
PROTOCOL

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

organised by CRL-*Salmonella*

FOOD STUDY III - 2009

Introduction

This protocol describes the procedures for the third interlaboratory comparison study on the detection of *Salmonella* spp. in a food matrix amongst the National Reference Laboratories (NRLs for *Salmonella*) in the EU. This study will have a comparable set-up as the earlier studies (food, veterinary and feed) on the detection of *Salmonella* spp. 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 perform PCR on the samples and/or use additional methods (routinely) used in their laboratories.

The samples will consist of minced chicken meat (*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).

The samples will be packed in 2 plastic containers in one large box together with cooling elements. One container will contain the capsules the other will contain the minced chicken meat. The container with the capsules 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 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. Do not forget to note your lab code before returning it to the CRL.

Each box with samples 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 3th of October 2009** (this is 4 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 a food matrix using different methods.

Outline of the study

Each participant will receive (in week 40 of 2009) one box containing 2 biopacks, packed with cooling elements. The containers contain:

Biopack 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 biopack 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 biopack 1 with capsules at -20 °C immediate after receipt.

Biopack 2:

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

Store biopack 2 with meat at 5 °C immediate after receipt.

The performance of the study will be in week 41 (starting on 5 October 2009).

The documents necessary for performing the study are:

- Protocol Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in food III (2009) (this document);
- SOP Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in food III (2009);
- Test report Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in food III (2009);
- 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 have to be reported in the test report and sent to the CRL-*Salmonella* **before 23 October 2009**. The CRL will prepare a summary report soon after the study to inform all NRLs on the overall results. **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 III (2009)

Week	Date	Topic
38	14 -18 September	Mailing of the protocol, Standard Operating Procedure and test report to the NRLs- <i>Salmonella</i>
40	28 September – 3 October	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"> - 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-<i>Salmonella</i> using the return envelope; - Store the capsules at -20 ± 5 °C - Store the meat at $+5 \pm 3$ °C If you did not receive the parcel at 3 October, do contact the CRL immediately. Preparation of: <ol style="list-style-type: none"> 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)
41	5 – 9 October	Performance of the study, following the instructions as given in the protocol and the SOP of study Food III (2009).
43	Before 23 October	Completion of the test report. Send the test report by e-mail to the CRL <i>Salmonella</i> Angelina.kuijpers@rivm.nl *.
44	26 - 30 October	Checking the results by the National Reference Laboratories.
	December 2009	Sending of the final results to the NRLs together with a short 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 CRL it is not necessary to sent the original test report as well, unless it is not legible (to be indicated by CRL-*Salmonella*)