EURL-*Salmonella* work-programme 2015

Introduction

The working programme of EURL-*Salmonella* consists of the following activities (the frequency 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 2015 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 animal feed matrix;
- One study on typing of *Salmonella*.

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 number 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, 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.

The way of artificially contaminating the samples, the choice of the *Salmonella* serovars as well as the contamination levels of the samples will be decided per study. The samples will either be artificially contaminated individually at the laboratory of the EURL-*Salmonella* or by combining reference materials with matrix at the laboratories of the NRLs.

For the reporting of the results by the NRLs for *Salmonella*, web-based test reports will be used, like for the interlaboratory comparison studies organised in the last two years.

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. A follow-up can exist of either one of the following activities, or by a combination of different activities:

- Sending extra samples which need to be tested according to a prescribed protocol;
- Training at the EURL for *Salmonella*;
- 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 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 third countries (like EU candidate countries and 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 is discussed at the annual workshop.

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

- Probable time period: February/March 2015.
- 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 2015 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 animal feed samples

- Probable time period: September/October 2015.
- Matrix: food or animal feed samples (to be decided at the annual workshop in 2015).
- *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 2015.
- 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;
  - PFGE (optional), with as advised method the SOP of EFSA, which is likely to be published in 2014;
  - Still to be decided: MLVA of *Salmonella* Typhimurium and *Salmonella* Enteritidis, with as advised method the SOP of EFSA, which is likely to be published in 2014.

Up to and including the typing study of 2014, also phage typing of *Salmonella* was included in the interlaboratory comparison studies. For this, the EURL-*Salmonella* cooperated with Public Health England (PHE), London (Colindale), UK. However, over the years, the number of laboratories performing phage typing is decreasing and in the study of 2013 only 7 of the 34 NRLs participated in the part of the study on phage typing. At the workshop of 2014 this situation was discussed with PHE and DG-Sanco and it was decided that the possibility of phage typing will no longer be offered in the annual typing studies. Serotyping of *Salmonella* will be retained in the studies and additional, more possibilities in testing the NRL performances in the field of molecular typing will be added.

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. Since the study of 2011, on request of the NRLs, 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 are not taken into account for the evaluation of the performance of the laboratory, but is used as additional information on the serotyping capacity of an NRL. 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 future typing studies as well.
The strains will be blindly coded and send to the NRLs for serotyping, one week before the performance of the study.

In 2013 a pilot interlaboratory comparison study for Pulsed Field Gel Electrophoresis (PFGE) analysis of different *Salmonella* serovars was organised. The experiences with this pilot study, as well as the experiences with the study organised in 2014, will be used for the organisation of the study on PFGE analysis in 2015.

As an alternative for phage typing, several laboratories use Multi Locus Variable number of tandem repeats Analysis (MLVA) for sub-typing of *Salmonella* Typhimurium and of *Salmonella* Enteritidis. It is considered to add MLVA typing for one or both serovars to the typing study as well. Before deciding to do so, this will be discussed with relevant parties, like NRLs for *Salmonella*, DG-Sanco, EFSA and Statens Serum Institute (SSI) in Denmark.
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 contributes to the 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 2015 and to participate in each other comparative tests.

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

Type interlaboratory comparison study	Planning study	Planning interim summary report	Planning final draft full report (including the possible follow-up study)[1]
Detection of *Salmonella* in samples from primary production	Feb./March 2015	May 2015	December 2015
Detection of *Salmonella* in food/feed samples	Sept./Oct. 2015	December 2015	July 2016
Typing of *salmonella*	Nov./Dec. 2015	February 2016	September 2016

[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 2015 is foreseen to be organised in May and will last 1,5-2 days. The location of the workshop is not yet decided, but it is quite likely to be organised in Berlin, Germany. Several NRLs for *Salmonella* have offered to help with organising the workshop in their country. The first offer was given by the NRL from Germany and it is worthwhile to explore the affordability of this offer.
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 2014 and 2015;
- 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: September 2015.

3. Supporting activities

Activities concerning standardisation of methods in ISO and CEN

The EURL-*Salmonella* is involved (as project leader or as member of working groups or task advisory 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.

For the following groups in ISO/TC34/SC9 and CEN/TC275/WG6, staff members of EURL-*Salmonella* have the leadership. Activities for these groups will be continued in 2015:

- *CEN/TC275/WG6 – TAG8 'Detection of Salmonella (EN ISO 6579-1)', group leader Kirsten Mooijman*. The CEN enquiry/ ISO DIS (Draft International Standard) vote of the amended draft document was launched on 5 June 2014 and will end on 5 November 2014. During the annual meeting of CEN/TC275/WG6 some comments were already received on the draft document, especially on the method for detection of *Salmonella* in samples from the primary production stage. According to a French study, 23% more samples were tested positive if a second selective enrichment medium is added to the current procedure. The results of this study will be further discussed with Kirsten and the French delegates in fall 2014. The results of the French study as well as the outcome of the voting on the draft document will be discussed in a meeting of TAG8, which is likely to be organised early 2015. Depending on the results of the French study and the possible need to amend the procedure for the detection of *Salmonella* in samples from the primary production stage, it may be needed to launch a second CEN enquiry/ISO DIS vote. This will also be discussed at the next meeting of TAG8. If necessary, additional literature search and/or experiments will be carried out. The outcome of the current DIS voting and the outcome of the discussions/additional experiments will be presented at the plenary meeting of CEN/TC275/WG6 and ISO/TC34/SC9 (June 2015) by the group leader (Kirsten) of TAG8.
- *ISO/TC34/SC9 – WG10 'Guide for serotyping Salmonella spp. (CEN ISO/TR 6579-3)', convener Kirsten Mooijman.* The final version of CEN ISO/TR 6579-3 ('Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* —Part 3: Guidelines for serotyping of *Salmonella* spp.') was published in July 2014. Additional to serotyping of *Salmonella* it was indicated at the annual meeting of CEN/TC275/WG6 in 2013, that it is important to come to an optimal, harmonised molecular method for typing of monophasic *Salmonella* Typhimurium. A listing of the existing methods will be done in CEN/TC275/WG6 – TAG3 ('Molecular methods') in close cooperation with ISO/TC34/SC9-WG10. Further discussion on possible standardisation of the chosen method will follow in 2015.
- *ISO/TC34/SC9 – WG3 'Method validation' – Drafting group of part 6 of EN ISO 16140 on 'validation of confirmation methods', project leader Kirsten Mooijman and co-project leader Wilma Jacobs.* In 2012 the EURL-*Salmonella* was approached to give its opinion on a possible procedure for validation of alternative confirmation/typing methods. Such a procedure is not yet available but is highly needed, especially to validate alternative methods for (sub-)typing of *Salmonella*. Wilma Jacobs and Kirsten Mooijman drafted a proposal for a procedure, based on available (limited) information and this was launched as New Work Item Proposal (NWIP) in 2014. The NWIP was approved with 100% positive votes, including several (mainly technical) comments. In fall 2014, these comments will be discussed with the other members of the (new) drafting group, after which the document will need to be amended before the next voting step can be made. It is quite likely to be

necessary to organise one or two meetings for the drafting group in 2015 to discuss the progress with the document.

- *ISO/TC34/SC9 – Ad hoc group 'Checklist to avoid ambiguity in drafting standards in food microbiology', project leader Wilma Jacobs and co-project leader Kirsten Mooijman*. At the annual meeting of 2013, the need of a checklist for writing standards for ISO/TC34/SC9 and CEN/TC275/WG6 was indicated. This would be a checklist for conveners and project leaders to make sure that standards in food microbiology will become as uniform as possible. The document will become an internal guidance document for SC9 and WG6. As Wilma Jacobs and Kirsten Mooijman have much experiences with writing standards, they were asked to become project leader and co-project leader respectively. To discuss proposals for this (draft) checklist, it may be necessary to organise a meeting with the ad hoc group in 2015.

In the following groups in ISO and CEN, a staff members of EURL-*Salmonella* participates. Activities for these groups will be continued in 2015:

- *CEN/TC275/WG6 – TAG9 'Improvement of the pre-enrichment step to enhance the recovery of Gram negative bacteria', member Kirsten Mooijman.* In 2012 this group was raised in trying to come to an optimal pre-enrichment medium for detection of several (Gram negative) pathogenic bacteria, to be able to resuscitate stressed or damaged cells. As convenor of CEN-TAG 8 on the revision of EN ISO 6579-1 ('Detection of *Salmonella'*), Kirsten Mooijman has become member of this TAG 9. In 2013 and in 2014, members of TAG9 have performed several experiments with growth of different strains in Buffered Peptone Water (BPW) prepared from different batches of peptones. The problem is that there does not exist a clear (chemical) definition for peptone. Therefore, priority is given to define performance characteristics for standardised peptone based broths. As soon as a new group leader is found for TAG9, a meeting may be organised for this group in 2015 and agreements will be made on possible additional tests. The EURL-*Salmonella* may also perform some of these (additional) tests in relation to the method for detection of *Salmonella* in food, animal feed and samples from the primary production stage.
- *ISO/TC34/SC9 – Ad hoc group 'Harmonisation of incubation temperatures', member Kirsten Mooijman.* During the drafting of CEN ISO/TR 6579-3 (serotyping of *Salmonella*) and EN ISO 6579-1 (detection of *Salmonella*), it was discussed whether the temperature range for incubation of non-selective media could be made broader (34-38 °C, instead of 37 °C ± 1 °C). This to (i) be able to use less stringent incubators and (ii) to have better harmonisation with methods used in (e.g.) USA and Canada. At the annual meeting in 2013, the broadening of the temperature ranges for incubation of non-selective media for culturing different bacteria (not only *Salmonella*) was agreed. However, after this agreement, an additional question was raised, during the drafting of prEN DIS 6579-1, whether this broader temperature range can be used for the incubation of selective media as well. As this question does not only relate to the culturing of *Salmonella*, but may be relevant to other bacteria as well, it was agreed at the annual meeting in 2014, to raise an ad hoc group which will have a closer look at data from predictive microbiology from databases. In these databases information may be available on the minimum and maximum growth temperatures of strains. It was agreed that several databases (in France, UK, USA and Canada) will be consulted, leaded by a project leader from France. Kirsten Mooijman will remain involved in this activity as the question originates from the method for detection of *Salmonella*. A meeting may be organised for this group in 2015.

The plenary meetings of both ISO/TC34/SC9 and CEN/TC275/WG6 are likely to be organised in Delft, the Netherlands in June 2015. From the EURL-*Salmonella,* Kirsten Mooijman will participate in these meetings. Additional, Wilma Jacobs may also participate in parts of these meetings.

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 at maximum 2 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 2015. Probable location: Delft, the Netherlands
- Meetings of several (working/ad hoc/task) groups of ISO/TC34/SC9 and CEN/TC275/WG6. Approximately 1 meeting per WG or TAG is foreseen in 2015. 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. Per meeting one, or occasionally two, staff members of the EURL-*Salmonella* will participate.

Output in relation to activity 3

ISO and CEN

- Update draft version of EN ISO 6579-1: fall 2015
- Proposal for a molecular method for typing of monophasic *Salmonella* Typhimurium. To be selected in cooperation with TAG3: fall 2015
- Update draft procedure on validation of confirmation/typing methods as part of the work of ISO/TC34/SC9 WG3 fall 2015
- Draft checklist for harmonisation of standards fall 2015
- Report of relevant items in relation to standardisation as discussed at the plenary meetings of ISO/TC34/SC9 and CEN/TC275/WG6: summer 2015

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 several 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 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.

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 (2015). March 2016

5. Communication

Any information relevant for the NRLs for *Salmonella* is published through the website of the EURL-*Salmonella*, www.eurlsalmonella.eu. One staff member of the EURL keeps the website up to date.

Since fall 2012, web based test reports are used for the reporting of the results of the interlaboratory comparison studies by the NRLs for *Salmonella*. These test reports were developed by the department on Communication/IT of the RIVM, in close cooperation with staff members of the EURL-*Salmonella.* In a questionnaire sent to the NRLs in 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 these web based test reports. However, as each interlaboratory comparison study may slightly differ from a former study (other matrix, other serovar, other number of samples, etc.), some amendments to each test report may be necessary. For this 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.

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: 04-2015; 07-2015; 10-2015; 01-2016

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 has 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 2015 and their evaluation, in the annual technical report of the EURL-*Salmonella.* March 2016

7. Molecular typing of *Salmonella* spp.

As a follow-up of the publication of the vision paper of DG-Sanco 'on the development of data bases for molecular testing of food-borne pathogens in view of outbreak preparedness' (fall 2012), two databases are developed. One database is managed by ECDC and is intended for the collection of molecular typing data from pathogens isolated from humans. This pilot database started early 2013. The other database is managed by EFSA and is intended for the collection of molecular typing data from pathogens isolated from food, animal feed and animals and its environment. The pilot for this latter database is planned to start in fall 2014.

The current molecular typing methods for *Salmonella* 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 and/or *Salmonella* Enteritidis is used by many laboratories and may be considered additional to PFGE.

In relation to the EFSA database, 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 this latter, the technical support of the EURL-*Salmonella* exists of curation of the PFGE data to be uploaded in the EFSA database. All submitted 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 are summarised in an EFSA-SOP which was drafted for a contract with EFSA in 2014. The information in this SOP was harmonised, as much as possible, with other parties involved, being: EURL-*E. coli* and EURL-*Listeria monocytogenes*, as well as ECDC and the curator of the ECDC database: Statens Serum Institute (SSI) in Denmark.
A part of the curation task is also to perform cluster analysis on the molecular *Salmonella* data in the EFSA database and/or in the data of the joint databases of EFSA and ECDC. 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). Details on the frequency of cluster analysis and on who will be responsible for which part of the cluster analysis is still under discussion in the EFSA working group on molecular typing and is intended to be clarified before the start of the pilot EFSA database in fall 2014.

To check the quality of the performance of PFGE by the NRLs for *Salmonella*, the EURL-*Salmonella* includes PFGE-typing in the interlaboratory comparison study on typing of *Salmonella* (see activity 1 'Interlaboratory comparison studies'). Participation in the PFGE part of the study is for the moment still optional. However, for future studies it may 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 results from earlier studies) 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'). For the interlaboratory comparison study on typing of *Salmonella* of 2015 it will also be considered to add MLVA typing for *Salmonella* Typhimurium and *Salmonella* Enteritidis. This will be further discussed with the parties involved (also see activity 1 'Interlaboratory comparison studies').

It may 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 in the future. 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 interest for the databases. Discussion on this has already started between EFSA, ECDC and the EURLs involved. 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. 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, including some practical testing of the method, like:

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

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

A meeting may be organised with one or more representatives of the Statens Serum Institute (SSI) in Denmark to harmonise information on curation of PFGE data and quality control of other molecular data. Furthermore, the set-up of interlaboratory comparison studies on molecular typing of *Salmonella* can be exchanged, including the selection of isolates as used in the studies.

Output in relation to activity 7

- 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 2016.

- 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/sequencing'.

Mrs. Drs. K.A. Mooijman
Head EURL-*Salmonella*
Bilthoven, 25 August 2014

Annexes (as separate documents):

- Estimated budget EURL-*Salmonella* 2015, including costs per activity (not to be published on the website)
- Performance Indicators activities EURL-*Salmonella* 2015