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# PROTOCOL

## INTERLABORATORY COMPARISON STUDY ON THE DETECTION OF *SALMONELLA* spp. IN FOOD

organised by EURL-*Salmonella*

FOOD STUDY V - 2011

### Introduction

This protocol describes the procedures for the fifth interlaboratory comparison study on the detection of *Salmonella* spp. in a food matrix amongst the National Reference Laboratories (NRLs for *Salmonella*) in the EU. In this study the number of samples to be tested will be comparable to earlier studies on the detection of *Salmonella* spp. but the type of reference materials will be different. 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 is also applicable for the analyses of food samples. Furthermore laboratories who are interested can also perform PCR on the samples and/or use additional methods (routinely) used in their laboratories.

The samples will consist of minced meat (*Salmonella* negative) artificially contaminated with reference materials. The reference materials (RMs) consist of lenticule discs containing *Salmonella* strains at different contamination levels. Each laboratory will examine 25 meat samples (25 g each) in combination with a *Salmonella* lenticule disc and 7 control samples (lenticule discs only).

The samples will be packed in 2 plastic containers in one large box together with cooling elements. One container will contain the lenticule discs the other will contain the minced meat. The container with the lenticule discs will also contain a temperature recorder to measure the temperature during transport of the samples. 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*, immediately after receipt of the parcel.** For this purpose a return envelope with a preprinted address label of the EURL-*Salmonella* has been included. Do not forget to note your lab code before returning it to the EURL.

Each box with samples will be sent as biological substance category B (UN3373) by door-to-door courier service. **Please contact the EURL-*Salmonella* when the parcel has not arrived at your laboratory on 22th of September 2011** (this is 4 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 using different methods.

## Outline of the study

In week 38 (starting on 19 Sept) of 2011 each participant will receive one box containing 2 biopacks, packed with cooling elements.

The containers contain:

Container 1:

one plastic bag with 32 numbered vials each containing one lenticule disc with or without *Salmonella*

-25 vials numbered **B1-B25**;

-7 vials numbered **C1-C7**;

This container 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* as soon as possible.**

**Store container 1, with its content, at  $(-20 \pm 5)$  °C immediately after receipt.**

Container 2:

one plastic bag with approximately 750 g of minced meat (free from *Salmonella*).

**Store container 2, with its content, at  $(5 \pm 3)$  °C immediately after receipt.**

The performance of the study will be in week 39 (starting on 26 September 2011).

The documents necessary for performing the study are:

- Protocol Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in food V (2011) (this document);
- SOP Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in food V (2011);
- Test report Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in food V (2011);
- ISO 6579 (2002). Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.;
- Amendment 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 used for the collaborative study will not be supplied by the EURL.

All data have to be reported in the test report and sent to the EURL-*Salmonella* **before 14 October 2011**. The EURL will prepare a summary report soon after the study to inform all

NRLs on their own results and on the overall results. **Results which will be received after the deadline can not be used in the analyses for the interim summary report.**

If you have questions or remarks about the interlaboratory comparison study please contact:

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### Time table of interlaboratory comparison study FOOD V (2011)

Week	Date	Topic
36	5 - 9 September	Mailing of the protocol, Standard Operating Procedure and test report to the NRLs- <i>Salmonella</i>
38	19 - 23 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 parcels at the laboratory: <ul style="list-style-type: none"> <li>- Check for any serious damages <b>(do not accept damaged packages);</b></li> <li>- Check for completeness;</li> <li>- Remove the <b>electronic temperature</b> recorder from the parcel (leave it in the plastic bag with lab code) and return it to <b>EURL-<i>Salmonella</i></b> using the return envelope;</li> <li>- <b>Store the lenticule discs at <math>-20 \pm 5</math> °C</b></li> <li>- <b>Store the meat at <math>+ 5 \pm 3</math> °C</b></li> </ul> <p><b>If you did not receive the parcel at 22 September, do contact the EURL immediately.</b></p> <p>Preparation of:</p> <ol style="list-style-type: none"> <li>1. Non selective pre-enrichment medium (see SOP 6.1)</li> <li>2. Selective enrichment media (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>
39	26 - 30 September	Performance of the study, following the instructions as given in the protocol and the SOP of study Food V (2011).
41	Before 14 October	Completion of the test report. Send the test report by e-mail to the EURL <i>Salmonella</i> <a href="mailto:Angelina.Kuijpers@rivm.nl">Angelina.Kuijpers@rivm.nl</a> *.
42	17 - 22 October	Checking the results by the National Reference Laboratories.
	December 2011	Sending of the final results to the NRLs together with an interim summary report. A follow-up will be discussed with NRLs who showed no good performance, according to pre-defined criteria.

\* If the test report is e-mailed to the EURL it is not necessary to send the original test report as well, unless it is not legible (to be indicated by EURL-*Salmonella*)