

# EURL-*Salmonella* work-programme 2012

## 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 research (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).

## **1. Interlaboratory comparison studies**

For 2012 it is planned to organise 3 interlaboratory comparison studies;

- One study on bacteriological detection of *Salmonella* in a veterinary 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 2012 will be planned with the NRLs-*Salmonella*, but an indication of the probable time period is given below.

In 2011 for the first time lenticules were used as reference materials in interlaboratory comparison studies for the detection of *Salmonella* in veterinary and food samples. In earlier years capsules were used for this purpose. The main advantage of the lenticules is that they are easier to dissolve, so that no special reconstitution procedure is needed. Because of this advantage, it is better possible to mimic the pre-treatment of routine samples in the interlaboratory comparison studies. Furthermore, the production process of the lenticules is more flexible than the production process of the capsules so that it may be possible to introduce other serovars in samples of the interlaboratory comparison studies, in stead of only *Salmonella* Typhimurium, *S. Enteritidis* or *S. Panama*. 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.

For the interlaboratory comparison studies on detection of *Salmonella* to be organised in 2012 it is planned to use again lenticules to artificially contaminate a matrix. The choice of the serovars as well as the contamination levels will be decided per study.

## ***Interlaboratory comparison study on bacteriological detection of Salmonella in veterinary samples***

Like for former studies, a *Salmonella*-free matrix will be selected which will be artificially contaminated with *Salmonella* reference materials. At the workshop of 2011, it was agreed that the EURL will explore the possibilities for using cattle faeces or pig faeces with *Salmonella* serovars most frequently found in these types of matrices, e.g. *S. Typhimurium* and *S. Dublin* for cattle faeces and *S. Typhimurium* and *S. Derby* for pig faeces.

The following is planned for the 2012 veterinary ring trial:

- Time period: February/March;
- Matrix: cattle faeces or pig faeces;
- Number of samples and level of reference materials to be decided later (depending on the outcome of the pre-tests);
- Methods: 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 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 2012 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 2012 will be discussed with the NRLs at the EURL-*Salmonella* workshop of 2012.

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 2012.

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***

Up to 2008, EnterNet laboratories (analysing human isolates) had the possibility to participate in the typing studies through a collaboration between the EURL-*Salmonella* and the Health Protection Agency (HPA), Colindale (UK). In fall 2008, this collaboration was formalised in a contract with the ECDC. This collaboration was also profitable for the NRLs, as it still gave them the opportunity to test their phage typing capacities additional to the serotyping of *Salmonella* in the interlaboratory comparison study on typing. However, the contract with the ECDC ended in August 2011, so that also the formal collaboration with HPA ended. To give the NRLs still the opportunity to test their phage typing capacities, a sub-contract with HPA will be needed to make sure that the expertise of HPA is still available for the study in 2012.

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. As not all NRLs are interested in such a type of serovar, it was agreed to add one 'reptile-serovar' as additional ('twenty-first') strain in the study of 2011. The results found with this serovar will not be taken into account for the evaluation of the performance of the laboratory. It will be discussed at the workshop of 2012 whether such a serovar shall be added to the typing study of 2012 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 2012.

In 2007 a definition on the evaluation of 'good performance' of the serotyping was agreed upon 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 2012, 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 will be needed 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 2012	May 2012	September 2012
Detection of <i>Salmonella</i> in food/feed samples	Sept./Oct. 2012	December 2012	May 2013
Typing of <i>salmonella</i>	Nov./Dec. 2012	February 2013	July 2013

<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**

The annual workshop is planned to be organised in the Netherlands in May/June 2012 and will last 1,5-2 days. The exact place of the workshop will be decided later.

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 2011 and 2012;
- 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 the draft regulation concerning the financial aid to the EU reference laboratories (SANCO/10536/2011 Rev.1), 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.rivm.nl/crissalmonella](http://www.rivm.nl/crissalmonella)): within a few weeks after the workshop.
- Draft report of the workshop, including a summary of the discussion performed per item at the workshop: within 2 months after the workshop (together with the cost statement).

## **3. Research**

### ***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 2012:

- *Detection of Salmonella (EN ISO 6579-1)*: In fall 2011, most likely, the first working draft of the EN/ISO document will be sent to the members of CEN/TC275/WG6 and ISO/TC34/SC9 for comments and for voting. It may be necessary to discuss the outcome of this voting in the CEN Technical Advisory Group (TAG) on *Salmonella* detection, in spring 2012. As a follow up, the document will need to be amended (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 2012) by the convenor (Kirsten) of the TAG group.
- *Enumeration of Salmonella (ISO 6579-2)*: Due to administrative problems in CEN, the launching of the final vote of the document is delayed. It is hoped that the document can be finished in 2012. Before the final publication, it may be necessary to introduce some last (editorial) amendments to the document (by Kirsten Mooijman).
- *Guide for serotyping Salmonella spp. (ISO 6579-3)*: In fall 2011, most likely, the first working draft of the EN/ISO document will be sent to the members of CEN/TC275/WG6 and ISO/TC34/SC9 for comments and for voting. It may be necessary to discuss the outcome of this voting in the ISO ad hoc group in spring 2012. As a follow up, the document will need to be amended (action by the convenor of the group – Kirsten Mooijman), before it can be sent around for a new voting round. The progress of the ad hoc group with the document needs to be presented at the plenary meeting of CEN and ISO (spring 2012) by the convenor (Kirsten) of the ad hoc group.

### *Other activities related to CEN*

CEN mandate on validation of microbiological methods: In 2007 the EURL-*Salmonella* was assigned as project leader for the validation of Annex D of ISO 6579. By then the EURL- *Salmonella* also submitted a work plan and budget forecast. By the end of 2010 the contract was signed between CEN and the EC. Next, subcontracts needed to be

made between CEN (Afnor, France) and each project leader. This will hopefully be arranged before the end of 2011, so that the validation study for Annex D of ISO 6579 can be performed in 2012. The main part of the validation study is the organisation of an interlaboratory comparison study in which at least ten laboratories should produce acceptable results. To be sure that sufficient data will be available, it is the intention to ask at least 15 laboratories to participate. The EURL-*Salmonella* will invite NRLs for *Salmonella* to participate in the study.

Although the work for this CEN mandate will be performed under a separate contract from the EURL-*Salmonella*, it is still the expertise of the EURL which is needed to perform the study. Therefore, information on this study is given here as well.

### **Methods**

If necessary, literature research 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.

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 are tested:

- Of each of the following three *Salmonella* serovars two strains are tested: Enteritidis, Typhimurium and 1,4,[5],12:i:-;
- The strains will be subjected to different types of stress: cold stress, freeze stress and heat stress;
- Four types of poultry meat will be tested: chicken and turkey meat with and without skin. At least five different samples of each type of meat will be tested;
- Dry pooling (pooling of sample units) as well as and wet pooling (pooling of pre-enriched cultures) will be tested;
- For the analyses ISO 6579 (2002) and Annex D of ISO 6579 (2007) are followed.

Due to the large study design, not all combinations may already be fully tested in 2011. Therefore, it may be necessary to continue the study in 2012.

### **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.

Research 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.

### **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 2012. Location: Belgium.

- Meetings of several working groups or ad hoc 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 2012
- Publication of ISO 6579-2: end 2012
- New draft version of EN ISO 6579-3: fall 2012
- Report of relevant items in relation to standardisation as discussed at the plenary meetings of ISO/TC34/SC9 and CEN/TC275/WG6: summer 2012

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 draft manuscript: end 2012

#### *Reference materials and matrices*

- Results of research performed to test optimal matrix and 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 with respect to certain issues (e.g., methods, participation in working groups, advices) are raised. In these 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 (2012).
- March 2013

## 5. Communication

A staff member of the EURL-*Salmonella* has been trained to keep the EURL-*Salmonella* website up to date ([www.rivm.nl/crlsalmonella](http://www.rivm.nl/crlsalmonella)).

In 2011, the RIVM website has been amended. Therefore, it is necessary to amend the EURL-*Salmonella* website as well. However, in August 2011, no start was yet made with the amendment of the English version of the RIVM website. It is therefore expected that the amendment of the EURL-*Salmonella* website will continue in 2012. With this amendment of the site, it will also be investigated whether it is possible to change the name in the url (from 'CRLSalmonella' to 'EURLSalmonella'). Furthermore, also the logo of the EURL-*Salmonella* will be reviewed.

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

Results on the interlaboratory comparison studies, the workshop and relevant research 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 research 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. An overview of 7 years of interlaboratory comparison studies on detection of *Salmonella* in veterinary and food samples was published in 2011. It will be investigated whether a publication of a similar summary for the typing studies is possible in 2012.

### Output in relation to activity 5

#### *Website*

- Keeping the EURL-*Salmonella* website up to date: continuously
- Amending the EURL-*Salmonella* website to the new RIVM demands, including investigation to the possibility of a new url: end 2012
- Investigation for a new logo of the EURL-*Salmonella*: end 2012

#### *Newsletter*

Publication of 4 newsletters through the website: 04-2012; 07-2012; 10-2012; 01-2013

#### *Trend analyses interlaboratory comparison studies*

- Draft review of several interlaboratory comparison studies on (sero)typing of *Salmonella*: end 2012



## 6. Training

On request of an NRL, the EURL can give a training for a specific need of an NRL. 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 2012, in the annual technical report of the EURL-*Salmonella*. March 2013

Mrs. Drs. K.A. Mooijman  
Head EURL-*Salmonella*  
Bitlhoven, 29 August 2011

### Annexes:

- Progress report EURL-*Salmonella* 2011 (up to August 2011)
- Estimated budget EURL-*Salmonella* 2012, including costs per activity (not to be published on the website)