined, a SOP for spent irrigation water manipulation will be prepared and a number of NRLs for *E. coli* will be voluntarily enrolled for an assessment of its performance parameters.

Objectives: *to provide to 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 suitable procedure to detect VTEC contamination in sprout irrigation water.*

Performance indicators: *SOPs, NRLs enrolled for the study on the performance parameters. See also FF.PT.5 in the PI spreadsheet.*

Duration*: 2015-2016*

1.2 Development of a MLVA protocol for molecular typing of non-O157 **VTEC*.***

Among the methods for molecular typing of VTEC and other enteric pathogens for outbreak detection and epidemiological surveillance, PFGE still represents the gold standard. However, multi-locus variable number of tandem repeat analysis (MLVA) has been successfully used to further differentiate strains of VTEC O157 and *Salmonella* Typhimurium that presented the same PFGE type. MLVA for VTEC typing has several advantages (rapid, not labour-intensive, amenable to inter-laboratory comparisons and to high-throughput), but also a major drawback: A suitable procedure is available for VTEC O157 typing only. The need to use different protocols for typing the different VTEC serogroups hindered the development of molecular surveillance programs based on this approach. An MLVA scheme was recently published for the typing of VTEC O26 strains (Løbersli et al, 2012). However, although scientifically plausible, such a scheme is far from being ready to be widely used within laboratory networks dedicated to surveillance activities. The EU-RL will evaluate the possibility to adapt some of the MLVA methods described in the literature for typing of *E. coli* types other than VTEC to the typing of non-O157 VTEC as a whole. Such a single method, to be set aside the VTEC O157 MLVA, could represent a complementary approach to PFGE for typing VTEC non-O157, in view of the shift of the typing technology from restriction fragment length polymorfism (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, partial evaluation by comparison with PFGE. See also FF.PT.5 in the PI spreadsheet.*

Duration*: 2015-2016*

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

The EU-RL 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. Collaborations have been established with laboratories of Public Health England (PHE), London, UK and the Oklahoma State University, Oklahoma city, OK, USA on the use of whole genomic sequencing to investigate the phylogenesis and the molecular epidemiology of VTEC strains belonging to the most pathogenic serogroups and the development of a MLVA scheme dedicated to non-O157 VTEC typing, respectively. The results of such collaborations should help in understanding the dynamics and the transmission routes of particular VTEC types, such as the VTEC O26 belonging to sequence type 29, and in deploying adequate methods for strain typing.

An additional study will be carried out with the University of Rome "*La Sapienza*" on the characterisation of a new clone of Enteroinvasive *E. coli* (EIEC), which is circulating in Europe. Such a study will have as the main objective the identification of the molecular features of such a virulent EIEC clone in order to understand its epidemiology and to set up the molecular target for its detection in food.

Objectives: *i) to improve the knowledge of the pathogenetic mechanisms of VTEC 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.*

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

Performance indicators: *papers on peer reviewed journals, whole genomes of VTEC and other pathogenic* E. coli *determined and released, procedures for whole genome-based cluster detection, typing methods for* E. coli *including VTEC.*

see FF.R&D.1 and "other activities" in the PI spreadsheet.

Duration*: ongoing*

2. Coordination of the application of analytical methods by the NRLs and organization of comparative testing

2.1. Support to the NRLs for the accreditation of methods for 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. A new version of the ISO 16654:2001 containing the performance parameters of the methods with all the matrices will be produced in 2015 for the publication by ISO.

The collaborative study on sprouts conducted in 2014 was also used to determine the performance parameters of the CEN ISO/TS 13136 method for the detection of VTEC in food. Those data will be included in a revision of the document "*EU-RL VTEC_Method_performance_CEN ISO/TS_13136:2012_Rev.0*" and published on the EU RL VTEC website.

The same document will also be updated with data on the performance of the method for the detection of *E. coli* genes specifying the serotype O104:H4, following an internal validation of the method.

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.ANA.1 and FF.ANA.2, in the PI spreadsheet.*

Duration*: 2015*

2.2. Proficiency tests (PTs)

Three PT rounds are planned for 2015: *i*) a study on the detection of VTEC in sprout samples; *ii*) a study on the identification and typing of pathogenic *E. coli* strains; *iii*) a 4th round of proficiency testing for PFGE typing, that will be conducted on the same *E. coli* strains of the previous *ii*) study.

2.2.1. Detection of VTEC in sprout samples

In March 2013, Reg. (EU) 209/2013, which amends Regulation (EC) 2073/2005 as regards microbiological criteria for sprouts, was published and introduced for the first time in the EU legislation a microbiologic criterion for VTEC. In particular, the regulation established that VTEC belonging to serogroups O157, O26, O103, O111, O145, and O104 must be absent in sprouts placed on the market. Reg. (EU) 209/2013 also prescribed that the CEN ISO TS 13136 method for the detection of VTEC in food has to be used for the analyses.

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. The 2014 study will be designed considering a sprouts mixture different from that used in the study run in 2013, in order to extend the sprout species assayed with the ISO TS 13136:2012 standard.

Objectives: *to build up the capacity of the NRLs to detect VTEC contamination in a matrix for which a microbiologic criterion has been included in the EU legislation.*

Expected output: *capacity to identify 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*: 2015*

2.2.2. Identification and typing of pathogenic E. coli

To verify and improve the performance of the NRLs in the identification and typing of VTEC and other groups of pathogenic *E. coli*, a study on strain identification and typing will be organized. The study will include the identification of VTEC strains, as well as the identification of strains belonging to other patho-groups.

The study will consist of 2 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
- *lt*, *sth,* and *stp* for ETEC
- *ipaH* for EIEC

2. Serogrouping of the VTEC strains identified.

Objectives: *to build up 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*: 2015*

2.2.3. PFGE typing of E. coli strains

As described at point 6, the EU-RL is supporting EFSA in the development of a database of molecular typing data on VTEC strains isolated from non-human 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 verify the quality of the PFGE profiles produced by the NRLs.

Therefore, a 4th PT round for PFGE typing (PT-PFGE4) will be conducted on the same *E. coli* strains sent for the above mentioned study on identification and typing.

The strains will be examined using the standard operating procedure (SOP) for PFGE produced by the EU-RL VTEC and based on the protocol in use in the PULSENET international network and adopted also by the European Centre for Disease Control and Prevention (ECDC) for its molecular surveillance program. The *E. coli* strains will be assayed together with a *Salmonella* Braenderup strain, provided by the EU-RL upon request, which is the control strain used for PFGE gels normalization and comparison of profiles obtained in different laboratories. The images of the gels will be submitted to the EU-RL and analyzed for the technical features affecting their suitability for inclusion in the general database and inter-strain

comparison, according to the criteria established in a specific SOP produced by the EU-RL VTEC.

Objectives: *to build up the capacity of the NRLs to produce PFGE profiles of* E. coli *suitable for inclusion in the European repository of molecular profiles and comparison managed by EFSA.*

Expected output: *capacity to produce 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*: 2015*

3. Training for the benefit of staff from the NRLs and of experts from developing countries

3.1. ***Annual Workshop with the NRLs***

The 10th annual workshop will be held in the second half of 2015 in Rome. In alternative, upon agreement with DG SANCO, one of the NRLs could host the workshop at its own Institute. The results of the 2014 -2015 inter-laboratory studies will be presented and discussed. The training program 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 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 ECDC will be invited. The workshop 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 6. The level of satisfaction of the participants toward the workshop organization, the proposed topics, and the quality of presentations will be evaluated by a questionnaire. 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, and FF.NRL.3 in the PI spreadsheet*

Duration*: 2015*

3.2. Training

3.2.1. Short-term 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 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 6). 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.

3.2.2. Basic training course on bioinformatics

The rapid development of next generation sequencing (NGS) platforms, which are becoming more and more affordable, and the parallel development of bioinformatics for NGS data management and analyses make the genome sequence-based typing approach a realistic alternative to PFGE and its designated successor for molecular surveillance systems of VTEC infections. Large discussion groups have been activated in the last few years, sponsored by organizations like the Organisation for Economic Co-operation and Development (OECD), or organized by EFSA at the European level, aiming at defining the framework for such a technology to be routinely applied to pathogens typing. ECDC is also activating a group, called NEXT, with the same purpose of defining the most appropriate context for the introduction of NGS as the standard technology for molecular surveillance in the field of human infections.

A recent inventory of the molecular typing methods and IT applications available within the network of the NRLs for *E. coli* showed that 7 NRLs already have the access to NGS facilities. To consolidate the knowledge on the NGS technology and to increase the level of skill and awareness within the network, a course on the use of bioinformatics for assembling, mining, and analysing NGS data will be organized. The course will be held in the second half of 2015 at the *Istituto Superiore di Sanità* (ISS) in Rome. The ISS IT Service will make available a didactic room equipped with 15 computer workstations. The course will have a hands-on approach and two days duration. It will be focused on the main features and the basic use of the open-source bioinformatics framework Galaxy, developed under the auspices of the *Generic Model Organism Database* project, a collection of open source software tools for managing, visualising, storing, and disseminating genetic and genomic data. The travel and accommodation costs for at least 8 participants from NRLs will be covered by the EU-RL funds. Additional participants, coming on their own expenses, may be accommodated to a maximum of 15.

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

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.*

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

Duration: *2015*

3.3. Assistance to NRLs

The EU-RL-VTEC will continue to assist the NRLs in the field of VTEC detection and typing, providing methods and standard operating procedures via the web site, 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 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.

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.PT.3, FF.PT.5, FF.PT.6, FF.ANA.1 in the PI spreadsheet.*
Duration: *ongoing*

4. Provision of scientific and technical assistance to the Commission 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:

4.1. Scientific and technical assistance to DG SANCO

The EU-RL VTEC will continue to provide scientific and technical support to DG SANCO 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 SANCO in managing any food safety issues related with* E. coli*.*
Expected output: *scientific and technical support to DG SANCO.*
Performance indicators: *see FF.COM.1 and FF.COM.2 in the PI spreadsheet.*
Duration: *ongoing*

4.2. 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 point 6).
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. 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 the molecular typing database of VTEC strains and 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: *ongoing*

4.3 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*.

The EU-RL VTEC will present the draft revision of the standard ISO 16654:2001 at the next CEN/TC/275/WG6 general meeting, scheduled for June 2015 in the Netherlands. CEN/TC275/WG6 also recommended to include the performance parameters obtained for the ISO/TS 13136 in the collaborative study on sprouts (see point 2.1) in a revision of the technical specification as an Annex. In order to make such performance parameters more readily available to the laboratories involved in the official control of food, CEN/TC275/WG6 also recommended to add them to the performance parameters of the ISO/TS 13136:2012 already listed in the technical document published in the EU-RL website (see point 2.1). The first draft of the Annex to be included in a revision of the ISO/TS 13136:2012 will be presented at the next 2015 CEN/TC275/WG6 general meeting.

An active participation of the EU-RL VTEC has also been requested for the production of a document containing the performance parameters of the DNA extraction procedures included in the ISO standards based on PCR, including the ISO/TS 13136:2012. Such a document will have the scope to standardise the quality of the nucleic acid to be used in the following steps of PCR amplification and to facilitate the accreditation of the PCR-based methods. In this respect, in 2015 the EU-RL VTEC will carry out a study in collaboration with the EU-RL for monitoring the viral and bacteriological contamination of bivalve molluscs and with the Netherlands Food and Consumer Product Safety Authority. The study will provide measurable

parameters for this specific method's segment, to be included in the new PCR-based ISO standards and in the revision of the existing ones.

Objectives: *to coordinate the CEN projects on methods dealing with pathogenic* E. coli*;*

Expected output: *i) validation report for the method EN/ISO 16654 for* E. coli *O157 in the sprout matrix; ii) performance parameters of the ISO/TS 13136:2012 for sprout.*

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

Duration: *2015*

4.4. 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 is developing for its own repository of molecular typing data on strains from human infections (see point 6).

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.

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*.

Expected output: *shared protocols for identification and typing of pathogenic* E. coli *of human and non-human origin.*

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

Duration: *ongoing*

5. Collaboration with laboratories responsible for analyzing feed and food in or carrying out investigations on *E. coli* in third countries

5.1. Central Laboratory of Residue analysis of Pesticides and Heavy Metals in Food (QCAP), Egypt.

The EU-RL VTEC began its relationship with QCAP after the outbreak of VTEC O104:H4 infections in 2011, associated with sprouts produced with seeds imported from Egypt. To improve the capability of testing food for VTEC, QCAP scientists performed training visits at the EU-RL in 2011 and 2014, and the laboratory received reference materials and was included in the PT organized between 2012 and 2014. Dr. Morabito, from the EU-RL VTEC, has now been invited to audit the microbiological testing activity of QCAP, with emphasis on *E. coli* testing, in order to assess the fulfilment of the prescriptions laid down in the ISO 17025. Additionally, he has been asked to carry out in house training sessions concerning the EU legislation on food safety and the technical aspects of molecular testing of food samples. The audit and the training visit are scheduled for the end of year 2014, but the collaboration with QCAP will be extended to 2015, with provision of advice, reference materials, external quality assessment and possible additional on-site visits in order to help QCAP in the establishment and accreditation of the methods for testing food for the presence of pathogenic *E. coli.*

In addition, Dr. Morabito will assume the tutorship of a PhD student from QCAP, who will be assisted in developing a doctoral project dealing with the detection and characterisation of pathogenic *E. coli* in the water of canals derived from the Nile River and used for crop irrigation.

The expenses related to these activities will be covered by QCAP funds and will not be included in the EU-RL budget.

5.2. The National Food Safety and Quality Service (SENASA), 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. In 2013, Dr. Susana Binotti, form the SENASA *Dirección General de Laboratorio y Control Técnico*, contacted the EU-RL to receive advice on the analytical methods

used in the EU for the detection and characterization of VTEC in food. The EU-RL provided protocols, reference materials and technical advice, and in 2014 SENASA participated in the PT organized by the EU-RL on the detection of VTEC in sprouts. The collaboration will continue in 2015, with provision of advice, reference materials, and external quality assessment, in order to help SENASA in the establishment and accreditation of the methods for testing food for the presence of pathogenic *E. coli.*

5.3. Ahmadu Bello University, Nigeria

In the period May-July 2014, the EU-RL VTEC hosted Mr. Kabiru Lawan, a PhD student from the Department of Veterinary Public Health and Preventive Medicine, of the Ahmadu Bello University, Zaria, Nigeria, for a three months stage as visiting scientist.

During the stage, the EU-RL offered Mr. Lawan training on the detection and typing of *E. coli* and collaborated in the characterisation of strains isolated from animals and from samples taken at one of the largest abattoirs in the region and the surrounding environment. During the visit, a manuscript concerning this piece of work has been prepared and submitted for publication. From this experience, a further scientific collaboration was planned for 2015. The aim is the study of the biological cycles of pathogenic *E. coli* in a developing country setting, characterised by the presence of a large slaughterhouse spilling effluents in a water stream used to irrigate crops by the nearby farms. The study could help in understanding the mechanisms that may favour the emergence of pathogenic *E. coli* with shuffled virulence features, such as the *E. coli* O104:H4 that caused the sprout-associated outbreak in Germany in 2011. The expenses generated from this scientific work will be supported by the Ahmadu Bello University, Zaria, Nigeria, with no charge on the EU RL Budget.

Additional expenses related to these activities, including possible new visits of Mr. Lawan to the EU-RL Rome, will be covered by Ahmadu Bello University funds and will not be included in the EU-RL budget.

Objectives: *i) to provide technical and scientific assistance to competent laboratories in third countries; ii) to support investigations on* E. coli *in third countries.*

Expected output: *harmonization of detection an typing protocols with third countries exporting food products to EU.*

Performance indicators: *methods for pathogenic* E. coli *accredited by partners,*

joint scientific papers. See also FF.NRL.5 and FF.CEN.1 in the PI spreadsheet.
Duration: *ongoing*

6. 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. Therefore, the EU-RL VTEC will support EFSA with the following initiatives.

6.1. 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 begin to provide molecular typing data to EFSA in the late 2014. The EU-RL shall take care of the curation of the database. The curation process will be accomplished according to the standard operating procedures (SOPs) specifically developed in 2014 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.

Objective: *to support EFSA in the management of the database of molecular profiles of VTEC of non-human origin*.
Expected output: *implementation of the PFGE database for VTEC strains from food and animals.*
Performance indicators: *all the suitable PFGE profiles submitted to EFSA by the NRLs included in the database. See also FF.COM.1 and FF.COM.2 in the PI*

spreadsheet.

Duration: *ongoing.*

6.2. Training for PFGE

To increase the number of NRLs capable to submit PFGE profiles and to improve the quality of the 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 3.2.1).

6.3. 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. A 4th PT round for PFGE typing (PT-PFGE4) will be conducted (see point 2.2.3). 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: *to build up the capacity of the NRLs to produce PFGE profiles of* E. coli *suitable for inclusion in the database and comparison.*

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

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

Duration: *2015 and ongoing*

7. 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.

7.1. 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.

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 in the PI spreadsheet.*

Duration: *ongoing*

7.2. 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 participating in the annual workshop and of the visiting scientists entitled to reimbursement for their 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).*

Duration: *ongoing*

7.3. Maintaining and implementing the EU-RL VTEC web site

The web site 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. A new section termed "EU-RL Services" will be available as an interface with NRLs to request services such as the provision of reference materials, training stages etc. The user interface will be based on a form to be filled on-line after choosing from a pop-up menu the service of interest.

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; iii) to collect requests for services.*

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

Performance indicators: *increase in the number of contacts with respect to 2014. See also FF.R&D.1 in the PI spreadsheet.*

Duration: *ongoing*

8. Missions

The following missions may be needed in 2014:

- 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 SANCO: two meetings in Brussels.
- A visit to one NRL is planned for 2015, upon agreement with the EC and the interested country.
- Participation of a scientist in the 20th CEN/TC275 WG6 annual plenary meeting (The Netherland, June 2015).
- Participation of a scientist in the 9th *International VTEC Symposium (VTEC 2015)* that will be held in Boston in September 2015 (http://vtec2015.com/), to present the activity of the EU-RL in the field of detection methods. VTEC Symposia are held every three years and represent the main international scientific event on VTEC infections.

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

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

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

Duration*: 2015*

9. Other activities not co-financed under the EURL budget

The EU-RL VTEC will continue to be available to cooperate with initiatives undertaken by two EC technical assistance and training instruments: the Technical Assistance and Information Exchange Instrument (TAIEX) and the Better Training for Safer Food (BTSF) initiative. In particular:

- Dr. A. Caprioli is registered in the TAIEX expert database for issues related with foodborne zoonoses and PTs, and will be available for initiatives in this field.
- Dr. A. Caprioli is included in the panel of the microbiologist tutor team of the DG Sanco "Better Training for Safer Food" course on Food-borne outbreaks, 2013-2015. He will participate in the course with the following presentations:
  - Agents and Outbreaks: *Salmonella*, *Campylobacter*, *E. coli* and *Listeria*. The presentation will include the description of agents and their microbiological potential to cause an outbreak, with examples from previous outbreaks.
  - Typing methods available for outbreak investigations. The presentation will include the use of molecular typing methods in outbreak investigations, the microbiological cluster definitions, the role of reference laboratories, the transfer of information between laboratories and epidemiologists.

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*: participation in at least one TAIEX or BTSF initiative. See also FF.CEN.1 in the PI spreadsheet.*

Duration*: ongoing*

August 27, 2014

Dr. Alfredo Caprioli
Director, EU-RL for *Escherichia coli*
Reparto Zoonosi Trasmesse da Alimenti
Dipartimento di Sanità Pubblica Veterinaria e Sicurezza Alimentare
Istituto Superiore di Sanità
Viale Regina Elena 299, 00161 Rome Italy