BACTERIOLOGICAL COLLABORATIVE STUDY VI (2002) ORGANISED BY CRL *SALMONELLA*

PROTOCOL

Introduction

The Community Reference Laboratory (CRL) *Salmonella* organises the sixth bacteriological collaborative study on the methods for the detection of *Salmonella* amongst the National Reference Laboratories (NRLs). In the first and second collaborative study (October 1995 and October 1996 respectively) no significant difference was found between the routine method and the reference method for the individual laboratories. The number of positive isolations was on average significantly lower with selenite/cystine compared to Rappaport-Vassiliadis (RV) as selective enrichment medium. In the third study the ability of the laboratories to detect different contamination levels of *Salmonella* in the presence of competitive organisms was tested. Some laboratories used a semi solid medium for selective enrichment. These media seemed to be superior to RV as selective enrichment, especially for the detection of *Salmonella* Enteritidis (SE). Therefore, in the fourth collaborative study all laboratories used Modified Semi solid Rappaport Vassiliadis (MSRV) as selective enrichment next to RV(S). In this study significantly better results were revealed with MSRV compared to RV. Results obtained with RVS were not significantly different from the results obtained with MSRV. The revised ISO 6579 will prescribe Muller Kauffmann Tetra Thionate + novobiocin (MKTTn) and Rappaport-Vassiliadis Soy (RVS) instead of RV. BGA as well as XLD will be used in this study as an isolation medium (see Workshop 2000). Laboratories that are interested can perform PCR on the samples.

For the performance of this study Reference Materials (RMs) produced by the CRL and poultry faeces will be used. The RMs consist of gelatin capsules containing sublethally injured *Salmonella* Typhimurium (STM) or SE.
Each laboratory will examine 25 faeces samples (10 g each) in combination with a capsule containing STM or SE and 10 control samples (no faeces added to the capsule). Next to the capsules, 20 faeces samples (25 g each) which are naturally contaminated with *Salmonella* will be examined (no addition of capsules). The results will be evaluated by the CRL.

Objectives

The main objective of the sixth bacteriological collaborative 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.

Outline of the study

The study will be carried out according to the newly adapted ISO 6579 method and optionally the routine method of a laboratory.
Each participant will receive a parcel containing:

- 25 numbered vials; each containing one *Salmonella* Typhimurium or one *Salmonella* Enteritidis capsule;
- 10 control vials; each containing one capsule with or without *Salmonella*;
- 300 g of frozen poultry faeces (free from *Salmonella*).
- 550 g of naturally contaminated (with *Salmonella*) frozen faeces.

The performance of the study will be in week 46 (starting on 11 November 2002). 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 VI (2002)

Week	Date	Topic
40	30 September - 4 October	Mailing of the protocol, standard operation procedure and test report to the NRLs
44	28 October - 1 November	In this week the airway bill number and other important information will be (e-)mailed to your institute.
45	4 - 8 November	Mailing of the parcels to the NRLs. The participants have to collect the parcel at the airport. The parcel will be mailed with cooling devices to keep the temperature low during transport and storage at the airport. Two cold chain monitors (for 10°C and 20°C) are included in the parcel (at the backside of the lid) to check the temperature during shipment. For collecting the parcel at the airport take your own cooling box with cooling devices or ice with you. Open the parcel at the airport and check the contents for damage. Put the contents of the parcel into your own cooling box. Check the cold chain monitor and *note on test report (a copy of the concerning page is enclosed in the parcel) the date, time, the colour of the different compartments and whether the complete compartment has become blue.* Place the reference materials with the cold chain monitors in the cooling box. Immediately after arrival at the laboratory **store all materials at -20°C ± 5°C**. Before placing the materials in the freezer check the cold chain monitor again and *note in test report date, time, the colour of the different compartments and whether the complete compartment has become coloured.* If you did not receive the parcel before or at 8 November, do contact the CRL immediately. Preparation of: 1. Non selective pre-enrichment medium (see SOP 5.1) 2. Selective enrichment media (see SOP 5.2) 3. Solid selective plating media (see SOP 5.3) 4. Confirmation media (see SOP 5.4)
46	11 - 15 November	Performance of the study
48	25 - 29 November	Completion of the test report and faxing or e-mailing it to the CRL. The original test report will be sent to CRL.
50	9 - 13 December	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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