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# Inventory of the expertise on molecular typing of Verocytotoxin-producing *Escherichia coli* (VTEC) within the National Reference Laboratories for *E. coli* of EU Member States

## CONTENT

## 1. INTRODUCTION

Molecular subtyping of bacterial isolates has been successfully applied to the detection of community-wide foodborne disease outbreaks, to aid their epidemiologic investigation, and to facilitate source attribution exercises. In this respect, the possibility to compare data referring to isolates of both human and food/animal origins represents an epidemiological added value.

A well-established molecular surveillance network for food-borne infections is *PulseNet International* (www.pulsenetinternational.org), a network of National and regional laboratory networks dedicated to tracking foodborne infections worldwide.

In Europe, the *PulseNet Europe* network has been active since 2006, then went into a stand-by state, due to lack of financial support. At present, the network is in the process of being taken over by the European Centre for Disease Prevention and Control (ECDC), as far as the isolates of Salmonella, Listeria and VTEC from human infections are concerned. In this perspective, the availability of typing data for isolates from food, animals, and environment would significantly improve the surveillance and trace-back of food-borne infections at the national, European and international level. Therefore, the possibility to launch similar networks for the same foodborne pathogens isolated from non-human sources has been the focus of a dedicated meeting organized by the EC DG SANCO on 15 December 2011, with the participation of representatives of the EU-RLs for Salmonella, Listeria, Campylobacter, *E. coli*, and antimicrobial resistance*,* and of ECDC and EFSA. The conclusion of the meeting was a declared interest in developing an harmonized sub-typing system for non-human isolates, following the *PulseNet International* guidelines, and in implementing pilot platforms based on new technologies, such as the next generation sequencing, for future developments.

Based on this background, the EU-RL for VTEC has included in its work program the development of a repository of molecular typing data on VTEC strains isolated from non-human sources by the EU NRLs. Such a repository should be linked with the 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.

The initiative was discussed and approved during the 2011 Annual Workshop with the NRLs, and the preliminary steps planned for the establishment of the repository of

molecular typing data on VTEC were:

1. To carry out an inventory of the molecular typing skills and activities currently existing in the NRLs.

2. To develop a training program to improve the molecular typing skills of the NRLs.

This document represents the inventory of the expertise, facilities, and activities currently available within the European network of NRLs for *E. coli* and has been used as a basis for selecting the NRLs more entitled to receive training at EU-RL VTEC.

## 2. METHODS

To carry out the inventory, a semi-structured questionnaire was administered to the NRLs for *E. coli* in the EU Member States in March 2012.

## 3. RESULTS

The questionnaire was sent to the 32 NRLs designated by the EU MS and a reply was obtained from all of them (response rate 100%).

### 3.1. Number of VTEC strains isolated/collected per year

The questions referred to the approximate number of strains belonging to the main serogroups isolated/collected each year from non-human sources and potentially available for molecular typing. The results indicated that the network of NRLs has a potential to isolate/collect up to a total of 6,000 VTEC strains per year (median: 20 strains per NRL), of which about 45% from animals, 30% from food, and 25% from environmental sources. The serogroups of the strains isolated in 2010 and 2011 are listed in Table 1.

**Table 1. Number of strains belonging to the serogroups mainly associated with human infections isolated from non-human sources by the EU NRLs.**

| Serogroup | No. of strains isolated in the year: | | |
|---|---|---|---|
| | **2010** | **2011** | **Average per year** |
| O157 | 1,122 | 1,143 | 1,133 |
| O26 | 224 | 377 | 300 |
| O103 | 94 | 216 | 155 |
| O145 | 43 | 75 | 59 |
| O111 | 25 | 25 | 25 |

### 3.2. Detection/typing of genes by PCR

The questions referred to both typing of *vtx* gene variants and detection of virulence genes. The results are reported in Table 1.

**Table 2. Detection/typing of genes by PCR at the NRLs (n= 32).**

| **Genes** | **No. of NRLs applying the method (%)** |
|---|---|
| *vtx*-genes (subtyping by the PCR method tested in the 6$^{th}$ PT of the EU RL VTEC) | 28 (87.5%) |
| *fliC* (H-genotyping) | 11 (34.4%) |
| *eae (*intimin*)* | 32 (100%) |
| *eHly* (entero-hemolysin) | 13 (40.6%) |

### 3.3. Molecular typing methods

The questions referred to the availability of the two main typing methods (PFGE and MLVA), the type of protocols adopted, the level of skills in applying the methods, the approximate number of strains belonging to the main serogroups typed each year.

*3.3.1. Pulsed-field gel electrophoresis (PFGE)*

The technique resulted available in 23 of the 32 NRLs (71.9%). Of these, 13 reported very good experience with methodology, and 6 of them obtained in 2006 an external quality assurance (EQA) certification from the *PulseNet Europe* network. The other 10 NRLs reported levels of skill ranging from poor to sufficient.

Between 2010 and 2011, over 950 VTEC strains isolated from non-human sources have been subjected to PFGE typing by the NRLs. The serogroups of those strains are listed in Table 3.

**Table 3. Number of VTEC strains isolated from non-human sources and subjected to PFE typing by the EU NRLs in the years 2010-2011.** Data refer only to the serogroups mainly associated with human infections

| Serogroup | No. of strains typed in the year: | | |
|---|---|---|---|
| | **2010** | **2011** | **Total** |
| O157 | 240 | 204 | 444 |
| O26 | 85 | 209 | 294 |
| O103 | 102 | 36 | 138 |
| O111 | 10 | 25 | 35 |
| O145 | 7 | 15 | 22 |
| **Total** | **444** | **489** | **933** |

*3.3.2. Multiple-Locus Variable number tandem repeat Analysis (MLVA)*

The technique resulted available in 9 of the 32 NRLs (29.4%). Of these, 5 reported very good experience with the methodology, while the other 4 NRLs reported levels of skill ranging from poor to sufficient. Seven of the 9 NRLs use of the protocol recommended by the *PulseNet International* network.

*3.3.3. Use of Bionumerics software for profile storage and analysis*

The software resulted available in 23 of the 32 NRLs (71.9%). Of these, 11 reported very good experience with the methodology, while the other 12 NRLs reported levels of skill ranging from poor to sufficient.

## 4. CONCLUDING REMARKS

1. PFGE still represents the molecular typing method most used for foodborne pathogens and is employed by the well established surveillance network for food-borne infections *PulseNet International*, dedicated to tracking foodborne infections world-wide.

According to the present inventory, PFGE is currently available in 23 EU NRLs, 13 of which reported very good experience with the method (6 of them obtained an EQA certification from the former *PulseNet Europe*). The other 10 NRLs reported levels of skill ranging from poor to sufficient.

The EU RL VTEC developed a standard training program dedicated to PFGE and 6 of the NRLs declaring poor skill in PFGE have already been included in the training program supported by DG SANCO that will be carried out at the EU-RL VTEC during 2012.

The number of VTEC O157 strains from non-human sources that could be typed by PFGE and included in the repository each year ranges between 200 and 1,000.

The number of VTEC non-O157 strains belonging to the main pathogenic serogroups that could be typed by PFGE and included in the repository each year ranges between 200 and 500.

In conclusion, this inventory of the expertise and activities on molecular typing of VTEC indicates that a good level of expertise on PFGE does exist in many of the EU NRLs, which also have the possibility to collect and type a significant number of isolates belonging to O157 and to the other main pathogenic serogroups. This would warrant the possibility to build up a repository of PFGE profiles of VTEC of non-human origin, available for any comparison with the database on human isolates that should be established within the ECDC Food and Waterborne Diseases Surveillance Network.