

The Activities and Role of the European Union Reference Laboratory for *Escherichia coli*, including Verotoxigenic *E. coli*

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VTEC/STEC are nasty bugs!!



One of the most dangerous foodborne pathogens

Life-threatening clinical manifestation

Very low infectious dose (10-100 CFU**)**

Very large community outbreaks



STEC: Zoonotic origin



Ruminants are reservoirs

Routes of transmission:

Foodborne - Primary contamination of meat and milk

Environmental contamination

Waterborne: Drinking and recreational water

Direct contact with infected animals



Shiga Toxin-producing *E. coli* (STEC)



Clinical manifestation

- **Intestinal**
 - Asymptomatic**
 - Watery Diarrhea**
 - Hemorrhagic Colitis**
- **Systemic**
 - Hemolytic Uremic Syndrome**



The "Zoonoses Directive" 2003/99/CE



Annex I A, First rank priority

Salmonella

***Escherichia coli* VTEC**

Campylobacter

Brucella

Listeria monocytogenes

Mycobacterium bovis

Echinococco

Trichinella

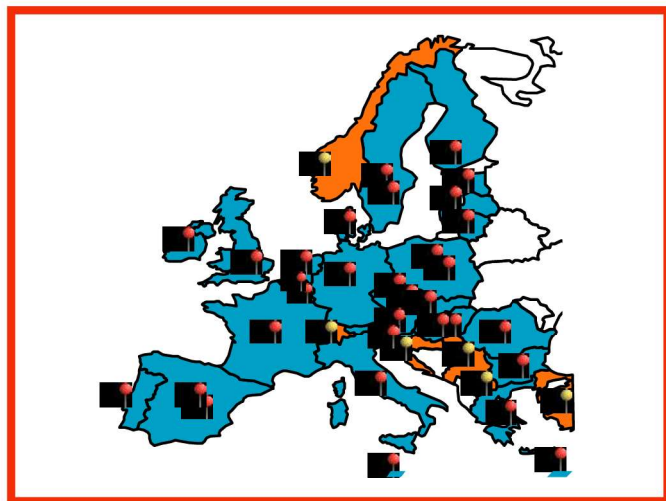


EURL for *Escherichia coli*



Appointed in 2006

Hosted in Rome, Italy, at the Department of Food Safety, Nutrition and Veterinary Public Health of the Italian Institute of Health



Pathogenic *E. coli*, including STEC

Operates as Italian NRL for *E. coli*



EURL for *Escherichia coli*



Network of 38 EU-NRLs from the 27 EU MS

4 NRLs from EFTA Countries:

1. Iceland
2. Norway
3. Switzerland (2 labs)

4 NRLs from EU Candidate Countries:

1. Republic of North Macedonia
2. Serbia
3. Turkey (2 labs)

10 NRLs from third Countries

1. Argentina (4 labs)
2. Chile (2 labs)
3. Egypt
4. Kosovo
5. UK
6. Uruguay



European Union Reference Laboratories

REGULATION (EU) 2017/625, Art 94



- European Union reference laboratories shall contribute to the improvement and harmonisation of methods of analysis, test or diagnosis to be used by official laboratories designated in accordance with Article 37(1) and of the analytical, testing and diagnostic data generated by them.
- To provide NRLs with analytical reference methods
- To produce and distribute reference materials
- To organise comparative testing (Proficiency Tests)
- To conduct training for NRLs, (annual workshop, courses, visits to the EU RL)
- To provide scientific and technical assistance to the Commission
- Collaborating within the scope of their mission with laboratories in third countries and with the European Food Safety Authority (EFSA), the European Medicines Agency (EMA) and the European Centre for Disease Prevention and Control (ECDC)



Providing NRLs with analytical reference methods



Laboratory methods

The laboratory methods for the detection, identification, and typing of STEC and other pathogenic *E. coli* developed by the EURL-VTEC are available in this section.

A report on the performance data of the ISO/TS 13136:2012 method for the detection of STEC in food, based on the results of the PTs organized by the EURL, is also available in the list. Such a report can be used as reference for the purpose of the method's accreditation.

Allegati

🔗 ISO TS 13136:2012 - Report on the primary validation of the PCR screening reactions and the determination of the performance parameters, based on the results of the analytical tests carried out within the EURL-VTEC proficiency testing program (2009-2020) (EURL-VTEC_Method_performance_CEN ISO/TS_13136:2012_Rev. 6)

🔗 Identification and characterization of Shiga toxin-producing Escherichia coli (STEC) by PCR amplification of the main virulence genes (EURL-VTEC_Method_01_Rev 1)

🔗 Identification and characterization of Shiga Toxin (Verocytotoxin)-producing Escherichia coli (STEC/VTEC) by Real Time PCR amplification of the main virulence genes and the genes associated with the serogroups mainly associated with severe human infections (EURL-VTEC_Method_02_Rev1)

🔗 Identification of the STEC serogroups mainly associated with human infections by conventional PCR amplification of O-associated genes (EURL-VTEC_Method_03_Rev2)

🔗 Detection and identification of Shiga toxin-producing Escherichia coli (STEC) O104:H4 in food by Real Time PCR (EURL-VTEC_Method_04_Rev 2)

🔗 Detection of Enteroaggregative Escherichia coli in food by Real Time PCR amplification of the aggR and aaiC genes (EURL-VTEC_Method_05_Rev 2)



ISO standard for the detection of STEC in foodstuff

ISO TS 13136

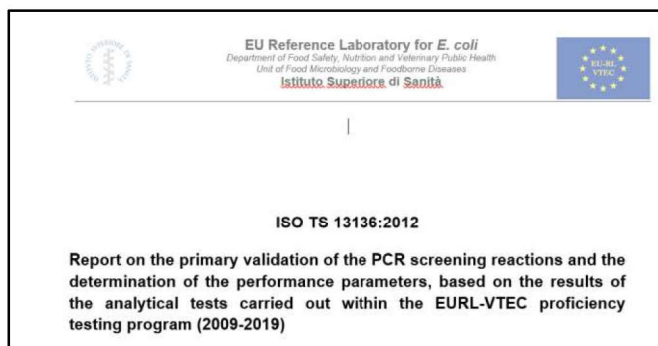


Leadership of the project to develop the standard appointed at the EURL for *E. coli* in 2006

Used since 2009 in the *E. coli* Proficiency Testing scheme of the EURL *E. coli*

Published in 2012

Document reporting the performance parameters calculated during the Proficiency Tests



Currently chairing the ISO TC34 SC9
WG38 group of experts – task: revision of
the ISO 13136



Development of a procedure of pre-treatment of spent irrigation water for the detection of STEC



- ✓ Regulation (EU) No 209/2013 has introduced for the first-time microbiological criteria for STEC in the EU legislation (sprouts)
- ✓ CEN/ISO/TS 13136:2012 and the EURL procedure for the identification of VTEC O104:H4 are prescribed for the detection of STEC in sprouts by Reg. 209/2013
- ✓ Reg. (EU) 209/2013 also gives the possibility to replace the sampling and testing of sprouts with the analysis of five samples of 200 ml of the water that has been used for their irrigation
- ✓ However, testing spent irrigation water for the presence of STEC or other enteric pathogens may pose technical problems, due to some characteristics of this particular matrix.

Support of the Network of NRLs



Organization of Proficiency Tests




- **Characterization of pathogenic *E. coli* strains**
- **Identification of STEC in carcass swabs**
- **Identification of STEC in milk and dairy products**
- **Identification of STEC in Vegetables**
- **Identification of STEC in water samples**
- **Identification of STEC in sprouts and SIW**
- **Identification of STEC in meat samples**
- **Identification of STEC in herbs**
- **Identification of STEC in flour**

- **Enumeration of *E. coli* in LBM**



Application to participate in the Proficiency testing program of the EURL-VTEC

Allegati

 Evaluation of the NRLs' performance and management of under performance (Rev.5-2021)

[PTs on the enumeration of E. coli in LBM](#)



[PTs on molecular typing of pathogenic E. coli strains](#)



[PTs on the detection of pathogenic E. coli in food and related matrices](#)



[PTs on the identification and typing of pathogenic E. coli strains](#)



All reports are available at the EURL for *E. coli* website



Training



[Request a training stage or course](#)

Allegati

- 📎 Program for a 4-days training at the EURL-VTEC on the identification and characterization of the different groups of pathogenic E. coli by Real Time PCR amplification of their virulence genes (Rev. 2)
- 📎 Program for a 5-days training at the EURL-VTEC on the identification and characterization of VTEC strains (Rev. 2)
- 📎 Program for a 5-days training at the EURL-VTEC on the detection of STEC in food matrices according to the ISO TS 13136:2012 and the characterization of the isolated STEC strains (Rev. 3)
- 📎 Program for a 5-days training at the EURL-VTEC on the design and preparation of PTs on the detection of VTEC in food matrices (Rev. 1)
- 📎 Program for a 3-days training at the EURL-VTEC on the use of bioinformatics tools for Next Generation Sequencing data mining for typing pathogenic E. coli (Rev. 0)
- 📎 Online Training on the detection of STEC in food matrices according to the ISO TS 13136:2012 and the characterization of the isolated STEC strains (Rev. 0)
- 📎 Online Training on the identification and characterization of the different groups of pathogenic E. coli by Real Time PCR amplification of their virulence genes (Rev. 1)
- 📎 Online Training on the design and preparation of proficiency tests (PTs) on the detection of STEC in food matrices (Rev. 0)
- 📎 Online Training on the identification and characterization of the different groups of pathogenic E. coli by Real Time PCR amplification of their virulence genes (Rev. 1)



Training



- **145 NRL staff trained 2011-2019**
- **112 NRL staff trained 2020-2023**
- **Remote sessions exploited during CoViD-19 pandemics**
- **Provision of materials and reagents to the participants in advance**
- **Use of streaming sessions and films**
- **Hands-on sessions conducted by the participants in their labs and discussion with the class in dedicated sessions**
- **In 2021 EURL for *E. coli* introduced the assessment of the training efficacy (questionnaire and 80% compliance threshold to get a certificate of achievement of the training goals)**
- **Starting from 2022 face-to-face training sessions have been restored**



Information & Documents

<https://www.iss.it/en/vtec-about-us>

Contacts:

General requests: crl.vtec@iss.it

Proficiency tests: pt.eurlvtec@iss.it

Training programs: training.eurlvtec@iss.it



INTER EURLs WORKING GROUP ON NGS



AIM

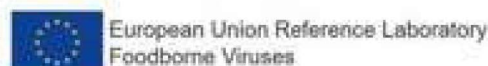
to promote the use of WGS across the EURLs' networks, build WGS capacity within the EU and ensure liaison with the work of the EURLs and the work of EFSA and ECDC on the WGS mandate sent by the Commission

Established
November 2017

Meeting twice a year
(14 meetings done)

- EURL *E. coli* (Chair)
- EURL *Listeria monocytogenes*
- EURL *Salmonella*
- EURL Coagulase Positive *Staphylococci*

- EURL Parasites
- EURL Foodborne viruses
- EURL AMR
- EURL *Campylobacter*





Documents prepared/1



- **Reference WGS collection**

Including STEC, *Salmonella*, *Campylobacter*, *L. monocytogenes*, AMR relevant strains

E. coli: Multiple sequences of the same six **O26 STEC strains** obtained during the 1st PT on WGS (anonymized)

Sequences available upon direct request to:

crl.vtec@iss.it

- **NGS laboratory procedures**

DNA extraction from bacteria

DNA extraction from parasites

RNA extraction from viruses

Library preparation



Documents prepared/2



- **Bioinformatics tools for basic analysis of NGS data**

Quality check, Trimming, Assembly, MLST, Virulotyping, Serotyping, AMR

Open source, commercial software, webservers

EFSA One Health WGS System and analytical pipeline (also available for download and setup through a dedicated repository)

- **Guidance document for NGS-Benchmarking**

Quality and quantity metrics

Wet and dry lab analyses

Accuracy, repeatability, reproducibility



Supporting document for DNA preparation and quality check



Following a request made by EFSA in the framework of the Joint EFSA-ECDC Steering Committee on Molecular Typing Data collection

AIM:

supporting the laboratories in the first steps of the NGS protocol, starting from the experiences on *E. coli*, *Listeria* and *Salmonella*, representing the first three pathogens for which WGS data collection has started

Suggestions for:

DNA extraction (isolated colonies; control of genetic features; plasmids; DNA manipulation; elution buffers)

DNA quality check

DNA concentration





Position paper published



- Adoption of NGS across NRLs
- Harmonization of procedures
- Proficiency tests
- Benchmarking guidelines
- Training and capacity building

MICROBIAL GENOMICS

SHORT COMMUNICATION

Michelacci *et al.*, *Microbial Genomics* 2023;9:001074
DOI 10.1099/mgen.0.001074



European Union Reference Laboratories support the National food, feed and veterinary Reference Laboratories with rolling out whole genome sequencing in Europe

Valeria Michelacci^{1*}, Adrien Asséré², Simone Cacciò³, Marina Cavaiuolo⁴, Kirsten Mooijman⁵, Stefano Morabito¹, Susanne Karlsmose Pedersen⁶, Maroua Sayeb², Bo Segerman⁷, Magnus Simonsson⁸, Hanna Skarin⁷, Rosangela Tozzoli¹, Angela van Hoek⁹ and Rene Sjøgren Hendriksen⁶

Abstract

The Inter European Union Reference Laboratories (EURLs) Working Group on Next Generation Sequencing (NGS) involves eight EURLs for microbiological food and feed hazards and has been working since 2017 to promote the adoption of NGS by the National Reference Laboratories (NRLs) in the European Union. This work illustrates the results of the first 5 years of activity. By working together, the EURLs involved have released guidance documents for assisting NRLs in all the steps of NGS, helping the transition from classical molecular methods towards whole genome sequencing while ensuring harmonization, with the final aim of improving preparedness in the use of NGS to characterize microbial hazards and trace the sources of infection.

Published on July 25th 2023





Work in progress



- Supporting document for **Quality Check of NGS data** (EURL *Listeria* lead) and **requirements for the accreditation of the dry lab** for responding to the Implementing Regulation on collection and transmission of molecular analytical data within the frame of epidemiological investigations of food-borne outbreaks in accordance with Directive 2003/99/EC of the European Parliament and of the Council (Text with EEA relevance)

The topic will have been discussed in a specific meeting of the WG in October to start drafting the document

- Supporting document for **Wet-lab part of sequencing viral genomes** (EURL Foodborne Viruses lead)

In preparation



Inter EURLs WG webpage



The webpages of each EURL contain the direct link to the **new webpage dedicated to the Inter EURLs WG on NGS**

zenodo Search records... Communities My dashboard Log in Sign up

Planned intervention: On Thursday 19/09 between 05:30-06:30 (UTC), Zenodo will be unavailable because of a scheduled upgrade in our storage cluster.

Inter Biorisks-EURLs WG on NGS

Organization French Agency for Food, Environmental and Occupational Health & Safety ROR, Istituto Superiore di Sanità ROR, National Institute for Public Health and the Environment ROR, Technical University of Denmark ROR, Swedish Food Agency - Livsmedelsverket, Swedish Veterinary Institute

New upload

Records Members

10 results found Sort by Newest

Versions

View all versions

Access status

Open

Resource types

Publication

January 16, 2024 (4) Other Open

Inventory of training supports for NGS
Cavaiuolo, Marina

From the survey sent in 2018 by the EURLs to the NRLs network, an inventory of training supports used by different organisations was performed and presented to the working group in November 2018. As all NRLs express their need for training in each step of WGS analyses (wet-lab and dry-lab), it was decided by the working group to draft a guidance...

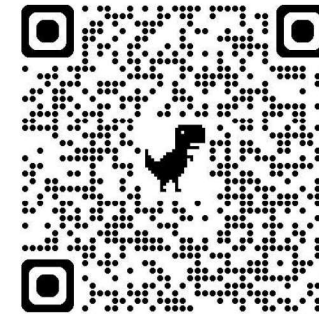
Part of Inter Biorisks-EURLs WG on NGS

Uploaded on January 22, 2024

3 more versions exist for this record

143 116

January 10, 2024 (3) Other Open



Direct link!

https://zenodo.org/communities/eurls-biorisks_wg_on_ngs

All the versions of all the documents are always available on Zenodo
The new versions always appear as first choices



3rd joint training – June 25th-26th 2024, Uppsala (Sweden)



TUESDAY 25 JUNE 2024

- 8.45 **Registration (for entering the premises of SVA)**
- 09.00 Welcome and general overview of the joint training activities (Hanna Skarin, EURL-*Campylobacter*)
- 09.15 Introduction of the inter EURLs Working Group on NGS and the guidance documents released (Valeria Michelacci, EURL-VTEC)
- 09.30 Introduction to WGS
1. Sequencing platforms & output data (20') (Maroua Sayeb, EURL-*Listeria monocytogenes*)
 2. Verification of the integrity of the raw data files (like md5sum) (10') (Angela van Hoek, EURL-*Salmonella*)
 3. Bioinformatics analysis of NGS data: approaches and opportunities (command-line tools, commercial software, webservers) (20') (Joakim Skarin, EURL-Foodborne viruses)
- 10.20 **Coffee break**
- 10.50 Introduction to quality check and trimming (Valeria Michelacci, EURL-VTEC)
- 11.10 **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)
Demo: Quality check and trimming with alternative tools (Bo Segerman, EURL-*Campylobacter*)
- 12.30 **Lunch break**
- 13.40 Introduction to 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 (Luca De Sabato, EURL-VTEC)
- 15.00 Introduction to data alignment (Paolo Vatta, EURL-Parasites)
- 15.20 **Hands-on exercises – Mapping approach**
Hands-on E. coli virulotyping using a mapping approach (Rosangela Tozzoli, EURL-VTEC)
Demo: Mapping with Seqsphere (Maroua Sayeb, EURL-*Listeria monocytogenes*)
- 16.20 Amplicon-based sequencing of viral genomes (Luca De Sabato, EURL-VTEC)
- 17.00 **End of the first day**
- 19.00 Optionally, a group dinner will be organised (at own costs)

WEDNESDAY 26 JUNE 2024

- 8.45 **Registration (for entering the premises of SFA)**
- 9.00 Introduction to gene detection using BLAST approach (Marina Cavaiuolo, EURL-CPS)
- 9.15 **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 webservice (Lauge Holm Sørensen, EURL-AMR)
Demo: Salmonella virulotyping with Seqsphere (Angela van Hoek, EURL-*Salmonella*)
- 10.10 Introduction to genome comparisons: gene-by-gene vs SNP approach (Bo Segerman, EURL-*Campylobacter*)
- 10.30 **Coffee break**
- 11.00 Demonstration of gene-by-gene approach through different platforms: ARIES (Rosangela Tozzoli, EURL-VTEC) Starflow (Maroua Sayeb, EURL-*Listeria monocytogenes* and Marina Cavaiuolo, EURL-CPS) Seqsphere (Angela van Hoek, EURL-*Salmonella*)
- 11.45 Parasites WGS: opportunities and challenges (Simone Cacciò, EURL-Parasites)
- 12.15 **Lunch break**
- 13.15 **Hands-on exercises – Visualisation of clustering data**
Demonstration via Grapetree (Maroua Sayeb, EURL-*Listeria monocytogenes*)
- 13.45 The EFSA One Health WGS database and demo (Mirko Rossi, EFSA)
- 14.45 Wrap up (Magnus Simonsson, EURL-Foodborne viruses)
- 15.00 **Closure**

**Introduction to Bioinformatics for
genomic data mining**



Webinar dedicated to NGS PTs



Held on September 29th 2023 through Microsoft Teams

Proficiency Tests on Next Generation Sequencing: approaches in use at the European Union Reference Laboratories

Friday September 29th 2023, 10 am - 12 am

Online through Microsoft Teams

Organised by the Inter EURLs WG on NGS, involving:

EURL-AMR

EURL-*Campylobacter*

EURL-Coagulase Positive Staphylococci (CPS)

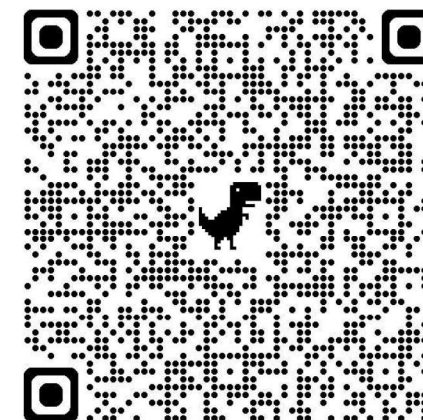
EURL-Foodborne viruses

EURL-*Listeria monocytogenes*

EURL-Parasites

EURL-*Salmonella*

EURL-VTEC



<https://www.iss.it/en/-/proficiency-tests-on-next-generation-sequencing-approaches-in-use-at-the-european-union-reference-laboratories-webinar-29-september-2023->

Presentations are published on EURL *E. coli* website and mirrored in all the websites of the other EURLs



“Science Meets Policy” conference



- First edition: virtual conference, September 2020: over 500 participants from 49 countries worldwide
- Second edition: “**EU initiatives towards the large-scale use of NGS to tackle foodborne threats**” organized by EFSA and the Inter EURLs WG on NGS
EFSA, Parma, Italy and online, September 5th-6th 2023



REGISTRANTS

• 844 registrants whose:

- 155 in-person registrants;
- 689 online registrants;

PARTICIPANTS

• In-person attendees: 102; No-show rate: 34.19%

• Online attendees:

- Day one peak viewers: 257;
- Day 2 peak viewers: 177;

98% of Survey participants declared that their event experience was **excellent (51%) or good (44%)**