PROTOCOL

INTERLABORATORY COMPARISON STUDY VIII (2004) ON THE DETECTION OF *SALMONELLA* spp. organised by CRL-*Salmonella*

Introduction

This 8th interlaboratory comparison study on the detection of *Salmonella* spp. amongst the National Reference Laboratories (NRLs) in the EU, will be slightly different when compared to the 7th study.

At the workshop of May 2004 it was discussed to simplify the 8th study by decreasing the number of methods and it was discussed whether other samples than faecal samples, like dust, could be introduced. For the latter it was unfortunately not possible to obtain and test dust samples at the CRL-*Salmonella* before the study. Therefore dust will not be introduced in this study. Concerning the methods it was agreed to prescribe only the method which will be introduced in the new (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).

The procedure in this Annex D is based on ISO 6579, with the only difference that both selective broths of ISO 6579 are replaced by one semi-solid agar: modified semi-solid Rappaport Vassiliadis (MSRV), to be incubated for in total 48 h. By prescribing the method of the new annex the study of 2004 is therefore 'simplified' when compared to the study of 2003. However, newly introduced will be different incubation times of the pre-enrichment (Buffered Peptone Water). Recent studies of the CRL where naturally and artificially contaminated chicken faeces were incubated in BPW for 4 h and 18 h revealed (much) more positive results with the shortly incubated BPW. Both incubation times of BPW will therefore be introduced in this 2004 study [(4 ± ½) h and (18 ± 2) h].

Another difference from the study of 2003 will be that the laboratories will not be informed in advance on the number per type of capsules. This may be better for the randomisation of the study.

Finally, similar to former studies, laboratories who are interested can also perform PCR on the samples and/or use additional methods (routinely) used in their laboratories.

For the samples again reference materials (RMs) and poultry faeces will be used. The RMs consist of gelatin capsules containing sublethally injured *Salmonella* Typhimurium (STM), *Salmonella* Enteritidis (SE) or *Salmonella* Panama (SPan) at different contamination levels.
Each laboratory will again 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). Next to the capsules, again 20 faeces samples (10 g each) which are naturally contaminated with *Salmonella* 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 faeces). 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.
The boxes (each 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 (25th of October 2004)

Objectives

The main objective of the eighth 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 naturally and artificially contaminated chicken faeces.

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

Parcel 3:
- 250 g of naturally contaminated (with *Salmonella*) faeces.

The performance of the study will be in week 46 (starting on 8 November 2004).

The documents necessary for performing the study are:

- Protocol Bacteriological Interlaboratory Comparison Study VIII (2004), on the detection of *Salmonella* spp.;
- SOP Bacteriological Interlaboratory Comparison Study VIII (2004), on the detection of *Salmonella* spp.
- Test Report Bacteriological Interlaboratory Comparison Study VIII (2004), 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 Annex D of ISO 6579 (Oct. 2004)

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

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

Time table of bacteriological interlaboratory comparison study VIII (2004)

Week	Date	Topic
42	11 – 15 October	Mailing of the protocol, standard operating procedure and test report to the NRLs
44	25 – 29 October	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 all materials at -20°C ± 5°C**. If you did not receive the parcel at 29 October, do contact the CRL immediately.
45	1 – 5 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)
46	8 - 12 November	Performance of the study, following the instructions as given in the protocol and the SOP of study VIII (2004).
48	22 – 26 November	Completion of the test report and faxing or e-mailing it to the CRL. The original test report will be sent to CRL.
49	29 November – 3 December	Data input at CRL-Salmonella and sending these data by CRL to NRLs for checking
50	6 – 10 December	Checking the results by the National Reference Laboratories.

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

Kirsten Mooijman (tel. number: + 31 30 274 3537) or
Hans Korver (tel. number: + 31 30 274 4263)
Fax. number: + 31 30 274 4434
E-mail: Kirsten.Mooijman@rivm.nl and Hans.Korver@rivm.nl