
PROTOCOL



EURL–*Salmonella* Proficiency Test Live Bivalve Molluscs 2020

Detection of *Salmonella* spp. in mussels

Introduction

This protocol describes the procedures for the Proficiency Test (PT) Live Bivalve Molluscs (LBM) 2020 on the detection of *Salmonella* spp. in mussels amongst the National Reference Laboratories (NRLs) for *Salmonella* in the EU.

The prescribed method is EN ISO 6579-1:2017 (Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Detection of *Salmonella* spp.) and the underlying EN ISO documents, e.g. the EN ISO 6887 series for preparation of test samples. Especially the procedure of EN ISO 6887-3:2017 should be respected.

Additionally, laboratories (who are interested) can also perform a second detection method (e.g. PCR, qPCR, ELISA, etc.) to analyse the samples, if this is (routinely) used in their laboratories. These results can be reported in addition to the results of the prescribed method in the result form. Only the results obtained with EN ISO 6579-1:2017 are used to assess the performance of the NRL. Please report relevant details of the method(s) used in the result form.

Objective

The main objective of the Proficiency Test is to evaluate the performance of the NRLs-*Salmonella* and their ability to detect *Salmonella* spp. in mussels.

Outline of the study

Each participant will receive in total two parcels: *Salmonella* reference materials and 2 kg of mussels. Each parcel will be sent as biological substance category B (UN3373) by door-to-door (for non-EU-MS sometimes door-to-airport) courier service DHL.

Parcel 1 contains *Salmonella* reference materials, which will be send with dry ice. The parcel contains four vials with or without *Salmonella*, which are resuspended in milk. The vials are coded with A, B, C and D.

Upon arrival of parcel 1: the four vials have to be stored at -20 °C until the day of analyses (18 March 2020).

Parcel 2 contains a packaging of 2 kg mussels (Modified Atmosphere Packaging conditions). Additional, the parcel will contain six (empty) sample bags. Four empty bags for the samples coded with A, B, C and D. Two empty bags are used for the control samples, being the procedure control (coded CTRL 1) and the positive control with *Salmonella* (coded CTRL 2). Finally one sample bag will contain a small electronic temperature recorder (coded with your labcode).

Upon arrival of parcel 2:

- **the packaging of 2 kg mussels have to be stored at 3 °C (\pm 2 °C) and the packaging should remained closed, until the day of analyses (18 March 2020).**
- **Please store the temperature recorder together with the mussels.**

The sample bag containing a small electronic temperature recorder will measure the temperature during transport and storage of the mussels at the laboratory. The safety bag and temperature recorder will be coded with your lab code. **You are urgently requested to return this complete sample bag with temperature recorder (and lab code) to the EURL-*Salmonella*, at the day your laboratory starts the study (18 March 2020).** For this purpose a return envelope with a preprinted address label of the EURL-*Salmonella* is included.

The performance of the PT will start on Wednesday 18 March 2020.

The media to be used for this study will not be supplied by the EURL-*Salmonella*

Extra instructions

For the correct use of the *Salmonella* reference material:

- Defrost the vials at room temperature for 30 minutes on 18 March (start of PT).
- Store in the refrigerator at 0 – 4 °C, until use.
- Mix well before use

Preparation of mussel samples (A, B, C and D):

- Per sample, open and pool at least 10 mussels.
- Weigh 25 gram of pooled sample in the supplied sample bag. Open more mussels, when needed.
- Repeat the preparation for the other three samples.

Spike the mussel samples with corresponding vial:

- Mix the *Salmonella* reference material well before use.
- All samples should be spiked with **100 µl** of the corresponding vial.

- Sample A should be spiked with 100 µl of vial A
- Sample B should be spiked with 100 µl of vial B
- Sample C should be spiked with 100 µl of vial C
- Sample D should be spiked with 100 µl of vial D

Continue with the procedure as described in EN ISO 6579-1:2017.

The sample bag coded with CTRL 1 have to be used for the procedure control (only Buffered Peptone Water) and the sample bag coded with CTRL 2 have to be used for the positive control (BPW spiked with own *Salmonella* control strain).

Relevant documents

- Protocol 'EURL-*Salmonella* Proficiency Test Live Bivalve Molluscs 2020. Detection of *Salmonella* spp. in mussels' (this document)
- Short guidance on the electronic submission of data in the result form for the EURL-*Salmonella* Proficiency Test
- EN ISO 6579-1:2017. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Detection of *Salmonella* spp.
- ISO 6887-1 & 3:2017 Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 1: General rules for the preparation of the initial suspension and decimal dilutions. Part 3: Specific rules for the preparation of fish and fishery products.

Reporting

All data have to be reported through the result form. The link will be sent by e-mail to the participants.

Submission of data has to be finalised on **10 April 2019** (23:59 h CET) at the latest. **Mind that the result form is no longer accessible after this deadline!** In case you foresee problems with the deadline, please contact us beforehand.

In case of deviating results, the EURL-*Salmonella* may ask the NRLs to send additional information or to perform additional tests. Therefore, we ask to conserve one *Salmonella* confirmed colony from each positive sample (A-D and CTRL 1 and 2).

The EURL will prepare a summary report soon after the study to inform all NRLs on the overall results.

Timetable
EURL - *Salmonella* Proficiency Test Live Bivalve Molluscs 2020
Detection of *Salmonella* in mussels

Week	Date	Subject
11	9 March	<p>Mailing of <i>Salmonella</i> reference materials to the NRLs as Biological Substance Cat. B (UN3373) by door-to-door courier service</p> <p>Mailing of the protocol and instructions for the result form to the NRLs by e-mail</p> <p>Sending the link for the result form to the participants by e-mail</p> <p>Preparation of media by the NRLs</p>
12	16 March	Mailing of parcels with 2 kg of mussels to the NRLs as Biological Substance Cat. B (UN3373) by door-to-door courier service
12	18 March	Performance of the Proficiency Test
15	At the latest 10 April	<p>Deadline for completing the electronic submission of results: 10 April 2020 (23:59h CET)</p> <p>After this deadline the result form will be closed</p>

If you have questions or remarks about this Proficiency Test, or in case of problems, please contact:

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