PROTOCOL



EURL-Salmonella Proficiency Test Food-Feed 2023 Detection of Salmonella spp. in flaxseed

Introduction

This protocol describes the procedures for the Proficiency Test (PT) Food-Feed 2023 on the detection of *Salmonella* spp. in <u>flaxseed</u> amongst the National Reference Laboratories (NRLs) for *Salmonella* in the EU. This PT will take place from Monday <u>27 March 2023 until Wednesday 19 April 2023</u>.

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 flaxseed.

Outline of the study

Samples

Each participant will receive one box containing 1 large plastic safety bag, packed with cooling elements. When a laboratory requested two sets of samples, the box will contain 2 large plastic safety bags.

The plastic safety bag contains 17 (coded) sample bags, consisting of:

- 14 samples of flaxseed (25 g per sample) artificially contaminated with different levels of a *Salmonella* serovar (coded A1-A14);
- 2 (empty) sample bags to be used for the own control samples. The sample bag coded with CTRL1 shall be used for the procedure control (add BPW only). The other sample bag is coded CTRL2 and shall be used for the (own) positive Salmonella control (add BPW to the bag and spike it with your own Salmonella positive control).
- 1 sample bag containing a small electronic temperature recorder (coded with your laboratory code). This sample bag will be between the flaxseed samples.

Note: the control samples can be performed either way, but reporting of the results should be as described: 'CTRL1' is the procedure control (BPW only) and 'CTRL2' is the positive control with own *Salmonella*.

Each box will be sent on Monday 20 March 2023 as biological substance category B (UN3373) by door-to-door (for non-EU-MS sometimes door-to-airport) courier service DHL. Please contact the EURL-Salmonella when the parcel has not arrived at your laboratory by 23 March 2023 (this is 3 working days after the day of shipping).

Upon arrival of the parcel:

- Store the complete (content of the) safety bag(s) at 5°C (± 3°C) until the start of analyses (27 March 2023).
- Please store the temperature recorder <u>together</u> with the flaxseed samples until the start of the study.

Temperature recorder

The small electronic temperature recorder will measure the temperature during transport and storage of the samples at the laboratory. The sample bag and the temperature recorder are coded with your laboratory code.

You are urgently requested to return this complete sample bag with temperature recorder (and laboratory code) to the EURL-Salmonella, at the day your laboratory starts the study (27 March 2023). For this purpose a return envelope with a preprinted address label of the EURL-Salmonella is included. Only a stamp is needed before sending it back.

Analyses of the samples

The performance of the PT will start on Monday 27 March 2023.

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 6887-4:2017 should be respected.

For this Proficiency Test the laboratories can follow their own procedure for preparation of the test samples for this type of matrix or follow the procedure below. This procedure is slightly adjusted from EN ISO 6887-4:2017 to avoid punctures in the plastic sample bags.

- 1. Add the BPW to the 25 gram test sample (instead of weighing accurately the sample into a pre-dispense volume of BPW);
- 2. Resuscitate the sample for 20 to 30 minutes at 18 °C to 27 °C (room temperature);
- 3. Mix for 30 seconds (± 5 seconds) by hand;
- 4. Continue with the non-selective pre-enrichment procedure as described in EN ISO 6579-1:2017(/A1:2020).

Note: mincing, grinding or crushing the test samples in advance is not necessary

Additionally, laboratories (who are interested) can also perform a second detection method (e.g. PCR, qPCR, 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.

The media to be used for this study will <u>not</u> be supplied by the EURL-Salmonella.

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.

When two laboratory codes were requested by one laboratory, it is possible to fill in two separate result forms (especially when results and information differ) or one result form (e.g. in case results and information are similar). If one result form is used, please indicate both laboratory codes. In both cases the EURL-*Salmonella* will produce a separate individual performance report per laboratory code.

Submission of data has to be finalised on **19 April 2023** (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 to conserve one Salmonella confirmed colony from each positive sample (A1-A14 and CTRL1 and CTRL2).

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 Food-Feed 2023. Detection of Salmonella spp. in flaxseed' (this document).
- Short guidance on the electronic submission of data in the result form for the EURL-Salmonella Proficiency Test.
- Screenshots of the result form: 'Result form EURL-Salmonella PT Food-Feed 2023'.
- 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/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 MSRV and SC.
- ISO 6887-1 & 4:2017 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 4: Specific rules for the preparation of miscellaneous products.

Timetable



EURL- *Salmonella* Proficiency Test Food-Feed 2023 Detection of *Salmonella* in flaxseed

Week	Date	Subject
8	24 February 2023	E-mailing the link to the registration form for the detecting study.
		Please register by 24 February 2023 at the latest.
10 - 11	06 March 2023 -	E-mailing the link for the result form to the participants.
	17 March 2023	E-mailing the protocol and instructions for the result form to the NRLs-Salmonella.
12	Monday 20 March 2023	Shipment of the parcels to the participants as Biological Substance Category B (UN3373).
		Preparation of media by the NRLs-Salmonella.
13	Monday 27 March 2023	Performance of the Proficiency Test.
16	19 April 2023 at the latest	Deadline for completing the result form:
		19 April 2023 (23:59h CET)
		After this deadline the result form will be closed.
	May 2023	Interim summary report

If you have questions or remarks about this Proficiency Test please contact:

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