PROTOCOL

INTERLABORATORY COMPARISON STUDY ON THE DETECTION OF *SALMONELLA* spp. IN FOOD organised by CRL-*Salmonella*

FOOD STUDY II - 2007

Introduction

This is the second 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 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 second study will have a comparable set-up as the first food study and the earlier studies on the detection of *Salmonella* spp. in veterinary samples.
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 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 analyse the samples with molecular methods and/or use additional methods (routinely) used in their laboratories.

The samples will consist of minced beef samples (*Salmonella* negative) artificially contaminated with 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) in combination with a capsule containing STM or SE and 10 control samples (capsules only).

For a better testing of the performance of the laboratories the contamination levels of the STM and SE capsules in this study are lower than in earlier studies. During the studies in

2006 the STM and SE samples were tested for almost 100% positive. In this study the contamination level of the low level capsules is at the detection limit of the method, and contain circa 5 colony forming particles (cfp) of *S.* Typhimurium (STM5) and 10 cfp of *S.* Enteritidis (SE10) instead of STM10 and SE100 in the first food-study. Besides these low level samples, also high level samples (approximately 5-10 times above the detection limit) are included, so that laboratories can be sure that *Salmonella* is present. The high level capsules contain circa 50 colony forming particles (cfp) of *S.* Typhimurium (STM50) and 100 cfp of *S.* Enteritidis (SE100) instead of STM100 and SE500 in the first food-study.

Like in earlier studies, the parcel will contain an electronic temperature recorder. This will give information on the temperatures and times during transport of the samples. The amount of materials can not be packed in one biobottel and will be divided over two biobottles (one containing capsules and one containing *Salmonella* negative meat). The two biobottles are packed in one box with cooling elements. Only one temperature recorder and will be included only in the biobottle 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 biobottles) will be sent as biological substance category B (UN3373) by door-to-door courier service. Please contact CRL-*Salmonella* when the parcel has not arrived at your laboratory at 15 of November 2007 (this is 4 working days after the day of mailing).

Objectives

The main objective of the second 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.
By comparing the results of this study with the results of the first food-study last year, further information may be obtained on a suitable contamination level of the reference material to test the performance of the laboratories, as the same matrix and methods are used in both studies.

Outline of the study

Each participant will receive (in week 46) one box containing 2 biobottles, packed with cooling elements. The biobottles contain:

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

This biobottle 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.**
Store biobottle 1 at (-20 ± 5) ºC immediate after receipt.

Biobottle 2:
- 300 g of minced meat (free from *Salmonella*).

Store biobottle 2 at (5 ± 3) ºC immediate after receipt.

The performance of the study will be in week 47 (starting on 19 November 2007).

The documents necessary for performing the study are:

- Protocol Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in food II (2007);
- SOP Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in food II (2007);
- Test report Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in food II (2007);
- 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 CRL.

All data will be reported in the test report and sent to the CRL-*Salmonella* and will be used for (statistical) analysis.
Please make sure to send your results to CRL-*Salmonella* before 7 December 2007. At the CRL a short report will be prepared 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.**

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

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Time table of interlaboratory comparison study FOOD II (2007)

Week	Date	Topic
44	29 October – 2 November	Mailing of the protocol, standard operating procedure and test report to the NRLs-*Salmonella*
46	12 – 16 November	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: - Check for any serious damages (**do not accept damaged packages)**; - Check for completeness; - Remove the electronic temperature recorder from the parcel (leave it in the plastic bag with lab code) and return it to CRL-*Salmonella* using the return envelope; - **Store the capsules at -20 ± 5 °C** - **Store the meat at +5 ± 3 °C** **If you did not receive the parcel at 15 November, do contact the CRL immediately.** Preparation of: 1. Non selective pre-enrichment medium (see SOP 6.1) 2. Selective enrichment media (see SOP 6.2) 3. Solid selective plating media (see SOP 6.3) 4. Confirmation media (see SOP 6.4)
47	19-23 November	Performance of the study, following the instructions as given in the protocol and the SOP of study Food II (2007).
49	Before 7 December	Completion of the test report. Send the test report, preferably by e-mail to the CRL Salmonella (Angelina.kuijpers@rivm.nl)*.
50	10-14 December	Checking the results by the National Reference Laboratories.
	December 2007 January 2008	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.

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