
PROTOCOL
INTERLABORATORY COMPARISON STUDY ON THE
DETECTION OF *SALMONELLA* spp. IN SAMPLES FROM THE
PRIMARY PRODUCTION
organised by EURL-*Salmonella*
STUDY XVI - 2013

Introduction

This protocol describes the procedures for the 16th interlaboratory comparison study on the detection of *Salmonella* spp. in samples from the primary production amongst the National Reference Laboratories (NRLs) for *Salmonella* in the EU. This study is a combined study with the CEN mandate study on validation of *Salmonella* in samples from primary production (Annex D of EN ISO 6579), as has been discussed at the EURL-*Salmonella* workshop in May 2012. The number of samples to be tested and the type of samples are different from earlier EURL-studies.

The prescribed method is, like in earlier EURL studies, the procedure as described in Annex D of ISO 6579 (EN-ISO 6579:2002/Amd1: 2007: Amendment 1: Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage). Additionally, laboratories (who are interested) can also perform an 'own' PCR method on the samples, if this is (routinely) used in their laboratories.

For the current study, it is no longer necessary to report results of 'own' culture media to the EURL-*Salmonella*. Results of an 'own' PCR method can still be recorded and will be used for comparison on the results of the bacteriological culture method.

The samples will consist of 24 pairs of boot socks to which environmental material (mainly faeces) from a laying hen flock is attached and 6 control pairs of boot socks (without faeces). The boot socks are artificially contaminated with three different levels (blank, low and high level) of a *Salmonella* serovar. Each laboratory has to examine 30 boot sock samples in total.

The samples will be packed in 2 plastic safety bags in one large box together with cooling elements. One safety bag will also contain a temperature recorder to measure the temperature during transport and storage of the samples at the laboratory. The recorder will be packed in a plastic bag, which will also contain your lab code. **You are urgently requested to return this complete plastic bag with recorder and lab code to the EURL-*Salmonella*, at the day your laboratory starts the study (11 March 2013).** For this purpose a return envelope with a preprinted address label of the EURL-*Salmonella* will be included. Do not forget to note your lab code before returning it to the EURL.

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 7th of March 2013 (this is 3 working days after the day of mailing).

Objectives

This current study has two objectives:

1. To evaluate the performance of the NRLs for *Salmonella* for their ability to detect *Salmonella* spp. at different contamination levels in samples from the primary production.
2. Determine precision characteristics of the prescribed method (Annex D of EN ISO 6579).

Outline of the study

Each participant will receive (in week 10 of 2013) one box containing 2 plastic safety bags, packed with cooling elements. The plastic safety bags contain:

30 numbered plastic 'Sodibox' bags each containing a pair of boot socks

- 24 plastic "Sodibox" bags numbered B1-B24;
- 6 plastic "Sodibox" bags numbered C1-C6;

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. **This recorder (in the plastic bag) should be returned to the EURL-*Salmonella* at the day your laboratory starts the study (11 March 2013).**

The performance of the study will be in week 11 (starting on Monday 11 March 2013).

The documents necessary for performing the study are:

- Protocol Interlaboratory comparison study on the detection of *Salmonella* spp. in environmental samples from the primary production XVI (2013) (this document);
- SOP Interlaboratory comparison study on the detection of *Salmonella* spp. in environmental samples from the primary production XVI (2013);
- Instructions for the web based test report Interlaboratory comparison study on the detection of *Salmonella* spp. in environmental samples from the primary production XVI (2013);
- 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.

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

This is the first EURL-*Salmonella* detection study for which all data have to be reported through an electronic result form. The link, also to become available on the EURL-*Salmonella* website and password for this form will be send by email to the participants. Submission of data has to be finalised on **29 March 2013** (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 primary production XVI (2013)

Detection of *Salmonella* spp. in boot socks

Week	Date	Topic
8	18 – 22 February	Mailing of the protocol, standard operating procedure (SOP) and instructions for the web based test report to the NRLs- <i>Salmonella</i>
9	25 – 28 February	Sending the link and the password for electronic results form to participants by email
10	4 March	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 7 March, do contact the EURL immediately. Preparation of: <ol style="list-style-type: none"> 1. Non selective pre-enrichment medium (see SOP 6.1) 2. Selective enrichment medium (see SOP 6.2) 3. Solid selective plating media (see SOP 6.3) 4. Confirmation media (see SOP 6.4)
11	11 – 15 March	Performance of the study, following the instructions as given in the protocol and the SOP of study XVI (2013). 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
13	Deadline 29 March (23:59)	Completion and submission of the web based test report.* http://www.eurlsalmonella.eu/
	April 2013	Data elaboration at EURL- <i>Salmonella</i>
	May 2013	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.

*A check-up of the submitted results by the NRLs is no longer needed when submitting the results by a web based test report. However, NRLs need to be sure to fill out the right results at once.

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

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