

# PROTOCOL

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

organised by CRL-*Salmonella*

FOOD STUDY I - 2006

### Introduction

This is the first interlaboratory comparison study on the detection of *Salmonella* spp. in a food matrix amongst the National Reference Laboratories (NRLs for *Salmonella*) in the EU. The research of *Salmonella* spp. in a food matrices is also an important task for the CRL, as well as for the NRLs-*Salmonella*. This is described in Commission Regulations EC No 882/2004 on official controls. This food-study will have a comparable set-up as the earlier studies on the detection of *Salmonella* spp. in veterinary samples. At the workshops of CRL *Salmonella* in 2005 and 2006 the detection of *Salmonella* in a food matrix was discussed and it was decided to start with meat.

The prescribed method is the procedure as described in ISO 6579 (Microbiology of food and feeding stuffs – Horizontal method for the detection of *Salmonella* spp. Fourth edition, 2002.) Beside ISO 6579 it is recommended also to use draft Annex D of ISO 6579. The method in this annex is especially intended for the detection of *Salmonella* spp. in animal faeces and samples of the primary production stage, but is also applicable to food samples. A copy of the latest version of draft Annex D (12 September 2006) will be provided with this study. Furthermore laboratories who are interested can also perform PCR on the samples and/or use additional methods (routinely) used in their laboratories.

Artificially contaminated (*Salmonella* negative) minced meat samples are tested by using reference materials. The reference materials (RMs) consist of gelatine capsules containing sublethally injured *Salmonella* Typhimurium (STM), *Salmonella* Enteritidis (SE) or *Salmonella* Panama (SPan) at different contamination levels. Each laboratory will examine 25 meat samples (10 g each and negative for *Salmonella* spp.) in combination with a capsule containing STM or SE and 10 control samples (no meat is added to the capsule).

Finally, to obtain more detailed information on the temperatures and times during transport of the samples we will include an electronic temperature recorder in the parcel. The amount of materials can not be packed in one parcel and will be divided over two parcels (one containing capsules and one containing *Salmonella* negative meat). The two parcels are packed in one box with cooling elements. We will include only one temperature recorder and only in the parcel containing the capsules. 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 CRL-*Salmonella*, immediately after receipt of the parcel.** For this purpose a return envelope with a preprinted address label of the CRL-*Salmonella* has been included.

Each box (containing 2 parcels) will be sent as diagnostic specimens by door-to-door courier service. Please contact CRL-*Salmonella* when the parcel has not arrived at your laboratory at 22<sup>nd</sup> of September 2006 (this is after 5 working days after the day of mailing).

### **Objectives**

The main objective of the first interlaboratory comparison study on the detection of *Salmonella* in a food matrix is to evaluate the results of the detection of different contamination levels of *Salmonella* in the presence of competitive micro-organisms in a food matrix, using different methods, among and within the NRLs.

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## Outline of the study

Each participant will receive (in week 38) one box containing 2 parcels, packed with cooling elements. The parcels contain:

Parcel 1:

- 25 numbered vials; each containing one *Salmonella* Typhimurium, one *Salmonella* Enteritidis or blank capsule (numbered 1-25);
- 10 control vials; each containing one capsule with or without *Salmonella* (numbered C1-C10).

This parcel will 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 CRL-*Salmonella* as soon as possible.**

Parcel 2:

- 300 g of minced meat (free from *Salmonella*).

**Parcel 1 should be stored at  $(-20 \pm 5)^{\circ}\text{C}$  immediate after receipt.**

**Parcel 2 should be stored at  $(5 \pm 3)^{\circ}\text{C}$  immediate after receipt.**

The performance of the study will be in week 39 (starting on 25 September 2006).

The documents necessary for performing the study are:

- Protocol Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in food I (2006);
- SOP Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in food I (2006);
- Test report Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in food I (2006);
- ISO 6579 (2002). Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.;
- Draft Amendment ISO 6579:2002/amendedDAmd 1 (2006-09-12) Amendment 1 Annex D: Detection of *Salmonella* spp. in animal faeces and in samples from the primary production stage.

The media used for the collaborative study will not be supplied by the CRL.

All data will be reported in the test report and sent to the CRL-*Salmonella* and will be used for (statistical) analysis.

In the time table of the interlaboratory comparison study Food I (see next page) on the bacteriological detection of *Salmonella*, a **strict deadline** for sending the results to the CRL-*Salmonella* is indicated (15 October 2006). We will prepare a short report to inform all NRLs within 1 to 2 months after the study on the overall results. We will start the first overall analyses immediately after the deadline. Results which will be received after the deadline can not be used in the analyses for the short report. It may still be possible to use late results in the analyses for the final report but results received after publishing the short report can not be incorporated in the final 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 I (2006)**

Week	Date	Topic
36	4 – 8 September	Mailing of the protocol, standard operating procedure, test report and draft Annex D of ISO 6579 to the NRLs-Salmonella
38	18 – 22 September	<p>Mailing of the parcels to the NRLs as diagnostic specimens by door-to-door courier service.</p> <p>Immediately after arrival of the parcels at the laboratory:</p> <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 electronic temperature recorder from the parcel (leave it in the plastic bag with lab code) and return it to CRL-<i>Salmonella</i> using the return envelope;</li> <li>- <b>Store the meat at +5°C ± 3°C</b></li> <li>- <b>Store the capsules at -20°C ± 5°C.</b></li> </ul> <p><b>If you did not receive the parcel at 22 September, do contact the CRL 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	25-29 September	Performance of the study, following the instructions as given in the protocol and the SOP of study Food I (2006).
41	Before 15 October	Completion of the test report and faxing or e-mailing it to the CRL. The original test report will be sent to CRL.
42	16-20 October	Checking the results by the National Reference Laboratories.
	November/ December 2006	Sending of the final results to the NRLs together with a short report. As a follow-up, actions will be undertaken for those NRLs which scored below the average results of all NRLs.