EU Reference Laboratory for *E. coli*

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Report of the 2nd proficiency test (PT) for pulsed field gel electrophoresis (PFGE) typing of Verocytotoxin-producing *E. coli* (VTEC) strains (PT-PFGE2) – 2013

SUMMARY

A total of 25 laboratories, including 23 NRLs representing 21 EU Member States and the NRLs of Norway and Switzerland, participated in the second proficiency test on pulsed field gel electrophoresis (PFGE) typing of Verocytotoxin producing *E. coli* (VTEC) organized by the EU Reference Laboratory for *E. coli* (EU-RL VTEC). The study was carried out on the 6 *E. coli* strains sent to the NRLs for the 11th inter-laboratory study on VTEC identification and typing (PT11), and the quality of PFGE images was evaluated using the criteria of the PulseNet International Protocol, by assigning ranks from poor to excellent to each of seven parameters and to the overall procedure.

The majority of the NRLs placed between “good” and “excellent” in the assessment of the overall procedure, even though weaknesses in the areas of image acquisition, band displaying, and gel background were recorded.

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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. 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 European Centre for Disease Prevention and Control (ECDC) is collecting molecular typing data, in particular pulsed field gel electrophoresis (PFGE) profiles of Salmonella, *Listeria monocytogenes* and Verocytotoxin-producing *E. coli* (VTEC) strains isolated from human infections.

In 2012, the EC DG SANCO decided to organize the collection of typing data for isolates 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 collection of data was initially focused on a restricted number of pathogens, namely Salmonella, *L. monocytogenes* and VTEC, and the responsibility of the management of the database on isolates from food and animals was assigned to The European Authority for Food safety (EFSA), with the scientific and technical support of the relevant EU-RLs. The strategy of this molecular surveillance system is described in the DG SANCO document "*Vision paper on the development of data bases for molecular testing of foodborne pathogens in view of outbreak preparedness*", available at the url: http://ec.europa.eu/food/food/biosafety/salmonella/docs/vision-paper_en.pdf.

According to the DG SANCO mandate, the bulk of molecular typing data on food/animal isolates of the selected food-borne pathogens will be primarily produced by the respective networks of National Reference Laboratories (NRLs). Therefore, the EU Reference Laboratory for *E. coli* (EU-RL VTEC) undertook initiatives to provide specific PFGE training opportunities to the *E. coli* NRLs and to set up a proficiency-testing (PT) scheme for PFGE.

Such PT scheme initiated in 2012, with the inclusion of PFGE typing in the 10th inter-laboratory study on VTEC identification and typing (PT10), which was performed jointly with the ECDC-FWD network involved in the typing of VTEC strains from human infections. The report of this first study (PFGE-PT1) is available in the EU-RL website (www.iss.it/binary/vtec/cont/Report_PT_PFGE1.pdf).

The second PT on PFGE (PFGE-PT2) was conducted on the *E. coli* strains sent to the NRLs in the framework of the 11th inter-laboratory study on VTEC identification and typing (PT11), with the purpose to assess the proficiency of the NRLs in PFGE typing. This document represents the evaluation report of the study.

2. OBJECTIVES

To best accomplish the duty of the *E. coli* NRL network of contributing PFGE profiles of VTEC strains to the forthcoming European database, the purposes of this PT were:

- To assess the level of preparedness of the NRL network with respect to the production of high quality PFGE profiles of *E. coli* strains.
- To identify the aspects of the pipeline for molecular data production and storage that still need improvement.

3. PARTICIPANTS

A total of 25 laboratories, including 23 NRLs representing 21 EU Member States and the NRLs of Norway and Switzerland, participated in the study. Each NRL received its own individual laboratory numerical code, which is reported in the result tables.

The NRLs participating in the study were:

- Austria, Österreichische Agentur für Gesundheit und Ernährungssicherheit (GmbH)
- Belgium, Scientific Institute of Public Health, also representing Luxembourg
- Cyprus, Laboratory for the Control of Food of Animal Origin (LCFAO), Cyprus Veterinary Services
- Czech Republic, Veterinary Research Institute
- Denmark, National Food Institute, Technical University of Denmark
- Finland, Finnish Food Safety Authority Evira, Kuopio
- France, VetAgro Sup Campus Vétérinaire de Lyon
- Germany, Federal Institute for Risk Assessment (BfR)
- Greece, National School of Public Health & Central Laboratory of Public Health
- Hungary, National Food Safety Office, Food and Feed Safety Directorate, National Food Microbiological Reference Laboratory
- Ireland, Central Veterinary Research Laboratory, Department of Agriculture
- Italy, Istituto Superiore di Sanità
- Lithuania, National Food and Veterinary Risk Assessment Institute
- Netherlands, Food and Consumer Product Safety Authority (NVWA)

- Norway, Norwegian Veterinary Institute
- Poland, National Veterinary Research Institute, Pulawy
- Romania, Institute for Hygiene and Veterinary Public Health
- Slovakia, State veterinary and food institute, Dolný Kubín
- Slovakia, NRC of Environmental Microbiology, Public Health Authority of SR, Bratislava
- Slovenia, Veterinary Faculty/ National Veterinary Institute
- Spain, Laboratorio Central de Veterinaria de Algete (MAGRAMA)
- Spain, University of Santiago de Compostela, Lugo
- Sweden, Livsmedelsverket/The National Food Agency
- Sweden, National Veterinary Institute (SVA)
- Switzerland, Institute for food safety and hygiene, University of Zurich

4. MATERIALS AND METHODS

The test material sent to the NRLs was constituted by 6 strains of *E. coli* (samples 1 to 6), belonging to different groups of pathogenic *E. coli* and sent to the NRLs in the framework of the 10th inter-laboratory study on VTEC identification and typing (PT10, report available at http://www.iss.it/binary/vtec/cont/PT10_Report.pdf).

4.1. Sample preparation

The *E. coli* strains were selected among those present in the EU-RL VTEC reference collection. The test samples were prepared on 14 June and consisted of freshly prepared bacterial cultures inoculated into microbank bacterial preservation system vials. The cultures were incubated 18 hours at 37 °C ± 1 °C and labeled with randomly generated numerical codes (3 or 4 digits), different for each NRL. The test samples were stored at room temperature until 17 June, when the samples were sent to the participating laboratories by courier.

4.2. Laboratory methods

The NRLs were requested to use the laboratory procedure for PFGE of *E. coli* O157:H7, *E. coli* non-O157 (STEC), Salmonella serotypes, *Shigella sonnei* and *Shigella flexneri* in use in the PulseNet international network, available at URL: http://www.pulsenetinternational.org/assets/PulseNet/uploads/pfge/PNL05_Ec-Sal-ShigPFGEprotocol.pdf. PFGE typing was one of the mandatory parts of the PT and the NRLs were expressly requested to use the running conditions for *E. coli* O157, despite the test strains were not *E. coli* O157.

4.3. Collection and evaluation of the NRL results

The NRLs were requested to submit by email the pictures of the PFGE gels as TIFF files, together with a scheme of sample loading in a separate word/excel file.

The quality of PFGE images was evaluated taking into account the criteria defined in the "Standard Operating Procedure for TIFF Quality Grading" in use in the PulseNet International network (Procedure PNQ01). The same criteria are adopted by the ECDC for evaluating the quality of gels of human strains submitted to the TESSy molecular surveillance system. The evaluation criteria are reported in Table1.

The performance of each NRL was evaluated by assigning ranks from "poor" to "excellent" to each parameter (Table 1). When a rank "poor" was assigned, the performance for the parameter was considered as unsatisfactory.

Table 1: Quality grading of PFGE images according to the PulseNet guidelines

Parameter	Image Quality Grading Guidelines			
	Excellent	**Good**	**Fair**	**Poor**
Image acquisition and running conditions	As per PulseNet protocol: Gel fills whole TIFF. Wells included on TIFF. Bottom band of standard is between 1.0 cm and 1.5 cm from the bottom of the gel.	Gel does not fill whole TIFF but band finding is not affected.	Not protocol: only one of the following: Gel does not fill whole TIFF, and band finding is affected. Wells not included on the TIFF. The bottom band of a standard is not between 1.0 and 1.5 cm from the bottom of the gel. Band spacing of standards does not match the global standard.	Not protocol: more than one of the following: Gel doesn't fill whole TIFF and this affects band finding. Wells not included on TIFF. The bottom band of a standard is not between 1.0 and 1.5 cm from the bottom of the gel. Band spacing of standards does not match the global standard.
Cell suspensions	The cell concentration is approximately the same in each lane.	Up to two lanes contain darker or lighter bands than the other lanes.	More than two lanes contain darker or lighter bands than the other lanes, or at least one lane is much darker or lighter than the other lanes, making the gel difficult to analyse.	The cell concentrations are uneven from lane to lane, making the gel impossible to analyse.

Parameter	Image Quality Grading Guidelines			
	Excellent	**Good**	**Fair**	**Poor**
Bands	Clear and distinct all the way to the bottom of the gel.	Slight band distortion in one lane, but this does not interfere with analysis. Bands are slightly fuzzy and/or slanted. A few bands (three or less) are difficult to see clearly (DNA overload), especially at bottom of gel.	Some band distortion (e.g., nicks) in two to three lanes but still analysable. Fuzzy bands. Some bands (four to five) are too thick. Bands at the bottom of the gel are light, but analysable.	Band distortion that makes analysis difficult. Very fuzzy bands. Many bands too thick to distinguish. Bands at the bottom of the gel too light to distinguish.
Lanes	Straight.	Slight smiling (higher bands in the outside lanes than on the inside). Lanes gradually run longer toward the right or left. Still analysable.	Significant smiling. Slight curves on the outside lanes. Still analysable.	Smiling or curving that interferes with analysis.
Restriction	Complete restriction in all lane.	One to two faint shadow bands on the gel.	One lane with many shadow bands. A few shadow bands spread out over several lanes.	Two or more lanes with several shadow bands. Lots of shadow bands over the whole gel.
Gel background	Clear.	Mostly clear background. Minor debris present that does not affect analysis.	Some debris present that may or may not make analysis difficult (auto band search finds too many bands). Background caused by photographing a gel with very light bands (image contrast was “brought up” in photographing gel-makes image look grainy).	Lots of debris present that may or may not make analysis difficult (auto-band search finds too many bands).
DNA degradation (smearing in the lanes)	Not present.	Minor background (smearing) in a few lanes but bands are clear.	Significant smearing in one or two lanes that may or may not make analysis difficult. Minor background (smearing) in many lanes.	Significant smearing in more than two lanes that may or may not make analysis difficult. Smearing so that a lane is not analysable (except if untypeable [thiourea required]).

To evaluate the performance of the NRLs in the whole procedure, an overall rank was assigned according to the following criteria:

- “Poor”: The profiles were not suitable for strain comparison and cluster analysis.

- "Fair": The profiles could still be used for strain comparison despite a low quality.
- "Good": The profiles contained analyzable bands between the top of the gel and the 33.3 Kb band of the molecular standard *S. braenderup* H9812.
- "Excellent": All the bands in the profiles were clear and easily analyzable.

An overall evaluation of "poor" identified the underperformance of the NRLs.

Each NRL received its own individual report with the critical evaluation, the breakdown of the ranks assigned to each parameter, and suggestions on how to improve the quality of the images, with respect to the specific points that generated underperformance.

5. RESULTS

The study was carried out by 25 NRLs. The overall ranks assigned to each NRL to evaluate the performance in producing PFGE images are reported in Figure 1.

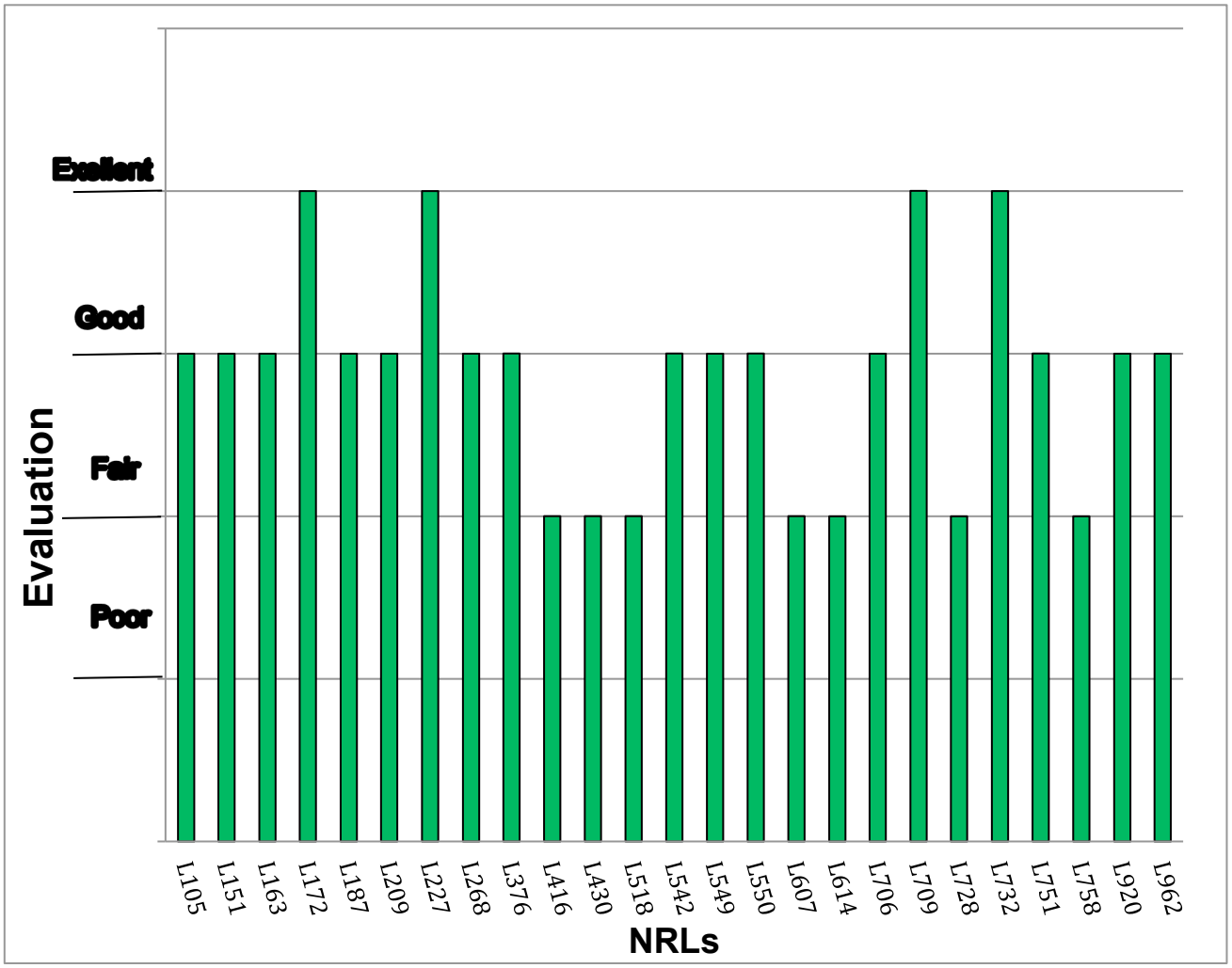

Figure 1. Overall evaluation of the performance in producing PFGE images for each NRL. Ranks were assigned according to the criteria described at paragraph 4.3.

None of the NRLs obtained a “poor” overall rank, 7 NRLs (28%) fell into the “fair” rank, 14 NRLs (56%) into the “good” rank, and 4 NRLs (16%) obtained an “excellent” rank. The overall evaluation is summarized in Figure 2.

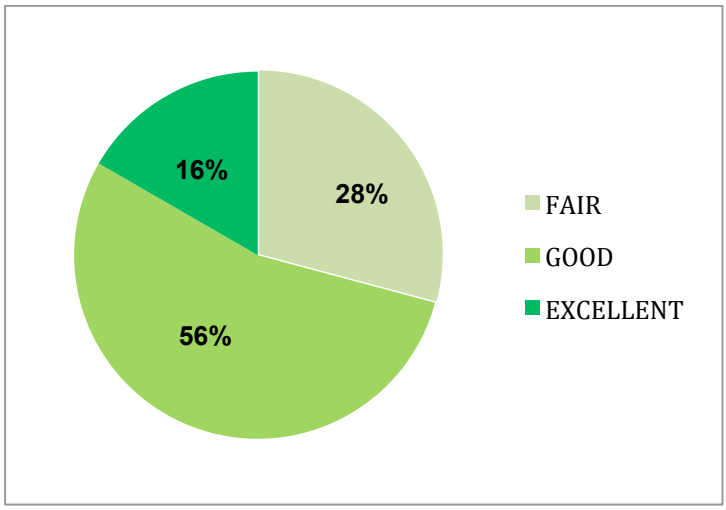

Figure 2: Overall evaluation of the NRL performance in producing PFGE images. Ranks were assigned according to the criteria described at paragraph 4.3.

Figure 3 summarizes the ranks obtained by the NRLs for each of the parameters listed in Table 1, according to the evaluation criteria described at paragraph 4.3.

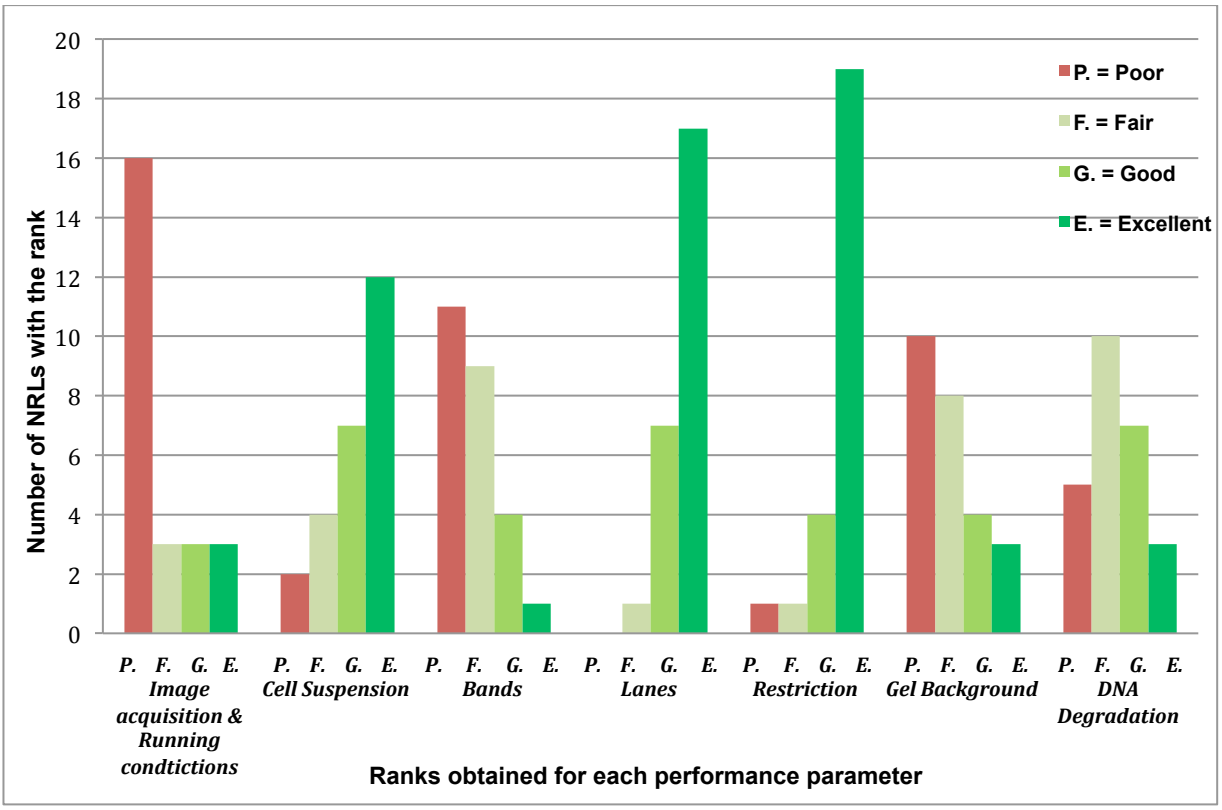

Figure 3. Number of NRLs obtaining a specific rank for each of the seven parameters (Table1) for the evaluation of the quality of PFGE images.

The participating laboratories obtained the best results for the parameters “restriction” and “lanes”, for which, respectively, 19 (76%) and 17 (68%) NRLs obtained the rank “excellent”. Conversely, problems were encountered with the parameters “image acquisition and running conditions”, “bands”, and “gel background”, for which, respectively, 16 (64%), 11 (44%), and 10 (40%) NRLs obtained the rank “poor”.

7. CONCLUDING REMARKS

PFGE typing of VTEC and other foodborne pathogens is a complex laboratory procedure, potentially affected by several troubles. As in the previous PT on PFGE, the protocol used in this PT was the same used for producing PFGE profiles of human strains in the ECDC-FWD network of public health NRLs, as well as in the international worldwide network PulseNet. Therefore, its adoption represents the key to the harmonization of the forthcoming molecular database for strains from food and animals, which will be managed by EFSA under the auspices and the mandate of the EC DG SANCO, with the other abovementioned networks.

Twenty-five NRLs, representing 20 EU Member States, Norway, and Switzerland participated in the study. This represented an increase with respect to the participation in the 1st PT on PFGE (report at http://www.iss.it/binary/vtec/cont/Report_PT_PFGE1.pdf), which was facultative and involved 19 NRLs, representing 16 EU Member States.

The participation in PT-PFGE2 was considered as mandatory, due to the decision of DG SANCO and EFSA to implement the database of molecular typing of VTEC strains from food and animals. Nevertheless, several NRLs did not participate in the PT, and this was mainly due to the lack of PFGE equipment. As a matter of fact, PFGE needs a specific equipment and well trained staff, that are usually possessed only by laboratories already involved in epidemiological networks.

In this second PT on PFGE typing, the network responded satisfactorily: none of the NRLs obtained a “poor” overall rank, while the majority of them had overall ranks between “good” and “excellent”. These results indicate a good level of preparedness of the NRLs in the PFGE typing of VTEC strains.

However, some weaknesses were identified in some specific technical areas, such as “image acquisition and running conditions”, “bands”, and “gel background”, for which the “poor” rank was assigned to a still high proportion of NRLs (Figure 3). For some NRLs, the performance in one or more of these specific parameters was poor even if their overall evaluation was considered as "excellent". In these cases, the poor performance was related with a wrong positioning of the standard lanes or with the lack of inclusion of the whole lanes in the picture, or even with the use of running conditions different form those specified in the guidelines provided with the invitation. These points will be addressed by providing specific advice and training, the tools mainly used by the EU-RL VTEC to manage the cases of NRL underperformance. In addition, the NRLs will be reminded to use the running parameters indicated in the provided procedures. Proper advice was given in the individual reports.

In conclusion, also this second PT on PFGE typing of *E. coli* provided useful information on the capability of the network to produce PFGE profiles suitable for the inclusion in the forthcoming molecular database managed by EFSA. In addition, the analysis of the results obtained in the PT will help the EU-RL VTEC in refining the training services provided to the NRLs. All these activities will contribute to the general objective of placing the NRL network in the condition to provide high quality PFGE profiles of VTEC strains from food and animals, suitable for comparison and cluster analyses with the analogue profiles of the human isolates collected within the ECDC-FWD network.