

# PROTOCOL

## INTERLABORATORY COMPARISON STUDY ON THE DETECTION OF *SALMONELLA* spp. IN CHICKEN FAECES organised by CRL-*Salmonella* STUDY XIII - 2010

### Introduction

This protocol describes the procedures for the 13<sup>th</sup> 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* strains at different contamination levels. Each laboratory will examine 29 faeces samples (10 g each) in combination with a *Salmonella* capsule and 6 control samples (capsules only). Two different batches of chicken faeces have to be tested: 25 samples with one batch and 4 samples with another batch of faeces.

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* will be 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 11<sup>th</sup> of March 2010 (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 2010) one box containing 2 plastic containers, packed with cooling elements. The containers contain:

Container 1:

contains one plastic bag with 35 numbered vials each containing one capsule with or without *Salmonella*

-4 vials numbered **A1-A4**;

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

-6 vials numbered **C1-C6**;

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  $(-20 \pm 5)$  °C immediately after receipt.**

Container 2:

contains two plastic bags

- 60 g of chicken faeces (free from *Salmonella*) marked with **A**.

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

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

The performance of the study will be in week 11 (starting on 15 March 2010).

The documents necessary for performing the study are:

- Protocol Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in animal faeces XIII (2010) (this document);
- SOP Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in animal faeces XIII (2010);
- Test report Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in animal faeces XIII (2010);
- 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 to be 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 **2 April 2010**. 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 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  
ANIMAL FAECES XIII (2010)**

Week	Date	Topic
7	15 - 19 February	Mailing of the protocol, standard operating procedure and test report to the NRLs- <i>Salmonella</i>
10	8 – 12 March	<p>Mailing of the parcels to the NRLs as biological substance category B (UN3373) 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 container (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 capsules at -20°C ± 5 °C</b></li> <li>- <b>Store the faeces at +5°C ± 3 °C</b></li> </ul> <p><b>If you did not receive the parcel at 11 March, 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>
11	15 – 19 March	Performance of the study, following the instructions as given in the protocol and the SOP of study Animal faeces XIII (2010).
13	Before 2 April	Completion of the test report. Send the test report, preferably by e-mail to the CRL <i>Salmonella</i> ( <a href="mailto:Angelina.Kuijpers@rivm.nl">Angelina.Kuijpers@rivm.nl</a> )* .
14	5 -9 April	Data input at CRL- <i>Salmonella</i> and sending these data to NRLs. Checking these results by the National Reference Laboratories.
	May - June 2010	Sending of the final results to the NRLs together with a short summary. As a follow-up, actions will be undertaken in case of poor performance.

\* 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*).