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 Programme

1st January - 31st December, 2013

Introduction

The EU-RL for VTEC (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.

In particular, the work programme of for the year 2013 will consist of the following activities:

1. Consolidating the EU-RL structures

1.1. Staff

1.2. Administration and Reporting

2. Coordination of the NRLs network and provision of technical assistance and training

2.1. Annual Workshop with the NRLs

2.2. Assistance to NRLs

2.3. Training

2.4. Creation of a repository of molecular typing data for VTEC strains from animal and food sources

3. Maintaining and Implementing the EU-RL-VTEC web site

4. Co-operation with EC structures or other bodies and projects 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

4.5. The U.S. Department of Agriculture (USDA)

5. Reference materials

5.1. Plasmids carrying DNA targets for PCR-based diagnostic assays

5.2. Preparation of toxin-negative variants of VTEC of public health relevance

6. Inter-laboratory studies

6.1 Identification and typing of pathogenic E. coli

6.2. Detection of pathogenic E. coli in food samples

6.3. Proficiency testing scheme for PFGE typing of E. coli strains.

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

8. Missions

The objectives, and the expected outputs of each action are indicated, as well as its duration, which will be either limited to 2013 or multi-annual (ongoing programme). Performance indicators are indicated where appropriate.

1. Consolidating the EU-RL structures

1.1. Staff

The permanent staff of ISS will continue to devote significant working time to the EU-RL's activities. Four persons, 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 laboratory technician skilled in quality assurance systems, equipment maintaining, and molecular typing methods, 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.*

Duration: *ongoing*

1.2. 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*

2. Coordination of the NRLs network and provision of technical assistance and training

2.1. Annual workshop with the NRLs

The 8th annual workshop will be held in the second half of 2013 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 2013 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 will also 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 and Prevention (ECDC) will be invited. The workshop will also represent an opportunity to evaluate the state of play of the initiative of the repository of molecular typing data on VTEC strains isolated from non-human sources launched in 2013. 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*: 2013*

2.2. 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. Drafts of standard operating procedures for detection of pathogenic *Escherichia coli* other than VTEC in animals, food, and in other relevant matrices and for molecular typing of the isolated strains will be developed. The *E. coli* pathogroups to be considered will be chosen based on: i) the epidemiological picture of human infections, according to ECDC and literature data; ii) specific requests from the NRLs; iii) specific prescription by the European legislation. 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 a dedicated survey.

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.5, FF.PT.6, FF.ANA.1 in the PI spreadsheet.*

Duration: *ongoing*

2.3. Training

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 in the under construction

repository of molecular typing data on VTEC strains isolated from non-human sources (see also point 2.4). 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.*

Expected output**:** *i) availability of standardized training programs at the EU-RL; ii) improved capability to detect and type VTEC in the laboratories receiving training.*

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

Duration: *ongoing*

2.4. Creation of a repository of molecular typing data for VTEC strains from animal and food sources

The EU-RL VTEC will continue the activities aiming at the development of a repository of molecular typing data on VTEC strains isolated from non-human sources: food, animals, and the environment. The main purpose of the initiative is to link such a repository with an ongoing similar initiative of ECDC on the molecular typing of strains from human infections. This will allow the comparison of VTEC strains isolated from human and non-human sources, improving the possibility of molecular epidemiology investigations, particularly in the case of international outbreaks.

To build up a repository representative of the EU epidemiologic situation, it is of the utmost importance that the whole NRL network is able to produce ad upload molecular data about the VTEC strains isolated in the member States. Therefore, on April 2012, an inventory of the expertise, facilities, and activities currently available within the European network of NRLs for *E. coli* was carried out and the results were used to select:

- 10 NRLs who should be already able to perform PFGE typing and should have the availability of VTEC strains isolated from non-human sources, belonging to the main pathogenic serogroups. Those NRLs will participate in a kick off meeting, planned for November 2012, to launch the initiative.
- The NRLs more entitled to receive training at EU-RL VTEC: six of those NRLs have already been enrolled in the 2012 training program.

The work plan will include the following steps:

1. Signature of a memorandum of understanding, presumably involving the 10 NRLs selected by the inventory.
2. Organization of an external quality assurance scheme to verify the quality of the PFGE profiles produced (see point 6.2).
3. Organization of the repository database, locally held at the EU-RL VTEC.
4. Building up the molecular typing capacity of other NRLs through dedicated training initiatives (see point 2.3).
5. Following the training, inclusion of new NRLs in the external quality assurance scheme and in the memorandum of understanding.
6. Maintaining the contact with similar initiative of ECDC on the molecular typing of strains from human infections.
7. Making the repository available to the qualified NRLs for direct uploading of the PFGE profiles and for consultation *via* web.

Points 1-6 will be achieved in the year of activity 2013.

The data collected and stored will be accessible by EC institutions involved in VTEC surveillance, monitoring and control, such as EFSA and ECDC. The database itself will remain property of the EC and the Member States.

Objectives**:** *i) to consolidate the initiative of a repository of molecular profiles of VTEC of non-human origin*; *ii) to begin the collection of molecular profiles from selected NRLs.*

Expected output: *i) improved capability of NRLs to type VTEC O157; ii) progress in the creation of the NRL network that will contribute molecular profiles to the repository.*

Performance indicators: *i) at least 10 NRIs participating to the first EQA program for PFGE; ii) >85% of acceptable results in the EQA program; see also FF.PT.1, FF.PT.2, FF.PT.3, FF.PT.4, and FF.PT.5 in the PI spreadsheet.*

Duration: *2013*

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.

A “news” section will be activated in the EU RL VTEC web site. The section will be updated at least quarterly with the latest scientific developments and epidemiologic reports. Timely communication of the posted communications will be given to the NRLs network.

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

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

Performance indicators: *i) see FF.R&D.1 in the PI spreadsheet; ii) increase in the number of contacts with respect to 2012.*

Duration: *ongoing*

4. Co-operation with EC structures or other bodies and projects related with food safety

The EU-RL will continue the cooperation with EC structures or other structures and projects active in the field of human and animal health and food safety.

The following liaisons will be maintained and implemented:

4.1. Scientific and technical support 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 during 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 will 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 advice to EFSA on food safety issues related with E. coli.*

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

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*.

4.3.1. The validation study of the method EN/ISO 16654 for E. coli O157.

The validation study of the method EN/ISO 16654 for E. coli O157, within the "Mandate for standardization M/381" addressed to CEN, was carried out in 2012 with the collaboration of 14 NRLs. The draft of the validation report will be discussed in a dedicated meeting with the representatives of the participating NRLs, to be held in Rome at the beginning of year 2013, and will be published later on.

4.3.2. CEN ISO TS 13136 for the detection of VTEC in food.

The publication by the ISO of the draft international standard CEN ISO TS 13136 for the detection of VTEC in food is expected within 2012. The EU-RL VTEC will continue to provide the CEN and ISO secretariats with any support possibly needed for such publication, and will maintain the leadership of the project until the final release of the TS.

Following the publication of the preliminary study of the validation of the Real Time PCR screening step, studies for the determination of the performances of the whole

method will be planned, including a collaborative study involving the NRLs network, based on the requirements of the ISO 16140 standard. The experimental design will be proposed and discussed as a new working item to the next CEN/TC275/WG6 2013 plenary meeting.

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

Expected output: *i) validation report for the method EN/ISO 16654 for E. coli O157; ii) experimental design of the validation project for of the method CEN ISO TS 13136 delivered to CEN.*

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

Duration: *2013*

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

The EU-RL will continue to take part into the Coordination Group of 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 repository 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 2.4).

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 assurance 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 repositories of molecular profiles of VTEC of human and non-human origin*.

Expected output: *i) 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: *2013*

4.5. The U.S. Department of Agriculture (USDA)

The collaboration with the Eastern Regional Research Center of USDA (Wyndmoor, PA, USA) will be maintained and strengthened during 2013, and will concern:

- the mutual exchange of information on the methodologies for VTEC detection in food, adopted in the US and the EU (FSIS Microbiology Laboratory Guidebook MLG chapters 5.06 and 5B.01 and ISO 16654 and CEN ISO TS 13136).
- the conduction of research programs aiming at improving the existing detection and typing strategies for VTEC.

In particular, the joint research program, started in 2011 and aimed at studying the VTEC sero-pathotypes most associated with severe human disease by means of whole genome automated sequencing, will be continued. The genomic sequence of six VTEC strains was completed in 2012 and further six VTEC strains will be fully sequenced in 2013. The comparison of the 12 complete genome sequences will be initiated as soon as the sequences will be assembled and uploaded in the genome sequences database at the GenBank (www.ncbi.nl.nih.gov) (see also point 7).

Objectives: *i) to strengthen the liaison with competent laboratories of third countries; ii) to share existing knowledge and to produce new data for the improvement of the detection strategies for VTEC.*

Expected output: *i) consolidation of the collaboration and the mutual exchange of information on the methodologies for VTEC detection in food; ii) scientific publications; iii) release of whole genome sequences of VTEC strain belonging to the main sero-pathotypes causing severe human disease.*

Performance indicators: *i) release of whole genome sequences of at least 6 VTEC strains.*

Duration: *2013*

5. Reference materials

The EU-RL VTEC has established collections of reference materials (RM) to be used as control in the analytical assays, as well as in quality assurance activities. These include reference *E. coli* strains and reference plasmids carrying DNA sequences representing PCR targets for genes relevant for the detection and identification of pathogenic *E. coli*. Such collections will be maintained and continuously updated and improved. The RM will be distributed to the NRLs, other official laboratories (OLs), and laboratories of third countries upon request.

5.1. Plasmids carrying DNA targets for PCR-based diagnostic assays

The collection of reference plasmids carrying the DNA targets for the real time PCR screening step of the ISO TS 13136 prepared in collaboration with the JRC (Ispra) has been created and made available for distribution to the NRLs since 2011. This collection has been integrated with other target genes, such as *aggR* and *aat*, needed for the identification of the VT-producing enteroaggregative *E. coli* (EAEC), including the VTEC O104:H4 strain associated with the large 2011 outbreak. The set of reference plasmids will be expanded with plasmids containing the target DNA sequences specific for the detection of pathogenic *E. coli* other than VTEC and EAEC. The availability of such plasmid-based reference materials has facilitated the implementation of detection methods for VTEC and will be of great value in the establishment of molecular methods for the identification of other pathogenic *E. coli*, being safe for handling and easy to distribute to the NRLs.

Objectives: *to implement and update the collection of reference materials for detection and typing of VTEC and other pathogenic E. coli.*

Expected output: *i) extended collection of reference plasmids for PCR-based pathogenic E. coli detection and typing;*

Performance indicators: *see FF.PT.4 in the PI spreadsheet.*

Duration: *2013*

5.2. Preparation of toxin-negative variants of VTEC of public health relevance

The production of toxin-negative variants of VTEC strains is a key point for the development of reference strains to be easily distributed and safely handled in analytical laboratories. As a matter of fact, the shipment of VT-producing *E. coli* is increasingly subjected to restrictions applied by the couriers worldwide with a parallel reduction of countries where such strain can be dispatched and a substantial increase in the shipment costs. Moreover, laboratory-acquired infections have been repeatedly documented and represent a further issue that makes important the availability of VT-negative control strains to be safely used as controls in the implementation of detection methods or for the quality assurance programs.

Non-toxigenic mutants from VTEC reference strains should be prepared by curing the VT-converting bacteriophage or by selective deletion of the VT-coding genes, to leave unaltered all the other metabolic, phenotypic and virulence-related characteristics of the strains. This is needed to make the strains suitable either for

the use as controls in food testing, for proficiency evaluation in quality assurance programs, and for experiments of pathogenicity or colonization in animal models.

The EU-RL has established a collection of reference strains that includes VTEC belonging to the top five serogroups most involved in human infections and, since the 2012 work program, has been working to produce non-toxigenic mutants from the two VT-producing enteroaggregative *E. coli* described so far: the VT-EAEC O111 and the VT-EAEC O104:H4. Till now, the collection has been expanded with a cured mutant of the VT-EAEC O111, while and the O104:H4 mutant should be obtained by the beginning of 2013.

The construction of cured mutants of VTEC belonging to the top five serogroups will also be initiated in the 2013, with VTEC O157 and VTEC O26, and will continue until mutants of all the VTEC reference strains of the serogroups of epidemiological importance will be obtained.

Objectives: *to implement and update the collection of reference materials for VTEC detection and typing.*

Expected output: *non-toxigenic variant of VT-EAEC O104:H4, to be used as non-class 3 reference strain; ii) non-toxigenic variant of VTEC O157 and VTEC O26, to be used as non-class 3 reference strain.*

Performance indicators: *production of 2 VT-negative mutant strains.*

Duration: *2013*

6. Inter-laboratory studies

Three studies are planned for 2013: i) a study on the detection of pathogenic *E. coli* other than VTEC in food samples; ii) a study on the identification and typing of pathogenic *E. coli* strains; iii) a proficiency testing scheme dedicated to PFGE typing of *E. coli* strains.

6.1. Inter-laboratory study on the identification and typing of pathogenic E. coli

VTEC are undoubtedly the group of pathogenic *E. coli* more relevant in food safety. However, the severe outbreak of HUS caused in Germany in 2011 by the VT-producing enteroaggregative *E. coli* O104:H4 brought to the attention of food safety professionals enteroaggregative E. coli (EAEC) and the other groups of diarrheagenic *E. coli*. EAEC, enterotoxigenic *E. coli* (ETEC) and enteroinvasive *E. coli* (EIEC) are well known causes of traveller diarrhea. However, they have been also reported in several foodborne outbreaks in industrialized countries, despite

insufficient laboratory surveillance data on human infections are usually collected, largely because suitable detection methods are not available in common diagnostic laboratories.

The objective of this inter-laboratory study will be to verify and improve the capacity of the NRLs to correctly identify the different groups of pathogenic *E. coli*.

The patho-group identification will be accomplished by the detection of the corresponding virulence genes by PCR amplification. The PCR procedures for detecting the different virulence genes and the *E. coli* strains to be used as positive controls in the PCR assays will be made available by the EU-RL.

The study could be performed jointly with the network of medical National Reference Laboratories for VTEC referring to the European Center for Disease Prevention and Control (ECDC).

Objectives: *i) to build up the capacity of the NRLs to detect food contamination by pathogenic E. coli other than VTEC.*

Expected output: *capacity to identify food items contaminated with pathogenic E. coli other than VTEC.*

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

Duration*: 2013*

6.2. Detection of pathogenic E. coli in food samples

As mentioned in the preceding paragraph, diarrheagenic *E. coli* other than VTEC probably play an under-estimated role in foodborne diseases and could be responsible for a proportion of outbreaks where the causative agent remains undetected. Testing food samples associated with presumptively foodborne outbreaks for the presence of different *E. coli* patho-groups represents therefore an important challenge for food and public health laboratories.

The proposed study will consist on the examination of artificially contaminated food samples for the presence of diarrheagenic E. coli strains belonging to the main patho-groups. In the absence of specific international standards, the standard operating procedures to be used for their detection and isolation will be developed and provided by the EU-RL.

Objectives: *i) to build up the capacity of the NRLs to detect food contamination by pathogenic E. coli other than VTEC.*

Expected output: *capacity to identify food items contaminated with pathogenic E. coli other than VTEC.*

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

Duration*: 2013*

6.3. Proficiency testing scheme for PFGE typing of E. coli strains

As described at point 2.4, the EU-RL is working at the development of a repository of molecular typing data on VTEC strains isolated from non-human sources. The work plan toward such a repository includes the organization of an external quality assurance (EQA) scheme to verify the quality of the PFGE profiles produced by the NRLs participating in the project.

The first round of this EQA scheme will include those NRLs (presumably 10) that are already able to perform PFGE typing and have the availability of VTEC strains isolated from non-human sources and belonging to the main pathogenic serogroups. However, the EQA scheme will be open to all the NRLs that will have the technical capacity to participate. A set of *E. coli* strains will be sent to the participating NRLs. The strains will be examined using an *ad hoc* guideline produced by the EU-RL and based on the protocol in use in the PULSENET international network and adopted also by the ECDC for its molecular surveillance pilot 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 run 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.

Objectives: *i) to build up the capacity of the NRLs to determine PFGE profiles of E. coli suitable for inclusion in the repository of molecular profiles and comparison.*

Expected output: *i) a shared protocol for the production of PFGE profiles of E. coli strains, harmonized with the procedures in use in the ECDC molecular surveillance pilot program and in the PULSENET international network; ii) 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*: 2013*

7. 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 genomic of pathogenic *E. coli*. The established scientific collaborations with EU and non-EU institutions will be maintained and reinforced, and new collaborations will be established. The research programs represent the basis for a better understanding of the epidemiology of the infections, for improving the detection of these pathogens in their animal reservoirs and food vehicles, and to increase the spectrum of the molecular tools available for strain typing. The research projects currently run at the EU-RL VTEC are focused on the identification of genes associated with the most pathogenic VTEC seropathotypes and representing candidates as single targets for the development of methods to detect VTEC in animals and vehicles of infections. Additionally, the EU-RL is involved in a study, in collaboration with ARS-USDA (see point 4.5), aiming at determining and comparing the whole genomic sequences of 12 VTEC strains belonging to the serogroups most involved in human disease, according to the epidemiological data managed by the US CDC and the ECDC. The results of this study will substantially contribute to our understanding of the structure of VTEC genomes, with important reflections on the identification of possible pathotype-associated genes and of chromosomal regions exploitable for the development of fast and reliable typing tools, such as the MLVA, currently available for VTEC O157 and O26 only. Other ongoing research is aiming at the definition of the mechanisms of acquisition of VT-converting phages by *E. coli* strains belonging to pathogroups other than VTEC, in particular the enteroaggregative *E. coli* (EAEC) and the atypical EPEC (aEPEC). The knowledge acquired on this subject could help in predicting the emergence of new pathogenic VTEC clones and in deploying adequate methods for their detection. These studies will be the subject of a collaboration between the EU-RL and the University of Barcelona, a center of excellence for the studies on VT-bacteriophages. The collaboration will be formalized within year 2012. The expenses related to these studies will be covered by ISS funds and will not be included in the EU-RL budget.

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: *i) development of innovative flexible methodologies for detection and typing of pathogenic E. col; ii) scientific publications.*

Performance indicators: *see FF.R&D.1 and "other activities" in the PI spreadsheet.*

Duration*: ongoing*

8. Missions

The following missions may be needed in 2013:

- Participation of a EU-RL representative in a meeting of the Coordination Group of the ECDC Food- and Waterborne Diseases and Zoonoses (FWD) Surveillance Network, presumably in Stockholm.
- 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: three meetings in Brussels.
- Participation of a scientist in the 19th CEN/TC275 WG6 annual plenary meeting (location and dates to be defined).
- A visit to one NRL is planned for 2013, upon agreement with the EC and the interested country.

Objectives: *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*: 2013*

August 30, 2012

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