

# PROTOCOL

## INTERLABORATORY COMPARISON STUDY ON THE DETECTION OF *SALMONELLA* spp. IN ANIMAL FEED

organised by CRL-*Salmonella*

FEED STUDY I - 2008

### Introduction

This protocol describes the procedures for the first interlaboratory comparison study on the detection of *Salmonella* spp. in a feed matrix amongst the National Reference Laboratories (NRLs for *Salmonella*) in the EU. In earlier studies the detection of *Salmonella* spp. in veterinary and food samples were studied. Additional to these matrices the detection of *Salmonella* spp. in animal feed is also an important task for the CRL-*Salmonella*, as well as for the NRLs-*Salmonella*. This is described in Commission Regulations EC No 882/2004 on official controls. The organisation of this interlaboratory comparison study was discussed with the NRLs for *Salmonella* at the annual CRL-*Salmonella* workshop in May 2008.

This first study will have a comparable set-up as the earlier studies on the detection of *Salmonella* spp. in food and 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 likely to be applicable for the analyses of feed samples. Furthermore, laboratories are allowed to additionally analyse the samples with other methods, like molecular methods and/or other methods (routinely) used in their laboratories.

The samples will consist of chicken feed (*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

feed samples (**25 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 feed. 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 labcode 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 25 of September 2008** (this is 4 working days after the day of mailing).

## Objectives

The main objective of this first interlaboratory comparison study on the detection of *Salmonella* in a feed matrix is to evaluate the results of the detection of different contamination levels of *Salmonella* in the presence of competitive micro-organisms in a feed matrix, using different methods, among and within the NRLs.

## Outline of the study

Each participant will receive (in week 39 of 2008) 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:

- Approximately 700 g of chicken feed (free from *Salmonella*).

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

The performance of the study will be in week 41 (starting on 6 October 2008).

The documents necessary for performing the study are:

- Protocol Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in feed I (2008);
- SOP Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in feed I (2008);
- Test report Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in feed I (2008);
- 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 **24 October 2008**. 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 FEED I (2008)**

Week	Date	Topic
37	8 – 12 September	Mailing of the protocol, standard operating procedure and test report to the NRLs- <i>Salmonella</i>
39	22 – 26 September	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"> <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 parcel (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 <math>-20 \pm 5</math> °C</b></li> <li>- <b>Store the feed at <math>+5 \pm 3</math> °C</b></li> </ul> <b>If you did not receive the parcel at 25 September, do contact the CRL immediately.</b>
40	29 September – 3 October	Preparation of: <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>
41	6 – 10 October	Performance of the study, following the instructions as given in the protocol and the SOP of study Feed I (2008).
43	Before 24 October	Completion of the test report. Send the test report, preferably by e-mail to the CRL Salmonella ( <a href="mailto:Angelina.kuijpers@rivm.nl">Angelina.kuijpers@rivm.nl</a> )*.
44	27 – 31 October	Checking the results by the National Reference Laboratories.
	December 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*)