

PROTOCOL

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

organised by CRL-*Salmonella*

STUDY X - 2006

Introduction

This is the 10th 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 draft Annex D of ISO 6579. The method in this annex is especially intended for the detection of *Salmonella* spp. in animal faeces and samples of the primary production stage. A copy of the latest version of draft Annex D (12 September 2006) will be provided with this study. Furthermore laboratories who are interested can also perform PCR on the samples and/or use additional methods (routinely) used in their laboratories.

Artificially contaminated (*Salmonella* negative) pig faeces samples are tested by using 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 faeces samples (10g each and negative for *Salmonella* spp.) in combination with a capsule containing STM or SE and 10 control samples (capsules only).

Finally, to obtain more detailed information on the temperatures and times during transport of the samples we will include an electronic temperature recorder in the parcel. The amount of materials can not be packed in one parcel and will be divided over two parcels (one containing capsules and one containing *Salmonella* negative faeces). The two parcels are packed in one box with cooling elements. We will include only one temperature recorder and only in the parcel containing the capsules. 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.

Each box (containing 2 parcels) will be sent as diagnostic specimens by door-to-door courier service. Please contact CRL-*Salmonella* when the parcel has not arrived at your laboratory at 17th of November 2006 (this is after 5 working days after the day of mailing).

Objective

The main objective of the tenth interlaboratory comparison study on the detection of *Salmonella* in pig faeces is to evaluate the results of the detection of different contamination levels of *Salmonella* in the presence of competitive micro-organisms among and within the NRLs.

Outline of the study

Each participant will receive (in week 46) one box containing 2 parcels, packed with cooling elements. The parcels contain:

Parcel 1:

- 25 numbered vials; each containing one *Salmonella* Typhimurium, one *Salmonella* Enteritidis or blank capsule (numbered 1-25);
- 10 control vials; each containing one capsule with or without *Salmonella* (numbered C1-C10).

This parcel will 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 parcel 1 at $(-20 \pm 5)^{\circ}\text{C}$ immediately after receipt.

Parcel 2:

- 300 g of pig faeces (free from *Salmonella*).

Store parcel 2 at $(5 \pm 3)^{\circ}\text{C}$ immediately after receipt.

The performance of the study will be in week 48 (starting on 27 November 2006).

The documents necessary for performing the study are:

- Protocol Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in animal faeces X (2006);
- SOP Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in animal faeces X (2006);
- Test report Interlaboratory comparison study on the bacteriological detection of *Salmonella* spp. in animal faeces X (2006);
- ISO 6579 (2002). Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.;
- Draft Amendment ISO 6579:2002/amendedDAmD 1 (2006-09-12) Amendment 1 Annex D: Detection of *Salmonella* spp. in animal faeces and in samples from the primary production stage.

The media used for the collaborative study will not be supplied by the CRL.

All data will be reported in the test report and sent to the CRL-*Salmonella* and will be used for (statistical) analysis.

Please make sure to send your results to CRL--*Salmonella* before 15 December 2006. At the CRL a short report will be prepared to inform all NRLs within 1 to 2 months after the study on the overall results. We will start the first overall analyses immediately after the deadline. 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 ANIMAL FAECES X (2006)

Week	Date	Topic
44	30 October – 3 November	Mailing of the protocol, standard operating procedure, test report and draft Annex D of ISO 6579 to the NRLs-Salmonella
46	13-17 November	Mailing of the parcels to the NRLs as diagnostic specimens 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 CRL-<i>Salmonella</i> using the return envelope; - Store the capsules at -20°C ± 5°C - Store the faeces at +5°C ± 3°C If you did not receive the parcel at 17 November, do contact the CRL immediately.
47	20-24 November	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)
48	27 November – 1 December	Performance of the study, following the instructions as given in the protocol and the SOP of study Animal faeces X (2006).
50	Before 15 December	Completion of the test report and e-mailing, faxing or mailing it to the CRL. If the test report is e-mailed or faxed 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- <i>Salmonella</i>).
51	18-22 December	Data input at CRL- <i>Salmonella</i> and sending these data by CRL to NRLs for checking
2	8-12 January	Checking the results by the National Reference Laboratories.
	January- February 2007	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.