PROTOCOL OF THE COLLABORATIVE STUDY (VII, 2002) ON SERO- AND PHAGE TYPING OF SALMONELLA STRAINS ORGANISED BY CRL-SALMONELLA

Introduction

The Community Reference Laboratory (CRL)-Salmonella organises the seventh collaborative typing study of *Salmonella* strains amongst the National Reference Laboratories for Salmonella (NRLs-Salmonella) and EnterNet laboratories (ENLs).

The main objective of this typing study is to compare the test results of sero- and phage typing of the participating laboratories with the results obtained by the CRL-Salmonella.

The performance of the study will take place in week 18 (starting on 29 April 2002) or one week earlier or later.

All data will be reported in the study report, send to the CRL-Salmonella and will be used for analysis. The data on phage typing will be send to CRL-Salmonella and to Linda Ward, PHLS, London.

The CRL-Salmonella has decided not to include the antibiotic resistance pattern typing in this study. This antibiotic resistance pattern typing will be discussed with several experts into detail and the CRL will present a new plan for the Collaborative Study of next year.

Transportation of the *Salmonella* strains

CRL will mail the parcels with the strains by cargo freight from Schiphol Airport (The Netherlands) to the airport of destination. The participants have to collect the parcels at their airport. To be able to collect the parcel from the airport you need the airway bill number. This number and other important information will be mentioned in a fax which will be send to you one week (=week 15) before mailing the parcels.

The transport costs from the airport of destination to the laboratory can not be paid by the CRL, so this will be at the expense of the participant.

Serotyping

In this study a total number of 20 *Salmonella* strains, supplied by the CRL, are tested. The method routinely performed in your laboratory will be used in this study. A NRL is allowed to send strains for serotyping to another reference laboratory in their country.

Phagetyping

Optionally the laboratories will receive a parcel containing 20 *Salmonella* cultures (supplied by PHLS, London) for phage typing:

10 strains of *S*. Enteritidis numbered E1-E10

10 strains of *S*. Typhimurium numbered M1-M10

Evaluation

The results will be evaluated by the CRL. Definite conclusions can only be based on agglutination with mono-specific antisera. Otherwise it is better to identify the strains by giving the antigenic formula as far as detected. The evaluation of the serotyping results will be performed according to Table 1.

Table 1: Guidelines for evaluation

Results	Evaluation	Abbreviation
Autoagglutination or Incomplete set of antisera (outside range of antisera)	Not typable	NT
Partly typable due to incomplete set of antisera or Part of the formula (for the name of the serovar) or No name serovar	Partly correct	+/-
Wrong serovar or mixed sera formula	Incorrect	-

The evaluation of the phage typing results will be done in collaboration with Linda Ward, PHLS, London.

If you have questions or remarks on the phage typing please contact:

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

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Timetable of the collaborative study on sero- and phage typing(VII, 2002)

Week	Date	Topic
11	11-15 March	Mailing of the protocol and test report 2002
15	8-12 April	The airway bill number and other important information will be mentioned in a fax which will be send to you in this week. Checking the presence of all necessary reagents and materials for the performance of the study
16	15-19 April	Mailing the strains to the participants. After arrival at the laboratory the strains need to be subcultured and stored until the performance of the typing. If the parcel has not arrived at the airport on 19 April, please do contact the CRL immediately.
18	29 April-3 May	Starting with the identification of the strains.
20	13-17 May	Completion of the test report. Sending of the complete report to the CRL by fax or e-mail. The original test report will be send to CRL by mail. Send the results of the phage typing also to PHLS, London. Keep a copy for your own information
21	21-25 May	A printed version of the individual results will be send to all NRLs. Checking of the results on this printed version will be done by the NRLs-Salmonella.
	June-September	Analysis and reporting of the results by CRL.

As an example the phage typing protocol from PHLS is included

Salmonella phage typing protocol from PHLS (London).

1. Media

1.1 Double strength nutrient broth

Bacto dehydrated nutrient broth (Difco)	20 grams
NaCl	8.5 grams
Distilled water	to 1000 ml

Autoclave for 10 minutes at 115°C and 15 lbs pressure

1.2 Nutrient agar

Bacto dehydrated nutrient broth (Difco)	20 grams
NaCl	8.5 grams
Bacto agar dyhydrated (Difco)	13 grams
Distilled water	to 1000 ml

Autoclave for 10 minutes at 115°C and 15 lbs pressure

The prepared agar is distributed in 30 ml volumes into 9 cm single vent petri dishes. The nutrient agar plates are incubated overnight at 37°C and then examined for contamination. Contaminated plates are discarded. The plates are further dried open at 37°C for 1.5 hours.

2. Procedure

2.1 By means of a sterile inoculating loop or plastic pastette, inoculate the test strain from the culture slope a-sceptically into a test tube containing 4 mls of double strength Difco nutrient broth. Heavy inoculum to give visible turbidity for *S.* Enteritidis and a very light inoculum for *S.* Typhimurium to give a barely visible turbidity.

2.2 Incubate the inoculated broth tubes on a horizontal shaker at 37°C for 1-1.5 hours for *S.* Enteritidis. For *S.* Typhimurium incubate at 37°C without agitation for 1.25 hours to obtain a very light growth in early log phase.

2.3 Flood the broth culture over the surface of a dried Difco nutrient agar plate using a flooding pipette or a plastic pastette. Remove the excess culture from the surface.

2.4 When the surface of the nutrient agar plate is dry, apply the appropriate typing phages at routine test dilution (RTD) to the dried surface. Suggested methods:

a) Multipoint inoculator
b) Sterile loops delivering approximately 0.01 ml phage lysate
c) Dropping pipettes delivering approximately 0.01 ml phage lysate

2.5 When the phage spots are dry, the Difco nutrient agar plates are incubated inverted at 37°C for 5-18 hours.

2.6 The phage typing plates are removed from the incubator and the phage reactions are read using a x10 aplanat hand lens (or alternative methods of magnification) through the bottom of the plates using both direct and oblique illumination.