18th Annual Worksop of the National Reference Laboratories for *E. coli* – 05-06 October 2023

The training program of the EURL for *E. coli* 2022-2023



Image from: https://accademiadomani.it/pacchetto/





Image from: https://viaggi.corriere.it/weekend/roma-segreta-gliitinerari-insoliti-e-i-classici-come-non-li-avete-mai-visti/



Istituto Superiore di Sanità, Food Safety, Nutrition and Veterinary Public Health Department European Reference Laboratory for *Escherichia coli*



Training activities of the EURL for *E. coli* in 2023

- Training course on characterization of pathogenic *E. coli* through NGS (two-days)
- STEC detection in food matrices based on the ISO TS 13136:2012 (five days stage)
- Identification and characterization of the different groups of pathogenic *E. coli* (four days stage)

The call was launched at the beginning of 2023, with the deadline for responding in mid-February

NRLs were informed that, in addition to the satisfaction survey, a final questionnaiere for evaluating the achievement of the training objectives would be administered to participants

Training course on characterization of pathogenic *E. coli* through NGS (two-days)

This course was held online in the days 11-12 July 2023

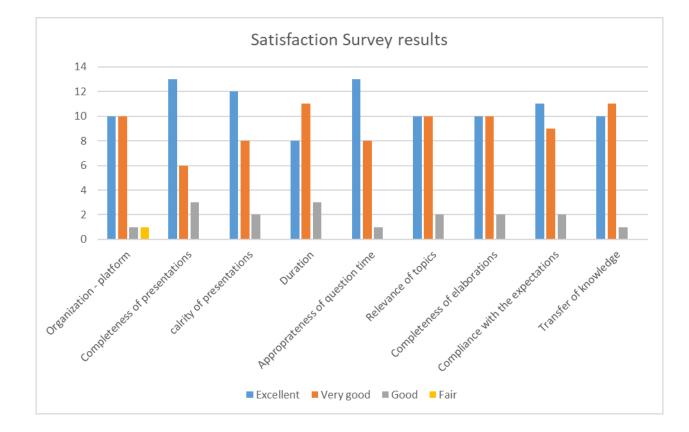
Presentations from EURL for E. coli staff

Assignments of excercises

Live-chat to provide assistance

22 participants – 16 from EU and non-EU NRLs and 6 from Italian OLs

86,4% of participants successfully met the training objectives reaching >80% compliance assessed through a dedicated questionnaire



Starting from 2022...

Scientists for training prgrams on laboratory procedures have been hosted in person at ISS ③



Training on the Identification and characterization of the different groups of pathogenic *E. coli* 17-21 April 2023



EU Reference Laboratory for E. coli Department of Food Safety, Nutrition and Veterinary Public Health Unit of Food Microbiology and Foodbome Diseases Istituto Superiore di Sanità



Program for a 4-days training at the EURL-VTEC, Istituto Superiore di Sanità, Rome, on the identification and characterization of the different groups of pathogenic *E. coli* by Real Time PCR amplification of their virulence genes

Day 1 (14:30-17:30)

- Overview of the activities and procedures in place at the EURL.
- Opening discussion on the work-plan and overview of the activities to be done during the stage.
- Preparation of the cultures of test and control strains.

Day 2 (9:30-17:30)

- Preparation of DNA samples for Real Time PCR from test and control strains.
- Real Time PCR for the identification of the E. coli pathogroups STEC and EAEC.

Day 3 (9:30-17:30)

- Real Time PCR for the identification of the E. coli pathogroups EIEC and ETEC.
- DNA template preparation for the identification of stx gene subtypes by conventional PCR.
- stx-subtyping by conventional PCR (EU-RL VTEC_Method_006_Rev 1).

Day 4 (9:30-17:30)

- Agarose gel electrophoresis to visualise the stx-subtyping PCR results.
- Analysis of the results obtained.
- General discussion on the activities carried out.
- Questionnaire on the trainee satisfaction toward the stage

All the three days include explanatory discussions 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.

Two scientists from EU NRLs participated



Both participants successfully met the training oblectives

Training on STEC detection in food matrices based on the ISO TS 13136:2012

6-10 November 2023 13-17 November 2023



EU Reference Laboratory for E. coll Department of Facel Safety, Number and Veterlany Public Nearth Unit of Facel Infractionary and Facebase Isofitutio Superiore di Sanita



Program for a 5-days training at the EU-RL VTEC, latitute, superiors of sanita, Rome, on the detection of STEC in food matrices according to the ISO T\$ 13136:2012 and the characterization of the isolated STEC strains

Day 1 (14:30-17:30)

- Overview on the activities and procedures of the EURL.
- Opening discussion on the work-plan and overview on the activities to be dope during the stage.
- Enrichment of food samples according to the ISO TS 13136:2012
- Introduction to the EURL Laboratory procedure for STEC O104 identification

Day 2 (9:30-17:30)

- DNA purification from food enrichment cultures.
- Real Time PCR for VTEC identification according to the ISO TS 13136:2012
- Real Time PCR for O104 identification
- Isolation of STEC from Real Time PCR-positive food enrichment cultures: streaking the plates

Day 3 (9:30-16:30)

- Picking up and pooling of colonies and backup onto solid media (nutrient agar)
- Confirmation of suspected colonies as STEC by conventional PCR (Annex C of the ISO TS 13136:2012)

Day 4 (5:30-17:30)

- Molecular serogrouping by conventional PCR (EU-RLVTEC_Method_003_Rev1 25/03/2014)

EU-RL VIEC-Judoka Program (SOT513136 Ray 3

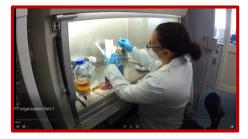
17/12/2018

Day 5 (9:30-13:30)

- Agarose gel electrophoresis to visualise the stx-subtyping PCR results
- Analysis of the results obtained
- General discussion on the activities carried out
- Questionnaire on the trainee satisfaction toward the stage

All the five days include explanatory discussions 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.

Hands-on



Seven scientists will be attending

17/12/2018

2nd joint training – June 20th-21st 2023 RIVM

Introduction to Bioinformatics for genomic data mining

TUESDAY 20 JUNE 2023

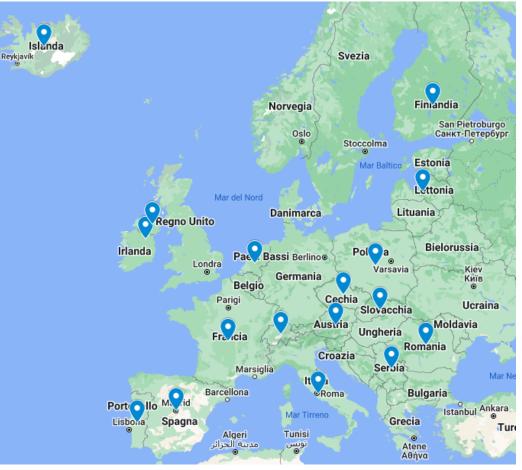
| 9.15 | Registration (for entering the premises of RIVM) |
|-------|---|
| 9.45 | Welcome and general overview on the joint training activities (Wilma Jacobs, EURL- Salmonella) |
| 10.00 | Introduction to WGS Sequencing platforms & output data (20') (Adrien Asséré, EURL-Listeria monocytogenes) Verification of the integrity of the raw data files (like md5sum) (20') (Angela van Hoek, EURL-Salmonella) Bioinformatics analysis of NGS data: approaches and opportunities (command-line tools, commercial software, webservers) (20') (Joakim Skarin, EURL-Viruses) |
| 11.00 | Coffee break |
| 11.20 | Introduction on quality check and trimming (Valeria Michelacci, EURL-VTEC) |
| 11.40 | Hands-on exercises – Quality check and trimming Quality check and trimming using different tools and platforms. Results interpretation. FastQC and Positional and Quality Trimming on ARIES (Valeria Michelacci, EURL- VTEC) Quality check and trimming with Seqsphere (Bo Segerman, EURL-Campylobacter) |
| 12.45 | Lunch break |
| 13.45 | Introduction on assembly and assembly statistics (Lauge Holm Sørensen, EURL-AMR) |
| 14.00 | Hands-on exercises – Assembly statistics Assembly statistics using different tools and platforms. Results interpretation. Hands-on SPAdes and Quast from ARIES (Federica Gigliucci, EURL-VTEC) Demo: Seqsphere Assembly Quality Check (Angela van Hoek, EURL-Salmonella) |
| 15.00 | From BAM to BCF and beyond, making sense of aligned data (Paolo Vatta, EURL- Parasites) |
| 15.30 | Hands-on exercises – Mapping approach Demonstration of mapping through different platforms. Hands-on E. coli virulotyping using a mapping approach (Federica Gigliucci,EURL- VTEC) Demo: Mapping with Seqsphere (Déborah Merda, EURL-Listeria monocytogenes) |

WEDNESDAY 21 JUNE 2023

| 8.45 | Registration (for entering the premises of RIVM) |
|-------|--|
| 9.15 | Introduction to gene detection using BLAST approach (Marina Cavaiuolo, EURL- CPS) |
| 9.30 | Hands-on exercises – Search of genetic features on contigs Identification of virulence and AMR genes using different tools and platforms. Results interpretation. Demonstration of genes identification on contigs through different platforms. Hands-on ResFinder on CGE webserver (Lauge Holm Sørensen, EURL-AMR) Demo: Salmonella virulotyping with Seqsphere (Angela van Hoek, EURL- Salmonella) |
| 10.15 | Parasites WGS: opportunities and challenges (Simone Cacciò, EURL-Parasites) |
| 10.45 | Coffee break |
| 11.00 | Introduction to genome comparisons: gene-by-gene VS SNPs (Guidance document for cluster analysis) (Bo Segerman, EURL-Campylobacter) |
| 11.30 | Demonstration of gene-by-gene approach through different platforms: ARIES (EURL-VTEC) Seqsphere (Angela van Hoek, EURL-Salmonella) Starflow (Déborah Merda, EURL-Listeria monocytogenes and Marina Cavaiuolo, EURL-CPS) |
| 12.15 | Hands-on exercises – Visualisation of clustering data Demonstration via Grapetree (Déborah Merda, EURL-Listeria monocytogenes) |
| 12:45 | Lunch break |
| 13.45 | Update on the EFSA OneHealth WGS database (Mirko Rossi, EFSA) |
| 14:15 | Information on activities of inter EURLs Working Group on NGS and guidance documents released (Valeria Michelacci, EURL-VTEC) |
| 14:45 | Wrap up (Wilma Jacobs-Reitsma, EURL-Salmonella) |
| 15.00 | Closure |



Participants – geographic distribution



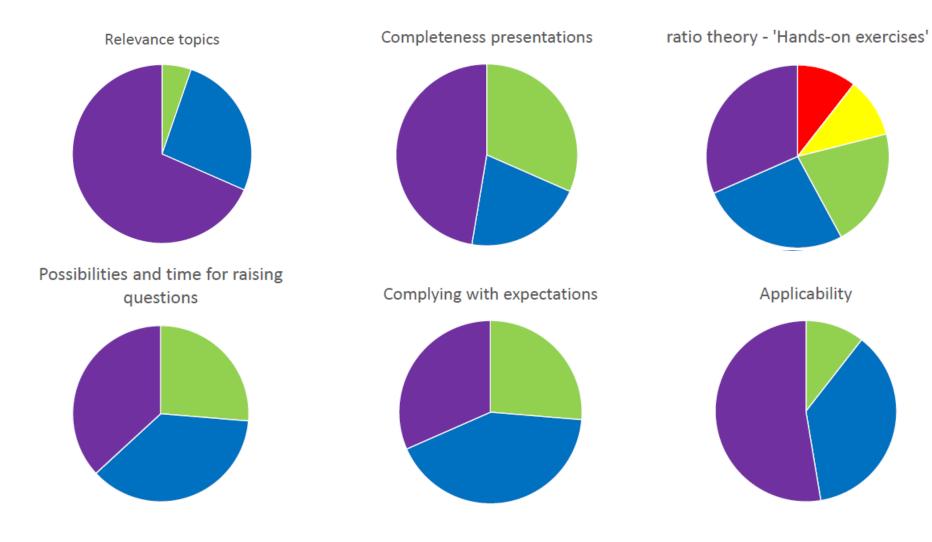
24 participants from different NRLs4 at own costs2 supported by EURL VTEC

17 different countries (13 Member States + 2 EFTA/EEA + Northern Ireland + 1 Candidate Country)

Many participants came from labs appointed as NRLs for multiple pathogens

2023 - Training satisfaction survey

■ Poor ■ Fair ■ Good ■ Very good ■ Excellent



Thank you for your attention!