# PROTOCOL OF THE NINTH COLLABORATIVE STUDY (IX, 2004) ON SERO- AND PHAGE TYPING OF SALMONELLA STRAINS AND TESTING OF ANTIMICROBIAL SUSCEPTIBILITY ORGANISED BY CRL-SALMONELLA

## Introduction

The Community Reference Laboratory (CRL) - *Salmonella* organises the ninth 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 and antimicrobial susceptibility testing of the participating laboratories with the results obtained by the CRL-*Salmonella*.

For the NRLs-*Salmonella* the performance of the study will take place in week 10 (starting on 1 March 2003) or one week earlier or later. For the ENLs the study will be performed a few weeks 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, Health Protection Agency (HPA), London, UK.**

## Transportation of the *Salmonella* strains to the NRLs, - and ENLs-*Salmonella*.

CRL-*Salmonella* will mail the parcels with the strains for serotyping and antimicrobial susceptibility testing 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 an e-mail which will be send to you one week (= week 8) before mailing the parcels. The transport costs from the airport of destination to the laboratory can not be paid by the CRL-*Salmonella*, so this will be at the expense of the participant. The shipment of the strains for phage typing to the NRLs and the shipment of all strains to the ENLs will be arranged by Linda Ward, HPA, London, UK.

**Serotyping**

In this study a total number of 20 *Salmonella* strains (numbered S-1 till S-20), supplied by the CRL-*Salmonella*, are serotyped. The method routinely performed in your laboratory will be used in this study. Each laboratory is allowed to send strains for serotyping to another reference laboratory in their country.

The results will be evaluated by the CRL-*Salmonella*. 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<br>Incomplete set of antisera<br>(outside range of antisera) | Not typable | NT |
| Partly typable due to incomplete set of antisera or<br>Part of the formula (for the name of the serovar) or<br>No name serovar | Partly correct | +/- |
| Wrong serovar<br>or<br>mixed sera formula | Incorrect | - |

**Phagetyping**

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

- 10 strains of *S.* Enteritidis numbered E1-E10
- 10 strains of *S.* Typhimurium numbered M11-M20

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

**Antimicrobial susceptibility testing**

The laboratories will receive 10 strains (different from the ones used for sero- and phage typing) for antimicrobial susceptibility testing. These strains are numbered from AST-1 till AST-10 and are to be tested according to **NCCLS guidelines** with one of the following methods: Minimal Inhibition Concentration (MIC) or disc diffusion method.

The strains should be tested against the following antibiotics:

1. Amoxicillin + clavulanate (30 µg)
2. Ampicillin (10 µg),
3. Cefotaxime (30 µg),
4. Chloramphenicol (30 µg),
5. Ciprofloxacin (5 µg) or Enrofloxacin (5 µg)
6. Florfenicol (30 µg),
7. Gentamicin (10 µg),
8. Kanamycin (30 µg) or neomycin (30 µg),
9. Nalidixic Acid (30 µg),
10. Streptomycin (10 µg),
11. Sulfamethoxazole + Trimethoprim (23,75 + 1,25 µg),
12. Trimethoprim (5 µg).

The numbers between brackets are the concentratiopns of antibiotics in the discs. The same antibiotics may be tested with the MIC if this method is your method of choice. If you do not have discs with the specified amount please omit this antibiotic from your list.

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

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

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# Timetable of the ninth collaborative study (2004) on sero- and phage typing and antimicrobial susceptibility testing.

| Week | Date | Topic |
|---|---|---|
| 5 | 26-30 January | Mailing of the protocol and test report 2004 (to NRLs and ENLs) |
| 8 | 16-20 February | The airway bill number and other important information will be mentioned in an e-mail which will be send to you in this week (only NRLs) |
| 9 | 23-27 February | Mailing the strains to the participants (NRLs)<br>After arrival at the laboratory the strains need to be subcultured and stored until the performance of the typing.<br>If the parcel has not arrived at the airport on 28 February, please do contact the CRL immediately. |
| 10 | 1-5 March | Starting with the identification of the strains. |
| 12 | 15-19 March | Completion of the test report. Sending of the complete report to the CRL by e-mail. The original test report will be send to the CRL by mail. Send the results of the phage typing also to HPA, London (o*nly printed versions of the test report will be accepted).*<br>**Deadline for NRLs: End of March 2004**<br>**Deadline for ENLs: End of April 2004** |
| 13 | 22-26 March and onwards | A printed version of the individual results will be send to all NRLs and ENLs by CRL. Checking of the results on this printed version will be done by the NRLs and ENLs. NRLs and ENLs will inform CRL whether their results are correct. If CRL does not receive a reaction within one week after receipt of the printed version the CRL will consider the results as correct. |

N.B. For the ENLs the data in the time table may be one or two weeks later.