EURL-*Salmonella* work-programme 2016 and 2017

Introduction

The annual work-programme of the EURL-*Salmonella* consists of the following activities (the frequency of the activities is 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. Trainings (duration dependent on the subject; yearly);
7. Molecular typing of *Salmonella* spp. (depending on the subject: yearly or for a limited period).

1. Interlaboratory comparison studies

Every year, the EURL-*Salmonella* organises 3 interlaboratory comparison studies. For 2016 as well as for 2017 the following studies are planned:

- 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 (relevant) *Salmonella* serovar will be used to artificially contaminate a matrix at the levels as indicated above. Additionally, two control samples are included (blank control and positive control of the NRL).

The choice of the *Salmonella* serovars, the contamination levels of the samples as well as the protocol for artificially contaminating the samples will be established for each study. Whenever possible, the samples will be artificially contaminated individually at the laboratory of the EURL-*Salmonella*.

For the transport of the samples to the NRLs, the materials are packed and shipped in accordance with the IATA rules for shipping UN 3373 materials (biological substance category B).

For the reporting of the results by the NRLs for *Salmonella* to the EURL, electronic (web-based) test reports will be used. These test reports are amended for each study.

Several years ago the EURL-*Salmonella* and the NRLs-*Salmonella* agreed on systems for the evaluation of the performance of the laboratories in the interlaboratory comparison studies. In the 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 the 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 these activities:

- Sending extra samples, which need to be tested according to a prescribed protocol;
- Training at the EURL for *Salmonella*;
- Visiting the poor performing NRL by staff members of the EURL-*Salmonella*.

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 as '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. In case of repeated moderate performance (like for poor performance), DG-Sante 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 (potential) candidate countries and EFTA countries) to participate in the interlaboratory comparison studies at 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 the primary production stage

- Probable time period: February/March each year.
- Matrix: samples from the primary production stage (e.g. animal faeces).
- *Salmonella* serovar: one serovar will be used. Which serovar will be decided in due course.
- 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). As soon as the revised EN ISO procedure on the detection of *Salmonella (*EN ISO 6579-1) is published, this will be the prescribed method (comparable to the current procedure as described in Annex D of ISO 6579 for samples from the primary production stage).

Since 2008, also reference laboratories of two third countries (from outside Europe) participated in the studies for detection of *Salmonella* in samples from the primary production stage, 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 will be Israel for 2016 and 2017.

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 each year.
- Matrix: food or animal feed samples (to be decided at the annual workshops).
- *Salmonella* serovar: one serovar will be used. Which serovar will be decided due course.
- Method: It is quite likely that by the time of the food/feed study of 2016, the revised EN ISO procedure on the detection of *Salmonella (*EN ISO 6579-1) is published, becoming the prescribed method. EN ISO 6579-1 prescribes selective enrichment in Muller Kauffmann Tetrathionate novobiocin (MKTTn) broth and in Rappaport Vassiliadis Soya (RVS) broth or on MSRV-agar.

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

- Probable time period: November/December each year.
- Samples: pure cultures of different *Salmonella* serovars.
- Methods:
  - Serotyping (obligatory), following the White-Kauffmann-Le Minor scheme as described in CEN ISO/TR 6579-3:2014 ('Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 3: Guidelines for serotyping of *Salmonella* spp.);
  - PFGE (optional), with as advised method the SOP of EFSA, which was published in 2014;
  - Still to be decided: MLVA of *Salmonella* Typhimurium and *Salmonella* Enteritidis, with as advised method the SOP of EFSA, which was published in 2014.

For the serotyping part of the typing study, the EURL-*Salmonella* will select twenty different serovars from *Salmonella enterica* subsp. *enterica*, including serovars with public health significance, serovars with antigens similar to those of public health significant strains and serovars that caused typing problems in previous studies. Since the study of 2011, on request of the NRLs, a 'twenty-first' strain is added to the set of strains. This concerns 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.
All strains will be blindly coded and send to the NRLs for serotyping, one week before the performance of the study.

For the PFGE-typing part of the study, ten different *Salmonella* strains will be selected. When possible, strains will be selected which are also used in Proficiency tests organised for the Food- and Waterborne Diseases and Zoonoses (FWD) Network in Europe (Public Health laboratories testing samples from human origin). Participants are requested to report their PFGE images as a (raw) TIFF file and/or after analysis of the profile in BioNumerics.

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, as phage typing is no longer offered in the typing studies. Before deciding to do so, this will be discussed with relevant parties, like NRLs for *Salmonella*, DG-Sante and EFSA.

In relation to the molecular typing parts of the typing study (PFGE and MLVA), the EURL-*Salmonella* has contact 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 the coming years and to participate in each other's comparative tests.

Missions in relation to activity 1
If necessary, a visit to a poor performing NRL of an EU-MS by two staff members of the EURL-*Salmonella* will be made. Time needed: approximately 2 days/year, 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 the primary production stage	Feb./March 2016 and 2017	May 2016 and 2017	December 2016 and 2017
Detection of *Salmonella* in food/feed samples	Sept./Oct. 2016 and 2017	December 2016 and 2017	July 2017 and 2018
Typing of *salmonella*	Nov./Dec. 2016 and 2017	February 2017 and 2018	September 2017 and 2018

[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. Workshops

Every year, the EURL-*Salmonella* organises a workshop for the NRLs for *Salmonella*. The workshops are mostly organised in May and will last 1,5-2 days. The locations of the workshops of 2016 and 2017 still need to be decided. Several NRLs for *Salmonella* have offered to help organising the workshop in their country. These offers will be considered, as well as the option to organise the workshop of 2016 in conjunction with the international *Salmonella* symposium (i3s) in France (Saint-Malo) in June 2016.
The programme of the workshops may contain the following items:
- Introductory presentations (e.g., by EU representative and EURL-*Salmonella*);
- Zoonoses in Europe (EFSA, DG-Sante);
- Results of (research) activities of EURL-*Salmonella*;
- Results of interlaboratory comparison studies as organised by EURL-*Salmonella*;
- 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. 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 each workshop.

- Report of the workshops, including a summary of the discussion performed per item at the workshops and the evaluation of each workshop. Draft report: September/October 2016 and 2017.

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 of the food chain.
- CEN/TC275/WG6: European Committee for Standardisation, Technical Committee 275 for Food analysis – Horizontal methods, Working Group 6 for Microbial of the food chain.

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 2016 and 2017:
- *CEN/TC275/WG6 – TAG8 'Detection of Salmonella (EN ISO 6579-1)', group leader Kirsten Mooijman*. It is expected that the voting for the Final Draft International Standard (FDIS) version of EN ISO 6579-1 on detection of *Salmonella* will be launched in 2015. The comments from this voting stage need to be discussed (quite likely through e-mail) with the members of the relevant CEN Task Group, TAG8. Next the document needs to be updated by the group leader (Kirsten). The group leader also has to check the proof version(s) of the updated ISO 6579-1 before it can be published as final EN ISO document.
- *ISO/TC34/SC9 – WG3 'Method validation' – Drafting group of part 6 of EN ISO 16140 on 'validation of confirmation methods', project leader Wilma Jacobs and co-project leader Kirsten Mooijman.* In 2014 a New Work Item Proposal (NWIP) was launched for a procedure for validation of proprietary alternative confirmation/typing methods. This procedure will become a part of the ISO 16140 series on validation of microbiological methods. Such a procedure is not yet available but is highly needed, especially to validate alternative methods for (sub-) typing of *Salmonella*. In 2015, two working draft versions have been prepared and discussed in the drafting group and in WG3. Several more amendments to the document will be needed before it will become more final. Comments to the different voting versions (Committee Draft – CD; Draft International Standard – DIS) will need discussion with the members of the drafting group and of WG3 in meetings in 2016 and 2017.
- *ISO/TC34/SC9 – Ad hoc group 'Checklist to avoid ambiguity in drafting standards in food microbiology', project leader Kirsten Mooijman and co-project leader Wilma Jacobs*. 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 convenors 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 Kirsten Mooijman and Wilma Jacobs have many experiences with writing standards, they were asked to become project leader and co-project leader respectively. The draft document as prepared in 2015 will need to be discussed with the members of the drafting group and next it need to be evaluated by the members of SC9 and WG6. Depending on the number and type of comments the document may be finalised in 2016 or 2017.

In the following groups in ISO and CEN, a staff members of EURL-*Salmonella* participates. Activities for these groups will be continued in the coming years:
- *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. In 2015 a new group leader was found for TAG9 (after one year without leader) and it is expected that meetings will be organised for this group in 2016 and 2017 to discuss 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. The project leader from France presented the results at the annual meeting of SC9 in 2015 and it was agreed to draft a protocol to ask the members to perform some additional experiments. The help of the EURL-*Salmonella* was asked with the drafting (and performing) of the protocol. The members of ISO-SC9 and CEN-WG6 are asked to perform some experiments between September 2015 and April 2016. The data will be evaluated by the project leader Daniele Sohier (France) and Kirsten Mooijman of EURL-*Salmonella.* The results will be presented at the annual meeting of SC9 and WG6 in 2016 and the next steps will be discussed.
- *ISO/TC34/SC9 – WG4 'Proficiency Testing', member Kirsten Mooijman*. ISO/TS 22117 was published in 2010 and it was decided in 2014 to revise the document for several reasons (e.g. to make it a full standard, to include PT schemes for viruses, parasites, primary production, yeasts and moulds, and molecular methods). The involvement of the EURLs in this working group is considered important as they have much experience with the organisation of PT schemes. For the optimisation of the document it may be necessary to meet with the other members of EG4 in 2016 and 2017.

The plenary meetings of both ISO/TC34/SC9 and CEN/TC275/WG6 of 2016 will be organised in France in May or June 2016. The location and dates of the annual meetings of SC9 and WG6 in 2017 are not yet known, but it may be quite likely that the location will be outside Europe. One representative from the EURL-*Salmonella* will participate in these meetings.

Comparison of (standard) methods for molecular typing of monophasic* Salmonella *Typhimurium

Although 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, a new activity in relation to this document was raised. At the annual meeting of CEN/TC275/WG6 in 2013 it was suggested to draft an annex to CEN ISO/TR 6579-3, describing a procedure for molecular typing of monophasic *Salmonella* Typhimurium. A listing of existing methods was made in close cooperation with CEN/TC275/WG6 – TAG3 ('Molecular methods') in 2015. No conclusions could be made on whether the method should be a gel based PCR or a Real Time PCR, as no data are available in which both methods are compared with a same set of strains. For that reason it is planned that the EURL-*Salmonella* will organize a comparison ('validation') study (quite likely in 2016) with a selected set of strains with a small group of (approximately 5) laboratories (NRLs for *Salmonella*). In this study the 'traditional' gel based PCR ('Tennant protocol') will be compared to a Real Time PCR. Additional it will be considered to also include for comparison a gel based PCR using the Real time PCR reagents. For the set-up of the study, the protocols of the methods to be tested, as well as for the selection of strains the EURL will cooperate with the

project leader of TAG3, Burkhard Malorny (NRL-*Salmonella* Germany), as well as with NRLs for *Salmonella* active with typing of monophasic *Salmonella* Typhimurium.
Depending on the outcome of the comparison study, it will be decided whether one or two procedures will be standardized (gel based PCR and/or Real time PCR) as an annex to CEN ISO/TR 6579-3.

Other standardisation activities
Within AOAC, an ISPAM (International Stakeholder Panel on Alternative Methods) working group on Harmonization of *Salmonella* methods was raised in 2013. The aim of this group is to provide recommendations for the process of harmonizing the US (BAM/MLG) and ISO *Salmonella* reference culture methods. Members of this group are, amongst others, representatives from AOAC, FDA, USDA and Health Canada. Kirsten Mooijman of the EURL-*Salmonella* also participates in this working group as representative for the ISO working groups on *Salmonella*. The meetings of the group concern mainly teleconferences and most contacts are through e-mail. The group has become more active in 2015 and it is foreseen that the work will continue for several years as it is a long and difficult process to come to one harmonised international standardised method (if ever possible).

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 (preferred procedure) 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.

<u>Missions in relation to activity 3</u>
- Participation of at a staff member of the EURL-*Salmonella* in the annual meetings of ISO/TC34/SC9 and CEN/TC275/WG6. Duration of each annual meeting: 5 days. Period of the year: May/June. Location 2016: France. Location 2017: quite likely outside Europe.
- Meetings of ISO/TC34/SC9-WG3 (method validation) and the ad hoc group on validation of confirmation and typing methods. Approximately 2 meetings per year. The meetings are not yet planned, but will be scheduled as soon as considered necessary. The locations of the different meetings still need to be decided, but will quite likely be located in an EU-MS. Per meeting one, or occasionally two, staff members of the EURL-*Salmonella* will participate.
- Meetings of ISO/TC34/SC9-WG4 (Proficiency Testing). Approximately 1 meeting per year. The meetings are not yet planned, but will be scheduled as soon as considered necessary. The locations of the different meetings still need to be decided, but will quite likely be located in an EU-MS. Per meeting one staff member of the EURL-*Salmonella* will participate.
- Meetings of CEN/TC275/WG6-TAG 9 (pre-enrichment). Approximately 1 meeting per year. The meetings are not yet planned, but will be scheduled as soon as considered necessary. The locations of the different meetings still need to be decided, but will quite likely be located in an EU-MS. Per meeting one staff member of the EURL-*Salmonella* will participate.
- Meetings with the relevant project leader of CEN/TC275/WG6-TAG 3 (molecular methods) at location or by teleconference in relation to the organisation of a comparison study between PCR methods for typing of monophasic *Salmonella* Typhimurium and for standardising the procedure in ISO format. Approximately 1 meeting per year. The meetings are not yet planned, but will be scheduled as soon as considered necessary. The locations of the different meetings still need to be decided, but will quite likely be located in an EU-MS. Per meeting one staff member of the EURL-*Salmonella* will participate.

Output in relation to activity 3

ISO and CEN

- Finalisation of EN ISO 6579-1 (approx.) fall 2016
- New draft versions of procedure on validation of confirmation and typing methods as part of the work of ISO/TC34/SC9-WG3 2016 and 2017
- Comparison study of PCR methods for typing of monophasic *Salmonella* Typhimurium. fall 2016
- Draft proposal for standardisation of molecular method(s) for typing of monophasic *Salmonella* Typhimurium. 2017
- Finalisation guidance document for drafting International Standards for microbiology of the Food chain 2016/2017
- Summary on comparison incubation temperatures (35 °C x 37 °C) summer 2016
  And follow-up 2017
- Report on relevant items in relation to standardisation as discussed at the plenary meetings of ISO/TC34/SC9 and CEN/TC275/WG6 in 2016 and 2017. summer 2016, summer 2017

Note: For the progress of the work with the EN ISO documents, the EURL-*Salmonella* is very much dependent on the cooperation and 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, (potential) Candidate Member States or (other) third countries, with requests for information or for participation in activities being organised. Also, requests for support from the European Commission (DG-Sante), 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-Sante and EFSA

When requested and when possible, one or two staff members of the EURL-*Salmonella* participate in working groups of DG-Sante 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-Sante and EFSA will be funded by DG-Sante 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-Sante will be used by DG-Sante 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 reports of the EURL-*Salmonella* over the years under review (2016 and 2017). March 2017, March 2018

5. Communication

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 electronic 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.* However, as each interlaboratory comparison study may slightly differ from a former study (other matrix, other serovar, other number of samples, amendments in the methods, etc.), some amendments to each test report may be necessary. For this the help of the IT department of the RIVM is needed for each study.

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 newsletters through the website: every quarter of the year

6. Training activities

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'), requests for practical trainings can be expected.

Additional to practical training on performing PFGE, trainings on the use of the software package BioNumerics for PFGE profile analysis may be needed. This latter is especially of importance to get good quality molecular typing data for storage in the (pilot) EFSA database (also see 7. 'Molecular typing of *Salmonella* spp.).
In 2016 as well as in 2017, 2-days' training sessions are foreseen on PFGE profile analysis, on the management of the metadata related with each isolate and on submission of the data to the (pilot) EFSA molecular typing database. Both training sessions will be organised in cooperation with the EURL-VTEC (ISS, Italy) and the EURL-*Listeria monocytogenes* (ANSES, France). The session of 2016 is planned at the premises of the EURL-*Listeria monocytogenes* (Maison Alfort, France) and the one of 2017 at the EURL-VTEC (Rome, Italy). A third session, in 2018, is forecasted to be organised at the premises of the EURL-*Salmonella* (the Netherlands). Each EURL will make available didactic rooms equipped with at least 12 computer workstations, and each course will be attended by representatives of 4 NRLs for

VTEC, 4 NRLs for *Listeria monocytogenes* and 4 NRLs for *Salmonella*. The trainings will be given by staff members of the three EURLs. The aim is to train the participants in correctly performing band assignment and profile analysis and identification of relatedness between PFGE profiles.

Training costs for participation of NRLs

- Travel and accommodation costs for approximately two practical trainings of individual NRLs for *Salmonella* per year. At maximum 2 members per NRL per training. Duration of each training: at maximum 5 days. Location: EURL-*Salmonella,* the Netherlands.
- Travel and accommodation costs for 4 members of NRLs-*Salmonella* per year for participation in BioNumerics trainings. Duration of each training: 2 days. Period of the year: June. Location 2016: EURL-*Listeria monocytogenes,* France. Location 2017: EURL-VTEC, Italy.

Missions in relation to activity 6

- Participation (acting as trainer) of at a staff member of the EURL-*Salmonella* in the trainings for BioNumerics. Duration of each training: 2 days. Period of the year: June. Location 2016: EURL-*Listeria monocytogenes,* France. Location 2017: EURL-VTEC, Italy.

Output in relation to activity 6

- Summary on the number and type of (individual) trainings performed in 2016 and 2017 and their evaluation, in the annual technical reports of the EURL-*Salmonella.* March 2017 March 2018

7. Molecular typing of *Salmonella* spp.

***Technical support to EFSA – Curation of EFSA Database for* Salmonella**

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 have been 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 was launched 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 was launched in December 2014 and is known as the EFSA Molecular Typing Data Collection system (MTDC).

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-VTEC and EURL-*Listeria monocytogenes*, as well as ECDC and the curator of the ECDC database: Statens Serum Institute (SSI) in Denmark.
To be able to exchange information in relation to the curation of molecular data and to try to perform the curation in a harmonised way between the 3 EURLs (for non-human strain profiles) and ECDC-curator (for human strain profiles), it is important to regularly organise

meetings ('curation trainings') with all parties involved. It is planned to organise every year a 'curation' meeting which may last 1 to 2 days. It is foreseen to plan these meetings in conjunction with the BioNumerics trainings (see '6. Trainings').

EFSA-ECDC Steering Committee

A staff member of the EURL-*Salmonella* participates in the joint EFSA-ECDC Steering Committee which is in charge of the management of the joint EFSA-ECDC molecular typing database. Additional to members of EFSA and ECDC also members of the EURLs for VTEC and *Listeria monocytogenes* participate, as well as the curator of the ECDC molecular typing database (SSI).
This steering committee will, amongst others, draft a Standard Operating Procedure (SOP) on the data analysis in both databases, including microbiological cluster evaluations on the molecular typing data. Information from these cluster evaluations can give information on whether certain types are found more frequently and it can be an important tool in foodborne outbreak situations. Furthermore, microbiological cluster evaluations can also be used to perform a regular check on the quality of data in the database(s).

Molecular typing of* Salmonella *by the NRLs

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-Sante (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. Additionally, 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. 'Trainings').
Additional to PFGE-typing it will be considered to also introduce MLVA typing for *Salmonella* Typhimurium and *Salmonella* Enteritidis in the interlaboratory comparison studies on typing of *Salmonella*. This will be further discussed with the parties involved (also see activity 1. 'Interlaboratory comparison studies').

It may be discussed in the EFSA-ECDC steering committee 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 studies 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.

Other molecular typing methods

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. However, for data analysis and data interpretation specific expertise (bio-informatics) is needed which may sometimes be a bottleneck in the use of the data. In principle, the 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 need to be discussed in the EFSA-ECDC steering committee and with DG-Sante. Furthermore, some practical testing of the method may be needed, 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.

Missions in relation to activity 7

- Participation of 1 to 2 staff members of EURL-*Salmonella* in annual meetings with curators of EFSA-ECDC molecular database (3 EURLs and curator ECDC database). Duration of each meeting: 1-2 days. The meetings may be organised in conjunction with the BioNumerics trainings (see ativity 6. 'Trainings'). Period of the year: June. Location 2016: EURL-*Listeria monocytogenes,* France. Location 2017: EURL-VTEC, Italy.
- Participation of a staff member of EURL-*Salmonella* in approximately 2 meetings of the EFSA-ECDC steering committee per year. Duration of each meeting: 1 day. Location: Parma, Italy.

Output in relation to activity 7

Information will be reported in the annual technical reports of the EURL-*Salmonella*, in March 2017 and March 2018. The following may be included:

- Information on experiences with curation of PFGE data for the pilot EFSA database.
- Information on activities in EFSA-ECDC steering committee.
- If applicable:
    - agreements in EFSA-ECDC steering committee on qualifying NRLs for uploading PFGE data in the database and on cluster analysis;
    - information on usefulness of a 'new' molecular typing method like whole genome sequencing.

Mrs. Drs. K.A. Mooijman
Head EURL-*Salmonella*
Bilthoven, 18 September 2015

Annex (as separate document):

- Estimated budget EURL-*Salmonella* 2016 and 2017, including costs per activity (not to be published on the website)