



**Program for a 5-days training at the EURL-VTEC, Istituto Superiore di Sanità, Rome,
on the design and preparation of proficiency tests (PTs)
on the detection of VTEC in food matrices**

General objective:

To improve the capacity of the NRLs in planning and delivering PTs to the network of official laboratories involved in the control of food in the EU member states.

Work Program

Day 1 (10:30-16:30)

- Overview on the procedures of the EURL.
- Opening discussion on the work-plan and overview on the activities to be done during the stage.

Requirements

- Terms of reference for PT organization: Reg. EC/882/2004 and the related ISO reference documents
 - ISO/IEC 17043:2010 Conformity assessment - General requirements for proficiency testing.
 - ISO/TS 22117:2010 Microbiology of food and animal feeding stuffs - Specific requirements and guidance for proficiency testing by inter-laboratory comparison.
 - ISO/TS 19036:2006 Microbiology of food and animal feeding stuffs - Guidelines for the estimation of measurement uncertainty for quantitative determinations.
- PT planning:
 - Identification of the objectives.
 - Selection of the analyte (STEC strains), the matrix, and the laboratory procedure.

Hands-on

- Preparation of the cultures of the STEC strains selected as analyte.

Day 2 (9:30-17:30)

Requirements

- Standardization of the spiking inoculum.
- Calculation of the of measurement uncertainty of the spiking inoculum.

Hands-on

- Refreshment of the STEC cultures (analyte).
- Growth curve (monitored by optical density).
- Dilution and plating of the 0.5 OD₆₀₀ STEC cultures for the determination of the bacterial titre and calculation of the measurement uncertainty.
- Preparation of the matrix and pre-testing:
 - evaluation of the endogenous microflora (direct plating);
 - absence of the target analyte (set up of enrichment culture and over-night incubation).

Day 3 (9:30-16:30)

Requirements

- Determination of the limit of detection (LOD) of the analytical method employed in the PT (combination matrix/analyte).

Hands-on

- Reading of the STEC culture plates for calculation of the of the measurement uncertainty.
- Evaluation of the matrix endogenous microflora (plates reading).
- Testing the matrix enrichment culture for the absence of the target analyte (Real Time PCR according to ISO TS 13136:2012).
- Preparation of the STEC cultures for sample spiking.
- Spiking of the samples with appropriate dilutions for LOD determination.
- Preparation of the enrichment cultures of the spiked samples.

Day 4 (9:30-17:30)

Hands-on

- Testing of the enrichment cultures for analyte target genes by Real Time PCR (ISO TS 13136:2012) and LOD determination for the screening step.
- Plating of the PCR-positive enrichment cultures for STEC isolation.

Requirements

- Planning of the stability tests, according to ISO/IEC 17043:2010.

- Planning of the homogeneity tests, according to ISO/IEC 17043:2010.

Day 5 (9:30-13:30)

Hands-on

- Identification of the target STEC colonies and LOD determination (isolation step).

Concluding session

- General discussion and concluding remarks.

All the five days include explanatory discussions (Requirements) driven by the EURL-VTEC experts and practical sessions carried out by the trainee with an hands on approach, under the supervision of the EURL-VTEC staff.