

# Protocol

## EURL-*Salmonella* Proficiency Test Live Bivalve Molluscs 2024

### Detection of *Salmonella* in mussels

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## 1. Introduction

This protocol describes the procedures for the Proficiency Test (PT) Live Bivalve Molluscs 2024 on the detection of *Salmonella* spp. in mussels amongst the National Reference Laboratories (NRLs) for *Salmonella* in the EU. The parcels will be shipped on Monday 26 February 2024 and the submission of data has to be finalised by 22 March 2024 at the latest.

## 2. Objective

The main objective of the Proficiency Test is to evaluate the performance of the NRLs-*Salmonella* on their ability to detect *Salmonella* at different contamination levels in mussels.

## 3. Outline of the Proficiency Test

### ***Mussels and Salmonella reference materials***

Each participant will receive two parcels:

- A parcel containing a packaging of 2 kg mussels (Modified Atmosphere Packaging conditions). The mussels will be send with cooling elements. Additional, the parcel will contain four (empty) sample bags coded with A, B, C and D. There will be also one sample bag containing a small electronic temperature recorder (coded with your laboratory code).
- A parcel containing four vials with or without *Salmonella*, which are resuspended in milk. The vials are coded with A, B, C and D. The *Salmonella* reference materials are send with dry ice. Use protective gloves when moving the materials from dry ice.

*Note: Sample bags for control samples will no longer be provided. Each NRL-*Salmonella* is expected to include (process) control samples according to its own Standard Operating Procedure and quality system. Still some information will be requested about the laboratory control samples used.*

The parcels will be sent on Monday 26 February 2024 by door-to-door courier service DHL (for non-EU MS sometimes door-to-airport). The parcel containing a packaging of mussels will be shipped as biological substance category B (UN3373). The parcel with *Salmonella* reference materials will also be shipped as biological substance category B (UN3373), but with dry ice (UN1845).

Please contact the EURL-*Salmonella* when the parcels have not arrived at your laboratory by the end of 28 February 2024 (this is 2 working days after the day of dispatch).

**Immediately after arrival of the parcel, or in the following days, the NRL-*Salmonella* can start the analysis of the samples. The latest date to start the analysis is by Wednesday 28 February 2024.**

**If not analysed immediately:**

- **the packaging of 2 kg mussels need to be stored at 5°C ( $\pm$  3 °C) and the packaging should remained closed, until the day of analysis.**
  - o **Please store the temperature recorder together with the mussels until the start of the analysis.**
- **the four vials of *Salmonella* reference materials need to be stored at -20 °C until the day of analysis. Use protective gloves when moving the materials from dry ice.**

***Temperature recorder***

The small electronic temperature recorder will measure the temperature during transport and storage of the samples at the laboratory. The EURL-*Salmonella* uses the temperature profile to monitor the quality of the samples received by each participant. The sample bag and the temperature recorder are coded with your laboratory code.

**You are urgently requested to return this complete plastic bag with recorder and lab code to the EURL-*Salmonella*, on the day your laboratory starts the PT (at the latest by 28 February 2024).**

For this purpose, a return envelope with a preprinted address label of the EURL-*Salmonella* is included.

***Preparation of the samples***

**The performance of the PT can start *immediately after receipt* of the samples but no later than by Wednesday 28 February 2024.**

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

- Defrost the vials at room temperature for 30 minutes at the start of the analysis.
- 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 **200 µl** of the corresponding vial.
  - o Sample A should be spiked with vial A
  - o Sample B should be spiked with vial B
  - o Sample C should be spiked with vial C
  - o Sample D should be spiked with vial D

Continue with the analysis of the samples.

***Analysis of the samples***

The prescribed method is EN ISO 6579-1:2017(/A1:2020) 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.

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

Additionally, laboratories (who are interested) can also perform a second detection method (e.g. PCR, real-time PCR, Vidas, 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(/A1:2020) are used to assess the performance of each NRL-*Salmonella*.

#### **4. Reporting**

All results and relevant details of the method(s) used 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 by **22 March 2024** (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-*Salmonella* to send additional information or to perform additional tests. Therefore, we ask you to conserve (at least) one *Salmonella* confirmed colony from each positive sample (A - D), as well as of the (own) positive control.

The EURL-*Salmonella* will prepare a summary report soon after the PT to inform all NRLs-*Salmonella* on the overall results.

## Relevant documents

- Protocol EURL-*Salmonella* Proficiency Test LBM 2024. Detection of *Salmonella* in mussels (this document);
- Short guidance on the electronic submission of data in the result form for the EURL-*Salmonella* Proficiency Test.
- Screenshots result form EURL-*Salmonella* PT LBM 2024.
- EN ISO 6579-1:2017. Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 1: Horizontal method for the detection of *Salmonella* spp.
- EN ISO 6579-1:2017/A1:2020. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Detection of *Salmonella* spp. - Amendment 1 Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSR/V and SC.
- 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.

# Timetable



EURL- *Salmonella* Proficiency Test Live Bivalve Molluscs 2024

Detection of *Salmonella* in mussels

Week	Date	Subject
4	Friday <b>26 January 2024</b> at the latest	Please register by <b>26 January 2024</b> at the latest.
6	Week of 5 February 2024	E-mailing the link for the result form to the participants. E-mailing the protocol and instructions for the result form to the NRLs.
7 – 8		Preparation of media by the NRLs.
9	Monday <b>26 February 2024</b>	Shipment of the parcel with mussels to the participants as Biological Substance Category B (UN3373). Shipment of the parcel with <i>Salmonella</i> reference materials to the participants as Biological Substance Category B (UN3373) and on dry ice (UN1845).
9	<b>Immediate after receipt of the parcel and at the latest on 28 February 2024</b>	<b>Start performance of the Proficiency Test.</b>
12	Friday <b>22 March 2024</b> at the latest	Deadline for completing the result form: <b>22 March 2024 (23:59h CET)</b> After this deadline the result form will be closed.
	May 2024	Interim summary report

If you have questions about this Proficiency Test, please contact:

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