
PROTOCOL
INTERLABORATORY COMPARISON STUDY ON THE
DETECTION OF *SALMONELLA* spp. IN FOOD
organised by EURL-*Salmonella*
FOOD STUDY VII - 2015

Introduction

This protocol describes the procedures for the seventh interlaboratory comparison study on the detection of *Salmonella* spp. in food samples amongst the National Reference Laboratories (NRLs) for *Salmonella* in the EU. The type of samples in this study concern whole liquid chicken egg samples, spiked with a *Salmonella* serovar.

The set-up of this study is comparable to the interlaboratory comparison studies on detection of *Salmonella* in food, animal feed samples and samples from the primary production stage as organised since 2013, but also some important changes have been introduced:

- We will no longer send a Standard Operating Procedure (SOP) summarising the detailed steps of the procedure for the *Salmonella* detection, but we ask the NRLs to follow EN ISO 6579 (and the underlying EN ISO documents, e.g. the EN ISO 6887 series for preparation of test samples) according to their normal routine procedure for detection (and confirmation) of *Salmonella* in 'official' samples. The Final Draft International Standard (FDIS) version of ISO 6579-1 is expected to be published in fall 2015. This document describes the (final) updated technical steps for the detection of *Salmonella* in food, animal feed and samples from the primary production stage. An important change in this document compared to the current version of ISO 6579, is the possibility to choose between RVS and MSRV for the selective enrichment of *Salmonella* from food and animal feed samples. For the current study we already want to introduce this choice, meaning that additional to MKTTn, either RVS or MSRV can be used for selective enrichment. It is also allowed to use all three selective enrichment media.
- We will ask in the test report for the procedure followed for detection and confirmation of *Salmonella*, including the selective enrichment media, isolation media and confirmation tests used.
- For the reporting of the results it will no longer be needed to report positive and negative results per combination of selective enrichment medium and isolation medium per sample. We only ask to report what would have been reported in case these samples would have been routine samples, meaning that the indication 'positive' (1) or 'negative' (0) per sample (after confirmation) will be sufficient

(independent of the combination of selective enrichment medium and isolation medium).

- We will no longer send an additional blank matrix control sample, as the set of samples already includes several blank matrix samples.

Additionally, laboratories (who are interested) can also perform an 'own' PCR method on the samples, if this is (routinely) used in their laboratories. Results of an 'own' PCR method can still be recorded and will be used to compare the results with the results found with the bacteriological culture method.

The samples consist of 18 samples of whole liquid chicken egg (25 g) and 2 control samples. The samples are artificially contaminated with three different levels (blank, low and high level) of a *Salmonella* serovar (B1-B18). The control samples consist of one sample with only BPW (C1) and one sample with an own positive control from the participating laboratory (e.g. a reference material (lenticule disc or capsule) or a (diluted) culture containing *Salmonella*) in BPW (C2). Each laboratory has to examine 20 samples in total.

Each box will be sent as biological substance category B (UN3373) by door-to-door (for non-EU-MS sometimes door-to-airport) courier service. Please contact EURL-*Salmonella* when the parcel has not arrived at your laboratory at 17th of September 2015 (this is 3 working days after the day of mailing).

Objective

The main objective of the interlaboratory comparison study is to evaluate the performance of the NRLs for *Salmonella* for their ability to detect *Salmonella* spp. at different contamination levels in a food matrix.

Outline of the study

Each participant will receive, in week 38 of 2015, one box containing 2 plastic safety bags, packed with cooling elements. The plastic safety bag contains:

20 numbered plastic 'Whirl-pack' bags:

- 18 plastic 'Whirl-pack' bags, each containing 25 g whole liquid chicken egg, numbered B1-B18;
- 2 plastic (empty) 'Whirl-pack' bags numbered C1 and C2.

Store the plastic bags at (5 ± 3) °C immediately after receipt.

One plastic safety bag will also contain the small electronic temperature recorder in a plastic bag with your lab code. Make a note of the lab code and **return the temperature recorder (in the plastic bag) to the EURL-*Salmonella* at the day your laboratory starts the study (21 September 2015)**. For this purpose the return envelope, with preprinted address label of the EURL-*Salmonella*, can be used.

The **performance** of the study will be in week 39 (starting on Monday 21 September 2015). Follow as much as possible your normal routine procedure for the detection and confirmation of *Salmonella* in 'official samples'.

Remarks:

- It is preferred to homogenize each egg sample (25 g) with BPW (225 ml) by hand, as mixing with a homogenizer (like a stomacher) will lead to unwanted foaming of the sample.
- Use bag C1 for the (blank) control of (225 ml) BPW.
- Use bag C2 for the culturing of your own positive control (reference material or (diluted) culture containing *Salmonella*) in BPW.
- After pre-enrichment in BPW, selective enrichment is performed in/on two selective enrichment media: MKTTn and RVS, ór MKTTn and MSR.V. It is also allowed to use all three selective enrichment media.
- For further details of the method, consult ISO 6579:2002 and ISO 6579:2002/Amd 1: 2007

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

Documents

The documents necessary for performing the study are:

- Protocol Interlaboratory comparison study on the detection of *Salmonella* spp. in food VII (2015) (this document);
- Instructions for the web based test report Interlaboratory comparison study on the detection of *Salmonella* spp.: Guidance on submission of results EURL detection studies and the web based test report;
- ISO 6579 (2002). Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.;
- ISO 6579:2002/Amd 1: 2007 Amendment 1 Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage.
- International Standard – ISO 6887-4: 2003 Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 4: Specific rules for the preparation of products other than milk and milk products, meat and meat products and fish and fishery products.

Reporting

As in earlier EURL-*Salmonella* interlaboratory comparison studies, all data have to be reported through an electronic result form. We updated the questions in the web based report resulting in a shorter test report than before. As all NRLs have to be accredited according to ISO 17025, we assume that additional information not requested in the test report will be available in their system. 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 (B1-B18 and C1-C2).

The link, also to become available on the EURL-*Salmonella* website, and password for this electronic result form will be send by email to the participants. Submission of data has to be finalised on **13 October 2015** (23:59 h CET) at the latest. **Mind that the electronic result form is no longer accessible after this deadline!** In case you foresee problems with the deadline, please contact us beforehand.

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

Timetable of interlaboratory comparison study food VII (2015)
Detection of *Salmonella* spp. in whole liquid chicken egg

Week	Date	Topic
37	7 – 12 September	Mailing of the protocol and instructions for the web based test report to the NRLs- <i>Salmonella</i> . Sending the link and the password for electronic reporting form to participants by email.
38	14 September	Mailing of the parcels to the NRLs as biological substance category B (UN3373) by door-to-door courier service. Immediately after arrival of the parcel at the laboratory: <ul style="list-style-type: none"> - Check for any serious damages and for completeness; (in case of deviations contact the EURL-<i>Salmonella</i>); - Store the samples at + 5 °C ± 3 °C If you did not receive the parcel at 17 September, do contact the EURL immediately. Preparation of media following ISO 6579: <ol style="list-style-type: none"> 1. Non selective pre-enrichment medium 2. Selective enrichment media 3. Solid selective plating media 4. Confirmation media
39	21 September	Performance of the study, following ISO 6579 (and ISO 6579/Amd 1) and the instructions as given in the protocol of food study VII (2015). Remove the electronic temperature recorder from the safety bag (leave it in the plastic bag with lab code) and return it to EURL- <i>Salmonella</i> using the return envelope. Note the lab code before sending the recorder.
42	Deadline 13 October 23:59	Completion and submission of the web based test report. http://www.eurlsalmonella.eu/
	November 2015	Data elaboration at EURL- <i>Salmonella</i>
	December 2015	Sending of the individual laboratory results to the NRLs together with an interim summary report. As a follow-up, actions will be undertaken in case of poor performance.

If you have questions or remarks about this study, or in case of problems using the electronic reporting form, please contact:

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