18 June 2024

Dear colleague,

This submission protocol describes the online submission of results for the Thirteenth External Quality Assessment (EQA-13) scheme for typing of **STEC in 2024-2025**.

This email is only sent to the contact person for the EQA, if additional persons need access to the submission form – forward the email.

NOTE: new virulence gene esta (STa)
If using WGS please read the WGS part thoroughly before starting your analysis. This year you have to use a specific strain/sequence when reporting allele differences/ SNP distances.

The deadline for submission of results is the 15th of October 2024.

Methods in EQA-13
Please see instructions in the Letter of invitation:
- **O and H serotyping**
  Results can be submitted for:
  - Either phenotypic serotyping **or** molecular serotyping
    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**
  Any combination of the different results for *stx1*, *stx2*, *eae*, *agg*R, *esta* **(STa)** and/or subtyping
    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 subtyping regardless the results of the screening and "not done" will by default be evaluated as an incorrect result.*

- **Molecular typing-based cluster analysis of closely related isolates**
  Results can be submitted for:
  - PFGE cluster analysis
  - Cluster analysis on derived data from WGS
  - Both PFGE cluster analysis and cluster analysis on derived data from WGS

If using WGS derived data for the cluster analysis:
Download the additional eight sequences (see below) before conducting the initial cluster analyses of all 20 strains/sequences.
Report the allele differences/SNP distances to the downloaded strain0018 sequence and report the strains/sequences which are a part of the identified cluster. If using reference-based SNP analysis – preferable use strain0018 sequence as reference. In addition, report the MLST, serotype (optional) and subtype (optional).
Report also the quality status and observations of all the sequences downloaded.

Instructions for online submission

From https://sit-ftp.statens-it.dk/ the EQA provided sequences should be downloaded prior to analysis. In addition an empty submission form, which includes all available fields and questions for preview. **Please keep in mind, depending on your answers in the form, you will not have to answer all the questions** (e.g. if reference based SNP analysis is selected, you can only add information on reference genome, read mapper, variant caller before you directly will get to the question "Cluster detected by analysis on data derived from WGS"). We strongly recommend that you look through the empty submission form before filling in online.

We recommend having a list with the isolates ID´s and the isolates no. 1-12 accessible for the further submission of results, because it will make the work easier.

Link for download the eight EQA provided sequences:
- o   Use link: https://sit-ftp.statens-it.dk/
- o   Password: EQA_STEC13_download

Option 1:
- o   Press the blue bottom "Download" for "EQA_STEC13_provided_seq.tar.gz" (1 file).
    - o   This file is a compressed zip archive and contains the provided strain. To extract the contents of the "EQA_STEC13_provided_seq.tar.gz" file, you can use the following command in a terminal:
      tar -xzvf EQA_STEC13_provided_seq.tar.gz

Option 2:
- o   Press the blue bottom "Download" for all 8 sequences which name match StrainXXXX_RX.fastq.gz (16 files).

Link for online submission form
> [SURVEY_LINK]
> You can also visit the Internet address https://www.survey-xact.dk/collect and enter:
> **KEY: [RESPONDENTKEY]**

If WGS is performed, please upload the raw reads to:
https://sit-ftp.statens-it.dk/

Remember that your files should be label with your strain ID and your Lab ID for easy recognition e.g.
- o   "**DK_SSI_1234**_S19_L555_R1_001.fastq.gz"
- o   "**DK_SSI_1234**_S19_L555_R2_001.fastq.gz"

Due to the many upload problems last year, we have decided to add another option 1 (zip archive) by using unix/linux system:
Option 1: (create a zip archive)
1.   Go to your folder which only contain the raw reads:
     cd <folder>
2.   Create the zip archive. Change **<Lab_ID>** to your Lab ID and remember the . in the end:
     tar -czvf **<Lab_ID** >.tar.gz --exclude=**<Lab_ID** >.tar.gz .
     example: tar -czvf DK_SSI.tar.gz .
3.   Go into the webpage: https://sit-ftp.statens-it.dk/

4. Password: EQA_STEC13_upload
5. Press "Choose Files"
6. Locate your **<Lab_ID>**.tar.gz and select file e.g. DK_SSI.tar.gz
7. Click "Open"

Option 2: (upload the raw reads one at the time)
1. Go into the webpage: https://sit-ftp.statens-it.dk/
2. Password: EQA_STEC13_upload
3. Press "Choose Files"
4. Locate your file(s) and select file(s)
5. Click "Open"

 Please contact ecoli.eqa@ssi.dk if you experience problems during submission.

Individual Evaluation Report & Certificate
Each participating laboratory will receive a participation certificate specifying the attended method(s) and an individual evaluation report during the beginning of 2024.

For participation in the serotyping part, the individual evaluation report will contain a score of number of correct assigned O group, H type and O:H serotype.
For participation in the virulence profile part the individual evaluation report will contain a score of number of correct identified virulence profiles based on all performed tests.

For participation in the cluster analysis the report will include an evaluation on the ability to detect a cluster of closely related isolates based on a pre-defined categorisation by the EQA-provider.

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 commented in the individual feedback report. In addition the quality status of the downloaded sequences will be assessed and compared with the EQA-providers data.

For submission of WGS data the individual evaluation report will include a Quality Assessment made by the SSI in-house quality control pipeline and based on PulseNet's Metrics QC parameters (e.g. coverage, N50, sequence length and number of contigs).

Summary Report
A summary report will be prepared in collaboration with ECDC and will include results on the EU/EEA countries, acceding country and candidate countries funded by ECDC. The summary report will show anonymous data and will include the different methods used in laboratories across Europe for obtaining results for this EQA-13. Furthermore, the results will be shown individually and compared between participating laboratories. Finally, the level of performance will be shown.