Protocol

INTERLABORATORY COMPARISON STUDY IX (2005) ON THE DETECTION OF *SALMONELLA* spp. organised by CRL-*Salmonella*

Introduction

The set-up of this 9th interlaboratory comparison study on the detection of *Salmonella* spp. is comparable to the set-up of the eighth study. Artificially contaminated (*Salmonella* negative) chicken 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 (**10 g each** and negative for *Salmonella* spp.) in combination with a capsule containing STM or SE and 10 control samples (no faeces added to the capsule). The prescribed method is the procedure as described in the 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 (like dust). As in the eighth study two incubation times of the pre-enrichment broth (Buffered peptone water (BPW)) will be used, being (4 ± ½) h and (18 ± 2) h. Further, similar to former studies, laboratories can also use their own method(s) besides the prescribed method, including PCR techniques.
Different from the eighth study is the fact that faeces will no longer be mixed with peptone/glycerol solution and will therefore be more representative to routine samples. The faeces samples should therefore be **stored at 5ºC** before use (and not at -20ºC). Also different from the former study is that the naturally polluted faces samples are replaced by naturally polluted dust samples (gathered at poultry flocks). For this, 10 dust samples of each 10 g will be examined (no addition of capsules).

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

only one recorder and only in the parcel containing the capsules. The recorder will be packed in a plastic bag, which will also contain your labcode. **You are urgently requested to return this complete plastic bag with recorder and labcode 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 3 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 within 5 working days after the day of mailing (14th of November 2004)

Objectives

The main objective of the ninth interlaboratory comparison study 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.
A derived objective of the study is to evaluate the influence of the incubation time of the pre-enrichment broth on the detection of *Salmonella* in artificially contaminated chicken faeces and in naturally contaminated dust.
Finally by comparing the results of this study with the results of the eighth study, further information may be obtained on the (negative) influence of peptone/glycerol on the detection of *Salmonella.* As the faeces of the eighth study was mixed with peptone/glycerol and the faeces of this ninth study is not mixed at all.

Outline of the study

Each participant will receive one box containing three separate parcels.

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 labcode. **This recorder (in the plastic bag) should be returned to the CRL-*Salmonella* as soon as possible**.

Parcel 2:

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

Parcel 3:

- ca 150 g of naturally contaminated (with *Salmonella*) dust.

Parcel 1 should be stored at (-20 ± 5)ºC immediate after receipt.
Parcel 2 and 3 should be stored at (5 ± 3)ºC immediate after receipt.

The performance of the study will be in week 48 (starting on 28November 2005).

The documents necessary for performing the study are:

- Protocol Interlaboratory Comparison Study IX (2005), on the detection of *Salmonella* spp.;
- SOP Interlaboratory Comparison Study IX (2005), on the detection of *Salmonella* spp.
- Test Report Interlaboratory Comparison Study IX (2005), on the detection of *Salmonella* spp.;
- ISO 6579 (2002). Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.;
- Draft Amendment ISO 6579:2002/DAmd 1 (2005) Amendment 1 Annex D: Detection of *Salmonella* spp. in animal faeces and in samples from the primary production stage.

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

All data will be reported in the test report and send to the CRL-*Salmonella* and will be used for (statistical) analysis.
In the time table of the interlaboratory comparison study IX (see next page) on the bacteriological detection of *Salmonella*, to be organised in fall 2005, a **strict deadline** for sending the results to the CRL-*Salmonella* is indicated.
The reason for setting this strict deadline (on 16 December 2005) is that we want to prepare a short report to inform all NRLs within 1 to 2 months after the study on the overall results. In earlier studies the NRLs received only their own results immediately after the study. The information on how they performed in comparison with the other NRLs was given ca half a year after the study (at the workshop). This is considered very late and with this short report we try to improve the information on the study. 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.
It may still be possible to use late results in the analyses for the final report but results received after writing the short report will not be incorporated in the final report.. However, this final (draft) report will not be available before ca half a year after the study.

If you have questions or remarks about the interlaboratory comparison study please contact:

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Time table of bacteriological interlaboratory comparison study IX (2005)

Week	Date	Topic
44	31 October – 4 November	Mailing of the protocol, standard operating procedure and test report to the NRLs
46	14 – 18 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: - 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 labcode) and return it to CRL-*Salmonella* using the return envelope; - **Store the dust and faeces samples at +5°C ± 3°C** - **Store the capsules at -20°C ± 5°C**. If you did not receive the parcel at 18 November, do contact the CRL immediately.
47	21 – 25 November	Preparation of: 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	28 November – 2 December	Performance of the study, following the instructions as given in the protocol and the SOP of study IX (2005).
50	Before 16 December	Completion of the test report and faxing or e-mailing it to the CRL. The original test report will be sent to CRL. **The deadline for sending the test report to CRL is 16 December 2005**
51	19 December – 6 January	Data input at CRL-*Salmonella* and sending these data by CRL to NRLs for checking
2	9 – 13 January	Checking the results by the National Reference Laboratories
	January/February 2006	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.