

**18 June 2024**

Dear colleague,

This submission protocol describes the online submission of results for the eleventh External Quality Assessment (EQA-11) scheme for typing of *Listeria monocytogenes* in 2024-2025.

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

If using WGS please read the WGS part thoroughly before starting your analysis. Again, this year you have to use a specific strain/sequence when reporting allele differences/ SNP distances.

**The deadline for submission of results is the 30<sup>st</sup> of August 2024.**

### Methods in EQA-11

Please see instructions in the Letter of invitation:

- Serotyping/grouping  
Results can be submitted for:
  - Both molecular and conventional serogrouping/typing
  - Only molecular serogrouping
  - Only conventional serotyping
  
- Molecular typing-based cluster analysis  
Results can be submitted for:
  - Both PFGE cluster analysis and cluster analysis on derived data from WGS
  - Only PFGE cluster analysis
  - Only cluster analysis on derived data from WGS

### If using WGS derived data for the cluster analysis:

Download the additional ten sequences (see below) before conducting the cluster analyses of all 17. Report the allele differences/SNP distances to the downloaded strain0013 sequence and report the strains/sequences which are a part of the identified cluster. If using reference-based SNP analysis – preferable use strain0013 sequence as reference. In addition, report the MLST, and optional serotype/group. Report also the quality status and observations of all the sequences downloaded.

### Instructions for analysis and submission

Use the link below to download the ten EQA provided sequences prior to analysis. In addition an empty submission form can also be downloaded, 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 you to look through the “empty submission form” before filling in online.

Link for download the ten EQA provided sequences:

- Use link: <https://sit-ftp.statens-it.dk/>
  - Password: EQA\_List11\_download
- Option 1:
- Press the blue bottom “Download” for “EQA\_List11\_provided\_seq.tar.gz” (1 file).

- This file is a compressed zip archive and contains the provided strains. To extract the contents of the "EQA\_List11\_provided\_seq.tar.gz" file, you can use the following command in a terminal:

```
tar -xvzf EQA_List11_provided_seq.tar.gz
```

Option 2:

- Press the blue bottom "Download" for all 10 sequences which name match StrainXXXX\_RX.fastq.gz (20 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]

Please upload your 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.

- "DK\_SSI\_1234\_S19\_L555\_R1\_001.fastq.gz"
- "DK\_SSI\_1234\_S19\_L555\_R2\_001.fastq.gz"

Due to the many upload problems last year, we have decided to add another option (zip archive) 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 to the webpage: <https://sit-ftp.statens-it.dk/>
  4. Password: EQA\_List11\_upload
  5. Press "Choose Files"
  6. Locate your <Lab\_ID>.tar.gz and select file e.g. DK\_SSI.tar.gz
  7. Click "Open"
- All of your raw reads will be uploaded combined in a zip file.

Option 2: (upload the raw reads one at the time)

1. Go into the webpage: <https://sit-ftp.statens-it.dk/>
2. Password: EQA\_List11\_upload
3. Press "Choose Files"
4. Locate your file(s) and select file(s)
5. Click "Open"

Please contact [list.eqa@ssi.dk](mailto:list.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 fall 2024.

For participation in serotyping/grouping the individual evaluation report will contain a score of number of correct serotypes/groups.

For participation in the cluster analysis the report will include an evaluation of 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 selected 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-11. Furthermore, the results will be shown individually and compared between participating laboratories. Finally, the level of performance will be shown.

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