# EURL-*Salmonella* work-programme 2014

## Introduction

The working programme of EURL-*Salmonella* consists of the following activities (the duration of the activities are indicated between brackets):

1. Organisation of interlaboratory comparison studies (yearly);
2. Organisation of a workshop with the NRLs-*Salmonella* (yearly);
3. Performance of supporting activities (depending on the subject: yearly or for a limited period);
4. Giving assistance to the Commission and ad hoc activities (yearly);
5. Communication (every 3 months and yearly);
6. Training (duration dependent on the subject);
7. Molecular typing of *Salmonella* spp. (depending on the subject: yearly or for a limited period).

## 1. Interlaboratory comparison studies

For 2014 it is planned to organise 3 interlaboratory comparison studies;

- One study on bacteriological detection of *Salmonella* in a primary production matrix;
- One study on bacteriological detection of *Salmonella* in a food or feed matrix;
- One study on typing of *Salmonella*.

In 2013, for the first time, the samples of the interlaboratory comparison studies for detection of *Salmonella* in a matrix were artificially contaminated individually at the laboratory of the EURL-*Salmonella* before sending them to the NRLs. This type of samples mimic better the routine samples of the NRLs than samples which have to be artificially contaminated with reference materials by the NRLs itself. However, artificial contamination of the samples in the laboratory of the EURL may not always result in sufficient homogeneous or stable samples. In that case it may be necessary to use again reference materials to artificially contaminate the samples. In case reference materials need to be used again, most likely lenticule discs as produced by Public Health England (PHE), United Kingdom, will be used. The way of artificially contaminating the samples, the choice of the serovars as well as the contamination levels of the samples will be decided per study.

For the set-up of the studies on detection of *Salmonella* in a matrix, EN ISO/TS 22117:2010 ('Microbiology of food and animal feeding stuffs – Specific requirements and guidance for proficiency testing by interlaboratory comparison') will be followed. In this EN ISO document the following numbers of samples are described:

- 6 negative samples, to check for the occurrence of false positive results;
- 6 low level samples, with a contamination level close to the detection limit of the method, so that ideally 50% of the samples are found positive and 50% negative;
- 6 high level samples, with a contamination level 10 times higher than the low level materials, representing the level at which all samples should be found positive.

For this set-up, only one *Salmonella* serovar will be used to artificially contaminate a matrix at the levels as indicated above. Additional to this set-up of EN ISO/TS 22117, it was agreed at the 2012 workshop to (still) include some control samples (pure cultures, or reference materials without matrix) as well.

Since fall 2012 more shortened test reports were introduced for the reporting of the results of the interlaboratory comparison studies. Furthermore, by the end of 2012 the reporting of the results of each interlaboratory comparison study became web-based. In a questionnaire sent to the NRLs in spring 2013, the majority of the NRLs indicated to be satisfied with the reporting of the results by web-based forms. Additionally, the use of web-based forms facilitates the analyses of the results by the EURL. It is therefore decided to continue the use of web-based forms for the reporting of the results of the interlaboratory comparison studies.

Several years ago the EURL-*Salmonella* and the NRLs-*Salmonella* agreed on a system for the evaluation of the performance of the laboratories in the interlaboratory comparison studies. This system is different for the studies on detection of *Salmonella* and for the studies on typing of *Salmonella*.
In studies for the detection of *Salmonella* in a matrix, the system is based on the contamination level of *Salmonella* in the samples and the expected number of samples to be found positive.
In studies for typing of *Salmonella*, especially the capacity of serotyping the different *Salmonella* serovars by the NRLs-*Salmonella* is evaluated. More stringent criteria are given to the serotyping capacities of the NRLs for the five most important health-related *Salmonella* serovars (as indicated in EU legislation).
The detailed criteria for the evaluation of the performance of the NRLs may vary slightly per study (depending on contamination level, type of matrix, level of background flora, choice of serovars, etc.) and is described per study.
The results of each NRL will be evaluated and compared with the pre-set definition of 'good performance' per study. In case of unexplainable 'poor performance', the follow-up will be discussed with the relevant NRL (e.g. sending extra samples which need to be tested according to a prescribed protocol, training at the EURL or visiting an NRL by members of the EURL-*Salmonella* staff). Additional to the judgement 'good performance', or 'poor performance', the results of an NRL can also be judged as 'moderate performance'. The criteria to define a performance 'moderate' are described per study. The actions after moderate performance are less stringent than after poor performance. In case of moderate performance, the performance of the NRL over several consecutive studies is judged. If moderate performance is seen in three consecutive studies, the NRL will be contacted by the EURL to discuss a proper follow-up. The type of follow-up will be considered on a case by case basis depending on the nature of the moderate performance. A visit of a staff member(s) of the EURL-*Salmonella* to the NRL can be considered as a possible follow-up. Also in the case of repeated moderate performance (like for poor performance), DG-SANCO will be informed.

Additional to the NRLs-*Salmonella* of the EU Member States, the EURL-*Salmonella* also offers a limited number of laboratories of EU candidate countries and of EFTA countries to participate in the interlaboratory comparison studies for their own costs. The results of all third countries will be analysed separately from the results of the NRLs of the EU Member States.

Some details of the aforementioned three studies are given below. Final details per study will be made, as much as possible, in agreement with the NRLs for *Salmonella* and may be discussed at the annual workshop.

***Interlaboratory comparison study on bacteriological detection of Salmonella in samples from primary production***

- Probable time period: February/March 2014.
- Matrix: samples from primary production (e.g. animal faeces).

- *Salmonella* serovar: one serovar will be used. Which serovar will be decided later.
- Method: Annex D of EN ISO 6579:2007 ('Detection of *Salmonella* in animal faeces and in environmental samples from the primary production stage'), implying modified semi-solid Rappaport Vassiliadis (MSRV) agar as selective enrichment medium, and own method(s).

Since 2008, also reference laboratories of two third countries (from outside Europe) participated in the studies for detection of *Salmonella* in samples from primary production, being: Tunisia and Israel. These countries participated on request of DG-SANCO. However, since 2011, Tunesia does not longer participate, as the EC did not agree on their monitoring plan. Therefore it is foreseen that the only third, non-European, country in this study of 2014 will be Israel.

The justification for participation of the third countries (from outside Europe) was given in the work-programme of 2008 and is repeated below:
*Salmonella control programmes in live poultry are introduced in the European Member States by Regulation (EC) No 2160/2003. The control programmes in breeding hens include the monitoring of Salmonella by the testing of faecal materials in accordance with the provisions in Regulation (EC) No 1003/2005. Third countries, who want to remain or be added to the list of third countries from which Member States may import breeding hens or hatching eggs, should submit a control programme equivalent to the control programmes of the Member States. In order to evaluate the equivalence of testing in these third countries, they should participate in the ring trials organised by the CRL. Tunesia, Canada, Israel and the United States forwarded their control programme for breeding hens and should therefore be included in the ring trial.*

***Interlaboratory comparison study on bacteriological detection of Salmonella in food or feed samples***

- Probable time period: September/October 2014.
- Matrix: food or animal feed samples (to be decided at the annual workshop in 2014).
- *Salmonella* serovar: one serovar will be used. Which serovar will be decided later.
- Method: The prescribed method will be EN ISO 6579: 2002 (Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.), with selective enrichment in Rappaport Vassiliadis Soya (RVS) broth and Muller Kauffmann Tetrathionate novobiocin (MKTTn) broth. The additionally requested method will be Annex D of EN ISO 6579 (2007), with selective enrichment on MSRV.

***Interlaboratory comparison study on typing of Salmonella***

- Probable time period: November/December 2014.
- Samples: pure cultures of different *Salmonella* serovars.
- Methods:
  - Serotyping (obligatory), following the White-Kauffmann-Le Minor scheme as described in draft EN ISO 6579-3;
  - Phage typing (optional), following the procedure of Public Health England (PHE);
  - PFGE (optional or obligatory, to be discussed), following the PulseNet protocol and/or the EFSA protocol, which may be published in 2014.

For the organisation of the interlaboratory comparison study on typing of *Salmonella* a subcontract with the Public Health England (PHE), London (Colindale), UK is foreseen. This subcontract will formalise the input of PHE on the organisation of the study for the part on phage typing. Thanks to this cooperation between the EURL-*Salmonella* and the PHE, the NRLs for *Salmonella* will have the opportunity to test their phage typing capacities again in the interlaboratory comparison studies on typing of *Salmonella* in 2014.

In 2014 it will be discussed with PHE whether it is worthwhile to continue with phage typing in the typing studies of the EURL-*Salmonella*, as the number of participants in this part of the study has decreased over the years. A decision will need to be made about continuation of offering the testing of phage typing capacities of NRLs in future typing studies.

Like in former studies the EURL-*Salmonella* will select twenty *Salmonella* strains for serotyping, including serovars with public health significance, serovars with antigens similar to those of public health significant strains and serovars that had caused typing problems in previous studies. In the studies of 2011 and in 2012, on request of the NRLs, for the first time a 'twenty-first' strain was added. This concerned a serovar from another subspecies than *Salmonella enterica* subsp. *enterica*. The results found with this 21st serovar were not taken into account for the evaluation of the performance of the laboratory. Most NRLs performed the typing of this 21st strain and its inclusion in the study was highly appreciated. It was therefore decided to continue with the inclusion of such a rare serovar in the future typing studies.
The strains will be blindly coded and send to the NRLs for serotyping, one week before the performance of the study.

The PHE will select twenty *Salmonella* strains for phage typing (10 *Salmonella* Enteritidis strains and 10 *Salmonella* Typhimurium strains). These latter strains will only be sent to the NRLs who have indicated to perform phage typing as well.

It is foreseen to organise a pilot interlaboratory comparison study for PFGE analysis of different *Salmonella* serovars by the end of 2013. The experiences with this pilot study will be used for the organisation of the study on PFGE analysis in 2014.
In 2013, contact has been made with staff members of the Statens Serum Institute (SSI) in Denmark, who organise the interlaboratory comparison studies on PFGE and MLVA typing for the ECDC programme on European Food,- and Waterborne diseases and Zoonoses. In these studies the European Public Health Laboratories participate, analysing samples from human origin. This contact may help in harmonisation of the organisation of comparative tests and interpretation of results of these studies for PFGE (and MLVA) analysis of *Salmonella* between the 'human sector' and the 'food and animal sector'. It is foreseen to continue this contact in 2014 and to participate in each other comparative tests.

Subcontract in relation to activity 1
A subcontract with Public Health England (PHE), London, United Kingdom is foreseen to hire the expertise of the PHE for phage typing of *Salmonella* in the interlaboratory comparison study on typing. For this, PHE will select and test strains to be used in the typing study and will send the strains to the EURL-*Salmonella*. Furthermore, PHE will contribute to the analyses and reporting of the phage typing results as found by the NRLs.

Missions in relation to activity 1
If necessary a visit to a poor performing NRL by two staff members of the EURL-*Salmonella* will be made. Time needed: approximately 2 days, country unknown.

Output in relation to activity 1

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|---|---|---|---|
| Type interlaboratory comparison study | Planning study | Planning interim summary report | Planning final draft full report (including the possible follow-up study)[1] |

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|---|---|---|---|
| Detection of *Salmonella* in samples from primary production | Feb./March 2014 | May 2014 | November 2014 |
| Detection of *Salmonella* in food/feed samples | Sept./Oct. 2014 | December 2014 | June 2015 |
| Typing of *salmonella* | Nov./Dec. 2014 | February 2015 | September 2015 |

[1]: The full reports will be published as 'RIVM-reports'. The publication of these reports takes some time-consuming administrative steps which can not be fully controlled by the author(s). Therefore, the planning of the (final) draft report is indicated in stead of the planning of the publication of the final report.

## 2. Workshop

The annual workshop of the EURL-*Salmonella* in 2014 is foreseen to be organised in May and will last 1,5-2 days. The location of the workshop is not yet decided. Several NRLs for *Salmonella* have offered to help with organising the workshop in their country. These different offers still need to be explored for the affordability. Furthermore, also the possibility of organising the workshop again in the Netherlands (after two years of organising it in another country) will be explored.

The programme of the workshop may contain the following items:
- Introductory presentations (e.g., by EU representative and EURL-*Salmonella*);
- Zoonoses in Europe (EFSA, DG-SANCO);
- Results of (research) activities of EURL-*Salmonella*;
- Results of interlaboratory comparison studies of 2013 and 2014;
- Experiences, problems, results in relation to monitoring surveys for *Salmonella*;
- Plans and results of (research) activities of the NRLs-*Salmonella*;
- Discussion on methods (e.g. typing, molecular, serological);
- Activities in ISO and CEN;
- Future working plan of EURL-*Salmonella*;
- Information on research in relation to *Salmonella* by one or more guest speakers.

According to Regulation (EU) 135/2013 concerning the financial aid to the EU reference laboratories for feed and food and the animal health sector, it will be possible to invite up to 3 invited speakers and up to 10 representatives of third countries additional to up to 32 representatives of NRLs of EU Member States (including the new Member State Croatia). Concerning the third countries, the EURL-*Salmonella* will (most likely) at least invite representatives of the following countries: Bosnia and Herzegovina, Iceland, Former Yugoslav Republic of Macedonia (FYROM), Norway, Serbia, Switzerland and Turkey.

Output in relation to activity 2

- Publication of the presentations of the workshop at the EURL-*Salmonella* website (www.eurlsalmonella.eu): within a few weeks after the workshop.
- Draft report of the workshop, including a summary of the discussion performed per item at the workshop and the evaluation of the workshop: within 2 months after the workshop.

## 3. Supporting activities

***Activities concerning ISO and CEN***

The EURL-*Salmonella* (Kirsten Mooijman) is involved (as project leader or as member of working groups) in several activities of ISO and CEN. More specific in:

- ISO/TC34/SC9: International Standardisation Organisation, Technical Committee 34 on Food products, Subcommittee 9 – Microbiology.
- CEN/TC275/WG6: European Committee for Standardisation, Technical Committee 275 for Food analysis – Horizontal methods, Working Group 6 for Microbial contaminants.

Kirsten Mooijman of the EURL-*Salmonella* is convenor of two groups in CEN/ISO dealing with methods for *Salmonella*. The activities for these groups will be continued in 2014:

- *Detection of Salmonella (EN ISO 6579-1)*: The launching of the CEN enquiry/ ISO DIS (Draft International Standard) vote of the amended draft document has been delayed due to the fact that the precision characteristics of the part on detection of *Salmonella* in samples from primary production still needed to be added. The validation study on this subject (under the CEN mandate project) was performed in March 2013 and the analysis of the data was performed in May 2013. It is foreseen to send the amended document of draft EN ISO 6579-1 to the secretariat of CEN/TC275/WG6 in fall 2013, so that the voting may be launched before the end of 2013. It may be necessary to discuss the outcome of this voting in the CEN Task Group (TAG) on *Salmonella* detection (TAG8), in 2014. As a follow up, the document may need to be amended again (action by the convenor of the group – Kirsten Mooijman), before it can be sent around for a new voting round. The progress of the group with the document needs to be presented at the plenary meeting of CEN and ISO (June 2014) by the convenor (Kirsten) of the TAG group.
- *Guide for serotyping Salmonella spp. (CEN ISO/TR 6579-3)*: The final voting for this document was launched in June 2013 and will last until the end of September 2013. It may be necessary to discuss the outcome of this voting in the ISO working group (WG10) in fall 2013 or early 2014 to finalise the document. The progress of ISO/TC34/SC9 WG10 with the document needs to be presented at the plenary meeting of CEN and ISO (June 2014) by the convenor (Kirsten) of the working group.

If necessary, literature search and experiments may be done in relation to the ISO/CEN activities in the field of *Salmonella*. The need and the nature for this will depend on the comments on the CEN/ISO documents which may be given during the voting rounds.

The plenary meetings of both ISO/TC34/SC9 and CEN/TC275/WG6 will be organised in Washington DC, USA in June 2014.

*Other activities related to ISO and CEN*

In 2012 the EURL-*Salmonella* was approached to give their opinion on a possible procedure for validation of alternative confirmation/typing methods. Such a procedure is not yet available but is highly needed. It has been agreed that the working group (WG3) in ISO/TC34/SC9 dealing with the revision of EN ISO 16140 will also draft a procedure for this type of validation studies. The EURL-*Salmonella* evaluated available (limited) information on (draft) procedures for validation of confirmation/typing methods and added its own opinion. This information was discussed with the convenor of WG3 and draft proposals for the procedure for validation of confirmation/typing methods were discussed at meetings of SC9 and WG3 in 2012 and in 2013. Members of SC9 as well as of WG3 agreed with the general set-up of the proposed procedure, but also indicated several comments. One of the comments was that it would be helpful to include examples for validation of confirmation/typing methods, especially for *Salmonella*. It was therefore agreed that

for the drafting of the document the input of (one or two) staff members of the EURL-*Salmonella* will be needed. For this reason, Kirsten Mooijman of the EURL-*Salmonella* was added to the list of members of WG3 to help with the drafting of the document. It may be the case that, for this purpose, participation in a meeting of WG3 may be needed in 2014.

In 2012 a new Technical Advisory Group (TAG 9) was raised in CEN/TC275/WG6 on the improvement of the pre-enrichment step to enhance the recovery of Gram negative bacteria. For the detection of several Gram negative bacteria like *Salmonella*, *Cronobacter*, STEC and *Enterobacteriaceae* a pre-enrichment step is included in the procedure. Unfortunately, so far little harmonisation exists between the different pre-enrichment broths of the different EN ISO methods, especially on the type of peptone to be used in the broths. The aim of TAG 9 is to harmonise the composition of the pre-enrichment broths to obtain the most optimal recovery of different Gram negative target organisms. As convenor of CEN TAG 8 on the revision of EN ISO 6579-1 on detection of *Salmonella*, Kirsten Mooijman has become member of this new TAG 9. By the end of 2012, the convenor of TAG 9 had drafted a protocol to test several compositions of pre-enrichment broths, based on the use of chemically defined products replacing peptone. However, growth in these chemically defined pre-enrichment broths was poor for most of the tested micro-organisms and a second protocol was prepared in which different peptones will be tested. If possible, the EURL-*Salmonella* will perform some experiments following this latter protocol to test the influence of different peptones on the growth of different *Salmonella* serovars.

***Samples for interlaboratory comparison studies***
Per interlaboratory comparison study on detection of *Salmonella* in a matrix, the serovar(s) and contamination levels in the samples will be chosen. For each study it will be evaluated whether the samples will be artificially contaminated with a diluted culture at the laboratory of the EURL or with reference materials at the NRLs. For each study it is necessary to test the homogeneity and stability of several samples after artificially contaminating them with different *Salmonella* serovars, either by using a diluted (pure) culture or by using reference materials. The homogeneity and stability of the samples may also be influenced by the matrix chosen and the amount of (natural) background flora in the matrix. Hence, these factors will also play a role in the preparation and control of the samples for the interlaboratory comparison studies.

Missions in relation to activity 3

- Participation of one staff member of the EURL-*Salmonella* in the annual meetings of ISO/TC34/SC9 and CEN/TC275/WG6. Duration of the meetings: approximately 5 days. Period of the year: June 2014. Location: Washington DC, United States of America.
- Meetings of several working groups (ISO) or TAG groups (CEN) of ISO/TC34/SC9 and CEN/TC275/WG6. Approximately 1 meeting per WG or TAG is foreseen in 2014. The meetings are not yet planned, but will be scheduled as soon as considered necessary. If possible, meetings of the different groups will be combined with each other or with the plenary meetings of SC9 and WG6.

Output in relation to activity 3

*ISO and CEN*

- New draft version of EN ISO 6579-1: fall 2014
- New draft/final version of EN ISO 6579-3: fall 2014
- Draft procedure on validation of confirmation/typing Methods as part of the work of ISO/TC34/SC9 WG3 fall 2014
- Report of relevant items in relation to standardisation

as discussed at the plenary meetings of ISO/TC34/SC9 and CEN/TC275/WG6: summer 2014

Note: For the progress of the work with the EN ISO documents, the EURL-*Salmonella* is very much dependent on the cooperation and on the speed of the administrative processes in CEN and ISO.

*Samples for interlaboratory comparison studies*

- Results of activities performed to test optimal matrix, inoculation and/or reference material combinations, will be published in the reports related to the interlaboratory comparison studies (see Activity 1. 'Interlaboratory comparison studies').

## 4. Giving assistance to the Commission and ad hoc activities

The EURL-*Salmonella* is regularly contacted by various parties, i.e. institutes in Member States, Candidate Member States or third countries, with requests for information or for participation in activities being organised. Also, requests for support from the European Commission (DG-SANCO), European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) with respect to certain issues (e.g., methods, participation in working groups, advices, help in international outbreaks) are raised. In all cases the EURL-*Salmonella* will in principle always react positively and will try to include the ad hoc work required in the working plan although it is difficult to plan the time needed to answer the different questions.

*Participation in Working Groups of DG-SANCO and EFSA*

When requested and when possible, one or two staff members of the EURL-*Salmonella* participate in the working groups of DG-SANCO and of EFSA for, among others, to give technical support in drafting EU legislation, for preparation of technical specifications of monitoring and control programmes, for drafting (EFSA) opinions for certain items. For 2014 participation in an EFSA working group on molecular typing is foreseen by one or two staff members of the EURL-*Salmonella*

Missions in relation to activity 4

Participation in working groups of DG-SANCO and EFSA will be funded by DG-SANCO and EFSA and will not be charged on the EURL-*Salmonella* budget.
For 'ad hoc' activities, no missions are foreseen.

Output in relation to activity 4

- Input in EFSA working groups are published by EFSA in for example EFSA opinions.
- Input in working groups of DG-SANCO will be used by DG-SANCO to prepare/amend specific documents (e.g. EU legislation).
- In case a question needs substantial input of the EURL-*Salmonella*, it will be summarised in more detail in the annual technical report of the EURL-*Salmonella* over the year under review (2014). March 2015

## 5. Communication

The new website of the EURL-*Salmonella*, www.eurlsalmonella.eu, was launched in 2012 and is kept up to date by one staff member of the EURL.

In fall 2012 the department on Communication/IT of the RIVM built, in close cooperation with staff members of the EURL-*Salmonella*, the first web based form for reporting of the results by the NRLs, of the interlaboratory comparison study on typing. In 2013 additional web based forms were prepared for the reporting of the results of the interlaboratory comparison studies on detection of *Salmonella* in samples from primary production and in food samples. These web based forms will be used for future interlaboratory comparison studies as well, but may need some amendments per study for which the help of the IT department of the RIVM is still needed.

The newsletter of EURL-*Salmonella* is published every quarter with information from the EURL-*Salmonella* relevant for the NRLs-*Salmonella* and/or from NRLs-*Salmonella* relevant for the EURL and for the other NRLs. Also, a literature search is included in each newsletter covering the previous 3-months period.

Results of the interlaboratory comparison studies, the workshop and relevant supporting activities will be published in RIVM reports. The reports will be distributed to the EC and to the NRLs and other interested bodies. Furthermore they will also become available at the EURL-*Salmonella* website. Summaries of several interlaboratory comparison studies and related supporting activities will be published (if possible) in the scientific literature. By comparing several studies over the years it is possible to determine the existence of trend analyses in the studies. It is planned to prepare a (draft) manuscript summarising the results of several interlaboratory comparison studies on typing of *Salmonella* in 2013. Finalisation of this manuscript may be done in 2014.

Output in relation to activity 5

*Website*

- Keeping the EURL-*Salmonella* website up to date: continuously
- Web based forms for reporting of results of interlaboratory comparison studies on typing and detection of *Salmonella* continuously

*Newsletter*

Publication of 4 newsletters through the website: 04-2014; 07-2014; 10-2014; 01-2015

*Trend analyses interlaboratory comparison studies*

- Preparing review of several interlaboratory comparison studies on (sero)typing of *Salmonella*: end 2013/early 2014

## 6. Training

On request of an NRL, the EURL can give a training for a specific need of an NRL, which can be on detection and typing of *Salmonella* (including serotyping and molecular typing). It is also possible that the EURL will advice an NRL to follow a training at the EURL or that staff members of the EURL give a training at the laboratory of the NRL, especially in case of (repeated) poor performance of the NRL in interlaboratory comparison studies.
As PFGE will become part of the interlaboratory comparison study on typing (see 1. 'Interlaboratory comparison studies'), it is quite likely that more trainings on this subject need to be organised than in former years (also see 7. 'Molecular typing of *Salmonella* spp.).

Output in relation to activity 6

- Short summary on the number and type of trainings performed

in 2014 and their evaluation, in the annual technical report of the EURL-*Salmonella.* March 2015

## 7. Molecular typing of *Salmonella* spp.

In fall 2012, DG-SANCO has published a vision paper 'on the development of data bases for molecular testing of food-borne pathogens in view of outbreak preparedness'. The purpose of this initiative is to encourage the collation of data on molecular testing so that the linkage of molecular typing data from humans to similar type of data from food and animals is possible. According to this paper, two databases will be developed: one for isolates from food and animals, which will be managed by EFSA, and one for human isolates, which will be managed by ECDC. In relation to this, technical support is requested from the EURL-*Salmonella* for coordination with the NRLs on the development and management of molecular typing methods and for the quality control of the molecular data of *Salmonella* isolates from food, animal feed and primary production.

For the development of the database on molecular typing of EFSA and the activities related to this, two staff members of the EURL-*Salmonella* participate in a working group of EFSA since spring 2013. This will continue in 2014.

The current molecular typing methods are mainly considered as sub-typing methods additional to serotyping. The molecular typing method which will be dealt with at first for (sub)typing of *Salmonella* is Pulsed Field Gel Electrophoresis (PFGE), as this is currently considered as the 'gold standard' for molecular typing of *Salmonella* spp. Furthermore also Multi-Locus Variable number of tandem repeats Analysis (MLVA) for subtyping of *Salmonella* Typhimurium or *Salmonella* Enteritidis is used by many laboratories and may be considered additional to PFGE.

It is foreseen that a pilot of the EFSA database may start in the second half of 2014. For this, technical support of the EURL-*Salmonella* is foreseen, especially for curation of the PFGE data to be uploaded in this database. All data need to be checked for their quality before entering them in the database, to make sure that the uploaded PFGE profiles are of good and uniform quality (as much as possible). The criteria for judging the PFGE data need to be agreed upon with EFSA, including the criteria for rejecting data and the way of reporting to the laboratories that sent poor quality PFGE profiles.
By the end of 2012/early 2013, the pilot for the ECDC molecular typing database has started. Hence, ECDC (or in fact SSI who performs this work under contract of ECDC) already has experiences in treatment and curation of PFGE data. Therefore, the quality criteria and the procedures for curation of the data will be discussed with ECDC (and SSI) as well.
Additional to the curation of the PFGE data, the EURL-*Salmonella* will also regularly perform cluster analysis on the data in the EFSA database. Information from cluster analysis can give information on whether certain *Salmonella* types are found more frequently and it can be an important tool in foodborne outbreak situations. Furthermore, cluster analysis can also be used to perform a regular check on the quality of data in the database(s).

To check the quality of the performance of PFGE by the NRLs for *Salmonella*, the EURL-*Salmonella* will include PFGE-typing in the interlaboratory comparison study on typing of *Salmonella* (see activity 1 'Interlaboratory comparison studies'). It will be discussed with DG-SANCO (and if necessary also with EFSA), whether participation of NRLs already performing PFGE need to become obligatory. From the results of the interlaboratory comparison study (in combination with the results of the pilot study from 2013) it may be decided to advise training for PFGE typing for (some) participating NRLs. Additional, training on PFGE may be organised for

NRLs-*Salmonella* not yet performing PFGE, but which are planning to introduce PFGE in their laboratories (also see 6. 'Training').

It will be discussed with EFSA and ECDC whether it is needed/possible to 'qualify' NRLs before they can upload data in the database and use existing data from the database. Results of the interlaboratory comparison study on PFGE may be used for qualification of an NRL. Regular participation in the interlaboratory studies, with good results may be a possibility to retain the qualification.

For the future, also other molecular typing methods can be of use for the databases and this may be discussed with EFSA. Additional molecular typing methods which may be considered are (not exhaustive):

- Multi-Locus Variable number of tandem repeats Analysis (MLVA), not only for subtyping of *Salmonella* Typhimurium or *Salmonella* Enteritidis, but also for subtyping of other *Salmonella* serovars;
- Multi-Locus Sequence Typing (MLST);
- Single nucleotide polymorphism (snp) analyses;
- Whole genome sequencing/mapping.

Although PFGE is currently considered as the 'Gold standard', it is still a relatively complex and time consuming method. It is generally more preferred to move from 'gel-based' methods to 'sequence-based' methods. New technological developments and declining costs are making whole genome sequencing available as a routine tool for bacterial typing. EURL-*Salmonella* wants to anticipate on these developments in typing of *Salmonella* in order to gain the required expertise to better fulfil its task to support EFSA, DG SANCO (and ECDC) on the management of typing data in the future. A (new) 'promising' method may be 'whole genome mapping'. For this, ordered restriction maps of the compete genome of a pathogen, usually some 300 restriction fragments, are created that can be used for comparison. Provided a bacterial culture is available, the isolate can be characterised and compared to other isolates in 24-48 h. Data obtained with whole genome sequencing can be compared with the current typing data retrieved from PFGE, MLVA and MLST. With other words, the data obtained with the current typing methods and stored in the EFSA database will still be of value even when these typing methods are replaced by whole genome sequencing. The potential usefulness of the method will be discussed with EFSA, ECDC and DG-SANCO. Furthermore, some practical testing of the method may be considered in the following way:

- Analyses of a set of reference strains both with PFGE and 'whole genome mapping';
- Comparison of data and if necessary optimisation of 'whole genome mapping';
- Analyses in parallel of a subset of strains (different serotypes, different sources, etc) with PFGE and 'whole genome mapping'.

<u>Missions in relation to activity 7</u>

Several missions are foreseen for participation in meetings of the EFSA working group on molecular typing. The costs for these missions will be funded by EFSA and will not be charged on the EURL-*Salmonella* budget.

Additional it may be necessary to have a meeting with one or more representatives of the Statens Serum Institute (SSI) in Denmark to obtain practical information on curation of PFGE data and other relevant information in relation to this subject.

<u>Output in relation to activity 7</u>

- Results of the interlaboratory comparison study on PFGE analysis. This will be part of the report of the full interlaboratory comparison study on typing of *Salmonella* (see activity 1 'Interlaboratory comparison studies'.

The following will be reported in the annual technical report of the EURL-*Salmonella*, in March 2015.

- Information on experiences with curation of PFGE data for the pilot EFSA database.
- If applicable:
  - agreements with EFSA and ECDC on qualifying NRLs for uploading PFGE data in the database and on cluster analysis;
  - report on training of one or more NRLs for PFGE analysis;
  - information on usefulness of a 'new' molecular typing method like 'whole genome mapping'.

Mrs. Drs. K.A. Mooijman
Head EURL-*Salmonella*
Bitlhoven, 26 August 2013

Annexes (as separate documents):

- Performance Indicators activities EURL-*Salmonella* 2014
- Estimated budget EURL-*Salmonella* 2014, including costs per activity (not to be published on the website)