PROTOCOL

BACTERIOLOGICAL COLLABORATIVE STUDY VII (2003) ON THE DETECTION OF *SALMONELLA* spp. organised by CRL-*Salmonella*

Introduction

This 7th collaborative study on the detection of *Salmonella* spp. amongst the National Reference Laboratories (NRLs) in the EU, will have a similar set-up as the 6th study.
At the workshop of May 2003 it was discussed whether the 7th study should be 'simplified' by decreasing the number of methods or whether again the (new) ISO 6579 (2002) should be tested as well. At the same workshop it was also agreed that the CRL and NRLs would like to have a standard (ISO) method for the detection of *Salmonella* spp. in poultry faeces. For this latter it would be necessary to show to the relevant ISO committee that a suggested new 'standard' method would perform better than the ISO procedure. At the workshop it was suggested that replacing a selective broth in the ISO procedure by a semi-solid medium would lead to a higher positivity rate of *Salmonella* spp. in faeces. In the collaborative studies of the CRL good experiences have been obtained with the semi-solid medium MSRV (Modified Semi Solid Rappaport Vassiliadis).
In the 6th collaborative study the performance of the new ISO 6579 (2002) was tested beside MSRV. However, in this study, not all laboratories were able to prepare the MKTTn medium (Muller Kauffmann Tetra Thionate with novobiocin) in accordance with the prescription as given in ISO. It was therefore decided to repeat the set-up of the 6th study in the 7th study. Thus testing the complete ISO 6579 (2002) beside the procedure with MSRV.
Furthermore laboratories who are interested can also perform PCR on the samples and/or use additional methods (routinely) used in their laboratories.

Still there are some (small) differences between study 6 and 7.
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).
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 which are naturally contaminated with *Salmonella* will be examined (no addition of capsules), but **in this study 10 g portions of faeces should be analysed instead of 25 g.** This was chosen to come to more uniformity in the study with the 'spiked' samples (faeces 'spiked' with capsules). Furthermore, the use of only 10 g portions of faeces might lead to less confusion in the amount of buffered peptone water (BPW) to be used for pre-enrichment. According to ISO 6579 a 1/10 dilution of the sample in BPW should be achieved. In former collaborative studies this 'rule' was not always followed (10 g of faeces was added to 225 ml BPW). As in this 7th we would like to follow the ISO as strictly as possible, we would also like to introduce this 'rule'. Meaning that **10 g of faeces should be added to 90 ml BPW**.
Furthermore, at the CRL we have performed some experiments, to try to find the most optimal procedure for dissolving the capsules in the presence of chicken faeces. Complete dissolution of the capsules is essential to bring all *Salmonella* bacteria in solution and give them a change to grow. The results of these experiments have lead to some improvements in the procedure for dissolving the capsules (see SOP Bacteriological collaborative study VII (2003)).
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 sent in one parcel and will be divided over three parcels (one containing capsules, one containing *Salmonella* negative faeces and one containing *Salmonella* positive faeces). 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.

Objectives

The main objective of the seventh bacteriological collaborative study is to evaluate the results of the detection of different contamination levels of *Salmonella* in the presence of competitive micro-organisms, using different methods, among and within the NRLs.

Outline of the study

Each participant will receive 3 parcels containing:

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 (frozen) poultry faeces (free from *Salmonella*).

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

The performance of the study will be in week 45 (starting on 3 November 2003).

The documents necessary for performing the study are:

- Protocol Bacteriological Collaborative Study VII (2003), on the detection of *Salmonella* spp.;
- SOP Bacteriological Collaborative Study VII (2003), on the detection of *Salmonella* spp.
- Test Report Bacteriological Collaborative Study VII (2003), on the detection of *Salmonella* spp.;
- ISO 6579 (2002). Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.;
- Newsletter CRL-*Salmonella*, Vol. 5, No. 2, June 1999 (for preparation of MSRV). See also on our website.

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 collaborative study will not be supplied by the CRL.

Time table of bacteriological collaborative study VII (2003)

Week	Date	Topic
41	6 – 10 October	Mailing of the protocol, standard operating procedure and test report to the NRLs
43 + 44	20 – 31 October	Mailing of the parcels to the NRLs. 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 31 October, do contact the CRL immediately. 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)
45	3 - 7 November	Performance of the study, following the instructions as given in the protocol and the SOP of study VII (2003).
47	17 - 21 November	Completion of the test report and faxing or e-mailing it to the CRL. The original test report will be sent to CRL.
48	24-28 November	Checking the results by the National Reference Laboratories.

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

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