

PROTOCOL

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

organised by CRL-*Salmonella*

STUDY XII - 2009

Introduction

This protocol describes the procedures for the 12th interlaboratory comparison study on the detection of *Salmonella* spp. in animal faeces amongst the National Reference Laboratories (NRLs for *Salmonella*) in the EU. This study will have a comparable set-up as the earlier studies on the detection of *Salmonella* spp. in veterinary samples. The prescribed method is the procedure as described in 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). 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 chicken faeces 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 faeces 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 container will contain the chicken faeces. 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.

Each box 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 5th of March 2009 (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 poultry faeces.

Outline of the study

Each participant will receive (in week 10 of 2009) one box containing 2 plastic containers, packed with cooling elements. The containers contain:

Container 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 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 CRL-*Salmonella* as soon as possible.**

Store container 1 at (-20 ± 5) °C immediately after receipt.

Container 2:

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

Store container 2 at (5 ± 3) °C immediately after receipt.

The performance of the study will be in week 12 (starting on 16 March 2009).

The documents necessary for performing the study are:

- Protocol Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in animal faeces XII (2009) (this document);
- SOP Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in animal faeces XII (2009);
- Test report Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in animal faeces XII (2009);
- 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 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 **3 April 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 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
ANIMAL FAECES XII (2009)**

Week	Date	Topic
7	9 - 13 February	Mailing of the protocol, standard operating procedure and test report to the NRLs- <i>Salmonella</i>
10	2 - 6 March	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 container (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°C ± 5 °C - Store the faeces at +5°C ± 3 °C If you did not receive the parcel at 5 March, do contact the CRL immediately.
11	9 - 13 March	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)
12	16 - 20 March	Performance of the study, following the instructions as given in the protocol and the SOP of study Animal faeces XII (2009).
14	Before 3 April	Completion of the test report. Send the test report, preferably by e-mail to the CRL <i>Salmonella</i> (Angelina.Kuijpers@rivm.nl)* .
15	6 -10 April	Data input at CRL- <i>Salmonella</i> and sending these data to NRLs. Checking these results by the National Reference Laboratories.
	May - June 2009	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 necessary to send the original test report as well, unless it is not legible (to be indicated by CRL-*Salmonella*).