



## **EURL-*Salmonella* work-programme 2013**

### **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.

### **1. Interlaboratory comparison studies**

For 2013 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*.

The exact timing of the studies in 2013 will be planned with the NRLs-*Salmonella*, but an indication of the probable time period is given below.

Since 2011 lenticule discs have been used as reference materials to artificially contaminate the matrices in the interlaboratory comparison studies for the detection of *Salmonella* in veterinary and food samples. So far, good experiences were gained with the lenticule discs and it is most likely that these reference materials will also be used in the studies on detection of *Salmonella* in 2013. The lenticules are produced by the Health Protection Agency in the United Kingdom, where the EURL-*Salmonella* orders the batches needed. The EURL-*Salmonella* has good contacts with the HPA, so that the possibilities can be discussed about amended contamination levels and new serovars. The choice of the serovars as well as the contamination levels will be decided per study.

Up to 2012, extensive test reports in Word or Excel format have been used by the NRLs for *Salmonella* for the reporting of their results of all interlaboratory comparison studies. By mid 2012 the test reports of all studies were reviewed and shortened by deleting several questions. The first shortened test report will be used in the study of September/October 2012 and its usefulness will be evaluated afterwards. Depending on the outcome of this evaluation, the test reports for the other studies will also be shortened accordingly. Furthermore, it will be explored whether web based forms of the shortened test reports can be used for the reporting of the results (see 5. Communication).

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

At the workshop of the EURL-*Salmonella* in May 2012 the possibility of combining the interlaboratory comparison study of the EURL-*Salmonella* with the one for the validation of Annex D of ISO 6579:2007 ('Detection of *Salmonella* in animal faeces and in environmental samples from the primary production stage') under the CEN mandate M/381 was discussed. It was agreed that the EURL will explore the possibilities for combining these studies. The item has also been discussed with DG-Sanco and a combination of studies was agreed upon by June 2012.

As the study is planned to be organised in February/March 2013, the 'pre-work' for the study already started in 2012. The following is planned for the study:



- Time period: February/March 2013.
- Matrix: pairs of boot swabs/socks mixed with a set amount (e.g. 10 g per pair) of *Salmonella*-free chicken faeces, collected (from the floor) at e.g. a flock of laying hens.
- The boot swabs/socks will either be artificially contaminated with a *Salmonella* culture at the laboratory of the EURL or again the protocol of earlier EURL-*Salmonella* studies will be followed. In the latter case, reference materials have to be added to the boot swabs/socks by the participants shortly before testing of the samples in their laboratories. The choice will depend on the outcome of the research on the stability and homogeneity of the samples (see 3. Supporting activities).
- Number of samples and contamination levels. For this the set-up for the (qualitative) validation studies under the CEN mandate will be followed, being:
  - Three levels of contamination: blank, low (at or slightly above the detection limit of the method) and high (5-10 times above the detection limit);
  - 8 blind replicates per level.
- *Salmonella* serovar: one serovar will be used. Which serovar will be decided later.
- Method: Annex D of ISO 6579 ('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).

The outcome of the study will be treated in two different ways. One way for testing the performance of the laboratories (the 'EURL-*Salmonella* part' of the study) and one way for testing the performance of the method (the 'CEN-mandate part').

The costs on the preparation, organisation, data treatment and reporting of the results of the study will be divided over the two projects. The experiments for testing the stability and homogeneity of artificially contaminated boot swabs/socks will be started in 2012 and will largely be charged on the budget of the CEN mandate. If tests continue in 2013 part of it will also be charged on the EURL-*Salmonella* budget of 2013. The costs for mailing of the samples for the study early 2013 will (most likely) be charged on the budget of the CEN mandate. The time spent for the different ways of treatment of the data and for preparing separate reports will be divided over the budget of both the CEN mandate and of the EURL-*Salmonella* of 2013.

For the evaluation of the performance of the laboratories (EURL-*Salmonella* part) the following is planned.

The results of each NRL will be evaluated and compared with the pre-set definition of 'good performance'. 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).

As agreed at the workshop of 2011, also 'moderate' performance of participating laboratories will be further taken into consideration. At the workshop of 2011 the following action for 'moderate' performing laboratories was proposed: 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 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, DG-Sanco will be informed.

Since 2008, also reference laboratories of two third countries (from outside Europe) participated in the 'veterinary' studies, being: Tunisia and Israel. These countries participated on request of DG-Sanco. However, since 2011, Tunisia 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 the veterinary study of 2013 will be Israel.

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 European Member States.



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. Tunisia, 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**

The choice of the matrix for the food/feed study in 2013 will be discussed with the NRLs at the EURL-*Salmonella* workshop of 2013.

The prescribed method will be ISO 6579 (2002: Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.), with selective enrichment in RVS and MKTTn. The additionally requested method will be Annex D of ISO 6579, with selective enrichment on MSRV.

The planning of the study is September/October 2013.

At the EURL-*Salmonella* workshop in May 2012, it was discussed to lower the number of samples in the interlaboratory comparison studies on the detection of *Salmonella*. It was agreed to follow for future studies, as much as possible, the set-up as described in EN ISO/TS 22117 ('Microbiology of food and animal feeding stuffs – Specific requirements and guidance for proficiency testing by interlaboratory comparison'). In short:

- 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 also agreed at the 2012 workshop to (still) include some control samples (lenticules without matrix) as well.

Like for the veterinary study, the results of each NRL will be evaluated and compared with the pre-set definition of 'good performance'. Actions will be taken in case of unexplainable 'poor performance', as well as in case of repeated 'moderate performance' (see above).

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

For the organisation of the interlaboratory comparison study on typing of *Salmonella* a subcontract with the Health Protection Agency (HPA), London (Colindale), UK is foreseen. This subcontract will formalise the input of HPA on the organisation of the study for the part on phage typing. Thanks to this cooperation between the EURL-*Salmonella* and the HPA, 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 2013.

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. At the workshop of 2011, the NRLs asked to include one or more 'reptile-serovars' as well. In 2011, 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 21<sup>st</sup> serovar were not taken into account for the evaluation of the performance of the laboratory. Most NRLs performed the typing of this 21<sup>st</sup> strain and its inclusion in the study was highly appreciated. At the workshop of 2012 it was agreed to include again such a 21<sup>st</sup> serovar of another subspecies in the typing study of 2012. Its usefulness will again be evaluated at the workshop of 2013 and by then it will be decided whether a 21<sup>st</sup> serovar will be included in the study of 2013 as well.



The strains will be blindly coded and send to the NRLs for serotyping, one week before the performance of the study.

The HPA 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.

The planning of the study is November/December 2013.

In 2007 a definition on the evaluation of 'good performance' of the serotyping was agreed with the NRLs and for the first time applied on the results of the study of 2007. The same criteria will be used for evaluating the results of the typing study of 2013, unless agreed otherwise with the NRLs and/or DG-Sanco.

#### Subcontract in relation to activity 1

A subcontract with the Health Protection Agency (HPA), London, United Kingdom is foreseen to hire the expertise of the HPA for phage typing of *Salmonella* in the interlaboratory comparison study on typing. For this, HPA will select and test strains to be used in the typing study and will send the strains to the EURL-*Salmonella*. Furthermore, HPA 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

Type interlaboratory comparison study	Planning study	Planning interim summary report	Planning final draft full report (including the possible follow-up study) <sup>1</sup>
Detection of <i>Salmonella</i> in veterinary samples	Feb./March 2013	May 2013	September 2013
Detection of <i>Salmonella</i> in food/feed samples	Sept./Oct. 2013	December 2013	May 2014
Typing of <i>salmonella</i>	Nov./Dec. 2013	February 2014	July 2014

<sup>1</sup>: 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**

At the annual workshop of the EURL-*Salmonella* in 2012, the NRLs indicated a preference for organisation of the annual workshop in conjunction with the international *Salmonella* symposium (i3s) in France (Saint-Malo) in May 2013. As this symposium last 3 days, the duration of the workshop may be shortened to 1-1,5 days instead of 1,5-2 days (as in earlier years) to facilitate the travelling of the participants of the NRLs. The EURL-*Salmonella* will explore the possibilities of organising the workshop in St. Malo again.

The programme 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 2012 and 2013;
- 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) 926/2011 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, Croatia, Iceland, Former Yugoslav Republic of Macedonia, Norway, Serbia, Switzerland and Turkey.

#### Output in relation to activity 2

- Publication of the presentations of the workshop at the EURL-*Salmonella* website ([www.euralsalmonella.eu](http://www.euralsalmonella.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 three groups in CEN/ISO dealing with methods for *Salmonella*. The activities for these groups will be continued in 2013:

- *Detection of Salmonella (EN ISO 6579-1)*: By fall 2012 it is planned that an updated version of the draft document of EN ISO 6579-1 will be sent to the secretariat of CEN/TC275/WG6. Most likely, the CEN enquiry/ISO DIS (Draft International Standard) voting of this document will then be launched in early 2013. It may be necessary to discuss the outcome of this voting in the CEN Technical Advisory Group (TAG) on *Salmonella* detection, in spring/summer 2013. 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 (spring 2013) by the convenor (Kirsten) of the TAG group.
- *Enumeration of Salmonella (EN ISO/TS 6579-2)*: It is expected that the final version of this document is published before the end of 2012. No further actions for this document are foreseen in 2013.
- *Guide for serotyping Salmonella spp. (CEN ISO/TR 6579-3)*: By fall 2012 it is planned that an updated version of the draft document of CEN ISO/TR 6579-3 will be sent to the secretariat of ISO/TC34/SC9. Most likely, the final voting of this document will then be launched by the end of 2012 or early 2013. It may be necessary to discuss the outcome of this voting in the ISO working group (WG10) in spring/summer 2013 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 (spring 2013) by the convenor (Kirsten) of the working group.



#### *Other activities related to ISO and CEN*

CEN-mandate M/381: In 2007 the EURL-*Salmonella* was assigned as project leader for the validation of Annex D of ISO 6579 (Detection of *Salmonella* in samples from primary production). By the end of 2011 a subcontract was signed between CEN (Afnor, France) and the RIVM, the Netherlands. In 2012 preparatory work is done for preparation and testing of samples to be used in the interlaboratory comparison study for the validation of the method. As described under '1. Interlaboratory comparison studies', the validation study under the CEN mandate will be combined with the EURL-*Salmonella* interlaboratory comparison study on the detection of *Salmonella* in samples from primary production in February/March 2013.

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 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 forwarded to the convener of WG3 after which it was presented at the plenary meeting of ISO/TC34/SC9 in June 2012. It was agreed that WG3 will draft a proposal for a procedure for validation of alternative confirmation/typing methods based on the information of the EURL-*Salmonella*. It was therefore agreed that Kirsten Mooijman of the EURL-*Salmonella* will be added to the list of members of WG3 to help with the drafting of the document. It is expected that for this participation in a meeting of WG3 may be needed in 2013.

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. The aim of TAG 9 is to harmonise the pre-enrichment broth for all Gram negative target organisms as far as possible. It was agreed that the project leader of TAG 9 will draft a protocol to test several compositions of pre-enrichment broths. As convener 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. If possible, experiments on optimising the pre-enrichment broth for the detection of *Salmonella* will be performed at the laboratory of the EURL-*Salmonella*, as soon as the protocol of TAG 9 comes available.

#### **Methods**

If necessary, literature search and experiments may be done in relation to the ISO/CEN activities in the field of *Salmonella* (see above). 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. Furthermore, as indicated above, the EURL-*Salmonella* will perform experiments to the optimisation of the pre-enrichment broth for the culturing of *Salmonella* spp., following the protocol of CEN TAG 9.

In 2011 a large study design was set-up to test the influence of pooling of poultry meat samples on the sensitivity of the *Salmonella* detection method. In the study design many combinations of *Salmonella* serovars, different ways of stressing the strains, type of matrices, different ways of pooling and different detection methods have been tested. The experimental work was finished in spring 2012, but the statistical analyses still need to be performed (planned in fall 2012). It will be considered to summarise the results of this study in a publication. Depending on when the statistical analysis is finished, the drafting of the manuscript may be done in 2013.

In 2012 a first inventory to alternative methods for typing of *Salmonella* was done, e.g. by inviting a guest speaker on this item at the EURL-*Salmonella* workshop. This will be continued in 2013 by



exploring literature and, if possible, by applying some practical comparison studies between 'classical' typing procedures and alternative procedures.

### **Reference materials and matrices**

Per interlaboratory comparison study the serovar(s) and contamination levels in the reference materials will be chosen. Before ordering lenticules with a 'new' *Salmonella* serovar, it may be necessary to test the interference of the matrix with the 'new' serovar. If the results are promising, the batch of lenticules will be ordered and the tests will be repeated in an interlaboratory comparison set-up with the relevant lenticules.

Supporting activities may be necessary for testing relevant matrices for the interlaboratory comparison study on the detection of *Salmonella*. The matrix will be tested with and without the addition of *Salmonella* reference materials. Also the amount of background flora will be analysed.

For the study on detection of *Salmonella* in primary production samples (boot swabs/socks with chicken faeces) of February/March 2013 it is planned to test the possibility of artificially contaminating individual samples at the EURL-*Salmonella* laboratory and sending these 'pre-contaminated' samples to the participating laboratories. To do so, it is necessary to test (at the laboratory of the EURL):

- different *Salmonella* serovars to find the most optimal strain to contaminate the samples;
- the most optimal inoculum of the culture of the chosen *Salmonella* serovar;
- the stability of the inoculated samples at storage temperature and at 'abuse' temperatures to check possible negative effects of mailing;
- the homogeneity of the inoculated samples;
- the repeatability of inoculating individual samples in a controlled way.

Most of the indicated test are planned to be performed in the second half of 2012, but it may be necessary to perform some repeatability tests in 2013 (shortly before the study).

### 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: spring/early summer 2013. Location: Germany.
- Meetings of several working groups or TAG groups of ISO/TC34/SC9 and CEN/TC275/WG6. Approximately 2 meetings per working group, with 1 meeting, for most of the groups, in conjunction with the meeting of ISO/TC34/SC9 and CEN/TC275/WG6. The meetings are not yet planned, but will be scheduled as soon as considered necessary.

### Output in relation to activity 3

#### *ISO and CEN*

- New draft version of EN ISO 6579-1: fall 2013
- New draft/final version of EN ISO 6579-3: fall 2013
- Draft procedure on validation of confirmation/typing Methods as part of the work of ISO/TC34/SC9 WG3 fall 2013
- Report of relevant items in relation to standardisation as discussed at the plenary meetings of ISO/TC34/SC9 and CEN/TC275/WG6: summer 2013

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.

#### *Methods*

- Summary of the results of the pooling experiment in a manuscript: summer 2013

#### *Reference materials and matrices*



- 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).

#### **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.

##### 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 (2013).

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#### **5. Communication**

In spring 2012 it was agreed to change the name of the website from [www.rivm.nl/crlsalmonella](http://www.rivm.nl/crlsalmonella) to [www.eurlsalmonella.eu](http://www.eurlsalmonella.eu). However, not only the name needed a change, but also the content of the website needed several amendments. During 2012 many changes in the 'new' website were introduced by a staff member of the EURL and the new site is expected to be launched for publication by the end of summer 2012. After the publication the staff member will continue improving the site as well as keeping the information up to date. This will continue in 2013 as well.

The possibilities for the use of web based forms for the reporting of the results of the interlaboratory comparison studies by the NRLs were explored in 2012. It is planned that the department on Communication/IT of the RIVM will prepare a first web based form in fall 2012. This form will then be used by the NRLs for the reporting of the results of the interlaboratory comparison study on typing of *Salmonella* as planned in November/December 2012. After this study, the web based form will be evaluated and the information will be used to build (new) web based forms for the studies on detection of *Salmonella* in veterinary and food/feed matrices in 2013. For the building, testing and evaluation of the web based forms, the IT department will keep in close contact with the staff members of the EURL-*Salmonella*.





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-month 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 fall 2012. Finalising of this manuscript may move to 2013.

#### Output in relation to activity 5

##### *Website*

- Keeping the (new) EURL-*Salmonella* website up to date: continuously
- Web based forms for reporting of results of interlaboratory comparison studies on typing and detection of *Salmonella* end 2013

##### *Newsletter*

Publication of 4 newsletters through the website: 04-2013; 07-2013; 10-2013; 01-2014

##### *Trend analyses interlaboratory comparison studies*

- Finalising review of several interlaboratory comparison studies on (sero)typing of *Salmonella*: mid 2013

## **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 advise 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.

#### Output in relation to activity 6

- Short summary on the number and type of trainings performed in 2013 and their evaluation, in the annual technical report of the EURL-*Salmonella*. March 2014

## **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.



Details on this technical support by the EURL-*Salmonella* will be discussed with the parties involved and worked out further in 2012 and later.

The current molecular typing methods are mainly considered as sub-typing methods additional to serotyping.

Items to be dealt with in relation to molecular typing methods (not exhaustive):

- Discussion with relevant parties (EFSA, ECDC, DG-Sanco) on choices to be made (e.g. what data of which methods should be included in the databases) to make sure that data in both databases can be compared.
- Giving advice on selection of preferred molecular typing methods.
- Giving advice on protocols for molecular typing.
- Judgment of quality of molecular data of the NRLs.
- Organisation of interlaboratory comparison studies for molecular typing of *Salmonella* spp.
- Training of NRLs for *Salmonella* for specific molecular methods.
- Contribute to the development and validation of standard protocols for specific molecular typing methods.
- Approaching the network of NRLs for *Salmonella* in case of requests for extra data, e.g. in case of outbreaks.

The choice on which molecular typing method should be dealt with at first will be discussed with EFSA, ECDC and DG-Sanco. As Pulsed Field Gel Electrophoresis (PFGE) is currently considered as the 'gold standard' for molecular typing of *Salmonella* spp. it is suggested to focus at first on PFGE. Suggested steps are:

1. Make an inventory of NRLs for *Salmonella* having PFGE operational, if necessary for a selection of serovars. The NRLs may also be asked what other molecular typing methods they perform regularly.
2. Make an inventory, together with EFSA and ECDC, of historical PFGE data in existing databases (e.g. PulseNet) and/or at NRLs and check whether quality control procedures exist for uploading data in these databases and decide on the usefulness of these quality control procedures.
3. Discuss with relevant parties (EFSA, ECDC, DG-Sanco) on the status of data in these existing databases and whether the data should be included in the (new) EFSA/ECDC databases or not.
4. Ask NRLs for *Salmonella* who already perform PFGE, for their PFGE protocol and check this for uniformity. If necessary give advice on the protocol (based on the protocol of PulseNet Europe and PulseNet USA).
5. Agree with EFSA and ECDC on the development of a quality control procedure, or on the use of an existing protocol, for the judgement of the quality of PFGE profiles. This protocol should be tested on a selection of PFGE profiles (e.g. obtained from some NRLs).
6. Organisation of a pilot interlaboratory comparison study on PFGE analysis on a selection of *Salmonella* serovars, for NRLs already performing PFGE. It is considered to organise this study in parallel with the interlaboratory comparison study on (sero)typing of *Salmonella* (fall 2013).
7. Discuss with EFSA and ECDC on the procedure to decide whether it is needed/possible to 'qualify' NRLs before they can upload data in the database and use existing data in the database. Results of the interlaboratory comparison study on PFGE (6) may be used for qualification of an NRL. Regular participation in the interlaboratory studies, with good results may be necessary to retain the qualification.
8. Discuss with EFSA and ECDC on the procedures to maintain the quality of uploaded data to a high level, e.g. by regularly checking the quality of uploaded profiles.
9. Training of NRLs for *Salmonella* in case they do not yet perform PFGE and/or in case of (repeated) problems with the quality of PFGE profiles. Before giving these trainings it will be discussed with EFSA and ECDC (and the NRLs) whether a training of NRLs for PFGE analyses is worthwhile or whether the focus should be on other molecular typing techniques.
10. Support NRLs with molecular typing of (a selection of) *Salmonella* isolates, especially in case of outbreak situations.



11. Support EFSA (and if necessary ECDC) with cluster analysis, especially in case of outbreaks. Furthermore, cluster analysis can also be used to perform a regular check on the quality of data in the database(s).

It is aimed to perform as many as possible of the above mentioned steps in 2013. The activities will be continued in the following years and extended where necessary.

Furthermore, in 2013 it will be discussed with EFSA and ECDC what other molecular typing methods can be of use for the databases. Molecular typing methods which may be considered are (not exhaustive):

- Multi-Locus Variable number of tandem repeats Analysis (MLVA);
- 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 fulfill 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 complete 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'.

#### Missions in relation to activity 7

It is foreseen to have a meeting with EFSA (and ECDC) to discuss details in relation to the database by two staff members of the EURL-*Salmonella*. Time needed: approximately 2 days (or two meetings of one day), country Italy.

#### Output in relation to activity 7

Summaries/reports in relation to PFGE, including: end 2013

- A list of NRLs for *Salmonella* using PFGE (and other molecular typing methods).
- A proposal for a quality control procedure of PFGE data and testing the usefulness of this protocol on a selection of PFGE profiles for *Salmonella*.
- The possible use of existing data in the (new) database.
- Check for uniformity of the PFGE protocol.
- Proposal for a pilot interlaboratory comparison study on PFGE analysis and organisation of the study.
- Agreements with EFSA and ECDC on qualifying NRLs for uploading PFGE data in the database and on cluster analysis.
- If applicable: report on training of one or more NRLs for PFGE analysis.
- If applicable: report on typing of (a selection of) *Salmonella* isolates.



Report on the progress of the feasibility studies in relation to: end 2013

- Usefulness of data obtained with other molecular typing methods for the database.
- Usefulness of a 'new' molecular typing method like 'whole genome mapping'.

Mrs. Drs. K.A. Mooijman  
Head EURL-*Salmonella*  
Bilthoven, 8 October 2012

Annexes (as separate documents):

- Performance Indicators activities EURL-*Salmonella* 2013
- Estimated budget EURL-*Salmonella* 2013, including costs per activity (not to be published on the website)