National Institute for Public Health
and the Environment
*Ministry of Health, Welfare and Sport*

# The 18th EURL-*Salmonella* workshop

*30 May 2013, St. Malo, France*

RIVM report 330604030/2013

K.A. Mooijman

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# Colophon



K.A. Mooijman

Contact:
K.A. Mooijman
Centre for Zoonoses and Environmental Microbiology
kirsten.mooijman@rivm.nl

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# Abstract

**The 18th EURL-*Salmonella* workshop**
30 May 2013, St. Malo, France

This report contains the summaries of the presentations of the 18th annual workshop for the National Reference Laboratories (NRLs) for *Salmonella*, held in St. Malo, France on 30 May 2013. The aim of this workshop is to facilitate the exchange of information on the activities of the NRLs and the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*). An important yearly item on the agenda is the presentation of the results of the annual ring trials organized by the EURL, which provide valuable information on the quality of the work carried out by the participating NRL laboratories. Another yearly item is the presentation of the most recent European summary report on Zoonoses by the European Food Safety Authority (EFSA). This latter report gives an overview on the number and types of zoonotic micro-organisms that were causing health problems in Europe in 2011. For several years, the number of health problems caused by *Salmonella* has been decreasing, but in 2011 it was still the second most significant cause, after *Campylobacter*, of zoonotic diseases in Europe.

Other presentations give information on the molecular typing databases which are built by EFSA and by the European Centre for Disease Prevention and Control (ECDC). The database of EFSA is intended for the storage of molecular typing data of pathogens isolated from food, animal feed or animals. The one of ECDC will contain information gathered from pathogens isolated from humans. Each strain has its unique molecular typing pattern. The molecular typing data in both databases can be useful for comparing strains from different sources. This knowledge can contribute to find the source of a European or national food-borne outbreak.

The workshop was organized by the EURL-*Salmonella* and is located at the Dutch National Institute for Public Health and the Environment. The main task of the EURL-*Salmonella* is to evaluate the performance of the European NRLs in detecting and typing *Salmonella* in different products.

Keywords:
EURL-*Salmonella*, NRL-*Salmonella*, *Salmonella*, workshop 2013

# Rapport in het kort

**De achttiende EURL-*Salmonella* workshop**

30 mei 2013, St. Malo, Frankrijk

In dit rapport zijn de verslagen gebundeld van de presentaties van de achttiende jaarlijkse workshop voor de Europese Nationale Referentie Laboratoria (NRL's) voor de bacterie *Salmonella* (30 mei 2013). Het doel van de workshop is dat het overkoepelende orgaan, het Europese Referentie Laboratorium (EURL) *Salmonella*, en de NRL's informatie met elkaar kunnen uitwisselen. Daarnaast worden de resultaten gepresenteerd van de ringonderzoeken van het EURL, waarmee de kwaliteit van de NRL-laboratoria wordt aangegeven. Een uitgebreidere weergave van de resultaten worden per ringonderzoek in aparte RIVM-rapporten opgenomen.

***Campylobacter* en *Salmonella* belangrijkste veroorzakers zoönosen**

Een terugkerend onderwerp is het jaarlijkse rapport van de European Food Safety Authority (EFSA) over zoönosen, oftewel ziekten die van dieren op mensen kunnen overgaan. Het verslag daarover bevat een overzicht van de aantallen en types zoönotische micro-organismen die in 2011 gezondheidsproblemen veroorzaakten in Europa. Hieruit blijkt dat *Salmonella* al een aantal jaren minder gezondheidsproblemen veroorzaakt, maar nog steeds, ná de *Campylobacter*-bacterie, de belangrijkste veroorzaker is van zoönotische ziekten in Europa.

**Databanken voor opslag van moleculaire typeringsdata**

Andere verslagen geven informatie over databanken die momenteel worden gebouwd door de EFSA en het European Centre for Disease Prevention and Control (ECDC). De EFSA-databank gaat informatie bevatten over moleculaire typering van ziekmakende bacteriën (pathogenen) die worden gevonden in voedsel, diervoeder en dieren. Die van het ECDC zal deze informatie bevatten van pathogenen gevonden bij de mens. Iedere bacteriestam heeft een eigen unieke moleculaire typering. Door de informatie uit de twee databanken te koppelen, kunnen bacteriestammen in producten en mensen worden achterhaald. Die kennis kan eraan bijdragen de bron te vinden van een, nationale of Europese, voedsel-gerelateerde uitbraak.

De organisatie van de workshop is in handen van het EURL voor *Salmonella*, dat onderdeel is van het RIVM. De hoofdtaak van het EURL-*Salmonella* is toezien op de kwaliteit van de nationale referentielaboratoria voor deze bacterie in Europa.

Trefwoorden:
EURL-*Salmonella*, NRL-*Salmonella*, *Salmonella*, workshop 2013

# Contents

# Summary

On 30 May 2013, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organized its annual workshop in St. Malo, France with the help of the organizer of the International Symposium for *Salmonella* and Salmonellosis (I3S), which was organized in the three days preceding the workshop. Participants of the workshop were representatives of the NRLs for *Salmonella* from all EU Member States, of three European Free Trade Association (EFTA) countries and of five EU candidate countries. Furthermore, representatives of the European Commission Directorate General for Health and Consumer Protection (DG-Sanco), as well as of the European Food Safety Authority (EFSA) were also present. A total of 50 participants were present at the workshop.

During the workshop, presentations were given on the several items.
The results of the interlaboratory comparison studies as organized by the EURL-*Salmonella* in the past year were presented. This concerned the studies on detection of *Salmonella* in animal feed (September 2012) and in samples from primary production, boot socks (February 2013) and the study for typing of *Salmonella* (November 2012).
A representative of EFSA gave a presentation on the most recent European summary report on Zoonoses as published by the European Food Safety Authority (EFSA). This latter report gives an overview on the number and types of zoonotic micro-organisms that were causing health problems in Europe in 2011. For several years, the number of health problems caused by *Salmonella* has been decreasing, but in 2011 it was still the second most important cause, after *Campylobacter*, of zoonotic diseases in Europe.
Three other presentations gave information on the molecular typing databases which are built by EFSA and by the European Centre for Disease Prevention and Control (ECDC). The presentations of EFSA and ECDC were introduced by a presentation of DG-Sanco, giving the views of the European Commission (EC) on these molecular typing databases. The database of EFSA is intended for the storage of molecular typing data of pathogens isolated from food, animal feed and animals. The one of ECDC will contain information from pathogens isolated from humans. Each strain has its unique molecular typing pattern. The molecular typing data in both databases can therefore be useful for comparing strains from different sources. This knowledge can contribute to find the source of a European or national food-borne outbreak. To be able to compare the data between the two databases, it is of major importance that EFSA and ECDC cooperate in building the databases.
Another presentation was given by the EURL-*Salmonella*, showing the results of a questionnaire sent to the NRLs-*Salmonella* in April 2013. Through this questionnaire, information was gained on a) the way the NRLs test the performance of the official national laboratories, b) molecular typing methods used by the NRLs and c) the opinion of the NRLs concerning several activities of the EURL.
The workshop was finished with a presentation obout the work programme of the EURL-*Salmonella* for the next year.

All presentations given at the workshop can be found at:
http://www.eurlsalmonella.eu/Workshops/Workshop_2013

# 1 Introduction

In this report, the abstracts of the presentations given at the EURL-*Salmonella* workshop of 2013 are presented, as well as a summary of the discussion that followed the presentations. The full presentations are not provided within this report, but are available at the website of the EURL-*Salmonella*: http://www.eurlsalmonella.eu/Workshops/Workshop_2013

In this report the following information can be found:
All abstracts of the presentations of the workshop are given in chapter 2.
The results of the questionnaire, as sent to the NRLs for *Salmonella* in April 2013, are given in Chapter 3 (the questionnaire itself can be found in Annex 3).
The evaluation of the workshop is summarized in Chapter 4 (the evaluation form can be found in Annex 4).
The list of participants is given in Annex 1.
The programme of the workshop is given in Annex 2.

# 2 Thursday 30 May 2013: the day of the workshop

## 2.1 Opening and introduction

*Kirsten Mooijman, head EURL-*Salmonella*, Bilthoven, the Netherlands*

Kirsten Mooijman, head of the EURL-*Salmonella*, opened the 18th workshop of the EURL-*Salmonella*, welcoming all participants in St. Malo, France.
At this workshop, representatives of all National Reference Laboratories (NRLs) for *Salmonella* from the EU Member States, candidate EU countries and EFTA countries were present, as well as representatives of the EC, Directorate General for Health and Consumer Protection (DG-Sanco) and the European Food Safety Authority (EFSA).
After a roll call of the delegates, the results of the evaluation of the last two workshops (2011 and 2012) were compared, showing an improvement in several general items for the workshop of 2012. The opinion on the scientific programme was the same in both workshops: good to excellent.
Next, the participants were informed that the EURL-*Salmonella* has gone more 'digital', expressed in the fact that for the reporting of the results of the last two interlaboratory comparison studies, web-based forms were used.

Finally, some information on developments in EN ISO standards was given.
A summary was given on the state of play with the *Salmonella* standard methods:

*Draft EN ISO 6579-1: Horizontal method for the detection of* Salmonella

- Early May 2013: an update of the draft document was sent to the CEN Task Group, Tag 8 to check the amended content.
- Next, validation data (obtained in the project on the CEN Mandate – see 2.8) of Modified Semi-Solid Rappaport Vassiliadis (MSRV) agar used as selective enrichment for detection of *Salmonella* in samples from primary production need to be added.
- The next voting round (CEN-enquiry/ISO-DIS voting) can then hopefully be launched by the second half of 2013.
- Because after this voting step yet another voting step is needed, the publication of the final document is not expected before the end of 2014/early 2015.

*EN ISO/TS 6579-2: Enumeration of Salmonella by a miniaturized Most Probable Number technique*

The final document of this standard was published in November 2012.

*EN ISO/TR 6579-3: Guidance for serotyping of* Salmonella *spp.*

- Early March 2013: an update of the draft document was sent to the ISO-secretariat to launch the final vote (expected by summer of 2013).
- As only one voting round is needed for a guidance document, it is expected that the final document will be published in 2014.

Information on other EN ISO standards of possible interest for the NRLs:
Published in March 2013:

- EN ISO 13307 Microbiology of food and animal feed – Primary production stage – Sampling techniques

- EN ISO 6887-6 Microbiology of food and animal feed – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 6: Specific rules for the preparation of samples taken at the primary production stage

Under preparation:
- EN ISO/TS 17728 Microbiology of food and animal feed – Sampling techniques for microbiological analysis of food and feed samples: final vote until mid-June 2013

The workshop started after explaining the programme and after giving some general information concerning the workshop.
The programme of the workshop is presented in Annex 2.

## 2.2 EU *Salmonella* monitoring data (Summary report 2011)

*Valentina Rizzi, EFSA, Parma, Italy*

The role of the European Food Safety Authority (EFSA) is to assess and communicate about all risks associated with the food chain. Within its remit, the Authority collects and analyses scientific data to ensure European food safety risk assessment is supported by the most complete scientific information available. EFSA's Biological Monitoring Unit is in charge of the collection of data on zoonoses. The Member States (MSs) and some other reporting countries submit data each year on zoonoses, zoonotic agents, antimicrobial resistance, microbiological contaminants, food-borne outbreaks and animal populations to the European Commission (EC) and EFSA. The Biological Monitoring Unit, in collaboration with the European Centre for Disease Prevention and Control (ECDC), analyses the data and produces the annual European Union Summary Reports (EUSRs). The Biological Monitoring Unit also analyses the results from EU-wide baseline surveys on zoonotic agents in animals and food. These are fully harmonized surveys carried out across the European Union (EU) MSs. The results have been published for *Salmonella* in holdings of laying hens, in flocks of broilers and turkeys, in slaughter pigs and in holdings with breeding pigs, and for *Salmonella* on broiler carcasses and *Campylobacter* in broiler batches and on broiler carcasses.
According the EUSR on zoonoses of 2011 (EFSA, 2013), salmonellosis was again the second most frequently reported zoonotic disease in humans, following campylobacteriosis. However, the incidence of salmonellosis continues to decrease in the EU with a statistically significant trend observed in the last four years. It is assumed that the observed reduction in salmonellosis cases is mainly a result of the successful *Salmonella* control programmes in poultry populations. Most MSs met their *Salmonella* reduction targets for poultry, and *Salmonella* is declining in these animal populations. In foodstuffs, *Salmonella* was mainly reported in fresh broiler meat and products thereof, and the food categories with the highest proportion of products not complying with the EU *Salmonella* criteria were foods of meat origin. *Salmonella* was also the major causative agent of the reported food-borne outbreaks, even though *Salmonella* outbreaks continued to decline in 2011. Many types of foodstuffs were implicated as food vehicles in the *Salmonella* outbreaks, but eggs and egg products were once again the main food vehicle reported.

**Discussion**

**Q:** What does 'unknown evidence of outbreaks' mean?
**A:** Analytical epidemiological evidence can show the cause of the outbreak, but no organism is isolated from the food.
**Q:** Is it possible to give more information on the outcome of the cost-benefit study for pigs?
**A:** The cost-benefit study is still ongoing. The study on slaughterhouses is expected to be published in June 2013. After this study is finalized, a decision will be made on possible studies in pigs.
**Q:** Do the figures in the report include the data of the outbreak in Germany of Shiga-toxine/Verotoxine producing *E. coli*?
**A:** Yes these data are also included, but are mainly shown in the number of human cases.
**Q:** In broilers, the number of positives for *Salmonella* Enteritidis (SE) or *Salmonella* Typhimurium (STM) is low, what about the other serovars?
**A**: Information on the other serovars is available in the report.
**Q:** Is it considered to change the current European regulations to include targets for more *Salmonella* serovars?
**A:** This is not under consideration by DG-Sanco. The targets for SE and STM were based on an EFSA opinion. These two serovars are found in all Member States (MSs) of the EU, while other, specific serovars may cause local problems and can (generally) be considered as single events. Therefore, the EC legislation will concentrate on SE and STM. In case of a local problem, e.g. in case of the persistent presence of a specific serovar, it is important that a MS takes additional measures on its own.

## 2.3 Results interlaboratory comparison study on detection of *Salmonella* in animal feed II - 2012

*Angelina Kuijpers, EURL*-Salmonella, *Bilthoven, the Netherlands*

In September 2012, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organized the second interlaboratory comparison study on detection of *Salmonella* in an animal feed matrix: poultry feed, mixed meal for laying hens. Participants were 34 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*): 29 NRLs from 27 EU Member States (MS), 2 candidate EU MSs and 2 NRLs from member countries of the European Free Trade Association (EFTA) and 1 NRL from a third country (non-Europe).

The most important objective of the study was to test the performance of the participating laboratories for the detection of *Salmonella* at different contamination levels in an animal feed matrix. To do so, chicken feed samples of 25 grams each were analysed in the presence of reference materials (being lenticule discs) containing *Salmonella* at various contamination levels. The performance of the laboratories was compared to criteria of good performance. In addition, a comparison was made between the prescribed methods (ISO 6579: Anonymous, 2002) and the requested method (Annex D of ISO 6579: Anonymous, 2007). For the prescribed method, the selective enrichment media were Rappaport Vassiliadis Soya broth (RVS) and Mueller Kauffmann Tetrathionate novobiocin broth (MKTTn). For the requested method, the selective enrichment medium was Modified Semi-solid Rappaport Vassiliadis (MSRV) agar. Optionally, a laboratory could also use an Own method, such as PCR, for the detection of *Salmonella.*

In comparison with former EURL-*Salmonella* interlaboratory comparison studies, a lower number of samples were tested, containing only one *Salmonella* serovar. For the number of samples and its contamination levels, CEN/ISO /TS 22117 (Anonymous, 2010) was followed.
Twenty-three individually numbered lenticule discs had to be tested by the participants for the presence or absence of *Salmonella*. Eighteen lenticule discs had to be examined in combination with each 25 grams of *Salmonella* negative chicken feed: six lenticule discs contained approximately eight colony-forming units (cfu) of *Salmonella* Enteritidis (SE8), six lenticule discs contained approximately 50 cfu of *S*. Enteritidis (SE50) and six lenticule discs contained no *Salmonella* at all (blank lenticule discs). The other five lenticule discs, to which no chicken feed had to be added, were control samples, comprising two lenticule discs SE8, one lenticule disc SE50 and one blank lenticule disc.

The laboratories found *Salmonella* in 94-97% of the (contaminated) samples, depending on the selective enrichment medium used. The accuracy rates for the prescribed selective enrichment media for food, MKTTn and RVS were 98% and 96% respectively. For the requested method (MSRV), the accuracy rate was 97%. A comparison between the different media did show a significant higher sensitivity rate for the low-level SE contaminated chicken feed samples when analysed with selective enrichment medium MKTTn.
Longer incubation (additional 24 hours) of MSRV resulted in more positive results, which was most clear for the low-level SE contaminated chicken feed samples (8% more positive results).
PCR was used as an own method by five participants. The laboratories scored all tested samples correctly with the PCR method used. One NRL found better results with the PCR than with the bacteriological culture methods.

Thirty out of 34 laboratories achieved the level of good performance at once. One NRL (EU-MS) did not perform the study due to organizational problems and this was considered as an incident. One NRL reported a positive result for a blank sample, which was indicated as transcription error after the reporting deadline. The performance of this NRL was indicated as moderate. Two laboratories, one EU-MS and one candidate EU-MS reported false positive blank control samples. For these two NRLs, a follow up study was organized in January 2013. One NRL (EU-MS) showed repeatedly deviating results in ring trials with animal feed as a matrix and the EURL-*Salmonella* visited this laboratory while they performed the follow-up study. Both poorly performing laboratories found false positive blank control samples again and did not reach the desired performance level. The EC, DG Sanco was informed about the deviations and the underperformances of both NRLs.

More details of the study can be found in the interim summary report (Kuijpers and Mooijman, 2012) and in the final report (Kuijpers et al., 2013a).

**Discussion**

**Q:** Did you observe any differences between different brands of MSRV?
**A:** No, we did not observe these kinds of differences.
**Q:** Can you explain why MKTTn performed somewhat better in this study than RVS or MSRV?
**A:** The samples of this study contained much disturbing background flora and caused problems with the isolation of *Salmonella* from the isolation media after selective enrichment. For some matrices, a certain selective enrichment medium or a combination of selective enrichment medium and isolation medium may

give better results than for other matrices. For the samples in the current study, MKTTn seemed to have been the optimal selective enrichment medium.

## 2.4 Results on serotyping of *Salmonella* of the 17th interlaboratory comparison study on typing (2012)

*Wilma Jacobs, EURL-*Salmonella*, Bilthoven, the Netherlands*

The 17th interlaboratory comparison study on serotyping and phage typing of *Salmonella* spp. was organized by the European Reference Laboratory for *Salmonella* (EURL-*Salmonella*, Bilthoven, the Netherlands) in cooperation with Public Health England (PHE, London, United Kingdom) in November 2012.
A total of 31 laboratories participated in this study. These included 28 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella)* in the 27 EU Member States, 1 NRL of an EU-candidate country and 2 NRLs of EFTA countries. The main objective of this study was to check the performance of the NRLs for typing of *Salmonella* spp. and to compare the results of typing of *Salmonella* spp. among the NRLs-*Salmonella*. All NRLs performed serotyping of the strains. NRLs of the EU member states which did not achieve the level of good performance for serotyping have to participate in a follow-up study.

A total number of 20 *Salmonella* strains had to be serotyped by the participants. As discussed at the previous EURL-*Salmonella* Workshop, one additional strain from an uncommon source and subspecies was included in the study. Serotyping of this strain was optional and results were not included in the evaluation. The strains had to be typed with the method routinely used in the laboratory, following the White-Kauffmann-le Minor scheme (Grimont and Weill, 2007).

The individual laboratory results were reported to the participants in January 2013. An interim summary report on the outcome of the study was prepared and sent to all participants in February 2013.
The serotyping results showed that the O-antigens were typed correctly by 24 of the 31 participants (77%). This corresponds to 99% of the total number of strains. The H-antigens were typed correctly by 19 of the 31 participants (61%), corresponding to 98% of the total number of strains. A total of 17 participants (55%) gave all correct serovar names, corresponding to 96% of all strains evaluated.
A completely correct identification by all participants was obtained for ten strains: *S.* Agama (S1), *S.* Infantis (S3), *S.* Poona (S5 and S12), *S.* Heidelberg (S11), *S.* Lexington (S13), *S.* Typhimurium (S14), *S.* Enteritidis (S16), *S.* Virchow (S17), and *S.* Orion (S18).
Most problems occurred with the serovar *S*. Galiema (S15). Seven laboratories had difficulties correctly assigning the correct serovar name to this strain, though this sometimes was caused by the (partly) nontypable nature of the strain.
All but one participant actually did serotype the additional strain S21, being a *Salmonella enterica* subspecies *houtenae* 44:$z_4$,$z_{32}$:-. However, the biochemical identification of the strain was disturbed by the presence of a non-*Salmonella* strain, which only became apparent after prolonged storage. In addition, over 50% of the participants also reported the presence of $z_{23}$ for this strain.

Two participants did not meet the level of good performance at the first stage of the study and these laboratories participated in the follow-up study in March

2013 by serotyping an additional ten strains. Both participating EU NRLs achieved a good performance on their results in the follow-up study.

More details of the study can be found in the interim summary report (Jacobs-Reitsma et al., 2013a) and in the final report (Jacobs-Reitsma et al., 2013b).

**Discussion**

**Q:** One strain (no 12) showed a deviating reaction with some antisera. Was this problem caused by antiserum of one manufacturer?
**A:** We were not able to trace the problem. The information we have received in relation to this problem will be summarized in the report on the study.
**Q:** We regularly observe problems with the serotyping of one or more strains. Do you have any suggestions on how to solve this problem?
**A:** This may be a problem with the quality of the antisera. Antisera of good quality are expensive and this may be a problem for some laboratories. Furthermore, it is important always to follow the instructions of the manufacturer carefully. Furthermore, it may be useful to test (new) antisera with control strains (e.g. to store the serovars from the interlaboratory comparison studies on typing).
**Q:** In our country, the serotyping of *Salmonella* is performed by another laboratory (analysing human samples). Can they take part in the EURL-*Salmonella* interlaboratory comparison study on typing?
**A:** For the analyses of the samples in the interlaboratory comparison studies, it is important to follow the normal routine procedures as much as possible. If this includes the fact that the isolates are sent to another (typing) laboratory, this can be done for the Proficiency Testing (PT) schemes as well. However, the NRL will remain responsible for the timely analyses and reporting, and will remain the contact for the EURL.

## 2.5 Results on phage typing of *Salmonella* of the 17th interlaboratory comparison study on typing (2012)

*Elizabeth de Pinna, Public Health England (PHE), London, United Kingdom*

The *Salmonella* strains for phage typing in the 17th interlaboratory comparison study on the typing of *Salmonella spp.* organized for the National Reference Laboratories (NRL) were provided by the Gastrointestinal Bacteria Reference Unit (GBRU) of Public Health England (PHE), London, United Kingdom. Ten strains of *Salmonella* Enteritidis and ten strains of *Salmonella* Typhimurium were selected from the culture collection of PHE.
Six NRLs performed phage typing of the *S*. Enteritidis strains and of the *S.* Typhimurium strains.
Three of the NRLs correctly phage typed all ten strains of *S*. Enteritidis. One of the NRLs correctly typed nine of the *S*. Enteritidis strains. One NRL correctly phage typed eight of the *S*. Enteritidis strains and one NRL correctly typed seven of the ten *S*. Enteritidis strains. Six of the ten *S*. Enteritidis strains were phage typed correctly by all the participating laboratories. Three strains, E4 (PT 11), E7 (PT 63) and E10 (PT 29) were incorrectly phage typed by two of the participating laboratories.
In the phage typing of *S*. Typhimurium by the NRLs, two of the participating laboratories correctly typed all ten strains. Three NRLs correctly typed nine *S*. Typhimurium strains and one NRL correctly phage typed eight of the ten *S*. Typhimurium strains. Seven of the *S*. Typhimurium strains were correctly phage typed by all the participating laboratories. One strain T6 (DT 2) was

incorrectly typed by one laboratory and one strain T9 (DT 141) was incorrectly phage typed by four laboratories.
Overall, 90% of the *S*. Enteritidis strains and 92% of the *S*. Typhimurium strains were correctly phage typed.
When compared to the previous two studies, the results of the NRLs for the phage typing of *S*. Enteritidis were better than in 2011, when 87% of the strains were correctly typed, but not quite as good as the 2010 study, when 98% of the strains were correctly typed. For the phage typing of *S.* Typhimurium, the results of this study were not quite as good as the studies in 2010 and 2011, when 98% of the strains were correctly phage typed. This was due to the problems with the phage typing of *S*. Typhimurium strain T9 (DT 141).

More details of the study can be found in the interim summary report (Jacobs-Reitsma et al., 2013a) and in the final report (Jacobs-Reitsma et al., 2013b).

**Discussion**

**Q**: We observed some problems with phage typing of *Salmonella* Typhimurium PT101. Do you have any explanations?
**A**: Sometimes there may be some low reactions with the phages, which can be caused by an inoculum that is too low or too high. For *S.* Typhimurium, it is often more difficult to get the right inoculum.
**Q:** Although phage typing is an important procedure to subtype isolates, it is only performed by six NRLs. Will it still be worthwhile to invest in this method, or may it disappear in the short term?
**A:** It is a typing method which is more often used in laboratories that analyse human samples. For the time being, phage typing is still used, often in combination with 'new' molecular typing methods. PHE has no intention of stopping the production of the phages in the short term. Whether or not a laboratory should invest in introducing phage typing is difficult to say. The procedure is not difficult, but the reading of the plates needs special expertise.
**Remark**: The Statens Serum Institute (SSI) in Denmark organizes ring trials for typing of *Salmonella* for the 'human laboratories' under contract with ECDC. In these studies, the number of laboratories participating in the part focused on phage typing is decreasing and therefore ECDC recently decided to stop including phage typing in the annual interlaboratory comparison studies.

## 2.6 Miscellaneous activities EURL and NRLs

*Kirsten Mooijman, head EURL*-Salmonella*, Bilthoven, the Netherlands*

In April 2013, a questionnaire was sent to all NRLs for *Salmonella* aiming to obtain more (detailed) information on some of the activities of the NRLs and to get the opinion of the NRLs on some of the activities of the EURL for *Salmonella*. The questionnaire was sent to a total of 36 NRLs for *Salmonella* and 24 completed questionnaires were returned to the EURL, which is a response rate of 67%. Details on the responses to the questionnaire are given in Chapter 3 of this report.

**Discussion**

**Q**: What is the opinion of DG-Sanco on the fact that some NRLs do not organize ring trials themselves but, for example, ask the official national laboratories to participate in a commercial Proficiency Testing (PT) scheme and ask them to send the results to the NRL to assess the performance.

**A**: In two EU Regulations, reference is made to the collaborative testing organized by the NRLs. Regulation 882/2004 (EC, 2004b) states in Article 33, as one of the tasks of an NRL: 'where appropriate, organize comparative tests between the official national laboratories and ensure an appropriate follow-up of such comparative testing'. While Regulation 2160/2003 (EC, 2003b) states in Article 12: 'Laboratories shall regularly participate in collaborative testing organized by the national reference laboratory'. The problem is that several NRLs are not able to organize comparative tests themselves, due to lack of funding and/or due to lack of knowledge. It could then be acceptable to use commercially prepared/organized comparative tests and for the NRL to receive the results of all official laboratories to monitor their performances.
**Q:** Is it a requirement that the organiser of the PT schemes is accredited?
**A:** For the selection of external (commercial) organizers, this could be a selection criterion. However, for organization by an NRL this is often not possible.
**Q:** To include molecular typing (like PFGE) in the EURL-*Salmonella* ring trials for typing is a good idea, but would enhance the amount of work of the NRLs. Would it perhaps be possible to reduce the number of strains for the part on serotyping?
**A:** The part on serotyping in the EURL-*Salmonella* studies is the only obligatory part. Phage typing and molecular typing will be optional. As the EURL typing study is organized only once a year, we came to this (high) amount of 20 strains for serotyping. However, the numbers may be reviewed again for the next study.

## 2.7 Results interlaboratory comparison study on detection of *Salmonella* in samples from primary production XVI - 2013

*Angelina Kuijpers, EURL-*Salmonella*, Bilthoven, the Netherlands*

In March 2013 the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organized the 16th interlaboratory comparison study on the detection of *Salmonella* in samples from primary production. The matrix of concern was boot socks, to which environmental material (mainly faeces) from a laying hen flock was attached.
This study was a combined study with the CEN mandate study (Validation of Annex D of EN ISO 6579 – see clause 2.8). The data are treated differently for the CEN mandate study (testing performance of the method) and for this EURL study (testing performance of the laboratories).
Participants were 36 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*): 28 NRLs from 27 EU Member States (MS), 4 candidate EU-MS: Bosnia and Herzegovina, Croatia, Former Yugoslav Republic of Macedonia (FYROM) and Serbia, 3 members of the European Free Trade Association (EFTA): Switzerland, Norway and Iceland and on request of DG-Sanco, 1 non-European NRL from a third country: Israel.

The most important objective of the study was to test the performance of the participating laboratories for the detection of *Salmonella* at different contamination levels in a matrix from the primary production. To do so, boot socks with environmental material, artificially contaminated with *Salmonella* Typhimurium at various contamination levels, were analysed. The performance of the laboratories was compared to the criteria for good performance. The prescribed method was Annex D of ISO 6579 (Anonymous, 2007), with selective enrichment on Modified Semi-solid Rappaport Vassiliadis (MSRV) agar.

The boot socks with environmental material (chicken faeces) were artificially contaminated at the laboratory of the EURL with a diluted culture of *S*. Typhimurium. This type of samples has not been used in earlier studies of the EURL-*Salmonella*. Before the start of the study, some tests were performed at the laboratory of the EURL to test the influences of: the addition of different fluids or no fluids to the boot socks, the background flora in the faeces, the ability to detect different levels of different serovars of *Salmonella* and the stability of the samples during storage at different temperatures.

In total, 30 individually numbered samples had to be tested by the participants for the presence or absence of *Salmonella*. Twenty-four of the 30 samples were boot socks with environmental material, of which eight were artificially contaminated with approximately 9 colony forming units (cfu) of *Salmonella* Typhimurium (STM low), eight with approximately 81 cfu of *S*. Typhimurium (STM high) and eight with no *Salmonella* at all (blanks). Six samples, consisting of boot socks to which no faeces was added, were control samples. Two of these control samples were artificially contaminated with STM low and two samples with STM high. To two samples no *Salmonella* was added (blank).

On average, the laboratories found *Salmonella* in 96% of the (contaminated) samples using the prescribed method, selective enrichment on MSRV. Nineteen of the 36 participants (53%) tested all boot socks with environmental material (chicken faeces) contaminated with *S*. Typhimurium positive.
Forty-eight hours of incubation of MSRV gave overall 3% more positive results compared to 24 hours of incubation.

All NRLs fulfilled the criteria of good performance.
The samples used in this study mimic better routine samples than the formerly used samples with reference materials. Furthermore, the current samples were easier to test by the participants and (very) good results were found overall. On the other hand, the preparation of the boot socks by artificially contaminating them with a diluted culture of *Salmonella* was more labour-intensive for the EURL than the preparation of the samples in the former studies.

More details of the study can be found in the interim summary report (Kuijpers and Mooijman, 2013b) and in the final report (Kuijpers and Mooijman, under preparation).

## 2.8 First results on the validation of Annex D of ISO 6579 (CEN mandate *Salmonella*)

*Kirsten Mooijman, head EURL*-Salmonella*, Bilthoven, the Netherlands*

In 2006, the European Commission (DG-Sanco) sent a mandate to CEN/TC275/WG6 (European Committee for Standardization, Technical Committee 275 for Food Analysis – Horizontal methods, Working Group 6 for Microbial contaminants). This mandate should result in the validation of 15 microbiological methods (mandate M/381). The mandate 'falls within the rules to ensure food safety in the whole food chain in relation to biological hazards'. The mandate is related to several EC Regulations, such as Regulation 882/2004 on food and animal feed control (EC, 2004b) and Regulation EC 2073/2005 on Microbiological criteria (EC, 2005). Annex D of EN ISO 6579

(Anonymous, 2007) is one of the methods to be validated under the project leadership of the RIVM.
The validation studies are not intended to compare methods, but are intended to set the performance characteristics of a method. For each validation study, an interlaboratory study (ILS) needs to be organized for which the set-up is based on the procedures as described in EN ISO 16140 (Anonymous, 2003).
For qualitative methods this includes that at least ten laboratories should participate, obtaining at least ten valid data sets per contamination level. Samples with three different contamination levels have to be tested: blank, low-level (at or slightly above the detection limit of the method) and high-level (five to ten times above the detection limit of the method). For each level, eight blind replicates have to be tested. For a horizontal method (applicable for e.g. food and feed) at least five different categories of matrices have to be analysed. For a vertical method (like primary production), only one category of matrix needs to be analysed.
Before the samples are used in the interlaboratory study, the organizer needs to have the samples tested for homogeneity and stability.

For the validation of Annex D of EN ISO 6579 (detection of *Salmonella* in primary production samples, Anonymous, 2007), the following was agreed:

- To use data of earlier organized EURL-*Salmonella* studies for the detection of *Salmonella* in animal faeces.
- To combine the study for the CEN mandate with the study of the EURL-*Salmonella* for the detection of *Salmonella* in veterinary (environmental) samples in February/March 2013. The data were treated differently for the EURL study (testing performance of the laboratories) and for the CEN mandate (testing performance of the method).

For setting the performance characteristics of Annex D of EN ISO 6579, the following, earlier organized EURL-*Salmonella* studies were selected:

- Study organized in 2008 for detection of *Salmonella* in chicken faeces (Kuijpers et al., 2008). In this study, samples of 10 g chicken faeces were each artificially contaminated with capsule reference materials containing *Salmonella* Typhimurium (STM) or *Salmonella* Enteritids (SE), both at low and high level. Also non-contaminated faeces samples were tested (blank samples). This study was selected because the results with the low-level SE samples showed 'fractional recovery' (approximately 50% of the samples were found to be positive for *Salmonella*), which is important for the calculation of one of the new performance characteristics 'LOD50' (the level of detection for which 50% of tests give a positive result). LOD50 is described in the revised (draft) version of EN ISO 16140-2 (Anonymous, 2013a).
- Study organized in 2012 for detection of *Salmonella* in pig faeces (Kuijpers and Mooijman, 2012). In this study, samples of 25 g of pig faeces were each artificially contaminated with capsule reference materials containing *Salmonella* Typhimurium (STM) or *Salmonella* Derby (SD), both at low and high level. Also non-contaminated faeces samples were tested (blank samples). This study was selected because of the use of faeces from another animal (pigs instead of chicken).
- The combined study organized in 2013. In this study boot swabs with artificially contaminated environmental material from a laying hen flock was used. The boot swabs were artificially contaminated with a diluted culture STM, at low and high levels. Furthermore, non-contaminated samples were also tested (blank samples). For more details on this study, see Clause 2.7.

Not all data from all participants could be used for the calculation of the performance characteristics. Some datasets were excluded because of technical deviations in the prescribed procedure, like deviations in incubation time or temperature of the (non-)selective enrichment step, or deviations in the novobiocin concentration of MSRV.
First, tables with the performance characteristics per matrix (per study) were prepared and the choices made will be discussed with the other project leaders of the CEN mandate. Depending on the outcome of this discussion, additional calculations will be made. Next, the performance characteristics need to be introduced in the amended draft version of EN ISO 6579-1, after which the next voting for this document can be launched.

**Discussion**

**Q**: Small deviations in pH of BPW or MSRV did not result in exclusion of data for calculation of the performance characteristics of the method. Is that still acceptable?
**A**: The pH of MSRV is most strict at the low side (should not become less than 5.1), but on the upper limit the influence on the growth of *Salmonella* may be marginal. The same is sound for small deviations in the pH of BPW. As BPW is a non-selective medium and the pH remains close to neutral (also in case of the small deviations), no problems were expected with the growth of *Salmonella*.
**Q:** Are lymph nodes considered as a 'primary production sample'? In other words, should it be analysed with Annex D of ISO 6579, with selective enrichment on MSRV (Anonymous, 2007) or with ISO 6579 (Anonymous, 2002)?
**A:** According to ISO 6887-6 (Anonymous, 2013b) and ISO 13307 (Anonymous, 2013c), lymph nodes should be considered as samples from primary production and should therefore be analysed using MSRV for selective enrichment.
**Q:** Is there a difference in quality between boot socks from different materials?
**A:** Some studies have been performed by the Animal Health and Veterinary Laboratories Agency (AHVLA) in the UK by testing different boot socks and hair nets as well, but no big differences were observed. Only the surface area seems to be of importance; the larger the surface, the better.

## 2.9 Information from DG-Sanco, including vision paper on molecular typing data

*Klaus Kostenzer, EC DG-Sanco, Brussels*

Food-borne outbreak management, investigation and reporting requires a multidisciplinary approach at the local, national and - if multinational - European level, and also across all the relevant sectors (public health and veterinary/food safety authorities). The Lisbon Treaty empowers the Union to support, coordinate or supplement actions of Member States in the areas of protection and improvement of human health, including combating serious cross-border threats.

The General Food Law (Regulation (EC) No 178/2002, EC, 2002) provides the basis for the Rapid Alert System for Food and Feed (RASFF) and for the management of emergencies and crises. The latter was further elaborated by a general plan on food/feed crisis management (Decision 2004/478/EC, EC 2004a). The Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents (EC, 2003a) is intended to ensure that food-borne outbreaks are properly monitored and subject to adequate epidemiological investigation.

On the human health side, the Union's network for the epidemiological surveillance and control of communicable diseases established under Decision No 2119/98/EC (EC, 1998) comprises the epidemiological surveillance of communicable diseases. Member States are required to provide information through the Early Warning and Response System (EWRS).

Both the European Centre for Disease Prevention and Control (ECDC) and the European Food Safety Authority (EFSA) substantially contribute to the EU approach to monitor and manage food-borne outbreaks.

One of the key elements in the EU strategy is 'prevention' of foodborne outbreaks. This is translated to the hygiene package as 'farm to fork', with the food business operators bearing the primary responsibility for the safety of the foodstuffs placed on the market. At the level of primary production, major efforts have led to the - highly successful - implementation of national control programmes to control *Salmonella* in poultry populations, which halved human salmonellosis in the EU within a couple of years.

Furthermore, 'preparedness' for foodborne outbreaks is also embedded in the General Food Law, where safeguard measures and a general plan for crisis management are provided for. The Commission has carried out a preparedness exercise on a multinational outbreak coordination in May 2013 in Luxembourg. The 'Better Training for Safer Food' programme of the Commission will begin with the first training modules on outbreak management in September 2013. A vision paper to encourage collection of molecular typing data of isolates from human cases, food and animals of foodborne pathogens has been presented by the Commission and will lead to the establishment of databases, both in ECDC and EFSA in collaboration with the EURL for *Salmonella*.

**Discussion**

**Q**: Would it be possible to include molecular typing results from *Salmonella* serovars isolated from animal feed as well?
**A**: Yes, it is the intention that these data will be included as well.
**Q:** Does your presentation on food-borne outbreaks caused by food from non-animal origin also include the number of deaths caused by the outbreak of STEC in sprouts?
**A:** No, these data were excluded.
**Q:** The mortality rate is about 10 times higher in cases of outbreaks caused by foods from non-animal origin. Do you know the reasons for this?
**A:** I do not know. It is an increasing trend, but this has not yet thoroughly analysed at EU level.
**Q:** What are the rules for decontamination of meat?
**A:** It is in general forbidden to use chemicals to decontaminate meat (or any food from animal origin) under the EU hygiene package. However, for specific purposes, proposals can be sent to the Commission, evaluated by EFSA and discussed at the SCOFCAH (Standing Committee on the Food Chain and Animal Health) and allowance may be possible. In this way, lactic acid for decontamination of beef has been submitted for evaluation by EFSA and since evidence could be shown that its use is not problematic under the EFSA assessment criteria, followed by the support of the Member States, it is currently allowed. More trials may be expected, e.g. for poultry and pig meat.

## 2.10 Activities by ECDC concerning molecular typing data from human samples

*Mia Torpdahl, Statens Serum Institut (SSI), Denmark*

The unit Foodborne Infections at Statens Serum Institut (SSI) in Denmark applied for tenders on two ECDC framework contracts concerning the European External Quality Assessment (EQA) programmes and Molecular typing database curator. The contracts were given to SSI for a period of four years and started in September 2012.
The EQA programme contains PFGE on *Salmonella*, MLVA on *S.* Typhimurium and phage typing of *S.* Typhimurium and *S.* Enteritidis. Protocols were provided to participants and trouble-shooting, individual reports, a summary report and a publication is expected from the first round of EQA. The first round is finished and 25 laboratories participated in the PFGE, 15 in MLVA and 11 and 12 laboratories in the two phage typing schemes. The results are encouraging but there is room for improvement, especially in regard to following in more detail the provided protocols. We also concluded that some laboratories could benefit from a training for the different methods.
ECDC has just started to integrate molecular methods into the surveillance at a European level with a five year plan (2012-2016) and has developed the human surveillance system for infectious diseases in Europe (TESSy system). The Objectives are to have a fast international cluster detection, detection of the emergence of new virulent strains, including resistance, and the identification of transmission routes, sources and risk factors. The data included in TESSy must be comparable between laboratories and data should be interpreted by typing experts or curators. The databases are managed by ECDC and SSI secures the quality and integrity. Senior curators at SSI are responsible for cluster analysis at least once a week, and for giving expert advice to ECDC on data interpretation, outbreak integrity, database setup, etc. Junior curators at SSI are responsible for the correct normalization of gels, for naming and managing profiles and for giving feedback to laboratories regarding gel quality and helping them to improve themselves.

### Discussion

**Q**: Would it be possible to have details on PFGE (e.g. by video) as there is no funding for training?
**A**: There may be some funding to visit a laboratory to give training. Additionally, it is also possible to give details on PFGE.

## 2.11 Activities by EFSA concerning molecular typing data from food and primary production samples

*Valentina Rizzi, EFSA, Parma, Italy*

In consultation with the European Centre for Disease Prevention and Control (ECDC), the European Food Safety Authority (EFSA) and the European Union Reference Laboratories (EURLs), the European Commission (EC) has prepared a proposal for integrating molecular typing data from different sources at the European Union (EU) level. The EC's Vision paper on the development of databases for the molecular testing of three food-borne pathogens (*Salmonella*, VTEC and *Listeria*) along the food chain and in humans has been formally approved by the Standing Committee on Food Chain and Animal Health (SCoFCAH) in December 2012. Based on this document, EFSA has been

requested by the EC to take responsibility for setting up and managing the molecular typing database on isolates from food, feed and animals at EU level in close collaboration with the three EURLs concerned and the relevant institutions (competent authorities and National Reference Laboratories (NRLs)) in the EU Member States (MSs). ECDC is in charge of collecting molecular typing data of food-borne pathogens isolated from human cases. The main objective is to facilitate the detection and investigation of multi-country, food-borne outbreaks due to *Salmonella*, Verotoxine producing *E. coli* (VTEC) and *Listeria* by comparing routinely molecular typing profiles of bacterial isolates of human and animal/food origin. The Pulsed Field Gel Electrophoresis (PFGE) will be used as a gold standard for all pathogens and Multiple-Locus Variable number tandem repeat Analysis (MLVA) will also be used for *S*. Typhimurium. Other pathogens and methods might be covered later on.
EFSA's Biological Monitoring Unit has set up a Working Group to define the structure of the data collection system for food and animal isolates and the integration with the human data. This includes common nomenclature and standardized procedures, policies for data submission, access, ownership and allowed use. The data would be submitted to EFSA primarily by the NRLs in the EU MSs. The three EURLs involved would act as curators of the data and would contribute to the data analyses. A pilot phase will be running in 2014.
Additionally, the EFSA's Biological Hazard Panel is working on a self-task on the evaluation of molecular typing methods for major food-borne microbiological hazards. The main objectives are to review information on current and prospective molecular identification and sub-typing methods; evaluate their appropriateness for outbreak investigation, attribution modelling and scanning surveillance; and consider specific requirements for surveillance activities and harmonized data collection.

**Discussion**

**Q**: Can you give an additional explanation of the meaning of the use of Whole Genome Sequencing to predict the outcome of pathogen-host interactions?
**A**: The intention is to try to get information on virulence genes of potential pathogens and to be able to intervene at an early stage.
**Q:** Do the curators of the ECDC database and of the EFSA database have contact with each other?
**A:** Representatives of 3 EURLs (who will become the curators of the EFSA database), as well as a representative from ECDC participate in the EFSA working group on molecular typing and have close contact. The intention is to draft harmonized protocols for the curation of the data of both databases.
**Q:** How many data can a curator handle on a day?
**A:** According to the EURL-*Listeria* (which already has a database for molecular typing), it is possible to manage approximately 100 profiles per day. Of course this may depend on the quality of the profiles and how they are submitted (as 'tiff' file or via Bionumerics). SSI has scheduled the curation of approximately 4000 isolates per year.

## 2.12 Work programme EURL-*Salmonella* second half 2013, first half 2014 and closure

*Kirsten Mooijman, EURL*-Salmonella*, Bilthoven, the Netherlands*

**Work programme**

Kirsten Mooijman summarized the information on the work programme of the EURL-*Salmonella* for the rest of 2013 and for early 2014.

*Interlaboratory comparison studies*

Three interlaboratory comparison studies are planned in the coming year:

- Detection of *Salmonella* spp. in food: September/October 2013. For this study it will be explored whether it is possible to use (chicken) minced meat inoculated with a diluted *Salmonella* culture at the laboratory of the EURL (instead of adding reference materials at the laboratories of the NRLs).
- Typing of *Salmonella* spp.: November/December 2013. Like in former typing studies, this study will contain an obligatory part for serotyping of 20 different *Salmonella enterica* serovars (and an additional 1 optional non-*enterica* isolate) and an optional part on phage typing of 10 STM isolates and 10 SE isolates. It was suggested that an optional (pilot) part for PFGE testing of 10 different *Salmonella* serovars be added to this study.
- Detection of *Salmonella* spp. in a sample from primary production: February/March 2014. The choice of the matrix will be decided later.

*Supporting activities*

The 'research' performed by the EURL-*Salmonella* always has a relation to the activities of the EURL. The following is planned or will be continued in the next year:

- Continuation of the activities for the standardization organizations, ISO (at international level) and CEN (at European level). If necessary, performing experiments for the revision of EN ISO 6579.
- Summarizing the results of the pooling experiments for a peer-reviewed publication.
- Testing different matrices in combination with different/new reference materials for ring trials.

Experts of the EURL-*Salmonella* regularly participate in working groups of EFSA and of DG-Sanco.
EURL-*Salmonella* will perform ad hoc activities (on its own initiative or on request) and, if needed, will support DG-Sanco or EFSA in case of outbreaks. Furthermore, training can be given by EURL-*Salmonella* at the EURL or at the laboratory of the NRL. Requests for training will be considered case by case.

*Molecular typing*

With the publication of the 'Vision paper on molecular typing data' by DG-Sanco (see clause 2.9), it is clear that the EURLs will be given an important role in judging the (quality) of molecular typing data to be entered in the new database of EFSA. Currently, staff members of the EURL-*Salmonella* participate in a newly raised working group on molecular typing of EFSA (started in April 2013). Activities foreseen are: organization of interlaboratory comparison studies on molecular typing of *Salmonella*, curation of molecular data (to start with PFGE) for the EFSA database, contribution to standardized protocols for molecular typing, training of NRLs for *Salmonella* on molecular typing.

*Other activities*

As before, the newsletter will be published four times a year through the EURL-*Salmonella* website. The NRLs are requested to provide any relevant information of interest for the other NRLs for publication through the newsletter.
The EURL-*Salmonella* website will be kept up to date with information on new activities/results.

*Workshop 2014*

The date and location of the EURL-*Salmonella* workshop to be organized in 2014 are not yet known. After organizing the workshop for two years in another country, consideration is being given to hold the next workshop in the Netherlands again. However, several NRLs kindly offered spontaneously to organize the workshop in their country (i.e. Germany, Sweden, Cyprus). These offers will be seriously considered and a further decision on date and place will be made in the second half of 2013.

**Other items**

In relation to molecular typing of *Salmonella* some items were raised:

- PCR for confirmation of the monophasic variant of *Salmonella* Typhimurium. To confirm that an isolate is a monophasic variant of *Salmonella* Typhimurium, a PCR is used by the majority of NRLs. An example of a PCR procedure is described in the EFSA opinion on '*Salmonella* Typhimurium-like strains' (EFSA, 2010), but the successfulness of the method varies per NRL. Some probable solutions to problems with the procedure were discussed at the workshop and were also discussed earlier by e-mail (e.g. concentration of primer, quality of some reagents may vary per manufacturer/batch). There was a general feeling that it would be helpful if advice on a harmonized procedure for this PCR would become available. It was agreed that the EURL-*Salmonella* will explore the possibility for preparing such a harmonized procedure. However, if the problems with the method are mainly caused by different qualities in reagents from different manufacturers or by different batches from one manufacturer, it may be hard to give advice without getting into a conflict of interests.
- Molecular serotyping. There is interest in a molecular serotyping method although, currently, only a limited number of NRLs are using such a method. The NRL from Denmark indicates the use of a multiplex PCR based on a method published by the Centre for Disease Control (CDC) in the USA. In this multiplex PCR, primers are used for the detection of O-antigens and H-antigens, so that results can still be linked to the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007). They also use this PCR for identifying monophasic variants of *Salmonella* Typhimurium.

**Closure**

Kirsten Mooijman closed the workshop, thanking all participants and speakers for their presence and contributions and thanking the staff members of the EURL-*Salmonella* and Genevieve Clement of ISPAIA-Zoopole (Ploufragan, France) for their help in organizing the workshop.

# 3 Results questionnaire EURL-*Salmonella* April 2013

## 3.1 Introduction

In April 2013, the EURL-*Salmonella* sent a questionnaire to all NRLs for *Salmonella* to obtain more (detailed) information on some of the activities of the NRLs for *Salmonella*. Furthermore, the EURL would like to get the opinion of the NRLs concerning some of the activities of the EURL-*Salmonella*.
The questions were related to three different areas and therefore the questionnaire consisted of three parts:

- Part A: Questions related to how NRLs-*Salmonella* test the performance of the official national laboratories in the relevant work field;
- Part B: Questions related to molecular typing methods used by the NRLs for *Salmonella*;
- Part C: Questions to gain opinions on some activities of the EURL-*Salmonella.*

The questionnaire itself can be found in Annex 3.

The questionnaire was sent to 36 NRLs for *Salmonella*, 28 of which were located in the EU Member States (MS), three in countries of the European Free Trade Association (EFTA) and five in the EU (potential) candidate countries (at the time of the questionnaire: Bosnia and Herzegovina, Croatia, Former Yugoslav Republic of Macedonia, Serbia and Turkey). In total, 24 completed questionnaires were returned, which is a response rate of 67%. The responses for the different groups were 21/28 NRLs of the EU-MS (75%), 3/3 NRLs of the EFTA countries (100%) and 0/5 NRLs of the candidate countries (0%).

The work fields in which the NRLs are active concern: samples from primary production, food and animal feed and typing of *Salmonella*.

## 3.2 Part A Questions related to how NRLs-*Salmonella* test the performance of the official national laboratories in the relevant work field

*A.1 How many 'official laboratories' are designated in your country for the different work fields?*

Figure 1 shows the number of official laboratories for the different work fields as reported by the NRLs (where pp stands for 'primary production'). In most of the countries the number of official laboratories is 2-10. Regularly it was also reported that the NRL is the only official laboratory for a certain work field, especially for typing of *Salmonella*.

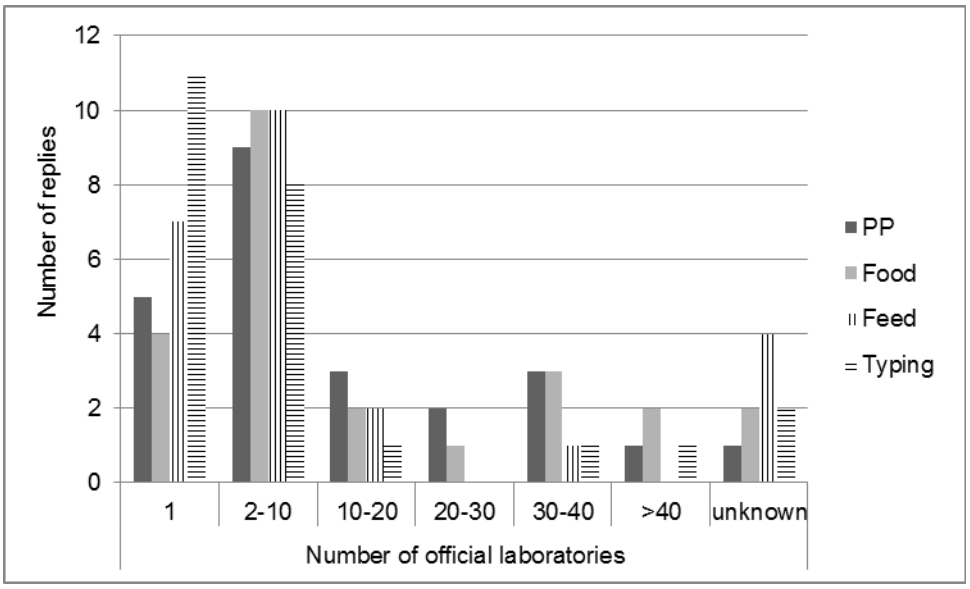


*Figure 1 Number of official national laboratories in different EU or EFTA countries, as reported by the NRLs for* Salmonella

*A.2 Do you organize comparative tests to test the performance of the official national laboratories?*

Fourteen NRLs reported that they organize comparative tests themselves for one or more work fields. In nine cases, the NRLs do not organize a comparative test themselves, but ask the official laboratories to participate in a Proficiency Test organized by another (commercial) organization and to provide the NRL with the results. In this way, the NRL is still able to check the performance of the official laboratories (for a certain work field). Four times it was reported that the NRL was the only official laboratory for a certain work field, making the organization of a comparative test unnecessary. Three NRLs reported that they do not test the performance of the official laboratories. For two NRLs, this was because they were only recently designated as an NRL and one NRL had the impression that testing the performance of the official laboratories by the NRL was not necessary since the official laboratories are accredited and meet the criteria of EN ISO 17025 (Anonymous, 2005). However, this latter fact may not be sufficient according to the tasks as described for an NRL in Regulations 2160/2003 (EC, 2003b) and 882/2004 (EC, 2004b) (also see 2.6).

*A.3 For what work field do you/other organizers organize comparative tests and with what frequency?*

Figure 2 shows the reported frequencies of the comparative tests for the different work fields. Studies are organized for all relevant work fields and, in most cases, the frequency varies between 1 to 4 times a year.

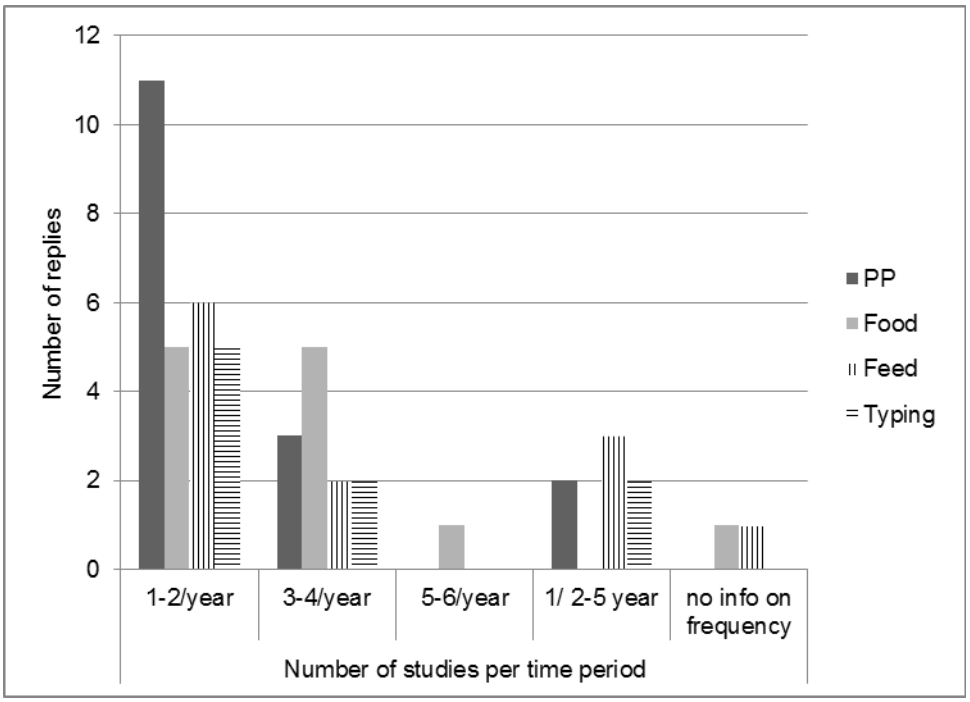


*Figure 2 Frequency of the comparative tests organized for the different work fields*

*A.4 Do you/other organizers test 'real matrices' in the detection study?*

The majority of the NRLs (13) indicated the use of a matrix in the comparative tests. The matrices most often reported were (number of reports between brackets): meat (5), animal feed (8), dairy products (6) and animal faeces (11). Other products used, but reported less frequently were: sponge/swabs (3), eggs (1), spices (2), food (general) (1) and bivalve molluscs (1).

*A.5 If 'real matrices' are used, how are they 'contaminated'?*

Generally, the contamination of the matrices was done either by inoculation with a culture (reported 11 times) or by mixing the matrix with a reference material (reported 8 times). Only one NRL reported the use of naturally contaminated samples.

*A.6 If (commercial) reference materials are used, where are they obtained?*

Many different reference materials or culture collections were reported. Lenticule reference materials from PHE (UK) and strains from the ATCC culture collection were reported most frequently.

*A.7 How many samples do you/other organizers include per study, and with what content, for the detection of Salmonella?*

Figure 3 shows the reported number of samples per study. In the majority of the comparative tests, 5-10 samples per study are used. The reported contamination levels of the samples varied largely, but in general 'low' (1-50 cfu/sample) and 'high' (≥100 cu/sample) contaminated samples are used in one study.

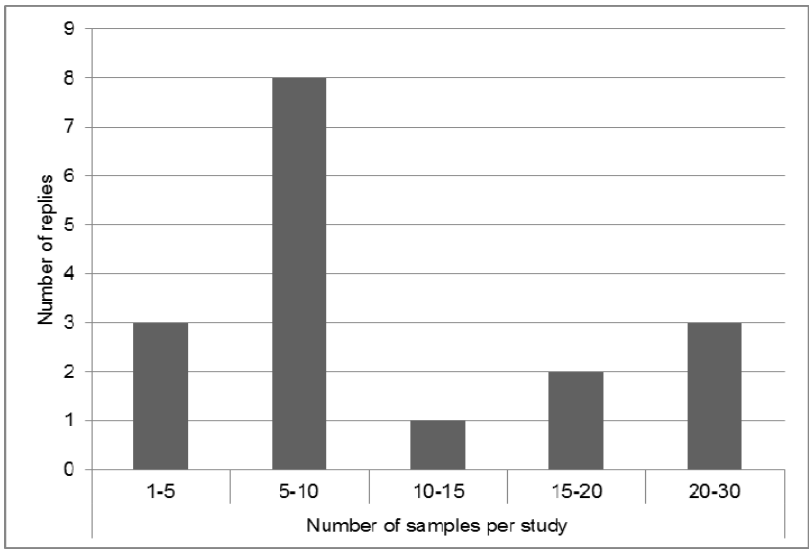


*Figure 3 Number of samples included per comparative test*

*A.8 How many Salmonella serovar(s) do you/other organizers most often include per detection study and which serovars are most often used?*

In most of the comparative tests for detection of *Salmonella* one or two different *Salmonella* serovars are included in the samples. A few NRLs reported that they include more than two different serovars, even up to a number of ten. Most often *Salmonella* Enteritidis or *Salmonella* Typhimurium are included, but the use of many other serovars are reported as well, albeit less frequently.

*A.9 If you/other organizers organize typing studies, for what typing procedures are the studies organized and how many strains are tested per study?*

If the organisation of a typing study is reported, this concerns most of the time a study for serotyping of *Salmonella*. However, a few NRLs also reported the organization of a typing study for PFGE. The number of strains to be tested is mostly 10 to 20 per study. The information is summarized in Figure 4.

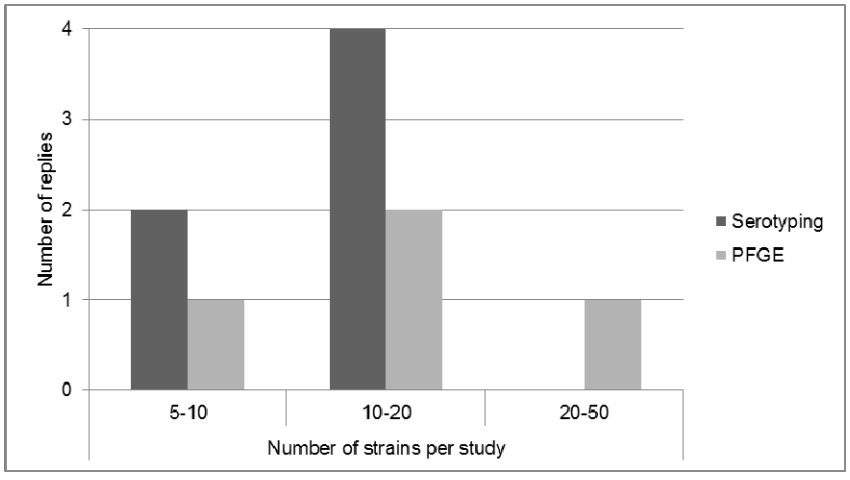


*Figure 4 Typing procedures included in comparative tests for typing of* Salmonella *and the number of strains included per study*

*A.10 Have you set criteria to judge the performance of the participating laboratories in the comparative tests?*

All, except one NRL, reported that they have criteria to judge the performance of the participating laboratories. A summary of the reported criteria are listed below.

- All samples 100% correct
- All control samples 100% correct
- All negative samples 100% correct
- Max. 1/5 wrong
- Same criteria as EURL
- Sensitivity >80%
- More than 60% correct
- Trend over more than 1 study
- Serotyping correct for top 5

All NRLs will also take actions in case of poor performance of the official laboratories, such as asking the relevant laboratory for the possible causes of the poor performance, organization of a follow-up study, or organization of a training in the relevant work field. Occasionally, poor performance can result in a (temporary) cancellation of the approval of a laboratory.

*A.11 Are you/other organizers accredited for organizing comparative tests*

Accreditation for the organization of comparative tests was mainly reported in case a (commercial) organization other than the NRL organizes the studies. Only two NRLs reported to be accredited for the organization of comparative tests itself.

## 3.3 Part B Questions related to molecular typing methods used by the NRLs for *Salmonella*

*B.1 Do you perform molecular typing of Salmonella isolates from food, feed and/or animals?*

Of the 24 NRLs completing the questionnaire, 16 reported that they perform molecular typing (67%).

*B.2 Do you perform molecular typing on a routine basis or only occasionally?*

In this question, 'routine and occasionally' were defined as follows:
Routine: all or an agreed proportion of isolates are typed yearly.
Occasionally: some isolates are typed without a special agreed plan.
The majority of the NRLs reported that they perform molecular typing occasionally, although seven NRLs reported that they perform the molecular typing routinely, depending on the source of the isolate or the molecular typing method to be applied.

*B.3 Where do the isolates come from?*

Figure 5 summarizes the number of replies to the different sources of the isolates. More than one answer could be given to this question, therefore the number of replies exceeds the number of NRLs which indicated to perform molecular typing for *Salmonella*.

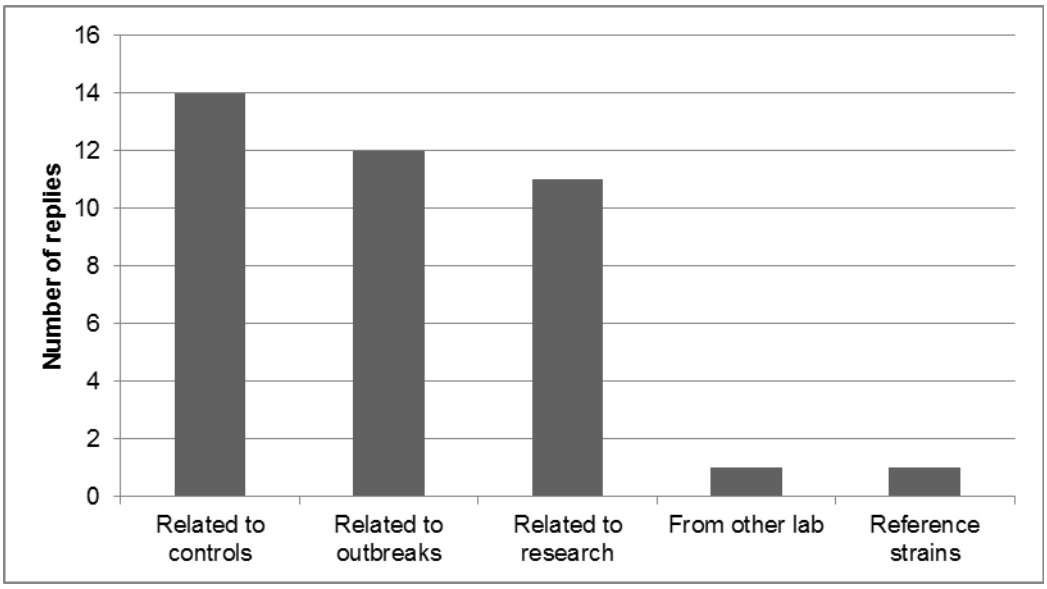


*Figure 5 Reported sources of the* Salmonella *isolates for which molecular typing is performed*

*B.4 What molecular typing methods for which Salmonella serovars are used and which protocols are followed?*

Figure 6 summarizes the molecular typing methods used by the NRLs. PFGE is used by many NRLs to type many different *Salmonella* serovars. MLVA is regularly used to type *Salmonella* Typhimurium (STM) or the monophasic variant of *Salmonella* Typhimurium and *Salmonella* Enteritidis. Furthermore, a PCR method for serotyping *Salmonella* serovars is also regularly reported, especially to type the monophasic variant of *Salmonella* Typhimurium. A few NRLs also reported the use of some other molecular typing methods: MLST (Multi Locus Sequence Typing), CRISPR (Clustered Regularly Interspaced short Palindromic Repeats) and WGS (Whole Genome Sequencing).
The majority of the NRLs reported that they follow the following protocols for the different molecular typing methods:
PCR for serotyping *Salmonella*, especially the monophasic variant of *Salmonella* Typhimurium: EFSA opinion on '*Salmonella* Typhimurium-like strains' (EFSA, 2010) and Tennant et al., 2010.
PFGE: PulseNet protocol (PulseNet, 2009).
MLVA: Larsson et al., 2009 and Lindstedt et al., 2004.
MLST: Achtman et al., 2012.
CRISPR: Fabre et al., 2012

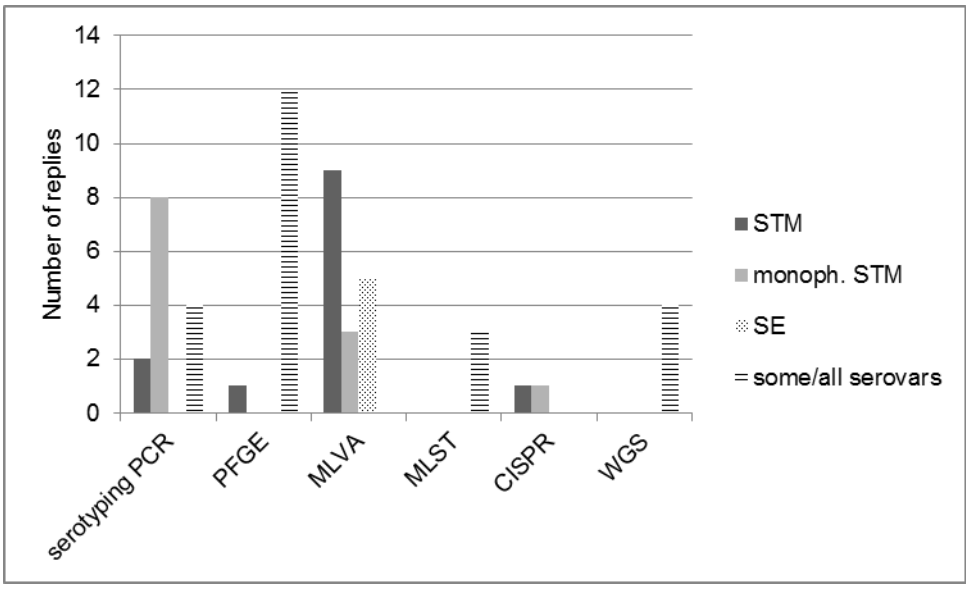


*Figure 6 Molecular typing methods used by the NRLs for* Salmonella

## 3.4 Part C Questions to gain opinions on some activities of the EURL-*Salmonella*

*C.1 Do you want to retain phage typing in the EURL-Salmonella interlaboratory comparison studies and/or do you want to add molecular typing to the studies?*

Thirteen NRLs indicated that they do not have an opinion on these subjects. Eight NRLs replied that they did not consider it necessary to retain phage typing in the comparative tests for typing of *Salmonella*. Three NRLs would like to retain the phage typing in the studies. These latter three laboratories are the only NRLs still performing phage typing and having completed the questionnaire. Twelve NRLs indicated that they are interested in adding molecular typing to the comparative tests, especially for PFGE and MLVA. For MLVA, this would only concern the *Salmonella* serovars Enteritidis and Typhimurium. Only one NRL had no interest in adding molecular typing to the studies.

*C.2 Would you like to keep receiving printed versions of the EURL-Salmonella reports or would you prefer digital versions only?*

Four NRLs indicated that they have no preference for either a printed or digital version of the reports. Eight NRLs would like to keep receiving the printed versions and fourteen NRLs would prefer a digital version only.

*C.3 Was the explanation on the use of the web-based test reports for reporting the results of the last two interlaboratory comparison studies clear/sufficient?*

All NRLs replied that this information was clear. Only one NRL gave no opinion.

*C.4 What is your opinion on the user-friendliness of the web-based forms?*

Sixteen NRLs considered the web-based test reports more user-friendly than the former test reports in MS Word or Excel. Four NRLs reported no differences, three had no opinion and only one considered the web-based forms less user-friendly. This latter NRL remarked that it was not possible to save data and that the instructions for the requested information were not visible before starting to complete the fields.

*C.5 Was it possible to report all relevant information concerning the study through the web-based form?*

All NRLs reported yes to this question.

*C.6 Did you have sufficient time to complete the form before the closing date?*

To this question also, only positive replies were given.

*C.7 If you have other suggestions to improve the web-based forms, please indicate below.*

Several NRLs used the opportunity to give some suggestions:

- 'It would be nice if the web-based form could be filled in 'online' daily (especially for a detection study), now we had to answer all questions at the same time because it could not be saved if not all questions are answered.'
- 'It would be nice if data could be entered and stored in web forms without submitting them immediately. Over time, data can be added or changed. When all is complete, a button should be pressed to submit the final data to the EURL.'
- 'It was not possible to save data and go back to the form or easily move between different parts of the form if one needed to go back. Thus, user-friendliness could be improved.'
- 'The print out of the web-based form (test report) does not contain the name of the study, can this be added?'
- 'Give more space for information regarding media used in laboratory, e.g. batch numbers, name of producers, etc.'

## 3.5 Conclusions

Based on the replies received, the performance testing of the official national laboratories can be summarized as follows:

- Almost all NRLs for *Salmonella* organize or coordinate comparative tests to test the performance of the official national laboratories.
- The comparative tests are organized for different work fields, generally at a frequency of one to four times a year.
- In almost all comparative tests for the detection of *Salmonella*, 'real matrices' (artificially contaminated) are tested.
- In most of the detection studies, 5-10 samples per study have to be tested, whereby the samples are often contaminated with one or two *Salmonella* serovars at low and high levels.
- Typing studies are organized to test the performance of the official laboratories for serotyping and/or PFGE typing.
- The NRLs have set criteria to judge the performances of the official laboratories and they organize a follow-up in the case of poor performances.

Molecular typing:

- More than half of the replying NRLs perform molecular typing of *Salmonella*.
- The molecular typing methods most often used are PFGE, MLVA (for *S.* Typhimurium and *S.* Enteritidis) and PCR for serotyping.

Activities of EURL-*Salmonella*:

- The majority of NRLs do not think it is necessary to retain phage typing in the interlaboratory comparison studies for typing.
- The majority of NRLs would like to add molecular typing (PFGE and/or MLVA) to the interlaboratory comparison studies on typing.

- All NRLs, except one, are satisfied with the web-based test forms for reporting the results of the interlaboratory comparison studies.
- Some NRLs gave suggestions for improvement of the web-based test reports. The EURL will review them for technical possibilities.

# 4 Evaluation of the workshop

## 4.1 Introduction

At the end of the workshop, an evaluation form was handed to all participants to ask for their opinion on the workshop (see Annex 4). A total of eleven questions were posed. For nine of these questions participants were asked to give a score ranging from 1 to 5 as an answer to the questions, with 5 as the highest score (excellent) and 1 as the lowest score (very poor). If they wished, it was also possible to give remarks to the questions. Two questions were 'open' questions, in which the participants were asked to give their opinion.
The evaluation form was handed to 49 participants of the workshop and 42 completed forms were returned, which is a response rate of 86%.

In Clause 4.2, the scores on each question are indicated and a summary of the remarks is given.

## 4.2 Evaluation form

*1. What is your opinion on the information given in advance of the workshop?*

Figure 7 shows that all respondents considered the information given in advance to the workshop to have been good or excellent (scores 4-5).

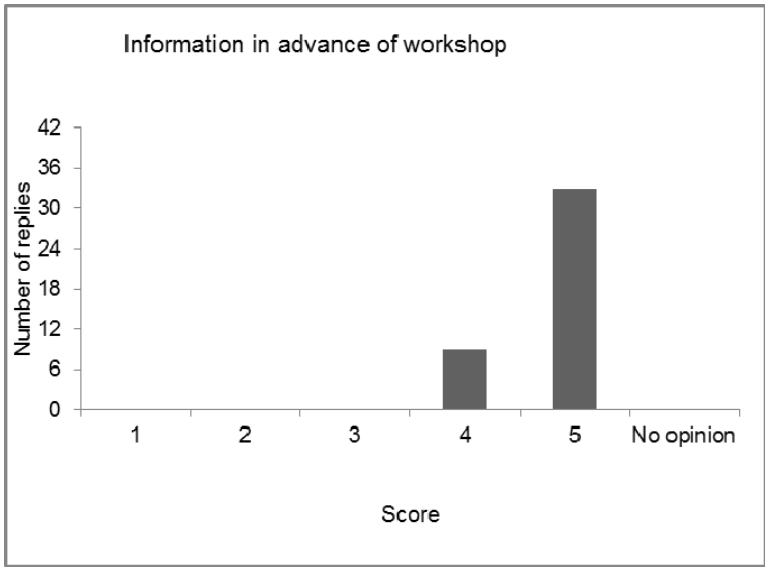


*Figure 7 Scores given to question 1 'Opinion on information given in advance of the workshop'*

*2. What is your opinion on the accessibility of the meeting venue?*

The opinions on the accessibility the meeting venue were varied.
Three participants remarked that it was nice to combine the workshop with the International Symposium on *Salmonella* and Salmonellosis (I3S) in St. Malo, but that the meeting venue was not easy to reach.
There is no airport close to St. Malo, which meant that many participants had to take the train from the airport in Paris, resulting in long travel times.

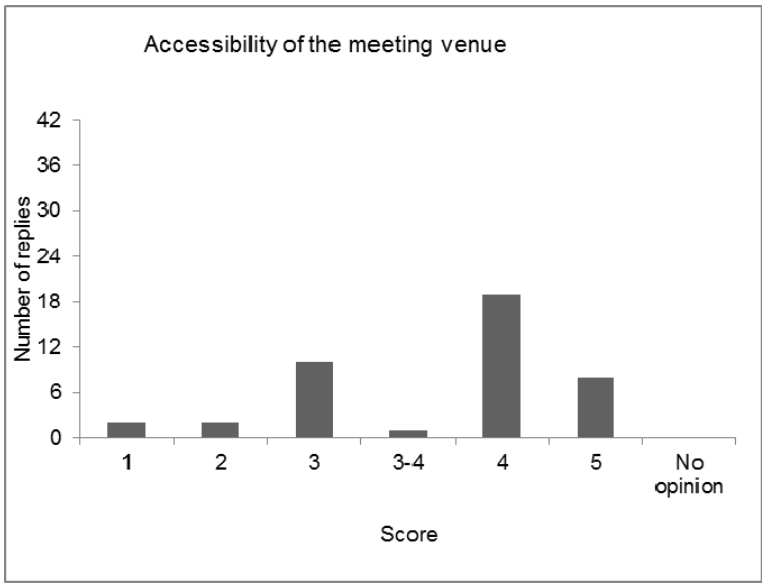


*Figure 8 Scores given to question 2 'Opinion on the accessibility of the meeting venue'*

*3. What is your opinion of the hotel room?*

The opinions of the hotel rooms were also varied. The participants were situated in three different hotels and it seems that the quality of one hotel was considered to be less than the other two. Remarks given were:

- 'Hotel was very basic. No internet connection in the room, only at the reception' (2x).
- 'Small room' (1x).
- 'Too dark, too cold, too old' (1x).
- 'Poor hotel' (1x).
- 'Fine hotel' (2x).

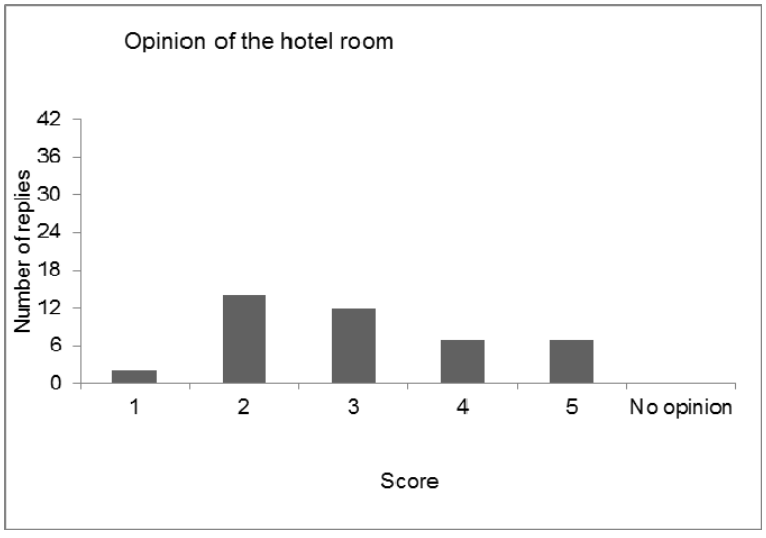


*Figure 9 Scores given to question 3 'Opinion of the hotel room'*

*4. What is your general opinion of the meeting room?*

The number of participants was high (50 participants in total) and the meeting room was relatively small. This is also reflected in the opinions of the participants as summarized in Figure 10. Remarks given were:

- 'Meeting room was too small and it was hard to see the screen from the third row or more backwards' (6x).
- 'Too many people for this small room, some people could hardly move' (1x).
- 'Pillars blocked the view of some people' (1x).
- 'It was nice to have a table to make notes' (1x).

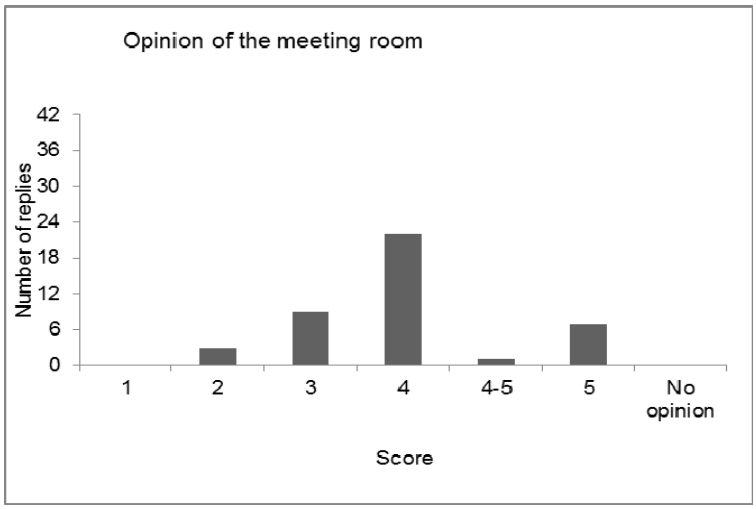


*Figure 10 Scores given to question 4 'Opinion of the meeting room'*

*5. What is your opinion on the readability of the presentations on the screen?*

In general, the readability of the presentations on the screen was considered to be good. However, due to the small screen and the crowded room, some people had difficulty reading the screen (see Figure 11). Remarks related to this question were:

- 'The screen was too low and too close to the participants' (1x).
- 'Could not see the bottom lines on the screen from the back rows' (3x).
- 'The speaker sometimes blocked the view on one side of the screen' (1x).

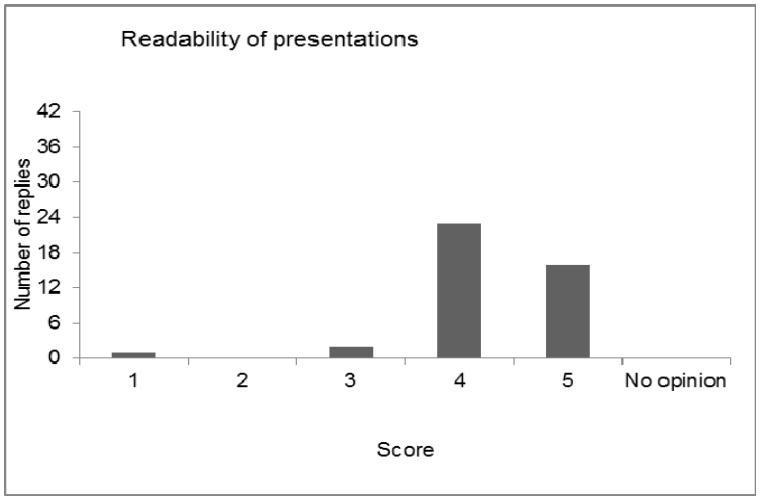


*Figure 11 Scores given to question 5 'Opinion on the readability of the presentations'*

*6. What is your opinion on the technical equipment in the meeting room (computer, screen, microphones, etc.)?*

The majority of the respondents considered the technical equipment to be good or excellent (scores 4-5), see Figure 12. Only two respondents considered the technical equipment to be moderate (score 3). Remarks related to this question were:

- 'No internet' (1x).
- 'It was difficult to move around with the microphone for questions. On the other hand, the room was so small that the speakers and the ones asking questions were easy to hear, even without a microphone' (1x).

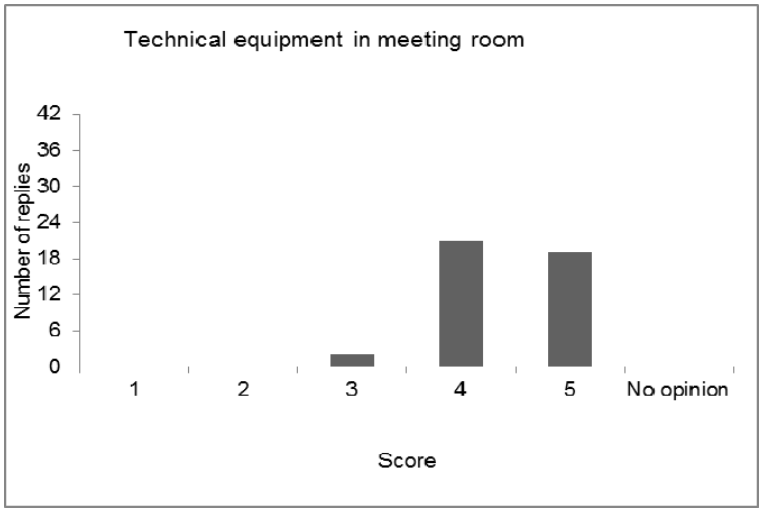


*Figure 12 Scores given to question 6 'Opinion on the technical equipment'*

*7. What is your opinion on the catering provided during the workshop (breakfast, coffee, tea, lunch, dinner)?*

The majority of the respondents considered the catering to be good or excellent (scores 4-5), see Figure 13. A few respondents indicated a moderate score (score 3), which was mainly related to the opinion of a few respondents who considered the breakfast to have been of poor quality.

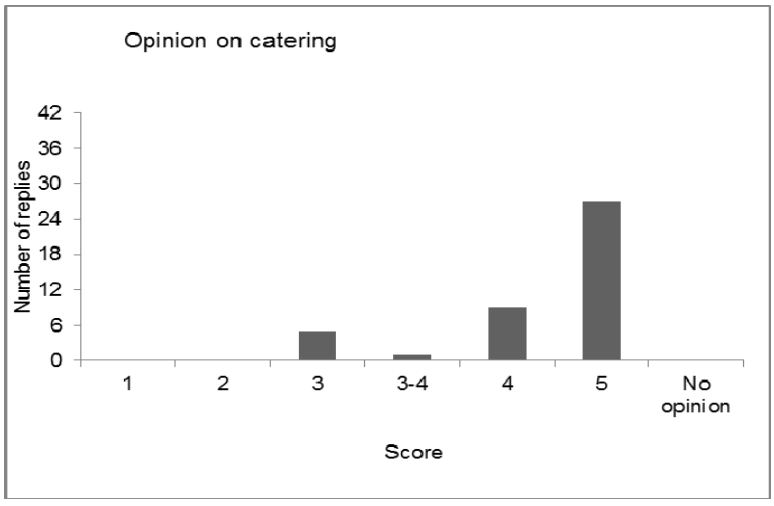


*Figure 13 Scores given to question 7 'Opinion on the catering'*

*8. What is your opinion on the scientific programme of the workshop?*

The majority of the respondents were very satisfied about the scientific programme of the workshop; good (score 4) or excellent (score 5) scores were given (see Figure 14). Only one respondent indicated a moderate score (score 3), remarking that the programme was limited due to limited time (only one day). However, it was also remarked that this was not considered to be a problem as the workshop was combined with the extensive scientific programme of the I3S symposium. One respondent did not give a score for this question.

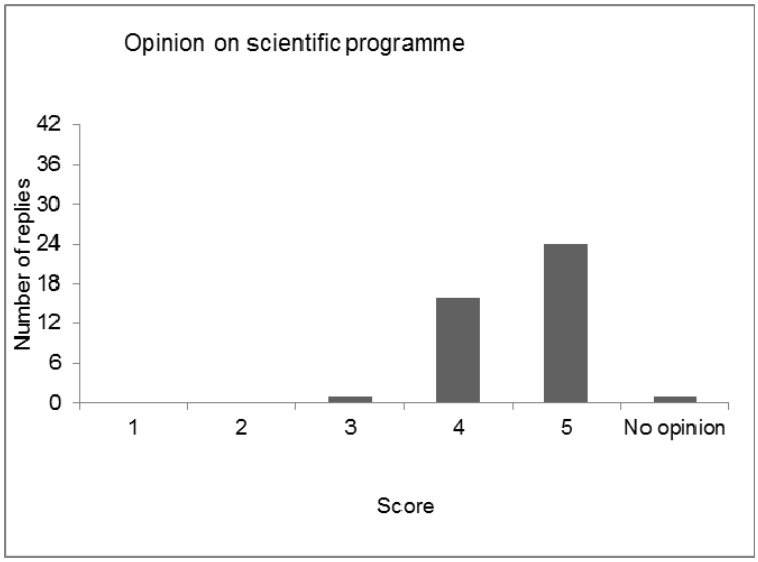


*Figure 14 Scores given to question 8 'Opinion on the scientific programme'*

*9. Are there specific presentations you want to comment on or did you miss information on certain subjects?*

This concerned an 'open' question and the following responses were obtained:

- 'We do not need so much detail on the preparation of ring tests. We know it is a lot of work. The afternoon session was more interesting' (1x).
- 'Missed the presentations of the Member States' (2x).
- 'Would like to have information on a harmonized protocol for PCR methods' (1x).
- 'Would like to have practical information on molecular typing of *Salmonella*' (1x).
- 'Maybe nice to have information on other/uncommon *Salmonella* sources (like reptiles, hedgehogs, plants, etc.)' (1x).

*10. What is your general opinion of the workshop?*

The respondents indicated that the workshop as a whole had been good (score 4) or excellent (score 5), see Figure 15. No further comments were given.


*Figure 15 Scores given to question 10 'General opinion of the workshop'*

*11. Do you have any remarks or suggestions which we can use for future workshops?*

This concerned an 'open' question and the following responses were obtained:

- 'Well organized, thank you' (2x).
- 'It would be nice to have more information on the results of the chromogenic media used in the proficiency tests, and not only on XLD and BGA' (1x).
- 'It would be nice to have a bigger meeting room and better visibility of the screen' (1x).
- 'Avoid the 17th of May please' (1x).
- 'It would be useful to include more outbreak situations in some countries' (1x).
- 'It would be nice to have more information about the molecular protocols used in some countries and their experiences' (1x).
- 'Ask NRLs early in the year if they want to present a topic or suggest topics of interest' (1x).
- 'It would be nice to get information from an industry representative on its views on *Salmonella* from primary production or food or both' (1x).
- 'Hand-outs are useful' (1x).

## 4.3 Discussion and conclusions of the evaluation

Despite some lower scores for some general aspects of the workshop, such as the accessibility of the meeting venue, the quality of the hotel rooms and the quality of the meeting room, the participants were generally satisfied with the workshop. The scientific programme was considered interesting, which is still the most important part of the workshop. It would however have improved the general feeling of the participants if the conditions under which the workshop was organized had been good as well. Although it is not always easy to get high scores on all aspects of the workshop, it is good to aim for better conditions for the workshop to be organized in 2014, while retaining the good quality of the scientific programme.

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# Acknowledgements

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# List of abbreviations

| | |
|---|---|
| A | Answer |
| ATCC | American Type Culture Collection |
| BGA | Brilliant Green Agar |
| BPW | Buffered Peptone Water |
| CEN | European Committee for Standardization |
| cfu | colony forming units |
| CRISPR | Clustered Regularly Interspaced short Palindromic Repeats |
| DG | Directorate General |
| DG-Sanco | Directorate General for Health and Consumer Protection |
| DIS | Draft International Standard |
| DT | Definitive Type |
| EC | European Commission |
| ECDC | European Centre for Disease Prevention and Control |
| EFSA | European Food Safety Authority |
| EFTA | European Free Trade Association |
| EQA | External Quality Assessment |
| EU | European Union |
| EURL | European Union Reference Laboratory |
| FBO | Food-borne outbreak |
| FYROM | Former Yugoslav Republic of Macedonia |
| ISO | International Organization for Standardization |
| LOD | Level of Detection |
| MKTTn | Mueller Kauffmann Tetrathionate broth with novobiocin |
| MLST | Multi Locus Sequence Typing |
| MLVA | Multi-Locus Variable number of tandem repeats Analysis |
| MS | Member State |
| MSRV | Modified Semi-solid Rappaport Vassiliadis |
| NRL | National Reference Laboratory |
| PCR | Polymerase Chain Reaction |
| PFGE | Pulsed Field Gel Electrophoresis |
| PHE | Public Health England |
| PP | Primary Production |
| PT | Proficiency Test |
| Q | Question |
| RIVM | National Institute for Public Health and the Environment |
| RVS | Rappaport Vassiliadis broth with Soya |
| SC | Sub Committee |
| SD(6) | *Salmonella* Derby (at a level of approximately 6 cfu) |
| SE(8) | *Salmonella* Enteritidis (at a level of approximately 8 cfu) |
| SSI | Statens Serum Institute |
| STM(10) | *Salmonella* Typhimurium (at a level of approximately 10 cfu) |
| S/VTEC | Shigatoxin/Verocytotoxin producing *E. coli* |
| TAG | Technical Advisory Group |
| TC | Technical Committee |
| TR | Technical Report |
| TS | Technical Specification |
| UK | United Kingdom |
| USA | United States of America |
| WG | Working Group |
| WGS | Whole Genome Sequencing |
| XLD | Xylose Lysine Deoxycholate |

# Annex 1 Participants

| | |
|---|---|
| European Food Safety Authority (EFSA) | Valentina Rizzi |
| EC DG-Sanco | Klaus Kostenzer |
| EURL – *Salmonella* | Kirsten Mooijman<br>Angelina Kuijpers<br>Wilma Jacobs |
| Guest speaker (Denmark) | Mia Torpdahl (SSI, Copenhagen) |
| Guest speaker (United Kingdom) | Elizabeth de Pinna (PHE, London) |

**National Reference Laboratories for *Salmonella***

| | |
|---|---|
| AUSTRIA | Christian Kornschober |
| BELGIUM | Pierre Wattiau<br>Katelijne Dierick |
| BOSNIA HERZEGOVINA | Sead Hadziabdic |
| BULGARIA | Gergana Mateva |
| CROATIA | Gordan Kompes |
| CYPRUS | Konstantinos Arsenoglou |
| CZECH REPUBLIC | Tomas Cerny |
| DENMARK | Karl Pedersen<br>Per Rasmussen |
| ESTONIA | Age Kärssin |
| FINLAND | Henry Kuronen |
| FRANCE | Renaud Lailler<br>Marylene Bohnert |
| FYROM | Mirko Prodanov |
| GERMANY | Istvan Szabo |
| GREECE | Aphrodite Smpiraki |
| HUNGARY | Erzsebet Adrian |
| ICELAND | Franklin Georgsson |
| IRELAND | John Egan |
| ITALY | Alessandra Longo<br>Antonia Anna Lettini |
| LATVIA | Jelena Avsejenko |
| LITHUANIA | Tatjana Kutyriova |
| LUXEMBOURG | Joseph Schon |
| MALTA | Gertrude Gatt Lanzon |
| NETHERLANDS | Wendy van Overbeek<br>Anjo Verbruggen |
| NORTHERN IRELAND | Gintare Bagdonaite |
| NORWAY | Bjarne Bergsjø |
| POLAND | Kinga Wieczorek<br>Magdalena Zajac |
| PORTUGAL | Patricia Themudo |
| ROMANIA | Luminita Monica Vanghele |
| SERBIA | Jasna Kureljusic |
| SERBIA | Zivka Ilic |
| SLOVAK REPUBLIC | Milan Sasik |

| | |
|---|---|
| SLOVENIA | Jasna Micunovic |
| SPAIN | Maria Christina de Frutos Escobar |
| SWEDEN | Lennart Melin |
| SWITZERLAND | Gudrun Overesch |
| TURKEY | Elcin Gunaydin |
| UNITED KINGDOM | Robert Davies |

# Annex 2 Programme of the workshop

**Programme of the EURL-*Salmonella* workshop XVIII**
**30 May, ST. Malo, France**

**General information**

**Meeting venue:**
*Hotel France et Chateaubriand:*
Place Chateaubriand 12, Saint Malo, France, http://www.hotel-chateaubriand-st-malo.com/index.php?lang=en

**Accommodation**
*Hotel Anne de Bretagne:*
Rue Saint Thomas 10-11, Saint Malo, France
http://www.hotel-annedebretagne.com/index.php?lang=en
*Hotel L'Univers:*
Place Chateaubriand, Saint Malo, France
http://www.hotel-univers-saintmalo.com/?lang=en
*Hotel Bristol Union:*
Place de la Poissonnerie, Saint Malo, France
http://www.hotel-bristol-union.com/?lang=en

Information for the people giving a presentation:

**Presentations**: To be able to make hand-outs for all participants, please send your (Power Point) **presentation** to Kirsten Mooijman (kirsten.mooijman@rivm.nl) **at latest on 23 May 2013**. Alternatively, bring your own hand-outs.

**Abstract:** To prepare the report for the workshop, it is also necessary to receive an abstract of your presentation (approximately one page). Please hand this over to Kirsten during the workshop or send it to Kirsten.mooijman@rivm.nl by **10 June 2013** at the latest

**Wednesday 29 May 2013**
Arrival of participants at St. Malo (if not already present for the I3S symposium)

**18.00 – 19.00 Registration and get-together in hotel France et Chateaubriand**

- Final information concerning the programme
- Administrative aspects

**Dinner information:** For participants whose costs of travel and accommodation are paid from the budget of EURL-*Salmonella*, the EURL will also cover the expenses of a dinner on Wednesday 29 May, up to a maximum of € 30.00 per person. A receipt will be needed in order to be able to reimburse you for this meal.

**Thursday 30 May 2013**

***Morning chair: Kirsten Mooijman***

| | | |
|---|---|---|
| 09:00 - 09:30 | Opening and introduction | Kirsten Mooijman, EURL-*Salmonella* |
| 09:30 - 10:00 | EU *Salmonella* monitoring data (Summary report 2011) | Valentina Rizzi, EFSA |
| 10:00 - 10:30 | Results interlaboratory comparison study on detection of *Salmonella* in animal feed II - 2012 | Angelina Kuijpers, EURL-*Salmonella* |
| *10:30 - 11:00* | *Coffee/tea* | |
| 11:00 - 11:30 | Results typing study XVII - 2012: serotyping | Wilma Jacobs, EURL-*Salmonella* |
| 11:30 - 11:45 | Resultstyping study XVII - 2012 : phage typing | Elizabeth de Pinna, PHE, UK |
| 11:45 - 12:15 | Miscellaneous activities EURL and NRLs | Kirsten Mooijman, EURL-*Salmonella* |
| *12:15 - 13:30* | *Lunch* | |

***Afternoon chair: Wilma Jacobs***

| | | |
|---|---|---|
| 13:30 - 14:00 | Results interlaboratory comparison study on detection of *Salmonella*-Primary production XVI-2013 | Angelina Kuijpers, EURL-*Salmonella* |
| 14:00 - 14:30 | First results validation of Annex D of ISO 6579 (CEN mandate *Salmonella*) | Kirsten Mooijman, EURL-*Salmonella* |
| 14:30 - 15:00 | Information from DG-Sanco, including vision paper on molecular typing data | Klaus Kostenzer, DG-Sanco |
| *15.00 - 15.30* | *Coffee/tea* | |
| 15:30 - 16:00 | Activities by ECDC concerning molecular typing data from human samples | Mia Torpdahl, SSI, Denmark |
| 16:00 - 16:30 | Activities by EFSA concerning molecular typing data from food and primary production samples | Valentina Rizzi, EFSA |
| 16:30 - 17:00 | Work programme EURL-*Salmonella* second half2013, first half 2014, discussion on general items and closure | Kirsten Mooijman, EURL-*Salmonella* |
| *19:00 -* | *Dinner at hotel France et Chateaubriand* | |

# Annex 3 Questionnaire EURL-*Salmonella* April 2013

**Introduction**

With this questionnaire, the EURL-*Salmonella* would like to obtain more (detailed) information on some of the activities of the NRLs for *Salmonella*. Furthermore, we would like to hear your opinion on some of the activities of the EURL-*Salmonella.*

As the questions are related to three different areas, this questionnaire also consists of three parts:

A. Questions related to how NRLs-*Salmonella* test the performance of the official national laboratories in the relevant work field;
B. An inventory on molecular typing methods used by the NRLs for *Salmonella*[1];
C. Questions to gain your opinion on some activities of the EURL-*Salmonella*.

At first we will ask you to complete two general questions to obtain more information of you as NRL.

It may be the case that in your country different laboratories/institutes act as NRL-*Salmonella* for different work fields (e.g. NRL for analysing food samples, another NRL for analysing samples from primary production, animal feed samples and/or for typing of *Salmonella*). If so, feel free to forward this questionnaire to the other 'NRL-laboratories', or agree with the other 'NRL-laboratories' to complete the questionnaire for them as well. It may also be the case that more than one person of an NRL-*Salmonella* for one work field receives this questionnaire. It would be preferable if we receive only one completed questionnaire per work field. Thank you in advance for coordinating the completion of the questionnaire between the relevant contacts.

We realise that it will take some of your time to complete this questionnaire, but your input is highly appreciated!

**Thank you in advance for taking the effort to complete this questionnaire. And thank you for returning your completed questionnaire to Kirsten Mooijman (Kirsten.mooijman@rivm.nl) before 6 May 2013!**

## General

I For which country are you NRL-*Salmonella*?

| |
|---|
| |

II For which work field are you NRL-*Salmonella*?
(More than one answer is possible)

| | |
|---|---|
| | Primary production – poultry |
| | Primary production – cattle |
| | Primary production – pigs |
| | Food |
| | Animal feed |
| | Typing |
| | Other, being: |

[1] For this inventory, several questions were copied from a questionnaire on molecular typing distributed by EFSA in 2008.

## A. Testing the performance of official national laboratories

According to Regulation 882/2004, Article 33, a task of a National Reference Laboratory is the following:
'where appropriate, organize comparative tests between the official national laboratories and ensure an appropriate follow-up of such comparative testing.'
With the questions stated in this part A, we would like to gain more information on the activities of the NRLs for this relevant task.

A.1 How many 'official laboratories' are designated in your country for the different work fields?

| Work field | Number of official laboratories |
|---|---|
| Analysis of samples from primary production | |
| Analysis of food samples | |
| Analysis of animal feed samples | |
| Typing | |
| Other, being: | |

A.2 Do you organize comparative tests to test the performance of the official national laboratories?

| | | |
|---|---|---|
| | Yes, we as NRL-*Salmonella* organize comparative tests ourselves | Please also answer questions A.3-A.11 |
| | No, we do not organize comparative tests ourselves, but we ask the official laboratories to participate in Proficiency Tests organized by another (commercial) organization and to provide us with their results | Please also answer questions A.3-A.11 |
| | No, we test the performances of the official laboratories in another way, namely: | Please go to part B of this questionnaire |
| | No, we do not test the performances of the official laboratories because:<br>□ The NRL is the only official laboratory<br>□ Other, namely: | Please go to part B of this questionnaire |

A.3 For what work field do you/other organizers organize comparative tests and at what frequency (for example 1x/year)

| | Work field | Frequency |
|---|---|---|
| | Detection of *Salmonella* in primary production samples | |
| | Detection of *Salmonella* in food samples | |
| | Detection of *Salmonella* in animal feed samples | |
| | Typing of *Samonella* | |
| | Other, namely: | |

A.4 Do you/other organizers test 'real matrices' in the detection studies?

☐ Not applicable (e.g. in case you organize only typing studies)
☐ No ☐ Yes
If yes, what types of matrices have been used in studies organised in the last 5 years?

| |
|---|
| |

A.5 If 'real matrices' are used, how are they 'contaminated'?

| | |
|---|---|
| | Use of naturally contaminated samples |
| | Inoculation of samples with a (diluted) culture or reference material by the organizer of the study |
| | Mixing of matrix and (commercial) reference material by the participant on the day of analysis |
| | Other, namely: |

A.6 If (commercial) reference materials are used, where are they obtained?

| **Type of reference materials** | **Prepared by:** |
|---|---|
| | |

A.7 How many samples do you/other organizers include per study, and with what content, on the detection of *Salmonella*?

| **Content** | **Approximate contamination level(s) in cfu/sample** | **Number of samples per study** |
|---|---|---|
| Total number of samples per study | | |
| Sterile matrix, (artificially) contaminated with *Salmonella* | | |
| Matrix with background flora, (artificially) contaminated with *Salmonella* | | |
| Sterile matrix samples | | |
| Matrix samples with background flora only | | |
| Pure *Salmonella* culture | | |
| Mixed culture of *Salmonella* and background flora | | |
| Culture with background flora only | | |
| Blank (sterile) samples | | |
| Other, namely: | | |

A.8 How many *Salmonella* serovar(s) do you/other organizers most often include per detection study and which serovars are most often used?

| **Number of *Salmonella* serovars per study** | ***Salmonella* serovars most often used** |
|---|---|
| | |

A.9 If you/other organizers organize typing studies, for what typing procedures are the studies organized and how many strains are tested per study?

| | **Procedure** | **Number of strains per study** |
|---|---|---|
| | Serotyping | |
| | Phagetyping | |
| | PFGE | |
| | MLVA | |
| | Other, namely: | |

A.10 Have you set criteria to judge the performance of the participating laboratories in the comparative tests?

☐ No ☐ Yes

If yes, please describe the criteria and the actions you as NRL take in case of poor performance? (More than one answer is possible)

| **Criteria:** |
|---|
| |

| **Actions in case of poor performance** | |
|---|---|
| | No further actions |
| | Ask laboratories for possible causes |
| | Organize a follow-up study |
| | Give training |
| | Other, namely: |

A.11 Are you/other organizers accredited for organizing comparative tests?

☐ No
☐ Yes

If yes, by which accreditation organization, under which reference number?

| |
|---|
| |

## B. Molecular typing

*Abbreviations*

| | |
|---|---|
| CRISPR: | Clustered Regularly Interspaced short Palindromic Repeats |
| MLST: | Multi Locus Sequence Typing |
| MLVA: | Multi Locus Variable-Number Tandem Repeat Analyses |
| PFGE: | Pulsed Field Gel Electrophoresis |
| WGS: | Whole Genome Sequencing |

B.1 Do you perform molecular typing of *Salmonella* isolates from food, feed and/or animals?

☐ No, please go to part C of this questionnaire
☐ Yes, please also answer questions B.2 – B.4

B.2 Do you perform molecular typing on a routine basis or occasionally[2]?

☐ On a routine basis
☐ Occasionally

B.3 Where do the isolates come from?
(More than one answer is possible)

| | |
|---|---|
| | Samples related to official controls, national control or monitoring programmes or surveys carried out by competent authorities |
| | Samples related to outbreak investigations |
| | Samples related to research |
| | Other, namely: |

B.4 What molecular typing methods, for which *Salmonella* serovars are used and which protocols are followed?

| | **Molecular typing method** | ***Salmonella* serovars** | **Protocol (please give reference)** |
|---|---|---|---|
| | Serotyping based on PCR | | |
| | PFGE | | |
| | MLVA | | |
| | MLST | | |
| | CRISPR | | |
| | WGS | | |
| | Other, namely: | | |

[2] Routine: all or an agreed proportion of isolates are typed yearly;
Occasionally: some isolates are typed without a special agreed plan.

## C. Your opinion on some activities of EURL-*Salmonella*

**EURL-*Salmonella* interlaboratory comparison studies on typing**

C.1 The current EURL-*Salmonella* interlaboratory comparison studies on typing include an obligatory serotyping part and a voluntarily phage typing. Do you want us to retain phage typing in the studies and/or do you want us to add molecular typing to the studies?

☐ No opinion, please go to question C.2

Retain phage typing:

☐ No
☐ Yes

Add molecular typing:

☐ No
☐ Yes

If yes, which molecular typing methods and which *Salmonella* serovars do you want to include?

| | **Molecular typing methods** | ***Salmonella* serovars** |
|---|---|---|
| | PFGE | |
| | MLVA | |
| | Other, namely: | |

**EURL-*Salmonella* reports**

C.2 The results of the EURL-*Salmonella* interlaboratory comparison studies and the abstracts and discussion of each annual workshop are published in (extensive) reports. All participants (of a study or a workshop) receive a printed version of the relevant report. Additionally, the reports are made available through the EURL-*Salmonella* website.
To save costs for paper, printing and mailing of the reports, we are discussing whether or not we should publish the reports in digital form only and no longer on paper. However, before deciding on this, we would appreciate to hear your opinion.

| | |
|---|---|
| | Prefer to retain the printed versions of the EURL-*Salmonella* reports |
| | Prefer to receive the EURL-*Salmonella* reports as digital reports only |
| | No preference |
| Remarks: | |

**Web-based test reports of the EURL-*Salmonella* interlaboratory comparison studies**

We would appreciate to hear your opinion on the reporting of results of interlabroratory comparison studies through web-based forms, newly introduced in November 2012 (typing study) and March 2013 (detection study).

C.3 Was the explanation on the use of the web-based forms clear/sufficient?

☐ No ☐ Yes

If no, please indicate what information was lacking.

| |
|---|
| |

C.4 What is your opinion on the user-friendliness of the web-based forms?

| | |
|---|---|
| | More user-friendly than the former test reports in Word or Excel |
| | Less user-friendly than the former test reports in Word or Excel |
| | No difference in user-friendliness |
| | No opinion |
| Remarks: | |

C.5 Was it possible to report all relevant information concerning the study through the web-based form?

☐ No ☐ Yes

If no, please indicate what information you could not report

| |
|---|
| |

C.6 Did you have sufficient time to complete the form before the closing date?

☐ No ☐ Yes

If no, please indicate your problems

| |
|---|
| |

C.7 If you have other suggestions to improve the web-based forms, please indicate below.

| |
|---|
| |

**--- End of questionnaire ---**
**Thank you for your time!**

# Annex 4 Evaluation form of the workshop

**Evaluation of the XVIII$^{th}$ EURL-*Salmonella* workshop**
**30 May 2013, St. Malo, France**

We would highly appreciate if you could give us your opinion on the 18$^{th}$ EURL-*Salmonella* workshop, organized in St. Malo, France on 30 May 2013. Thank you very much in advance for completing this questionnaire and returning it to the EURL-*Salmonella* team by the end of the workshop.

**Please give your opinion by indicating a score from 1 to 5, whereby 5 is the highest score (excellent) and 1 is the lowest score (very poor).**

1. What is your opinion on the information given in advance of the workshop?

| 1 (Very poor) | 2 | 3 | 4 | 5 (Excellent) | No opinion |
|---|---|---|---|---|---|
| | | | | | |

Remarks:

2. What is your opinion on how easy (high score) or difficult (low score) it was to reach the meeting venue?

| 1 (Very poor) | 2 | 3 | 4 | 5 (Excellent) | No opinion |
|---|---|---|---|---|---|
| | | | | | |

Remarks:

3. What is your opinion of the hotel room?

| 1 (Very poor) | 2 | 3 | 4 | 5 (Excellent) | No opinion |
|---|---|---|---|---|---|
| | | | | | |

Remarks:

4. What is your general opinion of the meeting room?

| 1 (Very poor) | 2 | 3 | 4 | 5 (Excellent) | No opinion |
|---|---|---|---|---|---|
| | | | | | |

Remarks:

5. What is your opinion on the readability of the presentations on the screen?

| 1 (Very poor) | 2 | 3 | 4 | 5 (Excellent) | No opinion |
|---|---|---|---|---|---|
| | | | | | |

Remarks:

6. What is your opinion on the technical equipment in the meeting room (computer, screen, microphones, etc)?

| 1 (Very poor) | 2 | 3 | 4 | 5 (Excellent) | No opinion |
|---|---|---|---|---|---|
| | | | | | |

Remarks:

7. What is your opinion on the catering provided during the workshop (breakfast, coffee, tea, lunch, dinner)?

| 1 (Very poor) | 2 | 3 | 4 | 5 (Excellent) | No opinion |
|---|---|---|---|---|---|
| | | | | | |

Remarks:

8. What is your opinion on the scientific programme of the workshop?

| 1 (Very poor) | 2 | 3 | 4 | 5 (Excellent) | No opinion |
|---|---|---|---|---|---|
| | | | | | |

Remarks:

9. Are there specific presentations you want to comment on or did you miss information on certain subjects?

| |
|---|
| |

10. What is your general opinion of the workshop?

| 1 (Very poor) | 2 | 3 | 4 | 5 (Excellent) | No opinion |
|---|---|---|---|---|---|
| | | | | | |

Remarks:

11. Do you have any remarks or suggestions which we can use for future workshops?

| |
|---|
| |

**Thank you very much!**

**National Institute for Public Health and the Environment**
P.O. Box 1 | 3720 BA Bilthoven
www.rivm.com