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, 2014

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 for the year 2014 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

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

3.1. Development of Standard Operating Procedures (SOPs) for EFSA

3.2. Inventory of the activities of the NRLs on VTEC molecular typing

3.3. External quality assessment (EQA) for PFGE

3.4. Curation of the EFSA database of PFGE profiles of VTEC from food and animals

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

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

5.1. Scientific and technical support to DG SANCO

5.2. The European Food Safety Authority (EFSA)

5.3. The European Committee for Standardization (CEN)

5.4. The European Centre for Disease Prevention and Control (ECDC)-Food- and water-borne diseases (FWD) program

5.5. EC training programs (TAIEX, BTSF)

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

6. Inter-laboratory studies

6.1. Detection of VTEC in sprout samples

6.2. Identification and typing of pathogenic E. coli

6.3. Proficiency testing scheme for PFGE typing

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 2014 or multi-annual (ongoing programme). Performance indicators are indicated where appropriate, making reference to the PI spreadsheet that is attached to this programme.

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, 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 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.

In addition, another Post Doc scientist with skills in molecular typing and bioinformatics will be hired in the second half of the year to cope with the new tasks that have been assigned to the EU-RL in the field of molecular typing (see point 3). Indeed, the EU-RL VTEC will have to support the European Food Safety Authority (EFSA) in the management of the database of molecular typing profiles of VTEC strains isolated from food and animals. Typing, mainly based on pulsed field gel electrophoresis (PFGE), will be performed by the NRLs. Around October 2014, the NRLs should begin to submit to EFSA the PFGE profiles, to be handled and included

in the database with the support of the EU-RL VTEC. In this respect, an increased effort will be also required to the EU-RL in terms of specific support and training to the NRLs (see points 2.3 and 3).

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 9th annual workshop will be held in the second half of 2014 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 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*: 2014*

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. 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

2.3.1. 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 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.

2.3.2. Training course on the use of the software package BioNumerics for PFGE fingerprints analysis

In the years 2012 and 2013, scientists from 11 NRLs visited the EU-RL to receive specific training on PFGE. Moreover, 15 EU NRLs participated on a voluntary basis in the first scheme of external quality assessment (EQA) of PFGE launched in 2013 (PT10), with an average good level of performance. Now that a relevant number of NRLs proved capable of producing acceptable PFGE gel images, the next step will be the training for the acquisition and normalization of PFGE images, including the bands assignment and the referencing to the gold standards. In this respect a course on the use of the software package BioNumerics for PFGE profile analysis will be organized. The software package BioNumerics offers an integrated platform for the analysis of PFGE fingerprints and allows the storage of gel images and epidemiological metadata in a single database. The course will be held in the second half of 2014 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 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 course will have a duration of 2 full days and it will focuses on the main features and the basic use of BioNumerics. An overview of the possibilities of the software package will be given to the participants through hands-on demonstrations and exercises. At the end of the course, the participants will be able to recognize the quality of the PFGE gels and to acquire them as Tiff images, to perform band assignment and profile analyses, to build up database for cluster analysis, in order to identify the level of similarity between the profiles.

The travel and accommodation costs for at least 10 participants 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.*

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: *ongoing; 2014 for the BioNumerics course.*

3. Creation of a database 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 database 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 database 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.

In 2012, DG SANCO assigned the responsibility of the management of the database on isolates from food and animals to EFSA, with the support of the relevant EURLs. Therefore, in agreement with EFSA, the EU-RL VTEC will continue the preparative work to build up the database of PFGE profiles, by providing specific training opportunities to the NRLs (see points 2.3.1 and 2.3.2) and with the following initiatives.

3.1. Development of Standard Operating Procedures (SOPs) for EFSA

The EU-RL VTEC will take care of the preparation of SOPs for the collection and analysis of the molecular typing data. In particular, the following SOPs will be delivered to EFSA:

- SOPs for PFGE typing of VTEC isolates from food, feed and animals.
- SOPs for the quality assessment of PFGE profiles/images of VTEC strains isolated from food, feed and animals.
- SOPs for the acquisition and normalization of PFGE images including the bands assignment and referencing to the gold standards
- SOPs for the "curation" of the molecular typing data of VTEC.

These SOPs will be based on the protocols and the procedures in use at the EU-RL VTEC, and will be reviewed in the light of the SOPs in use within the ECDC-FWD network for human VTEC infections. Such a revision will provide the basis for drafting SOPs ensuring the best comparability of the data on strains from human and non-human sources.

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

Objective**:** *to support EFSA in the creation of the database of molecular profiles of VTEC of non-human origin*.

Expected output: *harmonization of the collection and analysis of molecular typing data*

Performance indicators: *SOPs delivered to EFSA. See also FF.COM.1 in the PI spreadsheet.*

Duration: *2014*

3.2. Inventory of the activities carried out by the NRLs on VTEC molecular typing

According to the mandate given by the EC to EFSA for the collection of data on molecular typing of food/animal isolates of food-borne pathogens, the bulk of typing data will be primarily produced by the NRLs. Therefore, the EU-RL will carry out an inventory of the molecular typing methods used, the numbers of isolates tested and the IT applications available in the NRLs for *E. coli* for storing, managing and analyzing molecular typing data.

A structured questionnaire will be prepared to collect information on:

- The molecular typing methods used.
- The level of skills in preparing the PFGE gels.
- The IT platform and the skill available for storing, managing and analyzing molecular typing data.
- The number of VTEC isolates belonging to serogroup O157 and to the other most frequent serogroups that have been tested in the last 2 years and that could be tested in the near future.
- The participation in external quality assessment initiatives.

The questionnaire will be sent to the NRLs of EU MS, EEA countries, and other European countries, with a message explaining the purposes of the initiatives.

The results of the inventory will provide useful information on the NRLs' training needs and on the number of VTEC strains isolated per year from non-human sources that could be available for typing and could be included in the database. The results will be compared with those of a similar inventory carried out at by the EU-RL VTEC at the beginning of 2012.

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

Objectives**:** *to obtain information on the capacity of the NRLs in molecular typing;*

Expected output: *better calibration of the training initiatives, according to the NRLs' needs.*

Performance indicators: *85 % of the NRLs sending back the questionnaire. See also FF.COM.1 in the PI spreadsheet.*

Duration*: 2014*

3.3. External quality assessment (EQA) for PFGE

The external quality assessment (EQA) program, initiated in 2013 with the inclusion of PFGE in the PT10 and PT11 studies on strain identification and typing, will be continued (see point 6.2), to verify the capability of the NRLs to perform PFGE and the quality of the profiles produced, as well as the impact of the training visits on the NRL performance. If possible, the molecular typing EQA will be performed 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 determine 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: *i) 80 % NRLs participating to the EQA program; ii) >85 % of acceptable results in the EQA program; see also FF.PT.1, FF.PT.2, FF.PT.3, FF.PT.4 in the PI spreadsheet.*

Duration: *2014*

3.4. 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 the DG SANCO for the collection of data on molecular testing in food/animal isolates from food-borne infections, the NRLs should begin to provide molecular typing data to EFSA in the late 2014. When the submission of PFGE profiles will begin, the EUL-RL shall take care of the curation of the database. The curation process will be accomplished according to the SOP specifically developed (see point 3.1) 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: *i) all the PFGE profiles submitted to EFSA by the NRLs included in the database; ii) see also FF.COM.1 and FF.COM.2 in the PI spreadsheet.*

Duration: *ongoing.*

4. 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.

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 2013.*

Duration: *ongoing*

5. 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:

5.1. 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*

5.2. 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 point 3).

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*

5.3 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, carried out on the matrix milk between 2012 and 2013 within the "Mandate for standardization M/381" addressed to CEN, will be extended to sprouts, a matrix that became particulary relevant after the VTEC O104 outbreak in 2011 and the publication of Reg. (EC) 209/2013, which defines a microbiological criterion for VTEC in sprouts. Moreover, the ISO/TS13136 method for the detection of VTEC will be applied by the participating laboratories to the same enrichment culture, to obtain validation data also for this method, applied to the sprout matrix.

Such extensions have already been approved by CEN and will be carried out within the already granted CEN budget. Therefore, the related expenses will not be included in the EU-RL budget.

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 in the sprout matrix.*

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

Duration: *2014*

5.4. 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 3).

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: *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: *ongoing*

5.5. EC training programs (TAIEX, BTSF)

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 database of TAIEX experts for the issues related with foodborne zoonoses and proficiency testing, 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 investigations 2013-2014. He will participate in the course giving 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 outbreak investigations.
  - Typing methods available for outbreak investigations. The presentation will include the appropriate 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.

The expenses for participation in TAIEX and BTSF initiatives are covered by specific funds and will not be included in the EU-RL budget.

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.*

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

The collaboration with the Eastern Regional Research Center of USDA (Wyndmoor, PA, USA) will be maintained during 2014, 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 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 the VTEC strains completed in 2013 will be analyzed (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: 2014

6. Inter-laboratory studies

Three studies are planned for 2014: *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 proficiency testing scheme for PFGE typing, that will be conducted on the same *E. coli* strains of the previous *ii*) study.

6.1. Detection of VTEC in sprouts

In March 2013, four EC regulations on sprouts and seeds using for sprouting have been published. Among them, Reg. (EU) 209/2013, that amends Regulation (EC) 2073/2005 as regards microbiological criteria for sprouts, and introduces for the first time in the EU legislation a microbiologic criterion for VTEC. In particular, the regulation establish 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 prescribes 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.

Objectives: *i) 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*: 2014*

6.2. Inter-laboratory study on the 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.

6.3. Proficiency testing scheme for PFGE typing

As described at point 3, 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 such laboratories.

Therefore, a 3rd proficiency testing scheme for PFGE typing (PT-PFGE3) 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 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, according to the criteria established by the PULSENET international network and adopted also by the ECDC.

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: *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*: 2014*

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*, 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 collaboration with ARS-USDA (see point 5.6) on the comparison of the whole genomic sequences of VTEC strains belonging to the most pathogenic VTEC seropathotypes will continue, as well as the collaboration with the University of Barcelona on 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 results should help in predicting the emergence of new pathogenic VTEC clones and in deploying adequate methods for their detection.

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. coli.*

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 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: 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.

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*: 2014*

August 27, 2013

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