Evaluation of the performance of the NRLs participating in the PTs organized by the EURL for *E. coli* and management of underperformance

Foreword

The performance of the NRLs participating in the PT program of the EURL-VTEC is evaluated with the purpose of ensuring that the network of NRLs for *E. coli* is prepared in responding to the regulations concerning the food safety and to the increasing demand for testing food for the presence of pathogenic *E. coli* and Shiga toxin-producing *E. coli* (STEC) in particular.

The present document describes how the NRLs' performance is evaluated and how the underperformance is managed in the network coordinated by the EURL *E. coli*.

Introduction

The detection of pathogenic *E. coli* including STEC in food requires the use of a molecular methodology, such as PCR, being these microorganisms phenotypically indistinguishable from the other harmless individuals belonging to the same bacterial species. Therefore, the identification of the hazard is done by identifying the presence of virulence genes specifically associated with the different *E. coli* pathotypes.

As far as the identification of the genes associated to the *E. coli* virulence is concerned, the proficiency of the NRLs has been evaluated, in the first 10 PT schemes, by the determination of the Cohen's K value. The reason for choosing an indicator of the agreement of the results provided with the gold standard, rather than a more precise score-based scheme, lied in the need to introduce the use of such molecular methodologies among laboratories who were mostly involved in classical food microbiology and used to deal with culture-based methods. The adoption of an innovative and not-yet-established methodology was a Hobson's choice. In fact, to discriminate pathogenic strains from the ubiquitous commensal *E. coli* is a challenge that cannot be overcome by cultivating the microorganisms. Additional to the introduction of a new diagnostic approach, the identification of the different *E. coli* pathogroups, based on the detection of several genes associated with the ability to adhere to the host mucosa and to the production of toxins at the same time, further complicated the diagnostic scheme and the reporting of the results.

Therefore, the need to implement a state-of-the-art detection technology demanded the use of performance indicators that were at the same time directed towards the definition of the method's performance while allowing a certain degree of evaluation of the proficiency of the laboratories. While the Cohen's K value satisfied such a request, the adoption of a score-based scheme would not have allowed deploying an effective methodology and its widespread adoption by all the laboratories composing the NRL network for *E. coli*.

Now, the methodology is fully established and the international standard, ISO TS 13136:2012, based on the proposed approach, has been published and adopted in the EC Regulation 209/2013 on the safety of sprouts. Additionally, all of the NRLs now are able to perform correctly the molecular detection of the virulence genes of pathogenic *E. coli*. Therefore, from the PT11 and onwards, a different method for the evaluation of the NRLs proficiency has been adopted. The method used by the EURL-VTEC to evaluate the performance of the NRLs is illustrated to the participant NRLs in the final reports of each PT.

Reports of the PTs are available here.

Methodology

The new approach is based on the association of penalty points to the incorrect result reported. Such penalty points are used to identify the aspects of the NRLs proficiency to be improved and are assigned as detailed below.

- **4 penalty points**: Are assigned to each incorrect result concerning the identification of the stx genes. In fact, Stxs are the main virulence determinants of STEC and the detection of their coding genes represents the main objective of the ISO TS 13136:2012, the international standard aiming at the detection of these pathogenic *E. coli* in food. Moreover, there is a microbiological criterion for the presence of STEC in a food commodity in an EU regulation (Reg. EU 209/2013) and a negative result to this assay triggers the release of the food commodity.
- **2 penalty points**: Are assigned to each incorrect result concerning the identification of the genes encoding adhesion determinants such as the eae (EPEC), *aggR* and aaiC genes (EAggEC). The same penalty is assigned to each incorrect result concerning the identification of the genes encoding the heat labile (lt) and heat stabile (st) enterotoxins of ETEC. The rationale behind the choice of assigning 2 penalty points to the incorrect identification of these genes lies in the following considerations:

- The methods for their detection have been developed in house and are not validated yet (except for the eae gene, that has been partially validated in the ISO TS 13136:2012).
- There is not a microbiological criterion for the *E. coli* pathogroups identified by the presence of these genes (EPEC, EAggEC and ETEC) in food.
- *E. coli* displaying the presence of these genes in food are not expected to be frequently found (although the *eae* gene is present in some STEC or EPEC, the latter are not considered a hazard while all STEC are covered by the stx genes detection). Finally, the latest pathogenicity assessment of STEC published by EFSA (available here) states that these additional genes may represent aggravating factors but they are not essential for severe illness, therefore these may or may not be searched in food.

- **2 penalty points:** Are assigned to each incorrect result concerning the identification of the genes associated with the serogroup.
- **1 penalty point**: Assigned to each result reported as Not Done for all the virulence genes additional to the *stx* and those associated with the serogroup.

All the scores indicated are assigned to any assay for the detection of the mentioned genes either in the screening of the enrichment culture or in the characterization of the isolated strains

- **2 penalty points:** Are assigned to each unsuccessful attempt to isolate a STEC from a sample positive at the screening.

The sum of the penalty points obtained originates a total score used to evaluate the underperformance of NRLs. The total score determination will help in deciding which measures the EURL-VTEC needs to adopt to manage the under-proficient laboratories and will be evaluated by using the scheme depicted in the following table:

Score	Under-performance	Action
0 to 3	none	**No actions required**
4 to 8	Light	**Backup samples** are sent to the NRL in order to verify the sporadic nature of the error. Any error reported during the analysis of backup samples sum up to those reported during the PT. **The rank of the underperformance** is **downgraded** in case **no errors** are reported during the analysis of backup samples. **If new errors** are reported, the **rank of underperformance is upgraded** based on the present scheme. This will trigger the corresponding measures
9 to 15	Medium	**Interview** to the NRL with the aim of highlighting the problems encountered and identifying the adequate solutions. **Training stage at the EU RL VTEC.** A training program is selected among those developed by the EU RL VTEC that best fit the NRL's needs. Analysis of backup samples during the training session.
15 to 20	Heavy	**On-site visit** conducted to carry out a complete revision of the Laboratory's procedures. The Laboratory could be requested to perform the assay during the on-site visit if considered as necessary by the visit team. In this case it will be agreed with the laboratory and placed in the agenda before the visit is done.