24 May 2024

To: National contact points for STEC/VTEC in the ECDC-FWD Network

Dear colleague,

On behalf of the European Centre for Disease Prevention and Control (ECDC) you are hereby cordially invited to participate in the

Thirteen External Quality Assessment (EQA-13) scheme for typing of Shiga toxin-producing *Escherichia coli* (STEC) in 2024-2025

organised by the European Food- and Waterborne Diseases and Zoonoses Programme, ECDC (ECDC-FWD). The aim of the EQA-13 is harmonization of the typing methods used by the European laboratory network, in order to produce comparable typing data for STEC isolates between laboratories and to ascertain high quality data submissions to the European Surveillance System (TESSy). One Public Health National Reference Laboratory (PHNRL) in each of the EU/EEA, candidate country and potential candidate country will be funded by the ECDC.

Please reply whether or not you wish to participate within the 29th of April 2024 using the online form: individual link

Test isolates and methods

The selection of the test isolates for the EQA-13 programme is based on the following criteria:

- Strains primarily represent commonly reported STEC types in Europe.
- Strains remain stable during the preliminary testing period at the organising laboratory.
- Strains will be among the different subtypes of *stx1* and subtypes of *stx2*

The STEC EQA-11 includes three parts

- **O and H Serotyping**
  Purpose: Assign O group and/or H type
  Method: Suggested phenotypic protocol (link) or serotype finder by WGS (CGE Server (dtu.dk))
  Submission: O groups and H types in an online form
  Evaluation: The ability to assign correct O groups and H types
  Note that when participating in a test, all isolates must be tested using this test and that all non typable as well as "not done" will by default be evaluated as an incorrect result.

- **Virulence gene determination (*stx1*, *stx2*, *eae*, *esta*, *aggR* and subtyping)**
  Purpose: Assign virulence profile
  Method: No specific method is required, however protocol (link) is suggested for *stx1*, *stx2* and the *stx* subtyping.
  Submission: Presence/absence of *stx1*, *stx2*, *eae*, *aggR* genes and subtyping of *stx* genes (*stx1a, stx1c* and *vtx1d* and *stx2a* to *stx2g*) in an online form
  Evaluation: The ability to assign correct virulence profile
  Note that when participating in a test, all isolates must be tested using this test. Isolates that are negative in the initial screening of stx genes should therefore also be tested for stx *subtyping regardless of the results of the screening. Hence, "not done" will by default be evaluated as an incorrect result.*

- **Molecular typing-based cluster analyses**
  Purpose: Detection of a cluster of closely related strains
  Method: Participants can perform the cluster analyses by using whole genome sequencing (WGS) and another molecular typing method, e.g. PFGE. Extra sequences will be provided to evaluate, if these should be considered as part of the cluster. Participants must perform one joint analysis including both own sequence data from the received tests strains as well as the provided extra sequences (up to ten sequences)
  Laboratories can use their own analysis pipeline for the cluster analysis e.g. single nucleotide polymorphism analysis (SNP-based) or whole genome multi locus sequence typing (cgMLST/wgMLST).

  Submission: Report in the online form, the strains identified as cluster of closely related strains. SNP distances or allele differences between a sequence (selected by the EQA-provided) and all sequences in the joint analysis are submitted. As well as
    - 7-locus MLST
    - QC status and additional comments for each sequence
    - serotype can also be listed (will not to be evaluated in this part)
    - upload the raw reads (fastq-files) of your own data for QC evaluation by the SSI in-house quality control pipeline

  If PFGE or other methods is used a list of strains ID´s for the identified cluster is submitted in the online form.

  Evaluation: The ability to detect a cluster of closely related strains based on a pre-defined categorisation by the organiser (mimicking an outbreak situation). The cluster categorisation is based on WGS data and therefore the correct cluster delineation might be difficult to obtain by the use of less discriminatory methods, e.g. PFGE. This will be comment in the individual feedback report. In addition, the participant will get some feedback on the general quality of the sequences.

The EQA-13 will include **a participation/attendance certificate** and **an individual report** specifying the individual tests for each participant. All data will be stored and analysed in an EQA-database and the anonymised data will be made publicly available in a report published by ECDC.

<u>The package will contain</u>

- 10-12 blinded STEC strains for all three parts O and H Serotyping, Virulence gene determination and Molecular typing-based cluster analyses

<u>We kindly ask you to provide the following details</u>

- The exact shipping address and the contact e-mail address
- Specific laboratory and Institution/organization for receiving the official certificate
- Ordinary postal address if different from shipping address
- Please specify if you know deliverable of UN2814 will be an issue in your country
- Additional information regarding participation in EQAs in general
- By saying yes to participate, you also confirm to agree to the following terms:
  The strains that you receive in EQA are for internal use for quality assurance purposes in your laboratory and must not be distributed to other laboratories/institutions or used for additional purpose without permission from the EQA provider.

Dates to remember

29th of April 2024	**Deadline to respond to the invitation online**
21th -31th of May 2024	**Shipment of test isolates**
15th of October 2024	**Deadline for submission of results**

We encourage you to participate in all parts of the EQA-13. Participation in one part only is, however, also possible. The submission details will be distributed in connection with the isolates at shipment.

on behalf of the EQA-team

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Read more about how SSI processes personal data here: https://en.ssi.dk/about-us/information-about-processing-of-personal-data