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# Report of the 6th proficiency test (PT) for pulsed field gel electrophoresis (PFGE) typing of Verocytotoxin-producing *E.coli* (VTEC) strains (PT-PFGE6) – 2017

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## 1. INTRODUCTION

This document represents the report of the sixth study organized by EURL-VTEC on PFGE typing for the benefit of the network of NRLs (PT-PFGE6).

## 2. OBJECTIVES AND DESIGN OF THE STUDY

The main purposes of this PT were:

- A further assessment of the level of preparedness of the NRL network with respect to the production of high quality PFGE profiles of *E. coli* strains, suitable for the inclusion in the EFSA database of molecular typing data.
- To identify the aspects of the process of molecular data production and analysis that still need improvement.

In addition, the PT allowed a further evaluation of the capability of the NRLs to carry out the band assignment on of their PFGE profiles using the *BioNumerics* software.

The study was conducted according to the International Standard ISO/IEC 17043:2010 "Conformity assessment – General requirements for proficiency testing".

## 3. PARTICIPANTS

A total of 20 Laboratories joined the study. Each participant received an individual Laboratory code.

## 4. MATERIALS AND METHODS

### 4.1. Sample preparation

The test materials sent to the NRLs were constituted by 6 *E. coli* strains (samples 1 to 6).

As for the stability of the samples, previous experiences supported the assumption that the time range between the preparation of the specimens and the deadline for submission of results was short enough to assure the stability of the PFGE profiles.

The test samples were prepared between 7 and 8 November 2017. They consisted of freshly prepared bacterial cultures seeded into soft (0.3 %) nutrient agar in 2 ml glass vials. The cultures were incubated 18 hours at 37 °C ± 1 °C and labeled as "STRAIN 1_Lcode" up to "STRAIN 6_Lcode, for each NRL. The homogeneity of the test strains was assessed on 9$^{th}$ November, by testing two randomly selected sets of strains for the presence of known microbiological characteristics. The test samples were stored at room temperature until November 13$^{th}$, when the samples were sent to the participating laboratories by courier.

The PFGE profiles of the test strains were pre-determined at the EURL-VTEC and were considered as reference profiles to evaluate the acceptability of those submitted by the NRLs (Figure 1).

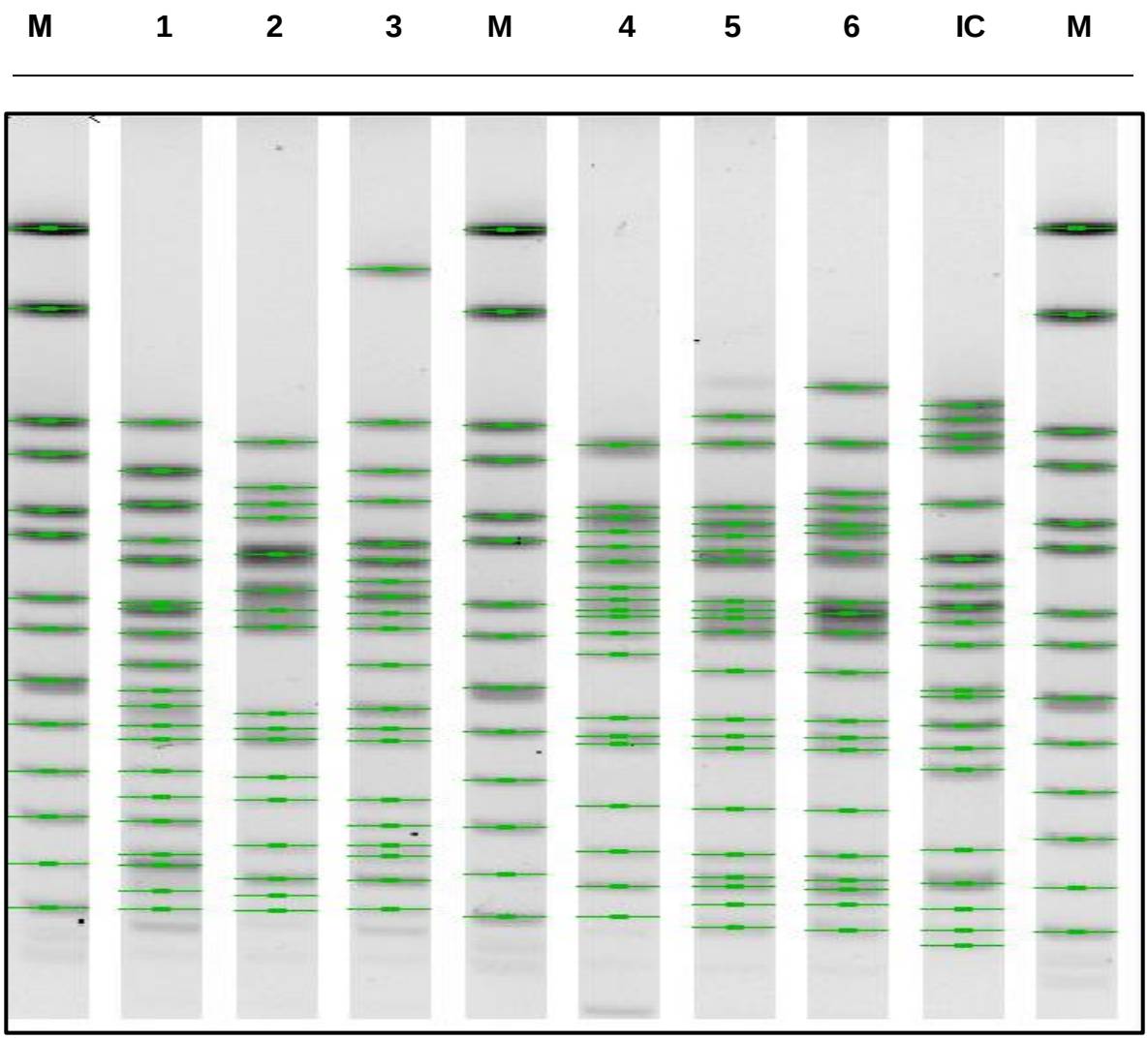


**Figure 1: PFGE profiles of the *E. coli* strains included in the study.** M: *S.* braenderup H9812 molecular weight standard.

## 4.2. Methods

### *4.2.1. Laboratory method for PFGE*

The NRLs were requested to use the standard operating procedure for the production of PFGE profiles of VTEC published by EFSA (http://www.efsa.europa.eu/en/supporting/doc/704e.pdf).

### *4.2.2. BioNumerics software analysis*

The NRLs that accepted to carry out this part of the study were requested to use the standard operating procedure for the profiles interpretation and curation published by EFSA (http://www.efsa.europa.eu/en/supporting/doc/704e.pdf).

### 4.3. Submission of the results

The results were uploaded in the Restricted Area of the Proficiency Tests Section of the EURL website, where the procedure for submitting the results was also available.

Briefly, the NRLs that did not perform the *BioNumerics* analysis were requested to submit the PFGE gel images as non-compressed TIFF format files, together with a scheme of samples loading.

The Labs carrying out the *BioNumerics* analysis were requested to submit the XML export files, prepared with the *BioNumerics* software, including normalization and band assignment.

### 4.4. Analysis of the submitted results: visual assessment of the gel images

The gel images submitted were visually inspected to evaluate their suitability for the further computer-assisted analysis. The parameters used for this evaluation were:

- The position of the gel in the image: the gel should fill the entire window screen, without cutting off wells or lower bands, and the end of the gel must be visible.
- A correct identification of the samples, through the matching with the sample codes assigned to the each NRL.
- A correct positioning of the *S. braenderup* H9812 standard, which had to be loaded in lanes 1, 5, 10 when using 10-well gels or in wells 1, 5, 10, 15 when using 15-well gels. The correct position of the standard is of the utmost importance, because it allows the comparison of PFGE profiles from different gels.
- The focus of the gel image, with no over-exposure of the bands.
- The position of the lowest band of the standard, at 1-1.5 cm from the bottom of gel.
- The intensity of the bands, which should be approximately the same in each lane.
- The absence of unrestricted DNA.
- The bands should be clear and distinct all the way to the bottom of the gel; some band distortion can be accepted, but it should not interfere with the analysis.
- The gel background, which should be clear.
- DNA degradation, which should not be present.
- The electrophoretic conditions.
- The resolution of the image file, which must be at least 8 bit in the color depth properties.

### 4.5. Computer-assisted analysis of the PFGE profiles: migration distortion analysis

The gel images that passed the visual inspection entered the instrumental analysis, which was carried out with the *BioNumerics* software, according to the standard operating procedures for PFGE profiles interpretation and curation published by EFSA (http://www.efsa.europa.eu/en/supporting/doc/704e.pdf); see Figure 2.

For the NRLs that submitted the pictures of the PFGE gels as TIFF files, this analysis was carried out by the EURL.

For the NRLs that made the *BioNumerics* analysis, the EURL used directly the XML files submitted. When problems (*i.e.* image area selection, assignment of the *S. braenderup* H9812 standard bands, background subtraction) were observed, the analysis was repeated by the EURL.

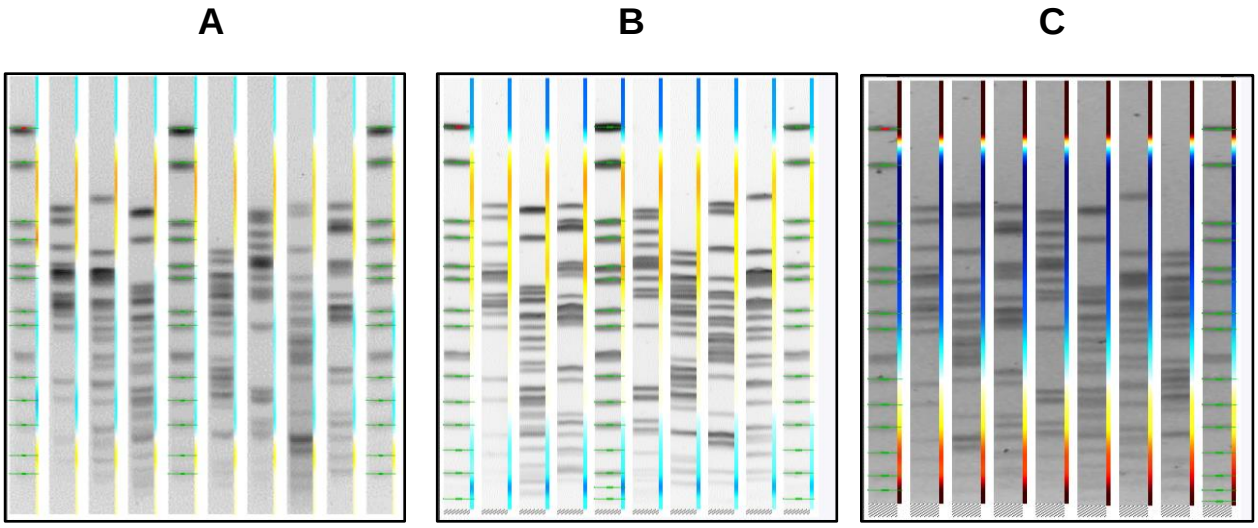


**Figure 2. Examples of migration distortion analysis.** Light colors (sky blue or yellow) indicate an acceptable level of distortion (A); darker colors (red or bright blue) indicate a stronger distortion, which may, however, be compensated by the software (B); black coloring indicates distortions too strong to be compensated by the software (C).

### 4.6. Computer-assisted analysis of the PFGE profiles: cluster analysis

The submitted images were considered acceptable after the migration distortion analysis entered the band assignment step followed by cluster analysis with the related reference PFGE profiles produced by the EURL (Figure 1). The similarity between a submitted profile and the corresponding reference profile was calculated using the Dice coefficient, which depends on the number of bands that are common to both profiles, with tolerance and optimization parameters set at 1.5 %. A single profile was considered acceptable for inclusion in a database when the cluster analysis returned at least 97 % of similarity with the reference

profile. Profiles showing a similarity rate lower than 97 % were considered as “not acceptable”.

For the NRLs that submitted only the pictures of the PFGE gels as TIFF files, both the band assignment and cluster analysis were carried out by the EURL.

For the participants that made the *BioNumerics* analysis, the band assignment was directly used for the cluster analysis. When errors in the band assignment were observed, the procedure was repeated by the EURL before evaluating the NRL’s performance and the modification on the band assignment of the profiles performed by the EURL were detailed in the Individual Report.

### 4.7. Evaluation of the NRL performance

#### *4.7.1. Evaluation of the PFGE profiles*

The performance of each NRL in producing PFGE profiles suitable for inclusion in a database of molecular typing data was evaluated by estimating the rate of not accepted profiles, according to the following scheme**:**

- **Excellent:** No rejected profiles
- **Good:** < 30 % of rejected profiles
- **Fair:** between 30 % and 60 % of rejected profiles
- **Poor:** > 60 % of rejected profiles

#### *4.7.2. Evaluation of the ability to carry out the BioNumerics analysis*

For the NRLs that submitted the XML files, the ability to correctly perform the *BioNumerics* analysis was assessed and the laboratories were assigned to categories from A to E, according to the following criteria:

- **A:** No modifications of the band assignment in the XML files were needed.
- **B:** Only some modifications of the band assignment were needed.
- **C:** Major modifications of the band assignment or complete re-assignment were needed.
- **D:** Both normalization and band assignment had to be repeated.
- **E:** The XML file was not usable for the cluster analysis.

#### *4.7.3. Individual Reports*

Each NRL received an Individual Report with the performance evaluation, the critical assessment of the gel image and suggestions on how to improve the quality of the profiles, with respect to the specific points that generated underperformance.

Starting from this round of EQA, we have also introduced a new section, including the details of the eventual recurring errors, in order to help the laboratories finding strategies to overcome the problems.

## 5. RESULTS

Twenty Laboratories out of the 22 that joined the study submitted PFGE profiles (both as TIFF files or XML export files, see Figure 3 for details), including 19 EU Member States and Norway.

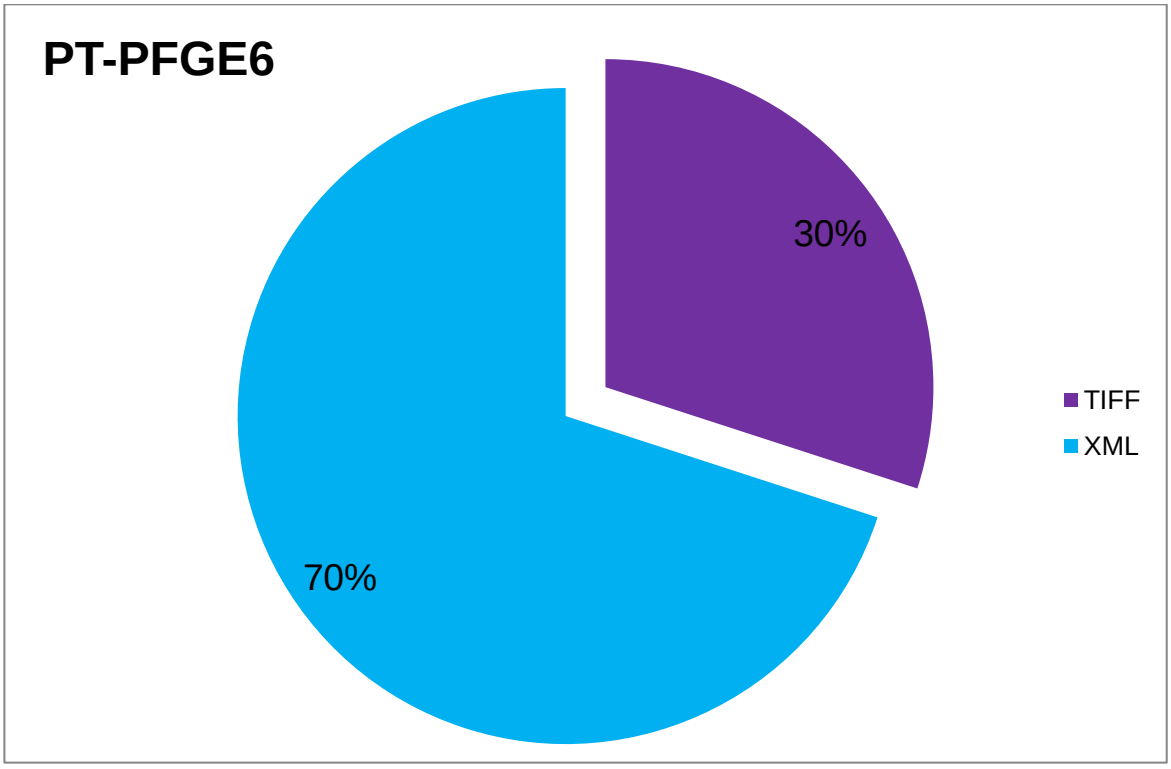


**Figure 3: Percentage of the laboratories submitting the gel images either as TIFF or as XML files (20 NRLs in total).**

### 5.1. Evaluation of the PFGE profiles submitted

The 20 PFGE gel images submitted were first evaluated by visual assessment, as described in paragraph 4.4: all the 120 submitted profiles passed this stage. At the following evaluation step of distortion bar analysis (paragraph 4.5), two images (including 12 profiles) presented excessive distortions to be compensated by the software and were considered as not acceptable. The remaining 108 profiles were subjected to cluster analysis and 105 of them (87.5 %) were considered as suitable for inclusion in the database of molecular typing data (more details included in Figures 4 and 5).

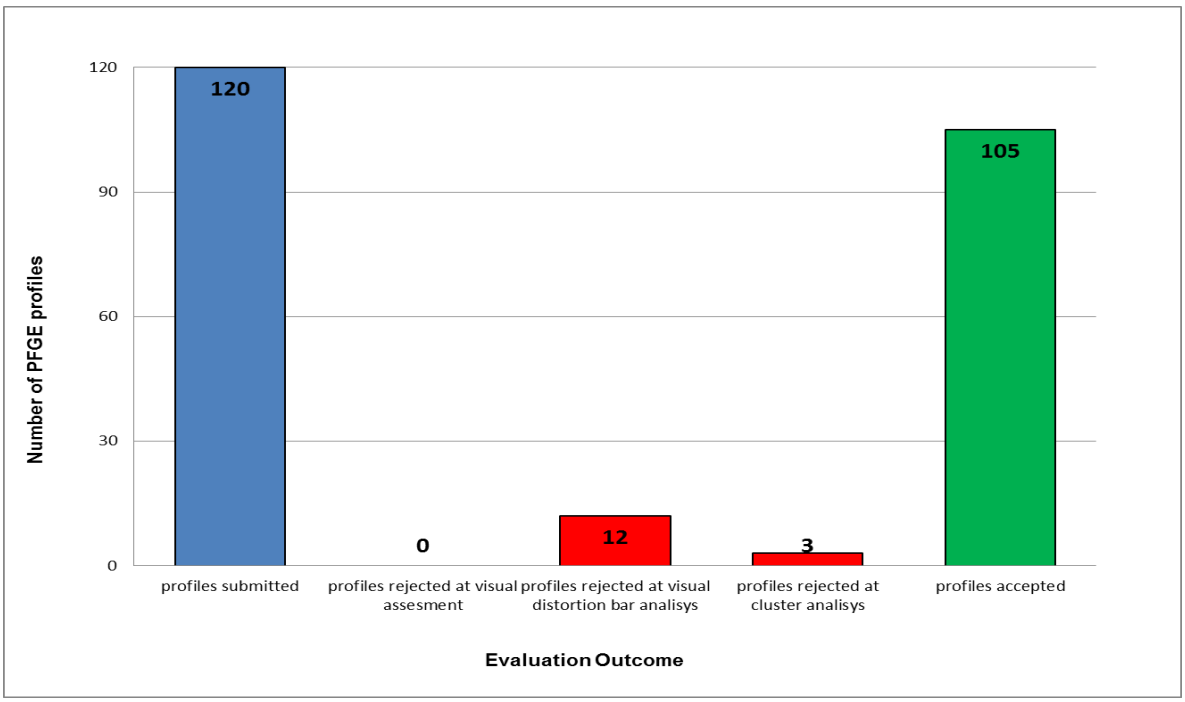


**Figure 4. Three-step evaluation of the submitted PFGE profiles.**

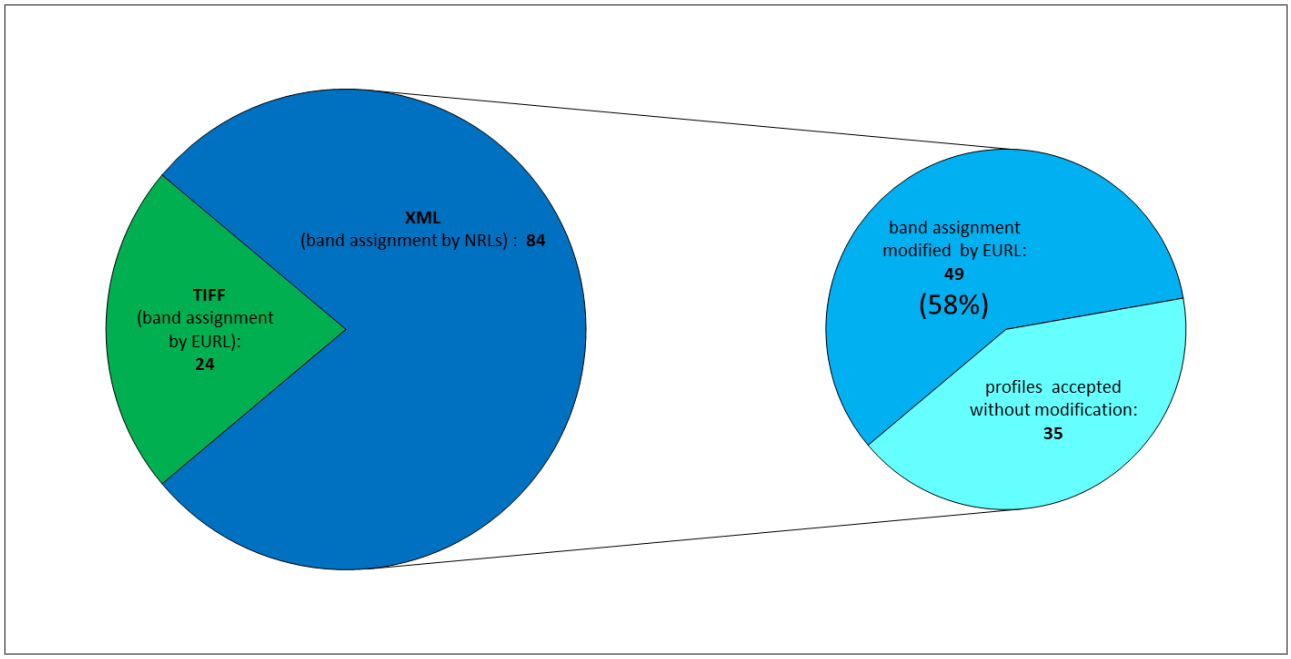


**Figure 5. Cluster analysis details.** Out of the 84 profiles submitted as XML, 49 were subjected to modification in the band assignment.

The similarity values obtained by the cluster analysis with the related reference PFGE profiles are shown in detail in Table 1.

**Table 1. Cluster analysis of the PFGE profiles submitted by the NRLs with the related reference PFGE profiles.** The green boxes indicate the acceptable profiles, the red boxes those considered as not acceptable. The numbers in the boxes indicate the percentage of similarity. NA: not analyzable (indicates that the quality of the profile was not suitable for cluster analysis).

| NRL | Similarity (%) of the PFGE profile submitted by the NRL with the related reference profile for: | | | | | | |
|---|---|---|---|---|---|---|---|
| | Strain 1 | Strain 2 | Strain 3 | Strain 4 | Strain 5 | Strain 6 | Nr. of modified profiles |
| L136 | 94.7 | 93.8 | 100 | 97.3 | 97.6 | 97.4 | 6 |
| L178 | 97.6 | 100 | 100 | 100 | 100 | 100 | N.A. |
| L230 | 97.4 | 100 | 100 | 97.3 | 97.6 | 100 | 0 |
| L322 | 100 | 100 | 100 | 100 | 97.7 | 97.6 | 4 |
| L404 | 100 | 97.1 | 100 | 100 | 100 | 100 | 6 |
| L419 | 97.4 | 97 | 95 | 100 | 97.6 | 97.4 | N.A. |
| L504 | 100 | 100 | 100 | 100 | 97.7 | 97.6 | 6 |
| L527 | 97.6 | 97.1 | 97.6 | 100 | 97.6 | 97.4 | 3 |
| L546 | 97.6 | 97.1 | 97.6 | 97.4 | 100 | 97.6 | 5 |
| L562 | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. |
| L600 | 100 | 100 | 97.6 | 100 | 100 | 97.6 | 2 |
| L607 | 100 | 97 | 100 | 97.3 | 97.6 | 97.4 | N.A. |
| L653 | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. |
| L712 | 97.6 | 100 | 100 | 100 | 97.6 | 97.6 | 2 |
| L723 | 100 | 97.1 | 97.6 | 100 | 100 | 97.6 | 2 |
| L792 | 100 | 100 | 100 | 100 | 100 | 100 | 0 |
| L843 | 100 | 100 | 100 | 100 | 97.6 | 100 | 5 |
| L894 | 100 | 100 | 100 | 100 | 97.7 | 100 | 6 |
| L944 | 97.4 | 97.1 | 100 | 100 | 100 | 100 | N.A. |
| L979 | 97.6 | 100 | 100 | 100 | 100 | 97.4 | 2 |

The performance of each NRL in producing PFGE profiles suitable for inclusion in a database of molecular typing data was evaluated according to the criteria described in section 4.7.1. Figure 6 shows the score obtained by each NRL and Figure 7 the number of NRLs grouped according to their score. The performance was classified as “poor” for two NRLs (10 %), “good” for two NRLs (10 %) and “excellent” for 16 NRLs (80 %).

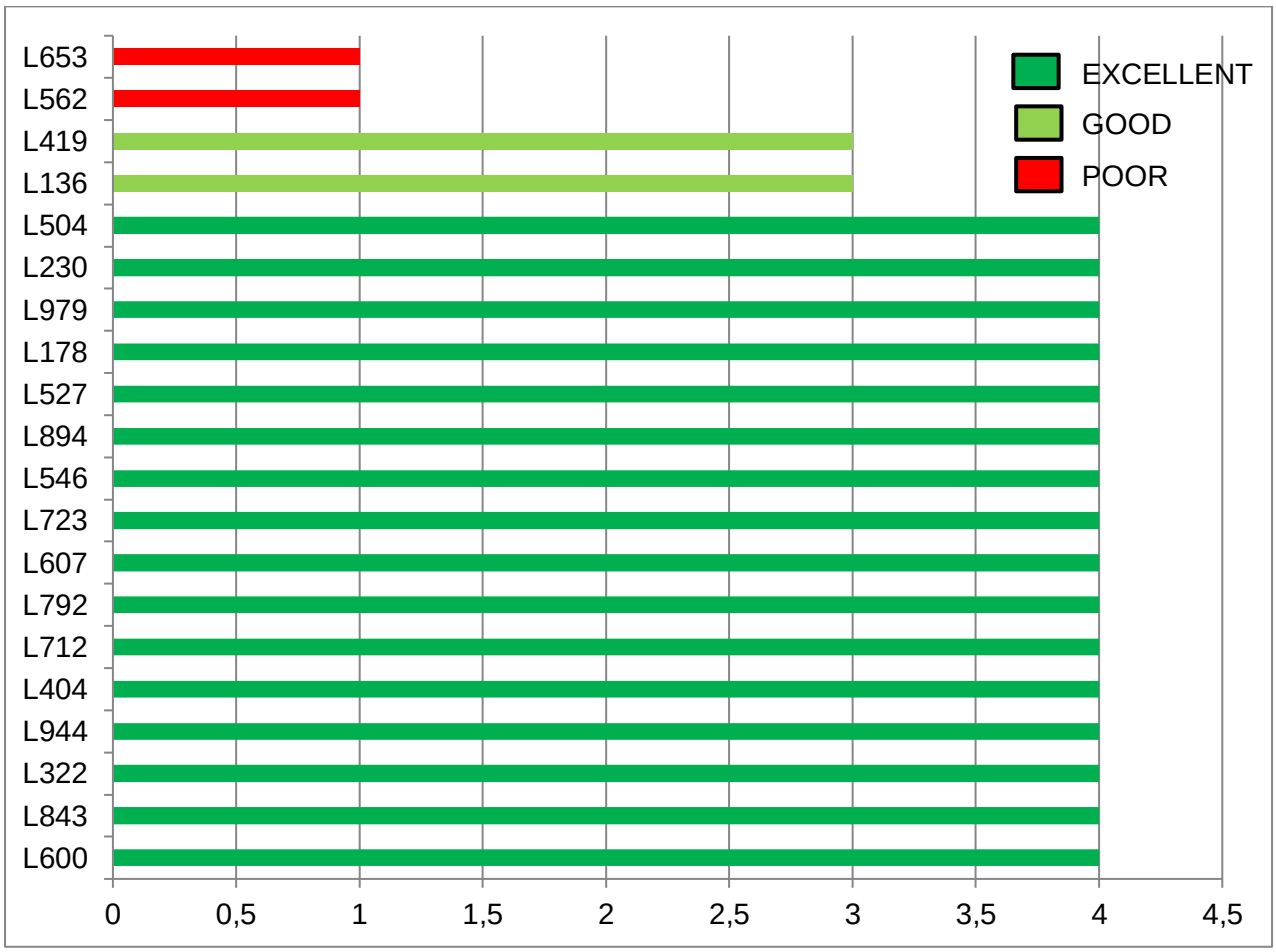


**Figure 6. Evaluation of the performance of each NRL in producing PFGE profiles.**


**Figure 7. Evaluation of the NRLs' performance in producing PFGE profiles.** The red bars indicate the NRLs whose performance was considered as "poor".

The problems most commonly encountered by the NRLs in producing PFGE profiles are shown in Figure 8. The most frequent problems were related with the band assignment performed by the NRLs submitting the XML file and the samples identification. Each NRL received proper advice to overcome specific problems in the individual report.

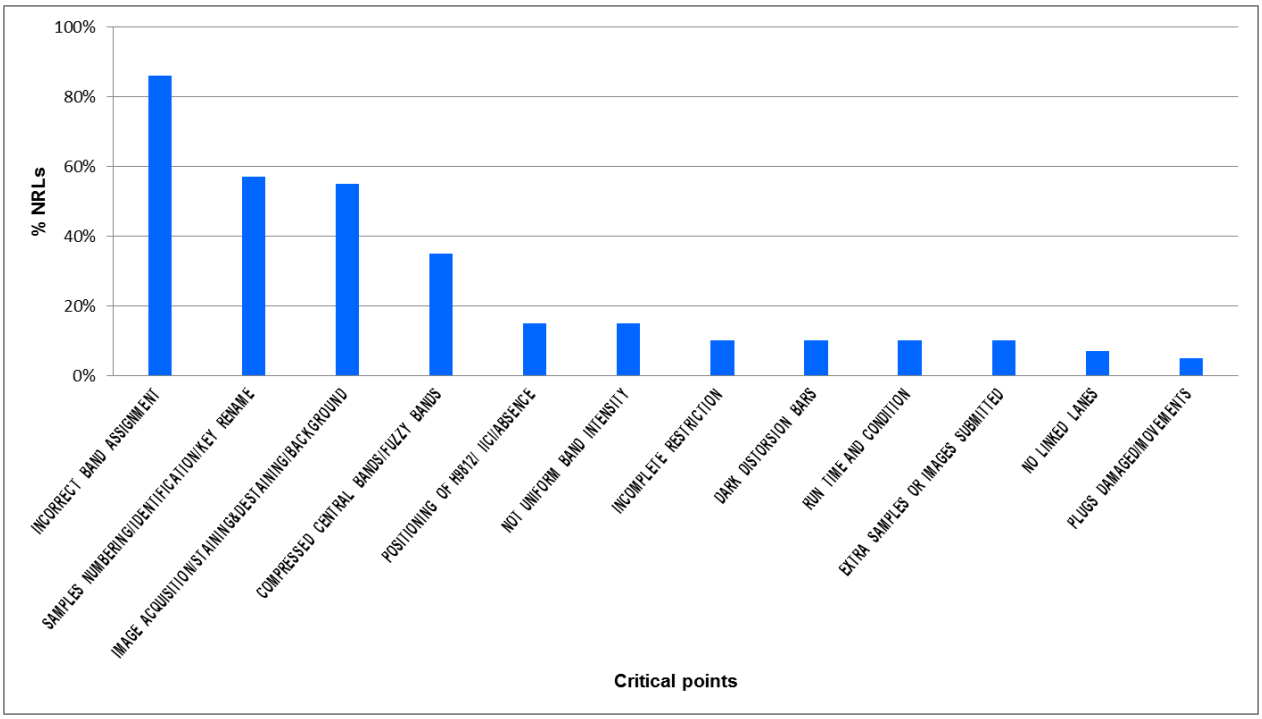


**Figure 8. Critical points encountered by the NRLs in PFGE profile production.**

### 5.2. Evaluation of the ability of the NRLs to carry out the *BioNumerics* analysis

This evaluation was performed on the basis of the modifications required for the profiles of the 14 NRLs that submitted the XML files, according to the criteria described in section 4.7.2. The category assigned to each NRL is shown in Figure 9, while Figure 10 shows the number of NRLs grouped in each category. Eight NRLs (57 %) were categorized as “C”, meaning that major modifications of the band assignment or complete re-assignment were done, and indicating improper analysis of PFGE profiles with BioNumerics software was carried out. Four NRLs, representing 28.6 % of the total, fell into “B” category and the remaining two Laboratories obtained the category “A” (no modifications of the band assignment in the XML files were needed).

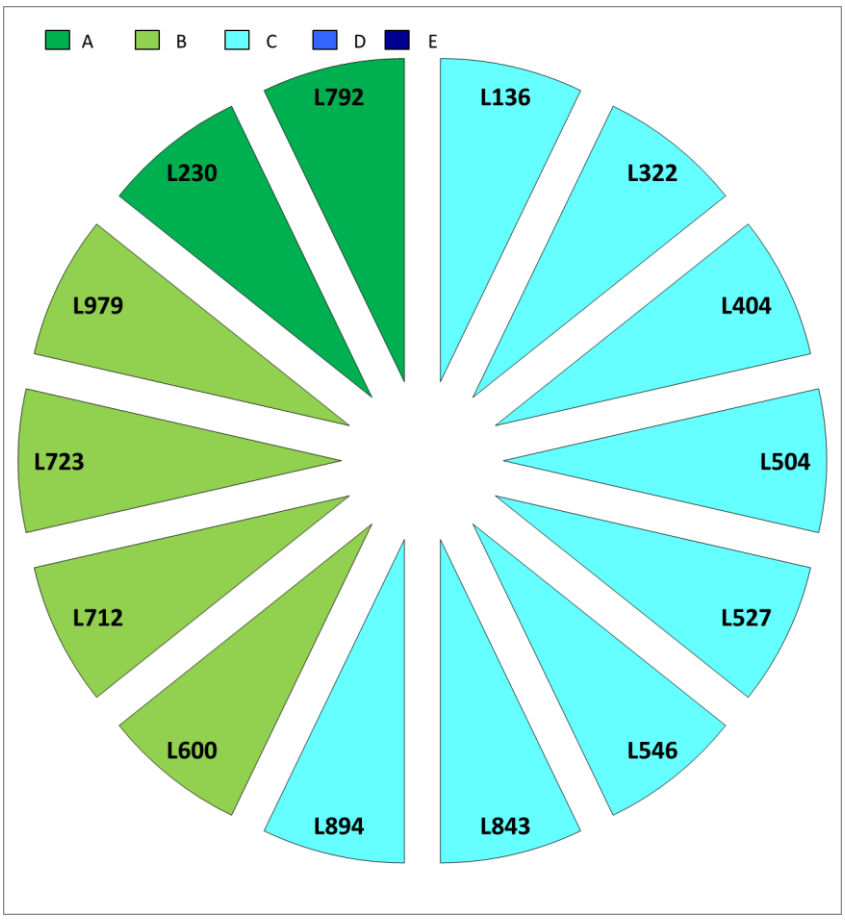


**Figure 9. Evaluation of the ability of the NRLs to carry out the *BioNumerics* analysis.**

Categories are defined in section 4.7.2.

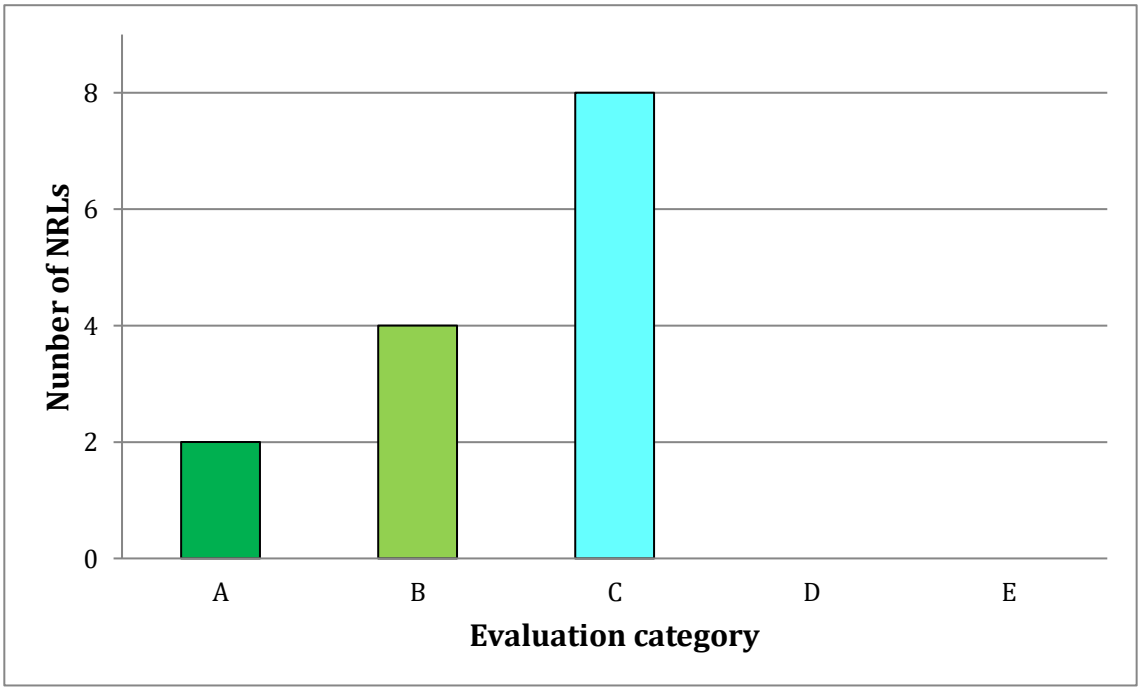


**Figure 10. Ability of the NRLs that submitted XML files to carry out the *BioNumerics* analysis, grouping by categories.**

## 6. CONCLUDING REMARKS

A good EQA program is meant to improve the performance to produce good quality profiles to be uploaded in a common database, monitoring this capability continuously.

This program has reached the sixth round and a network of laboratories producing suitable PFGE profiles has been established.

A total of 20 laboratories, including more than 70 % of the EU Member States and Norway, joined this round of PT, demonstrating the interest of the NRLs in the molecular typing by PFGE.

In order to find the areas needing dedicated training sessions and to better improve the performance, we made for the first time the identification of the recurring errors. Fifty percent of the participants presented 15 recurring errors, mainly associated to the visual assessment (Figure 11). This reflects the complexity of the procedure. However the evaluation of the profiles through a codified procedure (http://www.efsa.europa.eu/en/supporting/doc/704e.pdf) allowed the identification of the critical points that should be taken under control.

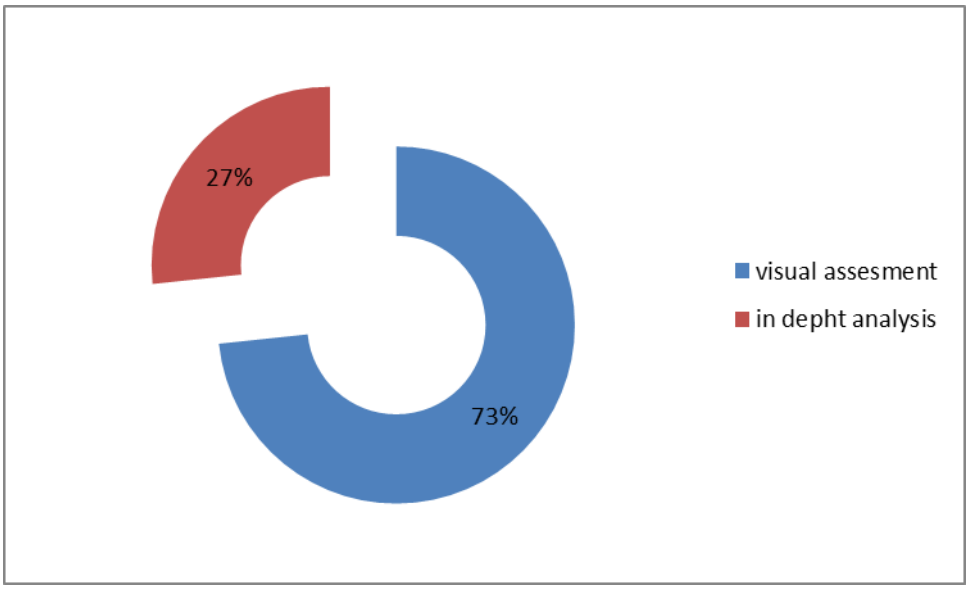


**Figure 11: Recurring errors: visual assessment *vs* in depth analysis.**

For this round of EQA, an unprecedented proficiency has been observed, with 80 % of laboratories obtaining “excellent” as overall evaluation. The band re-assignment played a crucial role for this achievement, as it had to be corrected for more than 80 % of the NRLs that submitted XML files (see Figure 8), representing 70 % of the total participants.

Taking into account the percentage of similarity with the reference profiles and the modification needed in the band assignment, we also identified the most critical strains.

The percentage of the modification needed in the band re-assignment is shown in Figure 12. Strain 4 required major modifications (11 Laboratories on 14 assigned a different PFGE profile).

The majority of the labs was able to proficiently analyze strains showing less complicated profiles, while the majority of the errors in band assignment concentrated on a couple of strains having more difficult profiles.

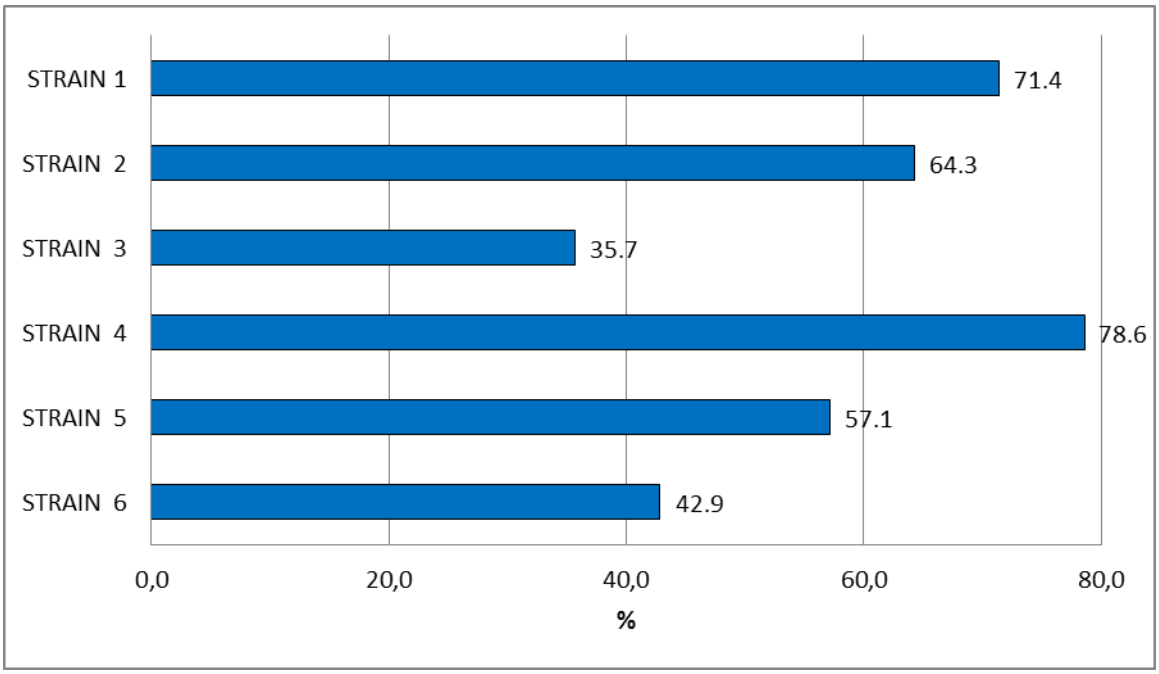


**Figure 12: Percentage of modification in the band re-assignment for each strain.**

In conclusion in this 6$^{th}$ PT on PFGE typing of *E. coli*, the percentage of the NRLs obtaining an excellent rate has been further increasing, in comparison to the previous rounds, confirming that a good network has been established and that the EQA program is a good way for improve the performance.