

# PROTOCOL

## INTERLABORATORY COMPARISON STUDY ON THE DETECTION OF *SALMONELLA* spp. IN CHICKEN FAECES organised by EU-RL-*Salmonella* STUDY XIV - 2011

### Introduction

This protocol describes the procedures for the 14<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. In this study the numbers of samples to be tested will be comparable to earlier studies on the detection of *Salmonella* spp. in veterinary samples, but the type of reference materials will be different. The prescribed method is, like in earlier studies, 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 lenticule discs containing *Salmonella* strains at different contamination levels. Each laboratory will examine 25 faeces samples (25 g each) in combination with a *Salmonella* lenticule and 7 control samples (lenticules only).

The samples will be packed in 2 plastic containers in one large box together with cooling elements. One container will contain the lenticule discs; the other container will contain the chicken faeces. The container with the lenticules 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 EU-RL-*Salmonella*, immediately after receipt of the parcel.** For this purpose a return envelope with a preprinted address label of the EU-RL-*Salmonella* will be included. Do not forget to note your lab code before returning it to the EU-RL.

Each box will be sent as biological substance category B (UN3373) by door-to-door courier service. Please contact EU-RL-*Salmonella* when the parcel has not arrived at your laboratory at 17<sup>th</sup> of February 2011 (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 7 of 2011) one box containing 2 plastic containers, packed with cooling elements. The containers contain:

Container 1:

One plastic bag with 34 numbered vials each containing one lenticule disc with or without *Salmonella*

-25 vials numbered B1-B25;

-7 vials numbered C1-C7;

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 EU-RL-*Salmonella* as soon as possible.**

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

Container 2:

One plastic bag with 900 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 9 (starting on 28 February 2011).

The documents necessary for performing the study are:

- Protocol Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in animal faeces XIV (2011) (this document);
- SOP Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in animal faeces XIV (2011);
- Test report Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in animal faeces XIV (2011);
- 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 EU-RL.

All data have to be reported in the test report and sent to the EU-RL-*Salmonella* before **18 March 2011**. The EU-RL 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 XIV (2011)**

Week	Date	Topic
4	24 - 28 January	Mailing of the protocol, standard operating procedure and test report to the NRLs- <i>Salmonella</i>
7	14– 18 February	<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 EU-RL-<i>Salmonella</i> using the return envelope;</li> <li>- <b>Store the lenticules at <math>-20^{\circ}\text{C} \pm 5^{\circ}\text{C}</math></b></li> <li>- <b>Store the faeces at <math>+5^{\circ}\text{C} \pm 3^{\circ}\text{C}</math></b></li> </ul> <p><b>If you did not receive the parcel at 17 February, do contact the EU-RL 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>
9	28 February – 4 March	Performance of the study, following the instructions as given in the protocol and the SOP of study Animal faeces XIV (2011).
11	Before 18 March	Completion of the test report. Send the test report, preferably by e-mail to the EU-RL <i>Salmonella</i> ( <a href="mailto:Angelina.Kuijpers@rivm.nl">Angelina.Kuijpers@rivm.nl</a> )*
12	21 -25 March	Data input at EU-RL- <i>Salmonella</i> and sending these data to NRLs. Checking these results by the National Reference Laboratories.
	May - June 2011	Sending of the final results to the NRLs together with an interim summary. As a follow-up, actions will be undertaken in case of poor performance.

\* If the test report is e-mailed to the EU-RL, it is not necessary to send the original test report as well, unless it is not legible (to be indicated by EU-RL-*Salmonella*).