
PROTOCOL
INTERLABORATORY COMPARISON STUDY ON THE
DETECTION OF *SALMONELLA* spp. IN PIG FAECES
organised by EURL-*Salmonella*
STUDY XV - 2012

Introduction

This protocol describes the procedures for the 15th 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 number of samples to be tested and the type of reference material (lenticule discs) will be comparable to the last study on the detection of *Salmonella* spp. in veterinary samples. 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 pig faeces samples (*Salmonella*-negative) artificially contaminated with reference materials. The reference materials (RMs) consist of lenticule discs containing different *Salmonella* strains at different contamination levels. Each laboratory will examine 25 faeces samples (25 g each) in combination with a *Salmonella* lenticule disc and 7 control samples (lenticule discs 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 pig faeces. 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 lab 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* will be included. Do not forget to note your lab code before returning it to the EURL.

Each box will be sent as biological substance category B (UN3373) by door-to-door courier service. Please contact EURL-*Salmonella* when the parcel has not arrived at your laboratory at 16th of February 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 faeces.

Outline of the study

Each participant will receive (in week 7 of 2012) one box containing 2 plastic containers, packed with cooling elements. The containers contain:

Container 1:

One plastic bag with 32 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 EURL-*Salmonella* as soon as possible.**

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

Container 2:

One plastic bag with 750 g of pig faeces (free from *Salmonella*).

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

The performance of the study will be in week 8 (starting on 20 February 2012).

The documents necessary for performing the study are:

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

All data have to be reported in the test report and sent to the EURL-*Salmonella* before **9 March 2012**. The EURL 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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**Timetable of interlaboratory comparison study
ANIMAL FAECES XV (2012)**

Week	Date	Topic
5	2 – 3 February	Mailing of the protocol, standard operating procedure and test report to the NRLs- <i>Salmonella</i>
7	13 – 17 February	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 EURL-<i>Salmonella</i> using the return envelope; - Store the lenticules at - 20°C ± 5 °C - Store the faeces at + 5°C ± 3 °C If you did not receive the parcel at 16 February, do contact the EURL immediately. 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)
8	20 – 24 February	Performance of the study, following the instructions as given in the protocol and the SOP of study Animal faeces XV (2012).
10	Before 9 March	Completion of the test report. Send the test report, preferably by e-mail to the EURL- <i>Salmonella</i> (Angelina.Kuijpers@rivm.nl)*
	March	Data input at EURL- <i>Salmonella</i> and sending these data to NRLs. Checking these results by the National Reference Laboratories.
	April - May 2012	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 EURL, it is not necessary to send the original test report as well, unless it is not legible (to be indicated by EURL-*Salmonella*).