PROTOCOL OF THE COLLABORATIVE STUDY ON TYPING OF *SALMONELLA* STRAINS (6) ORGANISED BY CRL *SALMONELLA*

Introduction:

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

In this study again a total number of 20 *Salmonella* strains, supplied by the CRL, have to be identified. The results will be evaluated by the CRL. Laboratories can also perform resistance pattern typing with the method routinely used by the laboratory.

For serotyping, the typing method routinely performed in the laboratory will be used in the study. 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 results by the CRL will be performed according to Table 1.

A NRL is allowed to send strains for serotyping to another reference laboratory in their country.

Also 20 *Salmonella* strains (10x *S.* Enteritidis and 10x *S.* Typhimurium), supplied by PHLS, London, can be send to the laboratories to perform phage typing. **As an example** the *Salmonella* phage typing protocol from PHLS (London) is included (page 4 and 5).

Table 1: Guidelines for evaluation

Result of laboratory	Evaluation
Autoagglutination Incomplete set of antisera (outside range of antisera)	Not typable (nt)
Partly typable due to incomplete set of antisera No name serovar Part of the formula (for the name of the serovar)	Partly correct (+/-)
Wrong serovar Mixed sera formula	Incorrect (-)

Objective:

The main objective of the fifth typing study is to compare the test results of sero- and resistance pattern typing of the participants with the results obtained at the CRL-*Salmonella*. Evaluation of the phage typing will be done by Linda Ward, PHLS, London.

Outline of the study:

Each laboratory will receive a parcel containing 20 *Salmonella* cultures (numbered 1 to 20) for sero- and optionally resistance pattern typing. On arrival the cultures must be subcultured on agar plates. Optionally the laboratories will receive a parcel containing 20 *Salmonella* cultures (numbered M1 to M10 and E1 to E10) for phage typing.

The performance of the study will be in week 10 (starting on 5 March 2001) or one week earlier or later. All data will be reported in the test report to the CRL-*Salmonella* and will be used for analysis. The data on phage typing will also be sent to PHLS.

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

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Time table of the collaborative typing study on of *Salmonella* strains (6)

The identification of the *Salmonella* cultures must take place in week 10 (starting on March 5th) or one week earlier or later.

29 Jan - 2 Feb	Mailing the protocol and test report to the participating laboratories.
19-23 February	Mailing the strains to the participants. CRL will mail the parcel by cargo freight from the Dutch airport (Schiphol) to the airport of destination. The participants have to collect the parcel at the airport. For this you need the airway bill number. This number and other necessary information will be indicated in an e-mail in the week before mailing.

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

After arrival at the laboratory the strains need to be subcultured and stored until the performance of the typing.

If the parcel did not arrive at the airport at the time mentioned in your flight details, please contact the CRL.

26 Feb - 2 March	Checking the presence of all necessary reagents and materials for the performance of the study.
5 - 9 March	Starting with the identification of the strains. **Note**: Each laboratory is free to identify the strains when they want as long as it will be done in the scheduled weeks.
19-23 March	Completion of the test report and faxing it to the CRL. The original test report will be send to the CRL. Results of phage typing will also be send to PHLS.
26 - 30 March	Checking the results by the NRLs.

Salmonella Phage typing protocol from PHLS (London).

1. Media

1.1 Double strength nutrient broth

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

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

1.2 Nutrient agar

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

to sterilise: 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 asceptically 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.