



National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport



EURL-*Salmonella* activities

Angela van Hoek



History

- The European Commission established the first European Union Reference Laboratories (EURL) in 1992 to ensure the safety of the food chain.
- One of the oldest EURLs is the EURL-*Salmonella*, which has been situated at RIVM in the Netherlands since 1992.

The screenshot shows the website for the European Union Reference Laboratory (EURL) for Salmonella. At the top, there is a navigation menu with links for Proficiency testing, Publications, Methods, Workshops, Network of NRLs, and About EURL. Below the menu is a search bar with the text 'Zoeken'. The main content area features a large image of laboratory equipment, including petri dishes with various colored agar, a rack of test tubes, and a rack of microcentrifuge tubes. Below the image, there is a section titled 'What's New' with two links: 'EURL-Salmonella Newsletter September 2024' and 'Final report PT PPS 2023'. At the bottom of the page, there is a logo for the European Union and the text 'Co-funded by the European Union'.

Here you can find information on the activities of the European Union Reference Laboratory for Salmonella (EURL-Salmonella) conducted on behalf of the European Commission. The EURL-Salmonella site is primarily intended to inform the National Reference Laboratories (NRLs) for Salmonella, but will also be of interest to other laboratories analysing Salmonella.

What's New

- [EURL-Salmonella Newsletter September 2024](#)
- [Final report PT PPS 2023](#)

Co-funded by the European Union



Tasks and Duties

- Are defined in European legislation, in short:
 - To ensure the availability and use of high-quality methods and performance by NRLs.
 - To provide scientific and technical assistance to NRLs.
 - To provide scientific and technical assistance to the European Commission and other organisations.
 - To maintain the quality of reagents and maintain reference strains and reference materials or have up-to-date lists available.



Harmonisation of methods

- EURL-*Salmonella* is project leader/member of several CEN/ISO working groups of
 - ISO/TC34/SC9: International Standardisation Organisation, Technical Committee 34 on Food products, Subcommittee 9 – Microbiology.
 - CEN/TC463: European Committee for Standardisation, Technical Committee 463 'Microbiology of the food chain'.



Activities in ISO and CEN

- Development harmonised ***Salmonella* methods**; EN ISO 6579 series - Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella*:
 - EN ISO 6579-1:2017. Detection of *Salmonella* spp.
 - EN ISO 6579-1:2017/Amd.1:2020. Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSR and SC.
 - CEN ISO/TS 6579-2:2012. Enumeration by a miniaturized most probable number technique.
 - CEN ISO/TR 6579-3:2014. Guidelines for serotyping of *Salmonella* spp.
 - CEN ISO/TS 6579-4: under development. Identification of monophasic *Salmonella* Typhimurium (1,4,[5],12:i:-) by polymerase chain reaction (PCR).



Activities in ISO and CEN

- Development harmonized procedures for **validation of alternative methods**; EN ISO 16140 series Microbiology of the food chain - Method validation:
 - Part 2:2016. Protocol for the validation of alternative (proprietary) methods against a reference method. 2020: start drafting amendment, followed by revision.
 - Part 3:2021. Protocol for the verification of reference and validated alternative methods implemented in a single laboratory.
 - Part 4:2020. Protocol for single-laboratory (in-house) method validation.
 - Part 5:2020 Protocol for factorial interlaboratory validation of non-proprietary methods.
 - Part 6:2019. Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures.



Activities in ISO and CEN

- Revision of EN ISO 17468:2016. Microbiology of the food chain - Technical requirements and guidance on **establishment or revision of a standardized reference method**. – Includes information on determining performance characteristics of EN ISO methods.
- Ad'Hoc group '**Validation status of ISO standards**'. – Making an inventory on whether EN ISO documents of the Food chain contain performance characteristics and if performance characteristics are included for a broad range of foods.
- Development of EN ISO 23418 Microbiology of the Food Chain - **Whole genome sequencing** for typing and genomic characterization of foodborne bacteria - General requirements and guidance. Voting for Draft International Standard (DIS) version took place in fall 2020.
- Ad'Hoc group on **harmonisation of ISO/CEN standards** for microbiology of the food chain



EURL-Salmonella website

- Procedures/protocols which are not published by ISO/CEN, but for which harmonization is needed, are published at www.eurlsalmonella.eu;

The screenshot shows the EURL Salmonella website interface. At the top, there is a logo for the National Institute for Public Health and the Environment (RIVM) and the text "National Institute for Public Health and the Environment, Ministry of Health, Welfare and Sport". Below this is a navigation bar with the following items: Proficiency testing, Publications, Methods (selected), Workshops, Network of NRLs, and About EURL. A search bar with the text "Zoeken" is also present. The main content area is titled "Methods" and contains a light blue box with the text: "Here, information is given on preparation of specific products, on analytical methods for detection, enumeration and typing of *Salmonella*, and on reference materials." To the right of this box is a vertical list of menu items, each with a right-pointing arrow: NGS, Detection of Salmonella, Enumeration of Salmonella, Serotyping of Salmonella, MLVA typing of Salmonella, Specific products, Reference materials, Validation and verification, and Miscellaneous.



Inter European Union Reference Laboratories (EURL) Working Group (WG) on Next Generation Sequencing (NGS)

- From November 2017, this WG meets twice a year
- EURLs operating in the field of the microbiological contamination of food and feed;
 - EURL-Antimicrobial Resistance, EURL-*Campylobacter*, EURL-Coagulase Positive Staphylococci, EURL-*Escherichia coli*, including Shiga toxin-producing *E. coli*, EURL-Foodborne Viruses, EURL-*Listeria monocytogenes*, EURL-Parasites, EURL-*Salmonella*
- Objectives
 - Promote the use of NGS/WGS across the network of the EURLs
 - Build NGS/WGS capacity within the EU
 - Ensure liaison with the work of the EURLs and the work of EFSA & ECDC on the WGS mandate sent by the Commission



Drafted guidance documents

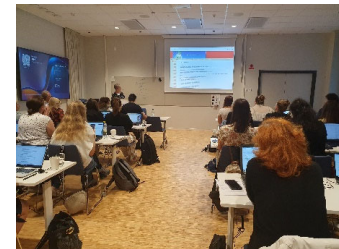
- Overview of conducted and planned proficiency tests (PTs)
- Reference WGS collection
- NGS laboratory procedures
- Preparing high quality DNA for WGS
- Bioinformatics tools for basic analysis of NGS data
- Cluster analysis of WGS data
- NGS-Benchmarking
- Inventory of training supports
- Survey on the use of NGS across the NRLs networks

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



Joint Trainings on NGS

- ANSES (Paris), EURL-Lm, October 2019 (**EURL-Lm, EURL-Salmonella, EURL-VTEC**)
'Analysis of molecular typing data, Bioinformatics tools for WGS data analysis'
12 participants from different NRLs from 10 countries
- ISS (Rome), EURL-VTEC, June 2022 (**All EURLs from the Inter EURL WG**)
'Introduction to Bioinformatics for genomic data mining'
24 participants from different NRLs from 20 countries
- RIVM (Bilthoven), EURL-Salmonella, June 2023 (**All EURLs from the Inter EURL WG**)
'Introduction to Bioinformatics for genomic data mining'
24 participants from different NRLs from 17 countries
- SVA & SFA (Uppsala), EURL-Campylobacter & EURL-Foodborne Viruses, June 2024
(**All EURLs from the Inter EURL WG**) 'Introduction to Bioinformatics for genomic data mining'
29 participants from different NRLs from 19 countries





Proficiency Tests

- The EURL-*Salmonella* regularly organises Proficiency Tests (PT) to test the performance of NRLs-*Salmonella* for detection and typing of *Salmonella* in samples from the food chain
 - 1) One study on detection of *Salmonella* in samples from the primary production stage.
 - 2) One study on detection of *Salmonella* in food or animal feed samples.
 - 3) One study on typing of *Salmonella* (serotyping, molecular typing).
 - Obligatory part on serotyping of *Salmonella* (NRLs can also use WGS).
 - Optional part on molecular typing of *Salmonella*. Since 2019 this part concerns cluster identification of a selected set of strains.
NRLs can use their 'routinely' used molecular methods: PFGE and/or MLVA and/or WGS.



Scientific advice and support NRLs and EC

- Perform confirmatory testing and/or typing (samples/isolates) for NRLs-*Salmonella* when needed.
- Perform WGS analysis suspect isolates from NRLs-*Salmonella* for outbreak investigations.
- Contact with EFSA in case of outbreaks; upload WGS data (and limited metadata) from NRLs-*Salmonella* in EFSA One Health WGS system.
- EC observer to the Joint Meeting of ECDC's FWD Network and EFSA's Zoonoses Network (WGS and FBO subgroups)



EURL-*Salmonella* in relation to AMR

- EURL-*Salmonella* personnel was/is involved in research on;
 - AMR genes detection in *Salmonella* Paratyphi B variant Java
 - Detection of antibiotic resistance genes in different *Salmonella* serovars by oligonucleotide microarray analysis
 - ESBL-producing *Enterobacterales*, among others *Salmonella*.
- Coipan et al., 2020, Genomic epidemiology of emerging ESBL-producing *Salmonella* Kentucky *bla*_{CTX-M-14b} in Europe (<https://www.tandfonline.com/doi/full/10.1080/22221751.2020.1821582>)
- Mughini-Gras et al., 2021, Prevalence, risk factors and genetic traits of *Salmonella* Infantis in Dutch broiler flocks (<https://www.sciencedirect.com/science/article/pii/S0378113521001437>)
- Expert in ISO/TC 34/SC 9/AHG 5 "Brainstorming on antimicrobial resistance"



Thank you for your attention

