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**PROTOCOL**  
**INTERLABORATORY COMPARISON STUDY ON THE**  
**DETECTION OF *SALMONELLA* spp. IN SAMPLES FROM THE**  
**PRIMARY PRODUCTION STAGE**  
**organised by EURL-*Salmonella***  
**STUDY XVI I- 2014**

### Introduction

This protocol describes the procedures for the 17<sup>th</sup> interlaboratory comparison study on the detection of *Salmonella* spp. in samples from the primary production stage amongst the National Reference Laboratories (NRLs) for *Salmonella* in the EU. The number of samples to be tested and the type of samples are comparable with the studies organised in 2013.

The following amendments were introduced in 2013, compared to the former studies:

- The chicken faeces samples are already spiked with *Salmonella* at the laboratory of the EURL-*Salmonella*. Hence, the samples can be analysed 'as such' and no reference materials need to be added at the laboratory of the NRL.
- Less control samples are included and therefore we ask each NRL to analyse the control sample(s) routinely used in the laboratory (e.g. a reference material or a (diluted) culture containing *Salmonella*).

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.

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 to the results of the bacteriological culture method.

The samples consist of 18 samples of chicken faeces and 3 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), one sample with chicken faeces and BPW (C2) and one sample with an own positive control from the participating laboratory in BPW (C3). Each laboratory has to examine 21 samples in total.

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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 to the laboratory 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 (31 March 2014).** 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 DHL. Please contact EURL-*Salmonella* when the parcel has not arrived at your laboratory at 27<sup>th</sup> of March 2014 (this is 3 working days after the day of mailing).

### Objectives

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 samples from the primary production stage.

### Outline of the study

Each participant will receive (in week 13 of 2014) one box containing 2 plastic safety bags, packed with cooling elements. The plastic safety bags contain:

21 numbered plastic 'Whirl-pak' bags:

- 18 plastic 'Whirl-pak' bags numbered B1-B18;
- 3 plastic 'Whirl-pak' bags numbered C1-C3;

**Store the plastic bags at  $(5 \pm 3)$  °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 (31 March 2014).**

**Note your lab code before returning the recorder!**

The performance of the study will be in week 14 (starting on Monday 31 March 2014).

The documents necessary for performing the study are:

- Protocol Interlaboratory comparison study on the detection of *Salmonella* spp. in samples from the primary production stage, PPS XVI I(2014) (this document);
- SOP Interlaboratory comparison study on the detection of *Salmonella* spp. in samples from the primary production stage, PPS XVII (2014);
- Instructions for the web based test report Interlaboratory comparison study on the detection of *Salmonella* spp. in samples from the primary production stage;
- ISO 6579 (2002). Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.;

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- 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**

All data have to be reported through an electronic result form. The link, which will also become available on the EURL-*Salmonella* website, and the password for this form will be sent by email to the participants. Submission of data has to be finalised on **16 April 2014** (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 stage PPS XVII (2014)

### Detection of *Salmonella* spp. in chicken faeces

Week	Date (2014)	Topic
11	In week of 10 March	Mailing of the protocol, standard operating procedure (SOP), lab code, questions and instructions for the web based test report to the NRLs- <i>Salmonella</i>
12	In week of 17 March	Sending the link and the password for electronic results form to participants by email
13	24 March	Mailing of the parcels to the NRLs as biological substance category B (UN3373) by courier service DHL. Immediately after arrival of the parcel at the laboratory: <ul style="list-style-type: none"> <li>- Check for any serious damages and for completeness; (in case of deviations contact the EURL-<i>Salmonella</i>);</li> <li>- <b>Store the samples at + 5°C ± 3 °C</b></li> </ul> <b>If you did not receive the parcel at 27 March, do contact the EURL immediately.</b> Preparation of: <ol style="list-style-type: none"> <li>1. Non selective pre-enrichment medium (see SOP 6.1)</li> <li>2. Selective enrichment medium (see SOP 6.2)</li> <li>3. Solid selective plating media (see SOP 6.3)</li> <li>4. Confirmation media (see SOP 6.4)</li> </ol>
14	31 March	Performance of the study, following the instructions as given in the protocol and the SOP of study XVII (2014). 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 your lab code.
16	<b>Deadline</b> <b>16 April (23:59)</b>	Completion and submission of the web based test report.* <a href="http://www.eurlsalmonella.eu/">http://www.eurlsalmonella.eu/</a>
	April / May 2014	Data elaboration at EURL- <i>Salmonella</i>
	June 2014	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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