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**PROTOCOL**  
**INTERLABORATORY COMPARISON STUDY ON THE**  
**DETECTION OF *SALMONELLA* spp. IN FOOD**  
**organised by EURL-*Salmonella***  
**FOOD STUDY VI - 2013**

### **Introduction**

This protocol describes the procedures for the 6<sup>th</sup> interlaboratory comparison study on the detection of *Salmonella* spp. in food samples amongst the National Reference Laboratories (NRLs) for *Salmonella* in the EU.

The number of samples to be tested and the type of samples are different from earlier interlaboratory comparison studies on detection of *Salmonella* in food samples. However, the study is comparable to the last study for the detection of *Salmonella* in samples from primary production, organised in March 2013.

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

-The minced chicken meat 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 studies, the procedure as described in ISO 6579 ('Microbiology of food and feeding stuffs – Horizontal method for the detection of *Salmonella* spp.' Fourth edition, 2002). Additional to ISO 6579 it is requested also to use 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'). The method in this annex is especially intended for the detection of *Salmonella* spp. in animal faeces and environmental samples from the primary production stage, but it is also applicable for the analyses of animal feed samples. 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 to compare the results with the results found with the bacteriological culture method.

The samples consist of 18 samples of minced chicken meat 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 minced chicken meat 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.

The samples will be packed in 1 plastic safety bag in one large box together with cooling elements. The 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 (30 September 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 26<sup>th</sup> of September 2013 (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 food samples using different methods.

### Outline of the study

Each participant will receive, in week 39 of 2013, one box containing 1 plastic safety bag, packed with cooling elements. The plastic safety bag contains:

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.**

The 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 (30 September 2013).**

The performance of the study will be in week 40 (starting on Monday 30 September 2013).

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The documents necessary for performing the study are:

- Protocol Interlaboratory comparison study on the detection of *Salmonella* spp. in food VI (2013) (this document);
- SOP Interlaboratory comparison study on the detection of *Salmonella* spp. in food VI (2013);
- Instructions for the web based test report Interlaboratory comparison study on the detection of *Salmonella* spp. in food VI (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 on a food matrix 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 **18 October 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 food VI (2013)**  
Detection of *Salmonella* spp. in minced chicken meat

Week	Date	Topic
37	9 – 13 September	Mailing of the protocol, standard operating procedure (SOP) and instructions for the web based test report to the NRLs- <i>Salmonella</i>
38	16 – 20 September	Sending the link and the password for electronic reporting form to participants by email
39	23 – 27 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"> <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 26 September, 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>
40	30 September	Performance of the study, following the instructions as given in the protocol and the SOP of food study VI (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
42	<b>Deadline</b> <b>18 October 23:59</b>	Completion and submission of the web based test report.* <a href="http://www.eurlsalmonella.eu/">http://www.eurlsalmonella.eu/</a>
	October 2013	Data elaboration at EURL- <i>Salmonella</i>
	November 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. Hence, 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 of problems using the electronic reporting form, please contact:

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