
PROTOCOL
INTERLABORATORY COMPARISON STUDY ON THE
DETECTION OF *SALMONELLA* spp. IN ANIMAL FEED
organised by EURL-*Salmonella*
FEED STUDY II - 2012

Introduction

This protocol describes the procedures for the second interlaboratory comparison study on the detection of *Salmonella* spp. in a feed matrix amongst the National Reference Laboratories (NRLs for *Salmonella*) in the EU.

The set-up of this study and the type of reference material (lenticule discs) are comparable to the interlaboratory comparison studies on detection of *Salmonella* in food samples and samples from primary production as organised in 2011 and 2012.

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.) Additional to 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 it is also applicable for the analyses of animal feed 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 chicken feed (*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 18 feed samples (25 g each) in combination with a *Salmonella* lenticule disc and 7 control samples.

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 will contain the chicken feed. The container with the lenticule discs 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 laboratory code. **You are urgently requested to return this complete plastic bag with recorder and lab code to the EURL-*Salmonella*, immediately after receipt of the parcel.** For this purpose a return envelope with a preprinted address label of the EURL-*Salmonella* has been included. Do not forget to note your lab code before returning it to the EURL.

Each box with samples will be sent as biological substance category B (UN3373) by door-to-door courier service. **Please contact the EURL-*Salmonella* when the parcel has not arrived at your laboratory on 20th of September 2012** (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 an animal feed matrix.

Outline of the study

In week 38 (starting on 17 Sept) of 2012 each participant will receive one box containing 2 biopacks, packed with cooling elements.

The containers contain:

Container 1:

one plastic bag with 23 numbered vials each containing one lenticule disc with or without *Salmonella*

- 18 vials numbered **B1-B18**;
- 5 vials numbered **C1-C5**;

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 EURL-*Salmonella* as soon as possible.**

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

Container 2:

one plastic bag with approximately 600 g of animal feed (free from *Salmonella*).

Store container 2, with its content, at (5 ± 3) °C immediately after receipt.

The performance of the study will be in week 40 (starting on 1 October 2012).

The documents necessary for performing the study are:

- Protocol Interlaboratory comparison study on the detection of *Salmonella* spp. in animal feed II (2012) (this document);
- SOP Interlaboratory comparison study on the detection of *Salmonella* spp. in animal feed II (2012);
- Test report Interlaboratory comparison study on the detection of *Salmonella* spp. in animal feed II (2012);
- 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 EURL.

All data have to be reported in the test report and sent to the EURL-*Salmonella* **before 19 October 2012**. The EURL will prepare a summary report soon after the study to inform all NRLs on their own results and on the overall results. **Results 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 FEED II (2012)

Week	Date	Topic
36	3 – 7 September	Mailing of the protocol, Standard Operating Procedure and test report to the NRLs- <i>Salmonella</i>
38	17 - 21 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"> - 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 EURL-<i>Salmonella</i> using the return envelope; - Store the lenticule discs at -20 ± 5 °C - Store the animal feed at $+ 5 \pm 3$ °C <p>If you did not receive the parcel at 20 September, do contact the EURL immediately.</p> 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. Selective plating agar media (see SOP 6.3) 4. Confirmation media (see SOP 6.4)
40	1 October	Start performance of the study, following the instructions as given in the protocol and the SOP of study Feed II (2012).
42	Before 19 October	Completion of the test report. Send the test report by e-mail to the EURL <i>Salmonella</i> Angelina.Kuijpers@rivm.nl *.
43	22 – 26 October	Checking the results by the National Reference Laboratories.
	December 2012	Sending of the final results to the NRLs together with an interim summary 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 EURL it is not necessary to send the original test report as well, unless it is not legible (to be indicated by EURL-*Salmonella*)