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

The 22nd EURL-*Salmonella* workshop

29 and 30 May 2017, Zaandam, the Netherlands

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Synopsis

The 22nd EURL-*Salmonella* workshop
29 and 30 May 2017, Zaandam, the Netherlands

This report gives a summary of the presentations held at the 22nd annual workshop for the European National Reference Laboratories (NRLs) for *Salmonella* (29-30 May 2017). The aim of the workshop is to facilitate the exchange of information on the activities of the NRLs and the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*).

Annual ring trials
A recurring item at the workshops is the presentation of the results of the annual ring trials organised by the EURL. These ring trials give information on the quality of the NRL laboratories tested. The NRLs had high scores in the 2016 studies. Detailed information on the results per ring trial is available in separate RIVM-reports.

Outbreaks
In some presentations, a number of outbreaks were reported, where a large number of people became ill due to *Salmonella*. It is often hard to detect the source of an outbreak, however, the source of a specific outbreak involving many people in several European Member States could be identified: Polish eggs contaminated with *Salmonella.*

Methods
Other presentations were held on the standardisation and harmonisation of methods, e.g. for testing food products on the presence of *Salmonella*. In this way, agreements on standard test methods can be made at European level, ensuring that Member States perform the tests uniformly. This enables a better comparison of results between different countries.

The annual workshop is organised by the EURL-*Salmonella*, part of 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 2017

Publiekssamenvatting

De 22e EURL-*Salmonella* workshop
29 en 30 mei 2017, Zaandam, Nederland

Het RIVM heeft de verslagen gebundeld van de presentaties van de 22e jaarlijkse workshop voor de Europese Nationale Referentie Laboratoria (NRL's) voor *Salmonella* (29-30 mei 2017). Het doel van de workshop is dat het overkoepelende orgaan, het Europese Referentie Laboratorium (EURL) voor *Salmonella*, en de NRL's informatie uitwisselen.

Een terugkerend onderwerp is de ringonderzoeken die het EURL jaarlijks organiseert om de kwaliteit van de NRL-laboratoria te controleren. De NRL's scoorden goed in de studies van 2016. In dit rapport staan de ringonderzoeken kort beschreven. Een uitgebreidere weergave van de resultaten wordt apart per ringonderzoek gepubliceerd.

Een aantal verslagen geeft informatie over grote aantallen mensen die ziek zijn geworden door *Salmonella*, zogenoemde uitbraken. Het is vaak moeilijk om uit te vinden wat de bron is van een uitbraak. Bij een uitbraak met veel zieke mensen in verschillende Europese lidstaten is de bron wel gevonden, namelijk eieren uit Polen die besmet waren met *Salmonella*.

Andere verslagen beschrijven de activiteiten om methoden te standaardiseren en te harmoniseren. Bijvoorbeeld over het testen van levensmiddelen op aanwezigheid van *Salmonella*. Op Europees niveau worden afspraken gemaakt over een methode, zodat de lidstaten een test op dezelfde wijze uitvoeren. Hierdoor kunnen resultaten tussen verschillende landen beter worden vergeleken.

De organisatie van de jaarlijkse 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.

Kernwoorden: EURL-*Salmonella*, NRL-*Salmonella*, *Salmonella*, workshop 2017

Contents

Summary

On 29 and 30 May 2017, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organised its annual workshop in Zaandam, the Netherlands. Participants of the workshop were representatives of the NRLs for *Salmonella* from 27 EU Member States, three European Free Trade Association (EFTA) countries, and two (potential) EU candidate countries. Also present were representatives of the European Commission Directorate General for Health and Food Safety (DG-Sante), and of the European Food Safety Authority (EFSA). In total, 3 participants of NRLs from one EU Member States (Malta), and two (potential) candidate countries (Bosnia and Herzegovina and Turkey), were unable to join the workshop. A total of 45 participants attended the workshop.

During the workshop, presentations were given on several items. The results of the interlaboratory comparison studies organised by the EURL-*Salmonella* in the past year were presented. This concerned the studies on detection of *Salmonella* in minced chicken meat (October 2016) and in samples from the primary production stage (March 2017) and the study on typing of *Salmonella* (November 2016).
An EFSA representative presented the most recent European summary report on Zoonoses, giving an overview of the number and types of zoonotic microorganisms that caused health problems in Europe in 2015. For several years, the number of health problems caused by *Salmonella* has declined, although last year it levelled. Still it remains the second most important cause of zoonotic diseases in Europe, after *Campylobacter*.

Additionally, the EFSA representative gave an update on the joint EFSA/ECDC molecular typing database and on the preliminary results of the (European) survey on the use of WGS for typing *Salmonella*.
A representative of EC DG-Sante informed the participants on an outbreak of *Salmonella* Enteritidis related to Polish eggs. Additionally, the representative of DG-Sante explained the new Official Control Regulation, published in April 2017.
A summary was given of the standardisation of methods in ISO and CEN, and more specifically on the validation of alternative microbiological methods.
A representative of the Greek NRL gave a presentation on the outbreak of a new *Salmonella* serovar, and a representative of the Swiss NRL gave a presentation on *Salmonella enterica* subsp. *diarizonae* in sheep. Five representatives gave a summary of the activities that they as NRL perform to fulfil the prescribed tasks and duties (the Netherlands, Serbia, Bulgaria, Cyprus and Romania).

The workshop concluded with a presentation on the EURL-*Salmonella* work programme for the current and coming year.

All workshop presentations can be found at:
http://www.eurlsalmonella.eu/Workshops/Workshop_2017

1 Introduction

This report includes the abstracts of the presentations given at the 2017 EURL-*Salmonella* workshop, as well as a summary of the discussion that followed the presentations. The full presentations are not provided in this report, but are available on the EURL-*Salmonella* website: http://www.eurlsalmonella.eu/Workshops/Workshop_2017

The layout of the report is consistent with the workshop programme.
Chapter 2 includes all the abstracts of presentations held on the first day.
Chapter 3 includes all the abstracts of presentations held on the second day.
The workshop is evaluated in chapter 4; the evaluation form template can be found in Appendix 3.
The list of participants is given in Appendix 1.
The workshop programme is given in Appendix 2.

2 Monday 29 May 2017: day 1 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 22nd workshop of the EURL-*Salmonella*, welcoming all participants to Zaandam, the Netherlands.

At this workshop, 45 participants were present, including representatives of the National Reference Laboratories (NRLs) for *Salmonella* from 27 EU Member States, two (potential) candidate EU countries, and three member countries of the European Free Trade Association (EFTA). Furthermore, representatives from the EC Directorate General for Health and Food Safety (DG-Sante), and the European Food Safety Authority (EFSA) were present. Apologies were received from representatives of NRLs from Malta, Bosnia and Herzegovina, and Turkey.

After a roll call of the delegates, the results of the evaluation of the last six workshops (2011-2016) were compared, showing variable results for the six workshops. The opinion on the scientific programme was the same in all workshops: very good to excellent.

The workshop started after the programme presentation and general information concerning the workshop.
The workshop programme can be found in Appendix 2.

2.2 *Salmonella* monitoring data and food-borne outbreaks for 2015 in the European Union

Valentina Rizzi, EFSA, Parma, Italy

The European Union (EU) Directive 2003/99/EC (EC, 2003) obligates the EU Member States (MSs) to collect data on zoonoses and zoonotic agents every year, and requests the European Food Safety Authority (EFSA) to analyse these data and to publish annual European Union Summary Reports (EUSRs) on zoonoses, foodborne outbreaks (FBOs) and antimicrobial resistance (AMR). EFSA is charged with the production of these annual EUSRs in collaboration with the European Centre for Disease Prevention and Control (ECDC) that collects and analyses human data. The most recent EUSRs on zoonoses, FBOs and AMR, related to 2015 data were published at the end of 2016 and the beginning of 2017 (EFSA and ECDC, 2016 and 2017). An update on data of *Salmonella* in humans, food and animals in the EU was given, as well as data on FBOs.

For 2015 data, the collaboration with DG SANTE units enable a cross-verification of data reported by MSs to both EFSA and EC in the context of specific programmes (i.e. *Salmonella* in poultry populations, *Salmonella* in pig carcases, bovine tuberculosis). Salmonellosis was confirmed as the second most frequently reported zoonoses in humans in the EU in 2015, after campylobacteriosis. The number of cases of

salmonellosis increased slightly, however, the decreasing EU trend in confirmed human salmonellosis cases observed in recent years has continued. Most MSs met their *Salmonella* reduction targets for poultry populations. In foodstuffs, the categories with the highest level of non-compliance to the microbiological criteria were minced meat, meat preparation, and meat products intended to be cooked before consumption. The reported EU level of *Salmonella* non-compliance in fresh poultry meat increased slightly. This was the first year that countries were required to report data on *Salmonella* in pig carcases at slaughter, according to Regulation 854/2004 (EC, 2004a); however, data may not be representative for the EU as they are based on the reports from a small number of MSs.

The analysis of the serovar distribution and trends in different animal populations and food categories shows an increase of *S*. Enteritidis in humans as well as in laying hens. The report also describes the overall distribution of the most common *Salmonella* serovars across different food, animal and meat sectors in the EU in 2015.
Salmonella was the first known causative agent of FBOs in 2015, representing 21.8% of all outbreaks reported in the EU. In total, 953 *Salmonella* FBOs were reported in the EU; a decrease of 40.6% compared to 2010. Of these outbreaks, 184 were supported by strong evidence. A new analysis provides a concise insight into the combinations of the causative agents and the food vehicles that were associated with the highest EU health burden in 2015. *Salmonella* in eggs continues to represent the most high-risk agent/food pair, being among the top-5 pairs for number of outbreaks, cases involved and hospitalizations. Other important food vehicles in strong-evidence *Salmonella* FBOs were 'pig meat and products thereof' and bakery products, but variations can be observed for different serovars.

The 2016 EUSR will include more detailed descriptive data analyses (analysis of domestic versus travel-related for human cases, and domestic versus imported for food-animal positive units), as well as improved data visualisation (joint maps for human and food-animal data).

Discussion
Q: I had some problems with downloading figures and tables.
A: A solution can be to first download the whole report and to save it; it should then be possible to view the figures and tables.
Q: The prevalence of *S.* Derby in pork seems to be equivalent to the prevalence of *S.* Derby in turkeys?
A: It is difficult to compare between countries as the data are very variable. It is only possible to compare results when countries have an official control programme in place for the specific parameter. Without this, it is up to the country to collect and report the data, meaning that not all countries report results.

2.3 Results of the 8th interlaboratory comparison study on detection of *Salmonella* in minced chicken meat (2016)

Angelina Kuijpers, EURL-Salmonella, Bilthoven, the Netherlands

In September 2016, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organised the eighth interlaboratory comparison study on the detection of *Salmonella* in food samples. The matrix of concern was minced chicken meat.
The participants were 34 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*): 30 NRLs from the 28 EU Member States (EU-MS), four NRLs from third countries within Europe (EU candidate MS or potential EU candidate MS, member of the European Free Trade Association (EFTA)) and one NRL from a non-European country.

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 minced chicken meat. The performance of the laboratories was compared with the criteria for good performance. The participants did not get a Standard Operating Procedure (SOP) but were asked to follow ISO/FDIS 6579-1 according to normal routine procedure for detection of *Salmonella* in 'official' samples. According to this document, it is possible to choose between Rappaport Vassiliadis Soya broth (RVS) or Modified Semi-solid Rappaport-Vassiliadis (MSRV) agar in addition to Mueller Kauffmann Tetrathionate novobiocin broth (MKTTn) for selective enrichment.
For the results, participants were asked to report what would have been reported should these samples have been routine samples. Therefore, the indication 'positive' (1) or 'negative' (0) per sample (after confirmation) was sufficient (independent of the combination of selective enrichment medium and isolation medium).

The samples consisted of minced chicken meat artificially contaminated with a diluted culture of *Salmonella* Stanley at a low level (approximately 15-20 cfu/25 g of meat), and at a high level (approximately 50-100 cfu/25 g of meat). Additionally, minced chicken meat samples without *Salmonella* (blank samples) had to be analysed. The samples were artificially contaminated at the laboratory of the EURL for *Salmonella*. Before the start of the study, several experiments were carried out to make sure that the samples were fit for use in an interlaboratory comparison study. For this, the stability of the *Salmonella* strain and the background flora in the meat was tested by storing the artificially contaminated meat samples at different temperatures:-20 °C, +5 °C and +10 °C. From the pre-test, it was concluded that the meat samples should be stored at -20 °C after preparation and after receipt at the participating laboratories, to stabilise *Salmonella* as well as the background flora. The pre-tests were performed with minced turkey meat. The choice of the matrix for this study was changed into minced chicken meat at the very last minute as it turned out that the batch of minced turkey meat was naturally contaminated with *Salmonella*.

Eighteen individually numbered blind samples with minced chicken meat had to be tested by the participants for the presence or absence of *Salmonella*. These samples consisted of six blank samples, six samples

with a low level of *S*. Stanley (inoculum 16 cfu/sample) and six samples with a high level of *S*. Stanley (inoculum 73 cfu/sample). Additionally, two control samples had to be tested: one blank control sample (procedure control (BPW)) and one own (NRL) positive control sample (with *Salmonella*).

Thirty-three of the 34 laboratories found *Salmonella* in all (contaminated) minced chicken meat samples, resulting in a sensitivity rate of 99%.

PCR was used as an own method by nine participants, and all found the same results as with the bacteriological culture method. Eight participants used a real-time PCR.

Nineteen participants used all three selective enrichment media (MKTTn, MSRV and RVS). Fifteen NRLs used two selective enrichment media, of which nine used MKTTn and MSRV and six used MKTTn and RVS.

For the positive control, the majority of the participants (21 laboratories) used a diluted culture of *Salmonella* Enteritidis (14), or *Salmonella* Typhimurium (7). The concentration of the positive control varied between 1and 10^4 cfu/sample. For the positive control it is advisable to use a concentration close to the detection limit of the method and a *Salmonella* serovar not often isolated from routine samples (to more easily recognise possible cross-contamination).

Three laboratories found one blank sample, containing only minced chicken meat, positive for *Salmonella*. After additional serotyping by these laboratories, it was shown that these 'blank' samples contained *Salmonella* Infantis and not *Salmonella* Stanley, the serovar used to artificially contaminate the meat samples. A possible clarification is natural contamination of the chicken meat with *Salmonella* Infantis at a very low level, as all other blank meat samples tested by the NRLs and the EURL (>200 samples) were negative for *Salmonella*.

All laboratories achieved the level of good performance.

More details can be found in the interim summary report and full report (Kuijpers and Mooijman, 2016 and 2017).

2.4 Preliminary results of the 20th interlaboratory comparison study on detection of *Salmonella* in chicken faeces (2017)

Irene Pol, EURL-Salmonella, Bilthoven, the Netherlands

In March 2017, the twentieth EURL-*Salmonella* interlaboratory comparison study on the detection of Salmonella in samples from the primary production stage was organised. In total, 36 NRLs participated in this study: 29 NRLs from 28 EU-Member States (MS), 6 NRLs from third countries within Europe (EU (potential) candidate countries and members of the European Free Trade Association (EFTA)) and on request of DG- Sante, one NRL from a non-European country.

In this study, *Salmonella* free chicken faeces, originating from a specific pathogen free (SPF) laying hen farm, was used. The chicken faeces

samples were artificially contaminated with *Salmonella* Infantis at the EURL laboratory.

Each NRL analysed a total of 20 blindly coded samples: 18 chicken faeces samples, of which 6 were not inoculated with *Salmonella* (blank samples) and 12 samples were inoculated with two different levels of *Salmonella* Infantis: 6x low (17 cfu/sample) and 6x high (55 cfu/sample). Additionally, 2 control samples consisting of a procedure blank control sample and an own positive control had to be analysed. The samples were stored at 5 °C until the day of transport. On Monday 13 March 2017, the contaminated chicken faeces samples were packed and sent to the NRLs. On arrival, the NRLs were asked to store the samples at 5 °C until the start of the analysis.

All laboratories used the prescribed method (Annex D of ISO 6579:2007 or ISO 6579-1:2017) with selective enrichment on MSRV agar.

All laboratories scored well, analysing both the procedure control as well as their own positive control samples. Only 1 laboratory reported the procedure control to be positive and the positive control to be negative (lab code 16). However, this was a reporting error, and this laboratory scored a moderate performance.

Almost all laboratories detected Salmonella in the faeces samples artificially contaminated with a high level of *Salmonella*. Two laboratories (lab codes 3 and 21) scored 1 of the 6 high level samples negative. This is still within the criteria for good performance which allows for 1 negative sample. In addition, almost all laboratories detected *Salmonella* in all 6 low contaminated samples. Three laboratories (lab codes 9, 34 and 36) scored 1 of the 6 low level contaminated samples negative for *Salmonella*. This is well above the criteria for good performance which allows three negative samples out of 6. The sensitivity score was 99% for these samples.
The specificity of the study is given by the correctly scored blank samples, and reached 99% for this study. Only 1 laboratory did not score all 6 blank samples negative (lab code 18). This laboratory scored 3 of the 6 blank samples positive for *Salmonella* and scored a poor performance.

Overall, the laboratories scored well in this year's study with an accuracy of 99%. Thirty-four laboratories fulfilled the criteria of good performance, one laboratory scored a moderate performance, and one laboratory scored a poor performance. The EURL will contact the latter laboratory for an explanation of the underperformance.

More details can be found in the interim summary report (Pol-Hofstad and Mooijman, 2017).

Discussion
Q: In my laboratory, one high level sample was tested negative for *Salmonella*. The MPN 95% range of the high-level samples was 11-110 cfu. Could it be the case that this negative tested sample did not contain *Salmonella* due to the variation in the number of cfu?
A: There is a small chance that this is possible.

Q: Did you identify the different strains in the background flora and their interference with *Salmonella* detection? We have seen in our laboratory that there are different populations in background flora in faeces (many *Klebsiella* spp.) compared to the ones in meat (many *Serratia* spp.).
A: We did not test for the different strains, but we know from earlier experiments that a high amount of background flora can disturb the detection of *Salmonella*.

2.5 Results of the 21st interlaboratory comparison study on typing of *Salmonella* (2016) – serotyping and PFGE

Wilma Jacobs, EURL-Salmonella, Bilthoven, the Netherlands

In November 2016, the 21st interlaboratory comparison study on serotyping and PFGE typing of *Salmonella* was organised by the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*, Bilthoven, the Netherlands). A total of 34 laboratories participated in this study. These included 29 National Reference Laboratories for *Salmonella* (NRLs-*Salmonella)* in the 28 Member States of the European Union (EU), 2 NRLs of the EU-candidate-countries Former Yugoslav Republic of Macedonia (FYROM) and Serbia, and 3 NRLs of the EFTA countries Iceland, Norway and Switzerland. The main objective of the study was to evaluate whether typing of *Salmonella* strains by the NRLs-*Salmonella* within the EU was carried out uniformly, and whether comparable results were obtained.

All 34 laboratories performed serotyping. A total of 20 obligatory *Salmonella* strains and one additional optional *Salmonella* strain from an uncommon type were selected for serotyping by the EURL-*Salmonella*. The strains had to be typed with the method routinely used in each laboratory, following the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007).

The individual laboratory results on serotyping, as well as an interim summary report on the general outcome, were emailed to the participants in February 2017. The O-antigens were typed correctly by 30 of the 34 participants (88%). This corresponds to nearly 100% of the total number of strains. The H-antigens were typed correctly by 28 of the 34 participants (82%), corresponding to 99% of the total number of strains. A total of 24 participants (71%) gave correct serovar names to the full set of strains, corresponding to 99% of all strains evaluated. A completely correct identification by all participants was obtained for ten *Salmonella* serovars: Infantis (S5), Duisburg (S6), Bispebjerg (S12), Typhimurium (S13), Enteritidis (S14), Reading (S15), Hadar (S16), Rissen (S17), Mikawasima (S19), and Virchow (S20). Most problems occurred with serotyping *Salmonella* serovar Umbilo (S3). Six laboratories had difficulties assigning the correct serovar name to this strain, mostly due to problems with the O-antigens.

All but four participants serotyped the additional strain S21, being a *Salmonella enterica* subsp. *diarizonae* (IIIb). However, not all laboratories had access to the required antisera to finalise the serotyping of this serovar (60:r:z).

At the EURL-*Salmonella* workshop in 2007, criteria for 'good performance' of the NRLs regarding the serotyping were defined. Two participants, both non-EU NRLs, did not meet the level of good performance at the initial stage of the typing study. A follow-up study was organized (May 2017) for one participant, consisting of ten additional strains for serotyping.

The individual laboratory results on the PFGE typing part will be reported to the 15 participants shortly after the Workshop. The participants were asked to test 10 *Salmonella* strains using their own routine PFGE method for digestion with XbaI. The evaluation of the analysis of the gel in Bionumerics was optionally included. A total of 10 participants also sent in their analysed gel data for evaluation.
The PulseNet Guidelines were used for the quality grading of the PFGE gel images, based on scoring 7 parameters with 1 (poor) point to 4 (excellent) points. Some variation in the quality of the gel images was observed, but also some improvements were seen since the first study in 2013.
The analysis of the gel in Bionumerics was evaluated according to the guidelines as used in the EQAs for the FWD laboratories. These guidelines use 5 parameters which are scored with 1 (poor), 2 (fair/good) or 3 (excellent) points. All participants scored 'Excellent' for the parameters 'Strips', and 'Normalisation'. Improvement could mainly be made for the parameter 'Band assignment'; this was most likely also influenced by the inclusion of some strains showing several 'double bands', thereby making the analysis more difficult.

PFGE typing, concerning the quality of PFGE gel image and also optional gel analysis in Bionumerics, will be again be offered in the 2017 interlaboratory comparison study on typing of *Salmonella*. MLVA typing on *S*. Typhimurium and/or *S*. Enteritidis and even WGS on *Salmonella* will be considered for introduction into future interlaboratory comparison studies.

More details can be found in the interim summary reports (Jacobs et al., 2017a and 2017b).

Discussion
Q: Is the use of alternative (sero)typing methods allowed (e.g. PCR, WGS)?
A: If validated, this is allowed. The problem is that there is not yet an official procedure for validation of alternative confirmation/typing methods. For this, part 6 of ISO 16140 has been drafted, but this standard has not yet been published. The EC Regulation indicates that the White Kauffmann Le Minor scheme has to be followed for serotyping, but the method is not specified.
Q: Would it be possible that the EURL validate alternative methods?
A: This is not exactly the task of the EURL, but merely the task of validation organisations like Afnor and MicroVal.
Q: How is it possible that some laboratories find O:17 positive, while the strain is positive for O:28?
A: This can be related to the quality of the antisera, and/or not following the manufacturer's instructions.
Q: Would the EURL-*Salmonella* consider including WGS or MLVA typing in the interlaboratory studies?

A: This will indeed be considered in future studies.

2.6 Update on the joint EFSA/ECDC molecular typing database and preliminary results of the survey on the use of WGS for typing *Salmonella*

Valentina Rizzi, EFSA, Parma, Italy

Following the EHEC crisis, a vision paper on the development of databases for molecular testing of food-borne pathogens in view of outbreak preparedness was prepared by the European Commission (EC), in consultation with ECDC, EFSA and the EURLs, and endorsed by the Member States in December 2012 (EC, 2012). Thereafter, the Commission asked EFSA to provide technical support regarding the collection of molecular typing data of food, feed, and animal isolates of *Salmonella*, *Listeria monocytogenes* and VTEC, and a similar request was made to ECDC on molecular typing data of human isolates. In addition, the Commission asked EFSA and ECDC to establish a joint database for the molecular typing data of these foodborne pathogens of human and non-human origin. The aim of the joint EFSA-ECDC database is to collect molecular typing data so that the linkage of molecular typing data from humans to similar type of data from food and animals is possible. This will enable and support detection and investigation of outbreaks and will contribute to source attribution studies. The data collection covers molecular typing results obtained through Pulsed Field Gel Electrophoresis (PFGE) for *Listeria monocytogenes, Salmonella* and VTEC, and Multiple-Locus Variable number tandem repeat Analysis (MLVA) only for *Salmonella* Typhimurium and *Salmonella* Enteritidis.

The joint database is physically hosted at ECDC, and more specifically in the European Surveillance System (TESSy). Typing data on bacterial isolates from food/feed and animals and their environment (non-human data) are reported to EFSA by the food and veterinary authorities and laboratories of the MSs. A subset of these data is then submitted by EFSA to the joint database. Different rights for data accessibility are associated with the different users. Moreover, to further protect the confidentiality of data, a collaboration agreement has been signed between the main actors in the database (ECDC, EFSA and European Union Reference Laboratories). In addition, to avoid any improper or non-authorised use of the data, all data providers are asked to sign an agreement with EFSA or ECDC, based on their area of competence, before any data submission or access to the database.
In the context of this project, the MSs have been invited by EC to nominate their representatives for the food safety/veterinary sector and to sign the specific agreement with EFSA. Until now, 12 countries have nominated their representatives, and one country has successfully submitted its data to the joint database. To promote the participation of laboratories in the data collection, the Steering Committee of the Molecular Typing Data Collection Project has published a paper explaining all the technical and collaborative aspects of the data collection system (Rizzi et al., 2017).

Following the recent development of Whole Genome Sequencing (WGS) as a new tool to investigate, assess and manage microbiological food safety issues, the EC has sent MSs a questionnaire on the availability of WGS methods for foodborne and waterborne pathogens isolated from animals, food, feed and environmental samples. The scope is to collect information about the WGS capacity in the laboratories of seven EU networks (*Salmonella, Listeria monocytogenes, Escherichia coli* including VTEC, live bivalve molluscs, *Campylobacter*, coagulase positive staphylococci, antimicrobial resistance). Preliminary results for the *Salmonella* network were presented.

Discussion
Q: What will be arranged for storage of WGS data? Currently most NRLs use in-house storage, but will it be possible that EFSA offers a public cloud for data at EU level?
A: EFSA has received a mandate from the EC to investigate the possibilities to expand the molecular ECDC-EFSA database for WGS data. The first step is to evaluate possible solutions for how to collect, analyse and store the WGS data across Europe. It is important that confidentiality of the data is guaranteed.

2.7 *Salmonella* Enteritidis outbreak related to Polish eggs

Pamina Mika Suzuki, DG-Sante, Brussels, Belgium

The Commission is working to improve crisis preparedness and management in the food and feed area in order to ultimately ensure a more effective and rapid containment of food and feed-related emergencies and crises in the future. Threats, which may relate to accidental mismanagement within food production processes or even to intentional acts such as bio-terrorist attacks, may seriously undermine the established high level of protection for consumers within the EU single market and put into question their confidence in the safety of the overall system.

In 2016, two countries reported unusual increases of *Salmonella* Enteritidis cases with MLVA type 2-9-7-3-2: the United Kingdom in January and the Netherlands in August. Cases with the same MLVA type were reported from other European Union/European Economic Area (EU/EEA) countries. Cross-border investigations were initiated to identify the source so that measures could be taken by Competent Authorities to stop the outbreak.

A probable case was *S*. Enteritidis positive with MLVA type 2-9-7-3-2 or 2-9-6-3-2 and symptom onset after 1 May 2016. A confirmed case was characterized by whole genome sequencing (WGS). Patient interviews and epidemiological studies were performed at national level. Food/environmental investigations were carried out in 6 countries and information collected through the Rapid Alert System for Food and Feed (RASFF).

Patient interviews suggested exposure outside of the home. Dutch investigations revealed a common link to one Polish egg packing centre. Of 48 farms, 18 had 82 *Salmonella* positive flocks. Over

600 consignments with 97 million eggs were distributed to 18 EU/EEA and 30 million eggs to 12 third countries during the withdrawal period. As of 5 May 2017, 13 EU/EEA countries have reported 230 confirmed and 245 probable cases (two patients died).
This is an example of a good multi-sectorial approach and collaboration between public health authorities (follow-up of human cases), food safety authorities (investigations to source), laboratories, risk assessors and risk managers. The outbreak underlines the importance of cross-sectorial investigations both at national and EU level, which was also possible thanks to the systems and networks in place to manage foodborne outbreaks: notably the RASFF system was effective for coordinating targeted control measures in the food sector.

Molecular typing data (MLVA and WGS) together with epidemiological and traceability information were crucial to narrow down the investigations for source identification. The collection of molecular typing data provides valuable support to risk managers to enable them to quickly respond to challenges posed by threats such as multinational foodborne outbreaks.

Discussion
Q: What went wrong; how could this happen in the Polish packing centre?
A: Some shortcomings in control of *Salmonella* Enteritidis at farm level were identified during an audit in Poland. There are some learning points for Poland to ensure that legislation is applied correctly. Polish competent authorities are performing investigations to identify what exactly went wrong.

2.8 Outbreak of a new serotype *Salmonella enterica* subsp. *enterica* with the antigenic formula 11:z_{41}:e,n,z_{15} in Greece, 2016-2017

Aphrodite Smpiraki, NRL-Salmonella, Chalkida, Greece

In a two-month period between March to May 2016, eleven *Salmonella enterica* subsp. *enterica* isolates with an unusual antigenic type (11:z_{41}:e,n,z_{15}), not referred to in the White-Kauffman-Le Minor Scheme (Grimont and Weill, 2007), were identified by the National Reference Laboratory for *Salmonella* and *Shigella* (NRLSS) in Greece (Mandilara et al., 2016). Their PFGE profiles were uploaded to the European Surveillance System (TESSy) operated by the ECDC. No other isolates with a matching PFGE profile (XbaI.2460) have been reported to TESSy. An urgent inquiry (UI-358) was launched via the ECDC's Epidemic Intelligence Information System. None of the 15 countries that replied to the UI had identified the new serovar in the past. According to the database of the NRL-*Salmonella* and of the Hellenic Veterinary Reference Laboratory for *Salmonella*, the specific antigenic type had never previously been identified, neither from animals, animal products nor from food samples**.** According to Institute Pasteur, the isolates represent a putative new serotype of *Salmonella enterica* subsp. *enterica.*

During initial investigations based on the results from trawling questionnaires, no food item emerged as possible source of the infections. An analytical case-to-case study was further performed to identify the possible risk factors, and this showed an association

between infection and a sesame-based product (sesame paste, tahini). The hypothesis was supported later on by the epidemiological data from Germany and Luxembourg, where consumption of sesame-based products was associated with the new serotype.
Whole Genome Sequencing and PFGE analyses have confirmed that the isolates from the infected cases that occurred in the past year in four EU Member States (Greece, Germany, Czech Republic and Luxembourg) are genetically close (clustered) and probably share a common source of infection. Hence, it is likely that contaminated sesame batches are among the EU MS's food chain; attention should be paid to a possible occurrence of new cases.

Discussion

Remark: EFSA and ECDC are currently drafting a Rapid Outbreak Assessment (ROA) on this outbreak, and Greece will be consulted before this ROA is published.

2.9 Salmonellosis or *Salmonella* infection – high nasal colonization rates of *Salmonella enterica* subspecies *diarizonae* 61:k:1,5,(7) in Swiss sheep herds

Gudrun Overesch, NRL-Salmonella, Bern, Switzerland

Salmonella (*S*.) *enterica* subspecies *diarizonae* (IIIb) serovar 61:(k):1,5,(7) (*S*. IIIb 61:(k):1,5,(7)) is considered to be host adapted to sheep and is found regularly in faeces of healthy carriers.
Two cases of chronic proliferative rhinitis (CPR) in sheep have been described in association with *S*. IIIb 61:k:1,5,(7) in the USA and Spain, and for the first time in Switzerland. Three animals from a flock of Texel sheep suffering from chronic nasal discharge and dyspnea with subsequent death were necropsied. The pathological lesions are consistent with a severe proliferation of the nasal mucosae of the turbinates in association with severe chronic inflammation.
S. IIIb 61:(k):1,5,(7) was isolated from lesion by direct bacteriological culture, and the presence of *Salmonella* spp. was confirmed by immunohistochemistry. Sheep from the affected flock were systematically tested after the first occurrence of the diseases. Clinical investigation of all sheep (lambs n=28, adults n=31) in the flock revealed 38.7% (n=12) of the adult sheep with nasal discharge and 9.7% with severe dyspnea (n=3). Very high positivity of nasal mucosa (87.1%), but low prevalence in faeces (5.9%) for *S*. IIIb 61:k:1,5,(7) was found in the adult sheep. The results lead to the assumption of a long-term nasal colonization leading to chronic disease and death after several months to years.

Discussion

Q: The NRL-*Salmonella* in Germany regularly receives isolates from sheep; these are most often the monophasic variant of this type. Did you find the monophasic variant as well?
A: In Switzerland only the non-monophasic variant was found. However, some other Member States have also found the monophasic variant. It was indicated that it may be difficult to find H:k; it may take several attempts and incubation of e.g. 2 days to find it. This *Salmonella*

serovar has occasionally been found in animals other than sheep, although generally it is considered to be sheep-adapted. For instance, the NRL-*Salmonella* from Greece found this serovar in dogs that were kept along with sheep.

2.10 Update on activities in ISO and CEN

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

Kirsten Mooijman of the EURL-*Salmonella* presented an overview of activities in ISO and CEN in relation to *Salmonella*.
The relevant groups in ISO and CEN are:

- ISO/TC34/SC9: International Standardisation Organisation, Technical Committee 34 on Food Products, Subcommittee 9 – Microbiology;
- CEN/TC275/WG6: European Committee for Standardisation, Technical Committee 275 for Food Analysis – Horizontal methods, Working Group 6 Microbiology of the Food Chain.

At the time of the workshop, the annual meetings of both groups still had to be organised (19-23 June 2017), therefore no update on the outcome of these meetings could be given. However, throughout the year, members of ISO/SC9 and CEN/WG6 are regularly informed about ongoing and new activities, and a summary of relevant activities was presented at the workshop.

EN ISO 6579-1
Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Horizontal method for the detection of *Salmonella* (Anonymous, 2017a).
The first FDIS (Final Draft International Standard) voting took place from 12 November 2015 to 12 January 2016. The outcome was: 100% positive in CEN (20 approvals, 13 abstentions) and 96% positive in ISO (24 approvals, one disapproval). The total outcome was positive, with 13 pages of comments, mainly editorial. A few technical comments were given which had to be taken into account. For that reason, a written consultation of ISO Resolution No. 686 took place from 9 March to 20 April 2016. However, in June 2016, CEN decided that a second FDIS vote was needed, which took place from 31 October until 26 December 2016. The outcome in ISO was 31 approvals, one disapproval, and 11 abstentions. The last editorial comments were introduced in the document after which the final version of EN ISO 6579-1 was published on 28 February 2017. The changes compared to EN ISO 6579:2002 are considered as minor and have little to no effect on the performance characteristics. Still it may be necessary that individual laboratories discuss with the accreditation board in their country whether an internal re-verification of the performance characteristics is needed for accreditation. A summary of all the changes will be published in Food Microbiology (Mooijman, in press).

Draft ISO/TS 6579-4 PCR monophasic *Salmonella* Typhimurium (cooperation ISO and CEN)
In 2016, several draft versions of the standard were prepared by Burkhard Malorny (NRL-*Salmonella* Germany) and discussed with the

EURL-*Salmonella* and the experts of CEN-TAG3. Earlier it was agreed that the performance characteristics of the standard will be determined in an interlaboratory study with a 'standard set of strains', to be organised by the EURL-*Salmonella*. In November 2016, EURL-*Salmonella* made a call for test strains to create this 'standard set of strains'. By March 2017, the EURL had received approximately 400 strains. The identity of all strains was verified by the EURL. Next, a selection of the 400 strains will be used to verify the 3 PCR procedures described in draft ISO/TS 6579-4 by the NRL-*Salmonella* in Germany and by the EURL-*Salmonella*. After this, the draft document may need further amendments. When the technical work is finished, the work will be moved to ISO-WG10, after which the New Work Item Proposal (NWIP) will be launched. As soon as a final draft version of ISO/TS 6579-4 is available, the interlaboratory study will be planned to determine the performance characteristics. The timing of this ILS is unsure.

Harmonisation of incubation temperature

In 2014, at an annual meeting of ISO/TC34/SC9 and CEN/TC275/WG6, it was agreed to use a broader temperature range for incubation of non-selective media (34-38 °C instead of 37 °C ± 1 °C). To accept a broader temperature range for the incubation of selective media, data were needed showing no effects on the results when incubating at this broader temperature range. In 2014-2015, the laboratory of Adria in France performed experiments to test the influence of incubation temperature (35 °C or 37 °C) on the growth of *Salmonella* and on several *Enterobacteriaceae* species. These experiments showed no difference in growth of *Salmonella* spp. at both temperatures, but some impact on the growth of some (other) *Enterobacteriaceae* species. Therefore, it was proposed to set up a protocol to test the influence of the incubation temperature with a larger group of laboratories (members of ISO and CEN), especially to test the influence on the growth of E*nterobacteriaceae*. In 2016, a protocol was prepared for comparing incubation of MKTTn broth (for detection of *Salmonella*) at 35 °C and at 37 °C. The members of ISO and CEN were invited to perform experiments, following the protocol. By March 2017, results had been received from 7 laboratories, from different countries, and the data will be analysed before the next annual meeting of ISO-SC9 and CEN-WG6 (June 2017).

CEN mandate M381

This project started in 2007 with the aim of standardising and validating methods that are referred to in legislation, in order to support the EU food policy. The project concerned international standardisation and validation of 15 microbiological methods. One of these sub-projects concerns the validation of the method for detection of *Salmonella* in samples from the primary production stage (pps). The performance characteristics for detection of *Salmonella* in pps samples were determined from the EURL-*Salmonella* interlaboratory studies of 2008 (chicken faeces), 2012 (pig faeces) and 2013 (boot socks – combined EURL/CEN mandate study). The CEN mandate project ended in June 2017. By then, all 15 EN/ISO standards, including the performance characteristics had been published. The raw data of all studies will remain available for possible future recalculations and are likely to be stored at DG-Sante and at CEN. It has been agreed that each project leader will prepare a manuscript about

each validation study for publication in a special issue of the International Journal of Food Microbiology.

Pre-enrichment step
The CEN Task group, TAG9, was set up in 2012 with the aim of preparing an optimal pre-enrichment medium for detection of several (mainly Gram negative) pathogenic bacteria, in order to resuscitate stressed or damaged cells. The group is currently working on a protocol to evaluate pre-enrichment media performance characteristics. The objective of this protocol is to evaluate the performance characteristics of pre-enrichment media (mainly raw ingredients, composition, etc.) during the development stage, and not as routine control. In this protocol, information will be given on stressing strains and the minimum concentration (cfu/ml) to be obtained after pre-enrichment. The target organisms are *Salmonella, Enterobacteriaceae,* STEC, *Cronobacter,* and *Listeria*. A first draft version of the protocol for review by the members of WG6 is planned for the end of 2017.
TAG9 is also working on a second protocol to evaluate neutralizing procedures/ingredients (given for example in EN ISO 6887-4; Anonymous, 2017b) to be used when inhibitory substances are present in the sample during pre-enrichment. A first draft of this second protocol is expected to be available in April 2018.

ISO working group on WGS
In 2014, a new working group was set up under ISO/TC34/SC9 to take a closer look at the options for standardisation of protocols for Whole Genome Sequencing. The project leader of this group is located in the USA. The original plan of WG25 was to draft a standard in three parts:

- Part 1: Wet laboratory sequencing and analysis of sequence data.
- Part 2: Validation of data and methods.
- Part 3: Metadata and sequence repository (not to develop databases, but to give guidance on how to control the quality of databases and pipelines).

However, while drafting the document, it was noticed that there was an overlap between the three parts and that it would be better to merge the three parts in one document. A next draft document is expected by the end of 2017.

Miscellaneous
Early in 2017, the revised versions of parts 1 to 4 of EN ISO 6887 ('Microbiology of the food chain - Preparation of test samples) were published. These documents contain important information on the preparation of many different types of samples:

- Part 1: General rules for the preparation of the initial suspension and decimal dilutions.
- Part 2: Specific rules for the preparation of meat and meat products.
- Part 3: Specific rules for the preparation of fish and fishery products.
- Part 4: Specific rules for the preparation of miscellaneous products (e.g. animal feed, eggs, cocoa products, acidic products).

In March 2017, the revision of Part 5 ('Specific rules for the preparation of milk and milk products') started.

Since 2014, ISO/TS 22117 ('Specific requirements and guidance for proficiency testing by interlaboratory comparison') has been under revision. The revision of this document will (amongst others) include:

- to make the document a full standard (instead of a Technical Specification - TS), as a TS is not recognised in some countries;
- to take into account some new information on statistical aspects for Proficiency Tests (PTs);
- PT schemes for viruses, parasites, primary production, yeasts and moulds and molecular methods.

Discussion

Q: Is reverification of ISO 6579-1 needed for accreditation?
A: The modifications are considered to be minor and for that reason verification will not be necessary. However, the opinion of the accreditation body may differ per Member State. Some NRLs have already discussed this with their accreditation body, and for example in Greece it was indicated that reverification has to be done for everybody and also when enriched cultures are stored at 5 °C.
Q: Is it necessary to always perform Annex D of ISO 6579-1 (for detection of *S.* Typhi and *S.* Paratyphi)?
A: No, not for regular/routine samples. It is only necessary to perform it for special needs, e.g. in case of outbreaks.
Q: Is confirmation of only one suspect colony (ISO 6579-1) permitted?
A: Indeed, that is correct. If this colony is negative for *Salmonella*, then up to 4 more suspect colonies have to be confirmed.
Q: Is it possible to store pre-enriched/selective enriched cultures for all kinds of products (ISO 6579-1)?
A: This has been tested for many different products and has worked fine. The UK NRL-*Salmonella* noted having found good results with storage of pre-enriched cultures of animal samples (faeces, boot socks), instead of storage of the samples.
Q: Is it possible to read MSRV agar plates only after 48 h and not after 24 h (ISO 6579-1)?
A: This may generally be possible, but it could cause some problems due to overgrowth of background flora.
Q: Is it necessary to confirm for O-antigens as well as for H-antigens?
A: Yes. The number of biochemical tests in ISO 6579-1 has been reduced from 6 to 3, and therefore it is considered important to test for H-antigens (group level) in addition to O-antigens (group level).
Q: When will the interlaboratory validation study for determining the performance characteristics of ISO/TS 6579-4 be organised?
A: This is not yet known. First a selected set of test strains will be tested with the PCR protocols of draft ISO/TS 6579-4 by the NRL-*Salmonella* in Germany and by the EURL. Next a further selection of strains will be made for the interlaboratory study and, if necessary, the protocols of draft ISO/TS 6579-4 will be amended. Before the validation study can be organised, ISO/TS 6579-4 should be available as final draft version.
Q: How much time do laboratories have to introduce the new ISO 6579-1 in their laboratories?
A: In general one year, but the period may vary per accreditation organisation/country. For example, in France this period is only 6 months.

2.11 Validation of alternative microbiological methods – the ISO 16140 series

Paul in 't Veld, Netherlands Food and Consumer Product Safety Authority (NVWA), Utrecht, the Netherlands

The first version of ISO 16140, for the validation of alternative methods, was published in 2003 (Anonymous, 2003) after 10 years of development. The development started in a European project called EURECA. In 2005, it was decided to revise ISO 16140 and to develop additional standards for validation of methods. In ISO/TC34/SC9 a working group was raised (WG3) with the following mandate:

- Revision of ISO 16140:2003;
- Development of a standard for verification;
- Development of a standard for validation of reference methods;
- Development of a standard for single lab validation;
- Development of a standard for intermediate validation;
- Development of a standard for validation of confirmation methods.

The following standards, prepared by WG3 have been published or are in the process for publication:

- ISO 16140-1: 'Vocabulary', published in 2016 (Anonymous, 2016a);
- ISO 16140-2: 'Protocol for the validation of alternative (proprietary) methods against a reference method', published in 2016 (Anonymous, 2016b);
- ISO 16140-3: 'Protocol for the verification of reference and validated alternative methods implemented in a single laboratory', for DIS voting (Draft International Standard) by the end of 2017;
- ISO 16140-4: 'Protocol for single-laboratory (in-house) method validation, for DIS voting by the end of 2017;
- ISO 16140-5: 'Protocol for factorial interlaboratory validation for non-proprietary methods', for DIS voting by the end of 2017;
- ISO 16140-6: 'Protocol for microbiological confirmation and typing procedures', for DIS voting by the end of 2017;
- ISO 17468: 'Technical requirements and guidance on establishment or revision of a standardized reference method', published in 2016 (Anonymous, 2016c).

A scheme has been drafted to give directions for the choice of the standard to be used. This scheme will be published in each standard for validation/verification of microbiological methods.

ISO 16140-2:2016 is the successor of ISO 16140:2003. The basis is the comparison between a reference method and an alternative method. Protocols are given for validation of qualitative and quantitative alternative methods. Each protocol has two phases: 1) a method comparison study and 2) an interlaboratory study. The method comparison study is performed by one expert laboratory and focusses on testing a diversity of samples/matrices. The interlaboratory study is performed with a group of laboratories and establishes the 'reproducibility' of the method using a single matrix. Evaluation of the

data is performed using pre-set criteria. The alternative method should at least give comparable results to the reference method, but can also be better when this is proven.
ISO 16140-3 describes a procedure for verification of methods. The content of the final procedure is still under discussion. The difference between validation and verification is clarified in its definitions:
Validation: establishment of the performance characteristics of a method and provision of objective evidence that the performance requirements for a specified intended use are fulfilled.
Verification: demonstration that a validated method functions in the user's hands according to the method's specifications determined in the validation study, and that it is fit for its purpose.

ISO 16140-4 describes the single-lab validation. In this standard, two experimental designs are given: the classical approach and the factorial design approach. For both experimental designs, a protocol is described with and without the use of a reference method. It is important to know that the results of the validation study following ISO 16140-4 are only valid in the laboratory that conducted the study.

ISO 16140-5 describes a factorial interlaboratory study. By using the factorial design, fewer laboratories (≤ 4) are needed for the study in comparison to ISO 16140-2. However, the factorial approach cannot replace the interlaboratory study of an alternative (proprietary) method according to ISO 16140-6.

ISO 16140-6 describes the validation of a (proprietary) alternative confirmation/typing method against the confirmation/typing procedure of a reference method. The validation study starts with a suspect colony and not with a (food) sample. The study is based on the inclusivity/exclusivity study of ISO 16140-2, using well characterised strains. A differentiation is made between validation at family, genus, species or type level. A comparison is made between the reference method and the alternative method in a method comparison study and an interlaboratory study.

Discussion
Q: What has to be done to introduce the new ISO/TS 6579-4 for identification of monophasic *S.* Typhimurium in the laboratory?
A: In fact this is a verification and should be described in part 3 of ISO 16140. However, what information should be introduced for verification of confirmation/typing methods in the laboratory is still being discussed.
Q: Which part of the ISO 16140 series should be used to validate alternative molecular typing methods?
A: For this, part 6 of ISO 16140 should be followed. It has to be clear which part of the reference confirmation step will be replaced by the alternative method. For example, if the alternative confirmation method only indicates whether *Salmonella* spp. is detected or not, then the alternative method has to be validated against the confirmation procedure as described in ISO 6579-1 (Anonymous, 2017a). However, if the outcome of the alternative method is a *Salmonella* serovar, then the alternative method has to be validated (per serovar) against the serotyping method described in ISO/TR 6579-3 (Anonymous, 2014).

Q: In Regulation 2073/2005 (EC, 2005), it is indicated that ISO 16140 has to be followed for validation studies, 'or other internationally accepted similar protocols'. What other protocols exist?
A: In the Netherlands, only those validation studies performed by an independent organisation (Afnor or MicroVal) in accordance with ISO 16140-2 are accepted. However, in other countries, validation studies performed by AOAC or NordVal are also accepted; these organisations may use different protocols. For validation studies performed by these latter organisations, it is also important to check to which reference method the alternative method is validated. In AOAC validation studies, the reference method is often a US method and not an EN/ISO method.
Q: In draft ISO 16140-3 for verification, samples have to be inoculated with very low contamination levels (1-3 cfu/g) to test LOD_{50}. These low levels are hard to achieve.
A: In draft ISO 16140-3, information is given on how to do this. First, a suspension of the target strain is made and checked for its contamination level. Next, dilutions are made from this suspension and used for spiking the samples. This should be a feasible way to produce low level contaminated samples.

3 Tuesday 30 May 2017: day 2 of the workshop

3.1 Activities of the NRL-*Salmonella* to fulfil tasks and duties in the Netherlands

Kirsten Mooijman, NRL-Salmonella, Bilthoven, the Netherlands

The Dutch NRL-*Salmonella* is situated (like the EURL-*Salmonella*) at the Centre for Zoonoses and Environmental Microbiology (Z&O) of the National Institute for Public Health and the Environment (RIVM) in Bilthoven, the Netherlands. At RIVM-Z&O a total of 6 biological NRLs are located: NRL-*Salmonella* (since 1993), NRL-Parasites (since 2005), NRL-bivalve molluscs (since 2005), NRL-*E. coli* (since 2011), NRL-*Listeria monocytogenes* (since 2011) and NRL-coagulase positive staphylococci (since 2011). RIVM-Z&O is accredited for all NRL (and EURL) activities. The task and duties of the NRLs are defined in Regulation 882/2004 (EC, 2004b):

- *'Collaborate with the EURL in relevant area'*. This is well organised, as both the EURL and the NRL are located in the same institute. Sometimes care has to be taken to keep activities for EURL and NRL separate, e.g. to make sure that the decoding of samples for EURL interlaboratory studies are not known by technicians performing the study as NRL.
- *'Coordinate activities of official national laboratories for analysis of samples'.* The Dutch NRL-*Salmonella* does not perform sample analysis for monitoring programmes; this is done by the official laboratories in the Netherlands. The NRL supports these official laboratories, e.g. by giving advice and organise training courses. Occasionally, the NRL performs additional sample testing as a second opinion, and performs (sero)typing of *Salmonella* isolates which the official laboratories are not able to type, including confirmation of monophasic *Salmonella* Typhimurium.
- *'Organise comparative tests'*. In the Netherlands, there is only one official laboratory for the analysis of *Salmonella* in food and feed samples. This laboratory participates, together with the NRL, in the relevant EURL interlaboratory comparison studies. For analysis of *Salmonella* in samples from the primary production stage (PPS), the Netherlands has 22 officially approved (private) laboratories, of which 13 also perform serotyping of *Salmonella*. Up to approx. 2009, the NRL-*Salmonella* organised interlaboratory studies itself. However, after 2009, the ministry no longer provided budget for the organisation of interlaboratory studies for private laboratories. Therefore it was decided that all official laboratories had to participate and pay for the same Proficiency Tests (PT). The selected PT schemes are offered by a UK organisation accredited by UKAS. The official laboratories participate in PT schemes for detection of *Salmonella* in poultry samples 4 times per year. Some also participate in PT schemes for serotyping *Salmonella*. Each laboratory has forwarded its lab code to the NRL-*Salmonella*, so that the NRL can judge the performances of all official laboratories in the different PT schemes. This appraisal is mainly based on the results of the

trend analysis of successive studies per laboratory. Should a laboratory find unsatisfactory results in more than one study, the NRL will contact this laboratory to ask for an explanation of the poor results and to find out if the NRL can be of help. If no improvement is seen in the trend results of a laboratory in several successive PTs, the NRL will also contact the Competent Authority. The Competent Authority can decide to (temporarily) suspend the approval of an official laboratory.

- *'Disseminate information supplied by EURL to authorities and national laboratories'*. The EURL reports and Newsletters are forwarded to the Competent Authority. Technical information from the EURL is forwarded to the official laboratories.
- *'Assist the national Competent Authority'*. The NRL-*Salmonella* cooperates with the Netherlands Food and Consumer Product Safety Authority, e.g. for approval of official Dutch laboratories. Additionally, the NRL participates in committees/working groups at national level for e.g. introduction of new/amended EC legislation. When needed, the NRL assists the Competent Authority in case of outbreaks.

Discussion
Q: Who analyses the samples for primary production in the Netherlands?
A: This is performed by private official (approved) control laboratories.

3.2 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Serbia

Jasna Kureljusic, NRL-Salmonella, Belgrade, Serbia

Serbia is a country located in the Balkans, in Southern Europe, and has a total population of 7 million; Belgrade is its capital city.

The Scientific Veterinary Institute of Serbia was founded on 11 February 1926 under the name Central Veterinary Bacteriological Institute. The Department had the task of testing the vaccine (vaccination) and diagnosis of bacterial, viral and parasitic diseases of animals. Experts from the Institute established and managed livestock diseases in the field. In addition to their regular activities, the experts of the Institute advised farmers and regularly gave lectures on cattle infections on the radio station in Belgrade. After the end of World War II, on the initiative of the Ministry of Agriculture Republic of Serbia, the acting Chief Veterinary administration founded the Veterinary Bacteriological Institute NR Serbia. In 1947, the Institute moved to Vozdovac - street Bulevar Vojvode Stepe no. 295, where it remained until 1982. The Institute then moved to two locations in Belgrade. Today's Scientific Veterinary Institute of Serbia is one of the leading scientific and professional institutions in the field of veterinary medicine. It consists of the Institute for Health Care and the Institute of Food and Drug.
The Institute for Health Care contains: the Department for sampling, media preparation, and sterilization; the Department of Epizootiology for epizootiology clinical pathology, pathological morphology and reproduction; the Department of Bacteriology and Parasitology; the Department of Virology; the Department of Immunology; and the Department of Epizootiology for health protection of birds.

The Institute of Food and Drug contains: the Department for sampling, media preparation and sterilization; the Department of food and feed safety; the Department of Radiation Hygiene; and the Department of Chemistry and Biochemistry and testing of drugs.
The Scientific Veterinary Institute of Serbia has 19 PhD vet. med., 5 MSc vet. med., and 23 lab technicians. The National Reference Laboratories situated at the Virology Department are the NRL for classical swine fever (CSF), NRL for African swine fever (ASF), NRL for foot and mouth disease (FMD), NRL for bluetongue (BT), NRL for rabies, NRL for bovine leucosis, NRL for equine infectious anaemia (EIA), NRL for African horse sickness (AHS), NRL for Equine Influenza, NRL for swine vesicular disease (SVD), and NRL for fish diseases. The National Reference Laboratory located at the Bacteriology Department is the NRL for salmonellosis. The National Reference Laboratories situated at the Immunology Department are the NRL for brucellosis, NRL for glanders and NRL for dourine.

3.3 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Bulgaria

Gergana Mateva, NRL-Salmonella, Sofia, Bulgaria

The NRL-*Salmonella* is part of the National Diagnostic and Research Veterinary Medical Institute (NDRVMI) founded in 1901. NDRVMI is a specialized structure of the Bulgarian Food Safety Agency (BFSA) and conducts research, scientific, applied, reference, diagnostic and expert activities in the field of animal health, food and feed safety, and environmental issues. The National Diagnostic and Research Veterinary Medical Institute has two national centres: animal health and food safety, and two departments: Aquaculture and aquatic animal and bee diseases, and Exotic and Especially dangerous infections. Furthermore, there are two regional laboratories: Stara Zagora and Veliko Tarnovo.

The National centre of food safety has two departments:

- Microbiology of food, feed and farm and environmental samples;
- Physico-chemical analysis of food.
- The activities of the NRLs for *Salmonella*, *Campylobacter*, staphylococci and antimicrobial resistance are:
- Serotyping of *Salmonella* isolates from the National Control Programmes;
- Serotyping of *Salmonella* isolates from food, feed, environment and veterinary samples isolated in other laboratories;
- Confirmation and identification of *Campylobacter* spp. through biochemical tests;
- Detection of staphylococci enterotoxins, types SEA to SEE in food;
- Determination of Antimicrobial Resistance (MIC) for *Salmonella* spp., *Staphylococcus* spp., *E. coli* and *Enterococcus* spp.

Bulgaria has the following National control programmes for *Salmonella*:

- National control programme for *Salmonella* in breeding flocks *(Gallus gallus)*;
- National control programme for *Salmonella* in flocks of laying hens *(Gallus gallus)*;

- National control programme for *Salmonella* in flocks of broilers *(Gallus gallus)*;
- National control programme for *Salmonella* in flocks of turkey.

Since 2006, Bulgaria has an NRL-*Salmonella* accredited according to ISO 17025 (Anonymous, 2005) for detection of *Salmonella* (since 2006), serotyping of *Salmonella* (since 2009), for Antimicrobial Resistance testing (since 2014), and for staphylococci (enterotoxins; since 2014).

The NRL-*Salmonella* organize interlaboratory studies for laboratories in the food safety system and participating private laboratories.
From 2006 to 2016, 1995 *Salmonella* serovars were isolated. The most prevalent serovars were *S*. Infantis (33%), *S*. Typhimurium (16%) and *S.* Enteritidis (13%).

Our observations for 2006 – 2016 are that:
- *Salmonella* is mainly isolated from food (75%) and 25% of the isolates are isolated from animals, faeces and other sources;
- Of the *Salmonella* strains in food, the highest percentage is isolated from poultry meat (approx. 57%);
- The most common serovar for the country, obtained from poultry meat, is *S. Infantis*.

3.4 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Cyprus

Konstantinos Arsenoglou, NRL-Salmonella*, Nicosia, Cyprus*

The Laboratory for the Control of Food of Animal Origin (LCFAO) of Cyprus Veterinary Services is accredited according to ISO 17025 (Anonymous, 2005) for 30 methods since 2004.
It is the NRL of Cyprus for *Salmonella, Listeria monocytogenes, Campylobacter,* Coagulase Positive staphylococci*,* Verotoxinenic *Echerichia coli* and other *E. coli,* Milk and Milk products*, Trichinella,* and Marine Biotoxins.

In 2016, the LCFAO examined 1156 batches (x5 samples) of food samples of animal origin after official sampling. 753 were samples from meat and meat products and 403 from dairy products. Of the 1156, 66 were positive (5.7%), of which none were isolated from dairy samples and 52 out of the 66 were isolated from poultry related samples (79% of the positive samples). Moreover, the NRL examined 76 animal feed samples after official sampling, of which 6 were positive (7.9%).

Serotypes of *Salmonella* isolated in LCFAO in 2016:
- Salmonella Infantis
- Salmonella Virchow
- Salmonella Anatum
- Salmonella Mishmarhaemek
- Salmonella Kedougou
- Salmonella Telaviv
- Salmonella Kentucky
- Salmonella Give
- Salmonella Schwarzengrund

- Salmonella Seftenberg (feed)
- Salmonella Tennessee (feed)
- Salmonella Agona (feed)
- Salmonella Mbandaka (feed)
- Salmonella O:42:1
- Salmonella Group E4:1 (feed)
- Salmonella Typhimurium from a pigeon
- Salmonella Enteritidis from a hare

The Cyprus national *Salmonella* control programmes were redesigned in 2012. New manuals were developed and a list of approved laboratories was made after a procedure consisting of application, audit and evaluation. Eight private laboratories were included in the approved list, together with the one already existing governmental lab. Since then the approved laboratories are audited once a year by the NRL. Since 2016, seven private laboratories and one governmental laboratory remained.

Discussion

Q: Do you have a specific control programme for reduction of infection in animals?
A: The national control programme targets animals/farms and the official control targets foods. The prevalence of *Salmonella* in poultry is less than it used to be.
Q: Which method do you use for the detection of *Salmonella* in dairy samples?
A: We follow ISO 6579:2002 for analysis of food and animal feed.

3.5 Activities of the NRL-*Salmonella* to fulfil tasks and duties in Romania

Monica Vanghele, NRL-Salmonella, Bucharest, Romania

The National Reference Laboratory (NRL) for animal salmonellosis is part of IDAH (Institute for Diagnosis and Animal Health), and is the official laboratory of the National Sanitary Veterinary and Food Safety Authority of Romania. The laboratory performs a variety of techniques, including isolation and identification, serotyping, biochemical and molecular biology methods. All methods used by the NRL for determination of animal salmonellosis according to official control are accredited in accordance with EN ISO 17025:2005 (Anonymous, 2005). *Salmonella* detection is performed following ISO 6579 (Anonymous, 2002) and serotyping of *Salmonella* strains is performed by following the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007).

The major activities of the NRL-for animal salmonellosis are:
- *Salmonella* detection in samples from the official control;
- *Salmonella* serotyping of strains isolated from the National Programmes for Control of zoonotic salmonellosis and veterinary samples isolated in official county laboratories;
- Storage of *Salmonella* strains isolated from samples of the National Programmes for Control of zoonotic salmonellosis.

Annually, approximately 500 samples are examined by the NRL for animal salmonellosis in the National Programmes for Control of zoonotic

salmonellosis, and approximately 1200 isolates are serotyped by the NRL, including those received from the official county laboratories.
The NRL-for animal salmonellosis participates in the annual EURL-*Salmonella* Proficiency Tests, and collaborates with the EURL-*Salmonella* and other NRLs.
The NRL-for animal salmonellosis provides scientific and technical assistance to both the National Sanitary Veterinary Authority and the Food Safety Authority of Romania. It also assists the official county laboratories through participation (in field of competence) when revising the surveillance programmes for animal salmonellosis. Furthermore, the NRL:

- performs coordination, evaluation and technical advising of county laboratories;
- makes proposals for designation of laboratories by the National Sanitary Veterinary and Food Safety Authority of Romania for testing of samples from National Programmes for Control of zoonotic salmonellosis;
- organises training activities for 30 county laboratories designated for performing official controls;
- organises annual interlaboratory comparative tests for checking the diagnostic capability for detection of *Salmonella* in animals by the authorized county laboratories;
- provides the Competent Authority and EFSA with data of *Salmonella* serovars and antimicrobial resistance data.

Discussion

Q: Do you organise interlaboratory comparison studies? If so, how are the samples prepared?
A: Yes, we organise interlaboratory comparison studies. However, I do not know all details, but as far as I know we artificially contaminate animal faeces samples.

3.6 New official control regulation (revision of Regulation 882/2004)

Pamina Mika Suzuki, DG-Sante, Brussels, Belgium

The new official control regulation (OCR), Regulation (EU) 2017/625 (EC, 2017), was published in the Official Journal on 7 April 2017. It replaces Regulation (EC) No 882/2004 (EC, 2004b) and R 854/2004 (EC, 2004a). It represents the framework legislation on all controls carried out by competent authorities (including official sampling and analyses and requirements for official laboratories, NRLs and EURLs). It will be completed with 34 delegated acts and 51 implementing acts.

The specific provisions on EURLs contain a clearer and more detailed description of EURL tasks and responsibilities. In particular, this concerns the provisions on reference materials and on collaboration with EU agencies and third countries. The new Regulation is also more explicit on the responsibilities of National Reference Laboratories (NRLs). Its tasks and responsibilities include the obligation to observe biosecurity standards and to comply with ISO 17025 (Anonymous, 2005). The OCR clarifies the relationship between EURLs, NRLs and

Official Laboratories. Articles 92-101, under Title III 'Reference laboratories and reference centres' of Regulation 2017/625 (EC, 2017), will apply one year after the entry into force of the Regulation.

Discussion

Q: What will happen with the other hygiene Regulations?
A: These other Regulations will remain as they are as these are not part of Regulation No 882/2004, with the exception of Regulation No 854/2004.
Q: Is it correct that a Food Business Operator can request an additional sample if they query the test results?
A: According to article 11 of Regulation No 882/2004, the feed and food business operators have the right to take additional samples for a supplementary expert opinion, without prejudice to the obligation of competent authorities to take prompt action in case of emergency.

3.7 Work programme EURL-*Salmonella* second half 2017, first half 2018, discussion on general items and closure

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

Kirsten Mooijman summarised the information on the work programme of the EURL-*Salmonella* for the rest of 2017 and for early 2018.

Interlaboratory comparison studies
The EURL-*Salmonella* would like to change the order of the interlaboratory studies for detection of *Salmonella* in samples from the primary production stage (PPS; up to 2016 organised in February/March) and for detection of *Salmonella* in food or animal feed (up to 2016 organised in September/October). This because of regular problems with the choice of the matrix for PPS studies due to Avian Influenza related to migration of wild birds in autumn and winter. The problems with changing the order of the studies is that two studies with similar matrix may be organised closely after one another and that in one year, a study for either PPS or for food/animal feed is not organised. To overcome these problems, the solution would be to organise a study with a matrix which is tested in 'food/feed laboratories' as well as in 'PPS-laboratories'. The chosen matrix for this will be hygiene swabs. Therefore, the following three interlaboratory comparison studies for the coming year are foreseen:

- September/October 2017: Detection of *Salmonella* in hygiene swabs. This study will be open for NRLs-*Salmonella* for PPS and food and each NRL shall use the relevant method for its own work field.
- October/November 2017: Typing of *Salmonella*. As in former typing studies, this study will contain an obligatory part for serotyping 20 different *Salmonella enterica* serovars and additionally, one optional non-*enterica* isolate, and an optional part for PFGE testing 10 different *Salmonella* serovars.
- February/March 2018: Detection of *Salmonella* in animal feed.

From autumn 2018, the order of the detection studies will be changed and the first interlaboratory study on detection of *Salmonella* in PPS samples in the new order is foreseen in September/October 2018.

Summary results questionnaire MLVA
In January 2017, a questionnaire was sent to all NRLs-*Salmonella* (36 countries) to obtain updated information on the use of MLVA (Multi Locus Variable-Number Tandem Repeat Analyses) for subtyping of *Salmonella* Typhimurium and *Salmonella* Enteritidis by the NRLs for *Salmonella*. In total, 26 countries replied (72%) of which 11 indicated that they perform MLVA typing for non-human isolates, and 15 do not. Of the latter 15 NRLs, three indicated that they send isolates to other (e.g. human health) institutes for MLVA typing in case of outbreaks. Of the 11 positive reactions, all performed MLVA typing for *Salmonella* Typhimurium and 8 for *Salmonella* Enteritidis. MLVA typing is performed for official controls and research purposes, as well as for outbreak investigations. The number of isolates typed with MLVA per year varies from a few to more than 1000. All NRLs used 'standardised' protocols for the MLVA typing, either published by ECDC or by EFSA.

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

- Continuation of the activities for the standardisation organisations, ISO (at international level) and CEN (at European level).
- Finalising a guidance document for drafting ISO/CEN standards for microbiology.
- Laboratory activities for development and validation of the standard for PCR identification of monophasic *Salmonella* Typhimurium.
- Analysis of the results of experiments to test the influence of incubation temperature (35 °C versus 37 °C) on selective enrichment of *Salmonella* and background flora in MKTTn.
- Testing different matrices for use in interlaboratory comparison studies.
- Drafting a manuscript summarising the validation studies performed for EN ISO 6579-1.

Assistance to the Commission and communication

- If necessary/requested, EURL-*Salmonella* experts will participate in working groups of EFSA and of DG-Sante.
- EURL-*Salmonella* will perform ad hoc activities (on its own initiative or upon request) and, if needed, will support DG-Sante or EFSA in case of outbreaks.
- The EURL regularly receives questions for information or advice from NRLs, DG-Sante and third parties. Replies are given as quickly as possible, but may sometimes be delayed due to the fact that literature and/or other experts need to be consulted.
- 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 to the other NRLs for publication in the newsletter.
- The EURL-*Salmonella* website will be kept up to date with information on new activities/results.
- Results of interlaboratory comparison studies and workshops are summarised in (RIVM) reports. These reports are published on

the RIVM and EURL-*Salmonella* websites, and NRLs are informed about the publication of the reports by e-mail.

Training

- Training courses can be given by EURL-*Salmonella* at the EURL premises or at the NRL laboratory, either on request of an NRL or indicated by the EURL (in case of poor performance).
- In July 2016, a training course on the use of BioNumerics was organised in France, and another will be organised in Italy in July 2017. This training is organised in cooperation with the EURLs for *Listeria monocytogenes* (France) and STEC (Italy). Per EURL network, 4 NRLs can participate, resulting in a total of 12 NRLs per training. The 2018 the training course will be held in the Netherlands.

Molecular typing

Activities in relation to molecular typing foreseen for the coming year are:

- Including (again) PFGE typing in the EURL-*Salmonella* interlaboratory comparison study on typing of *Salmonella*;
- Continue participation in the EFSA steering committee on molecular typing database;
- Training NRLs on *Salmonella* molecular typing and use of BioNumerics;
- Curator meetings with the other EURLs involved in the EFSA molecular database (EURLs for STEC and for *Listeria monocytogenes*) and with the Statens Serum Institute (SSI) in Denmark, the curator of the ECDC database;
- Curation of molecular data (PFGE) for the EFSA (pilot) database, if uploaded;
- Perform WGS analysis and/or analysis of WGS data in case of outbreaks.

Other subject

The EURL-*Listeria monoctyogenes* (*Lm*) has prepared a guidance document 'on outsourcing part of proficiency testing trials by NRLs for national networks'. In this document, criteria are given for outsourcing parts of PT schemes organised and supervised by NRLs for their national networks. In addition, criteria are given to select PT providers, including steps of PT schemes that can be outsourced or not, frequency, details on methods used by participants, and minimum values (if possible). EURL-*Lm* proposed to make it a general guidance document for all biological EURL networks. Before doing so, the interest of the different EURL/NRL networks was investigated and the NRLs-*Salmonella* were asked if they saw a need/had an interest in this guidance document. The majority of NRLs-*Salmonella* present at the workshop were positive about having a guidance document for outsourcing part of PT schemes.

Workshop 2018

Thanks to the kind invitation of NRL-*Salmonella* in Sweden, the EURL-*Salmonella* workshop of 2018 will be organised in Uppsala, Sweden. Details of the exact location and dates will be decided later.

Discussion on combined Food-PPS interlaboratory study
Q: What to do if my laboratory analyses both food samples and samples from the primary production stage (PPS)?
A: You can choose for which product you participate or you can participate for both products.
Q: In my country there are two different laboratories analysing food samples and PPS samples, will you send two sets of samples?
A: Yes, in case of more than one NRL, we will send two sets of samples so that both NRLs can participate.
Q: Carcass swab samples have to be in the laboratory within 24h after sampling. Can you fulfil this criterion? What about stability of the samples?
A: We try to mimic the type of samples in our interlaboratory studies as much as possible with 'real life' samples. However, it may not always be possible to mimic all conditions. For the stability of the artificially contaminated hygiene swabs we have performed several experiments to mimic temperature abuse due to transport. For this, the samples were stored at 5 °C and 10 °C for three weeks, and we still could detect *Salmonella* in the samples.

Remarks:
- The hygiene swabs are relevant samples for both fields (food and PPS) and are an obvious choice, which I support.
- We take a lot of swab samples in the field and find them very satisfactory.

Discussion on guidance document for outsourcing PT trials by NRLs
Q: This document may be of value as it is sometimes difficult to choose a PT provider.
A: The guidance document may give general guidance for the choice of a PT provider. A possible requirement is, for example, that the PT provider is accredited for organisation of the relevant PT.
Q: Does this fit with what is happening with WGS, i.e. sequencing is done by the laboratory and data analysis is outsourced?
A: This is not what is intended with the guidance document and may be more relevant for the newly raised EURLs working group on WGS.
Remarks: This type of guidance document may be helpful, especially for having a set of (general) criteria for selecting PT providers.

General discussion
Q: Can we use WGS for serotyping of *Salmonella*?
A: EC DG-Sante indicates that they do not want to impede in the development of new methods, and they can see that WGS is very promising for serotyping of *Salmonella*. However, before any alternative method can be used, a proper validation needs to be performed to ensure the same quality of results as existing methods. For the validation of alternative confirmation/typing methods, the protocol is still under development and will become available as part 6 of ISO 16140. As it may take approximately two more years before part 6 is published, it was suggested that existing validation/verification data would be evaluated with the DIS version of ISO 16140-6 (likely to be published before the end of 2017).

Q: We have had some official samples from the primary production stage (faeces, boot socks) and found no growth at all on MSRV agar. Have other NRLs seen similar problems?

A (from several other NRLs):

- We have seen something similar in some cases; it has occurred that the matrix was inhibitory.
- We have seen similar things when testing samples from turtle farms, and we had the suspicion that a growth inhibitor had been added to the samples.
- Sometimes lime is used with cleaning, which kills everything so that MSRV plates remain clear.
- Disinfectant added to a sample also gives negative results. If this is suspected, then repeat sampling by an independent sampler is suggested.
- We have had the suspicion that some farmers microwave the samples so that they become sterile. We have tested the influence of microwave samples and found negative results. We therefore performed resampling by an independent (official) sampler.
- It is possible to check whether a sample is sterile or still contains background flora, by plating out on a non-selective agar medium (e.g. blood agar). No growth at all on a non-selective medium would be very suspicious for a faecal sample.

4 Evaluation of the workshop

4.1 Introduction

At the end of the workshop, an evaluation form was given to all participants to ask for their opinion of the workshop (see Appendix 3). A total of twelve questions were asked. For ten of these questions, participants were asked to answer using a score ranging from 1 to 5. The scores represent: very poor (1), poor (2), fair (3), good (4) and excellent (5).
In addition, it was possible to add comments. Two questions were 'open' questions, in which the participants were asked to give their opinion.
The evaluation form was handed to 44 workshop participants;
41 completed forms were returned, a response rate of 93%.

In section 4.2, the scores on each question are presented 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 1 shows that the respondents considered the information given in advance of the workshop as good or excellent (scores 4-5).

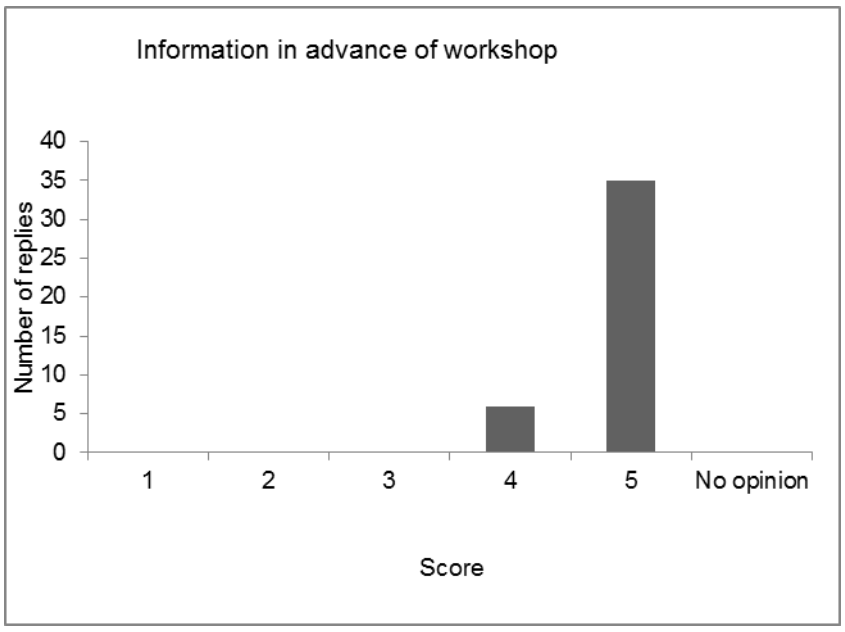

Figure 1 Scores given to question 1 'Opinion on information given in advance of the workshop'

2. What is your opinion on the booking of the tickets by the EURL-Salmonella?
The majority of the participants for who tickets were arranged by the EURL were very satisfied. Participants who booked their own ticket indicated 'no opinion' (see Figure 2).

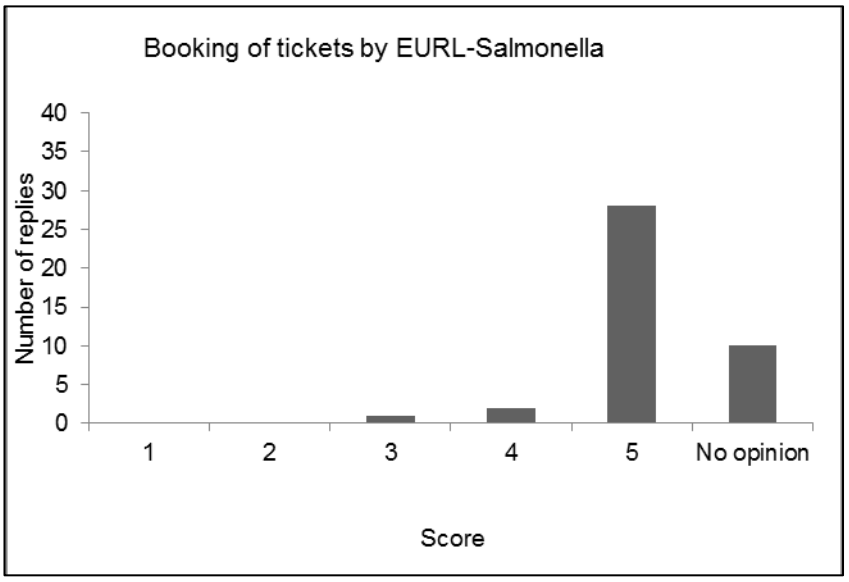

Figure 2 Scores given to question 2 'Opinion on the booking of the tickets by the EURL-Salmonella'

3. What is your opinion on the accessibility of the meeting venue?
Almost all respondents found access to the meeting venue excellent (40/41 scored 5; Figure 3).

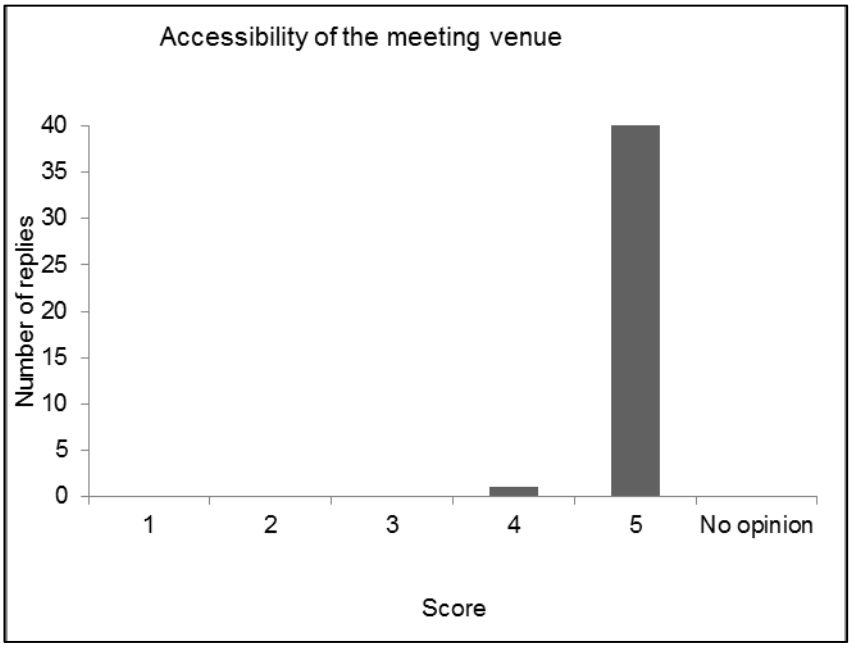

Figure 3 Scores given to question 3 'Opinion on the accessibility of the meeting venue'

4. What is your opinion on the hotel room?

The participants were satisfied with the hotel rooms; scores 4 (good) and 5 (excellent) were given (see Figure 4).

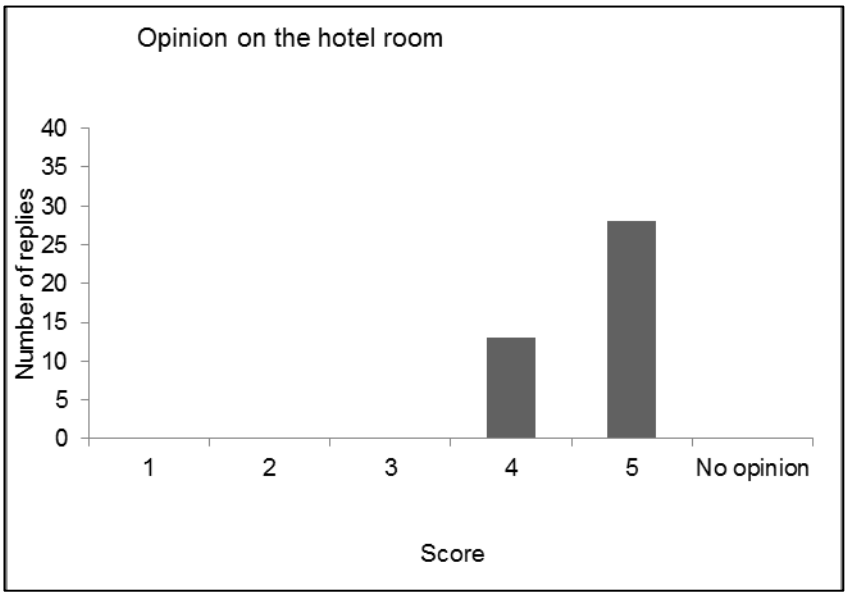

Figure 4 Scores given to question 4 'Opinion on the hotel room'

5. What is your general opinion on the meeting room?

The opinion on the meeting room was, in general, good to excellent (scores 4 and 5; see Figure 5). Still a few remarks were made:

- 'A little cold at times' (4x);
- 'The position of the tables was too academic, which did not allow efficient discussions' (1x);
- 'Last row a little too distant from the screen' (1x).

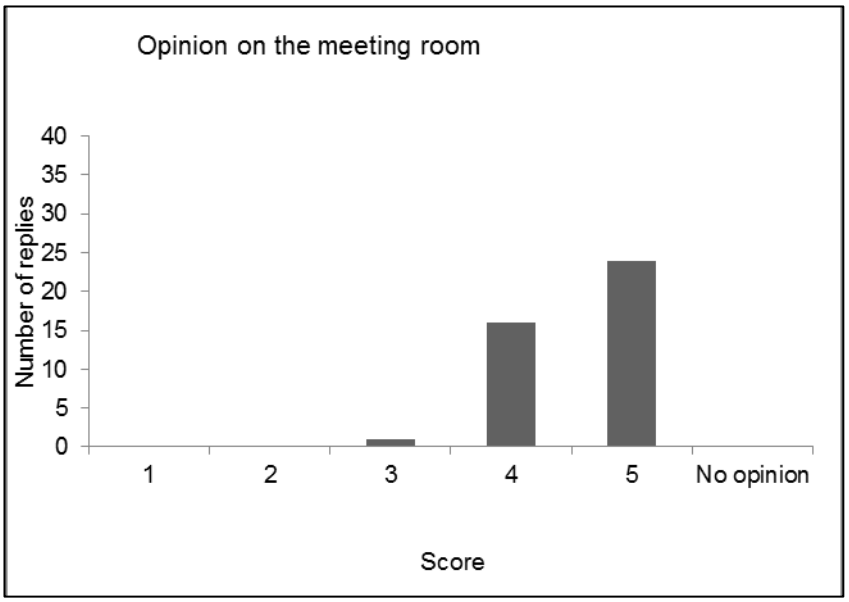

Figure 5 Scores given to question 5 'Opinion on the meeting room'

6. What is your opinion on the readability of the presentations on the screen?
Almost all respondents were satisfied about the readability of the presentations on the screen (see Figure 6).
One remark was made about the fact that small words in the presentations were hard to read from the last row.

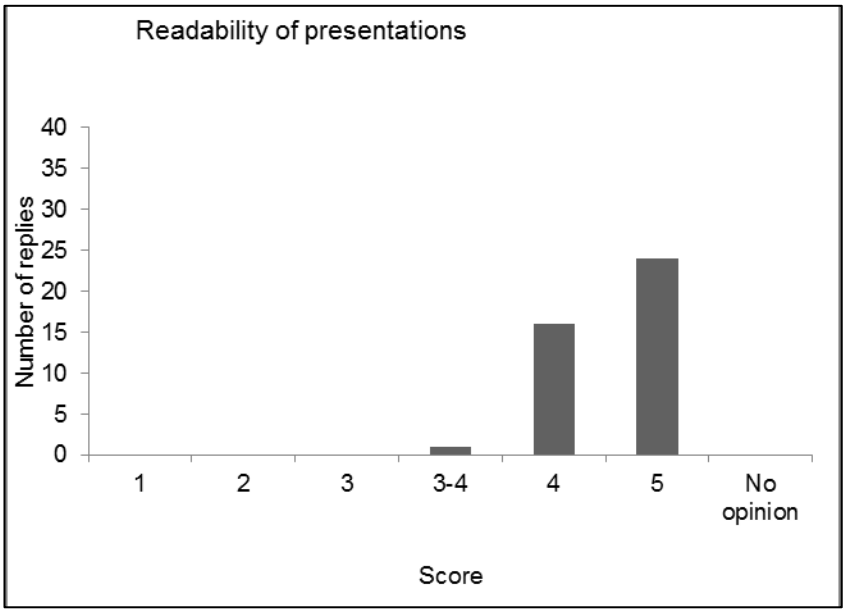

Figure 6 Scores given to question 6 'Opinion on the readability of the presentations on the screen'

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

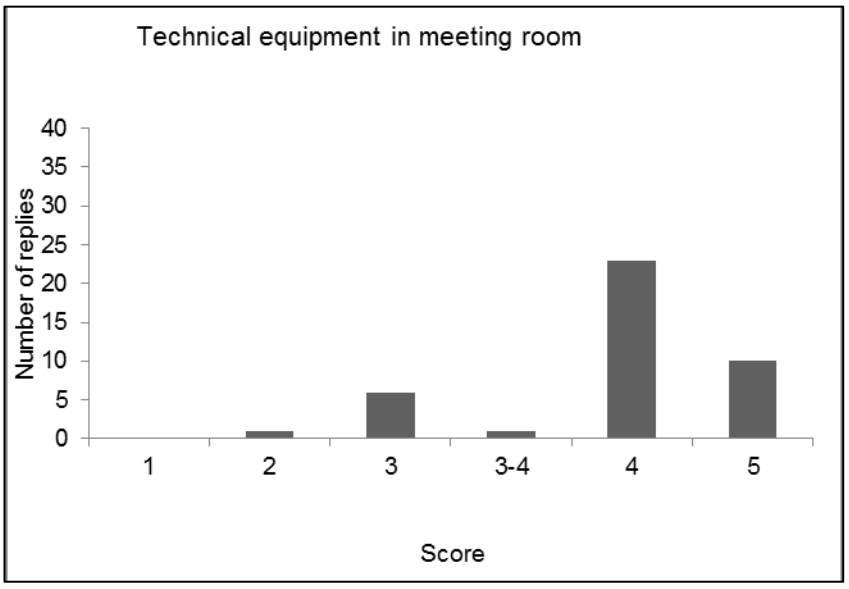

Figure 7 Scores given to question 7 'Opinion on the technical equipment'

The opinion on the technical equipment varied from poor (score 2; one respondent) to excellent (score 5; 10 respondents), as shown in Figure 7. It was noted that there were some problems with the projector on the first day, but after this problem was solved, it worked fine.

8. What is your opinion on the catering provided during the workshop (breakfast, coffee, tea, lunch, dinner)?
The respondents found the catering fair (score 3) to excellent (score 5), see Figure 8. It was noted that there was nothing to eat during the coffee/tea breaks and there was a lack of vegetables during the dinner.

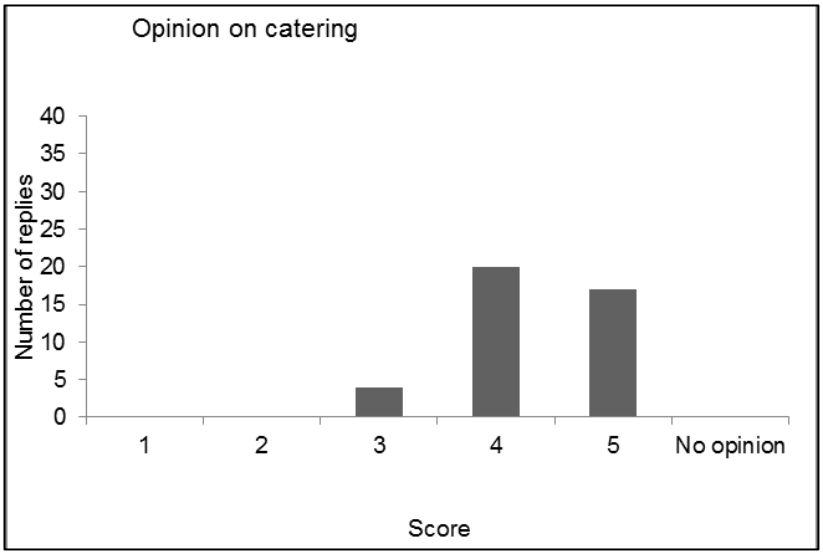

Figure 8 Scores given to question 8 'Opinion on the catering'

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

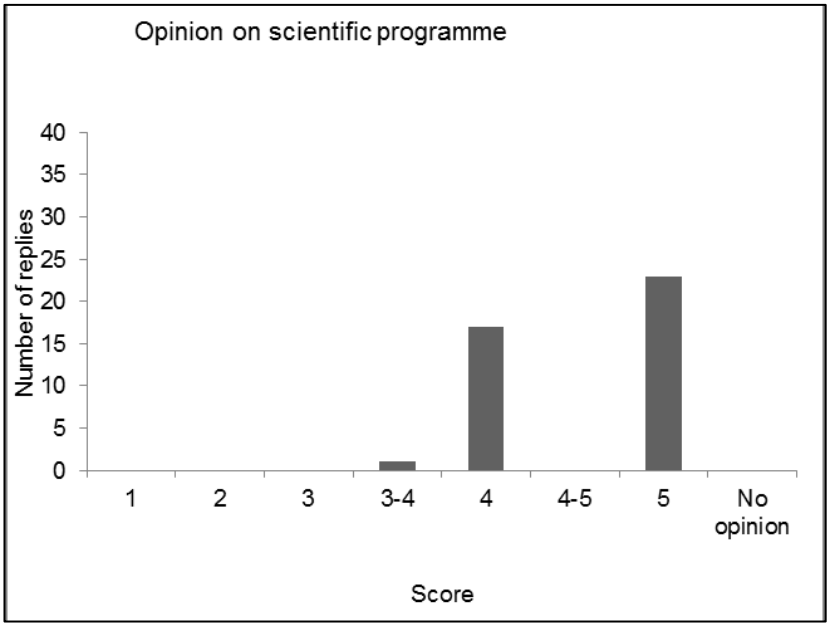

Figure 9 Scores given to question 9 'Opinion on the scientific programme'

The majority of the respondents were very satisfied with the scientific programme of the workshop: mainly good (score 4) or excellent (score 5) scores were given (see Figure 9).

10. 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:

- 'I liked the discussion.'
- 'The presentation on the validation of methods (ISO 16140 series) was very useful.'
- 'Still some questions left about validation.'
- 'Excellent presentation of Valentina Rizzi, EFSA about *Salmonella* monitoring data.'
- 'More focus on the results from EFSA. All their struggle with their reporting systems is not so interesting.'
- 'Excellent presentation by Kirsten Mooijman, EURL-*Salmonella* about the update on activities in ISO and CEN.'

11. 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 10.

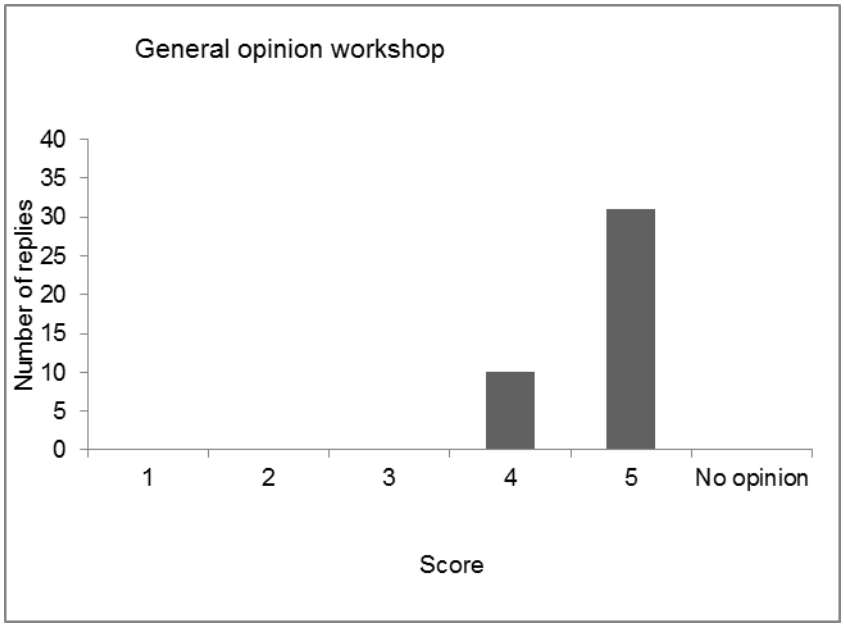

Figure 10 Scores given to question 11 'General opinion of the workshop'

12. 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:

- 'Nice presentations from different NRLs.'
- 'Discussion about ISO 16140 (validation of methods) was good.'
- 'It would have been more useful if the presentation on ISO 16140 was planned at the beginning of a session, when the audience is more willing to absorb all this information, and not at the end.'
- 'If manageable, arrival should not fall in a weekend.'
- 'Do not start the workshop on a Monday, to avoid traveling on Sunday.'
- 'Presentations from NRLs may be made on specific topics, which may better feed discussions.'
- 'Please indicate the participants' email addresses on the list of participants.'
- 'Organise the workshop every year at another location/country.'
- 'Noël is a great helper.'
- 'Thank you very much.'

4.3 Discussion and conclusions of the evaluation

In general, the participants were satisfied with the workshop. For almost all items 'good' (score 4) or 'excellent' (score 5) were given. A problem at the start of the meeting with the connection wire of the laptop to the projector resulted in a few low scores. Luckily the problem could easily be solved at the end of the morning session of the first day. Due to miscommunication with the hotel, no cookies were provided during the coffee and tea breaks; this was an unintended mistake. Some remarks were made that it would be preferred to prevent the organisation of the workshop on a Monday, as this would automatically result in traveling on Sunday. This suggestion will be taken into account for the future workshops.

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List of abbreviations

A	Answer
AMR	Antimicrobial resistance
BPW	Buffered Peptone Water
CEN	European Committee for Standardization
cfu	colony forming units
DG-Sante	Directorate-General for Health and Food Safety
DIS	Draft International Standard
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
EFSA	European Food Safety Authority
EFTA	European Free Trade Association
EPIS	Epidemic Intelligence Information System
EU	European Union
EURL	European Union Reference Laboratory
EUSR	European Union Summary Report
FBO	Food-borne Outbreak
FDIS	Final Draft International Standard
FWD	Food and Waterborne Diseases
FYROM	Former Yugoslav Republic of Macedonia
ISO	International Organization for Standardization
MKTTn	Mueller Kauffmann Tetrathionate broth with novobiocin
MLVA	Multi-Locus Variable number of tandem repeats Analysis
MPN	Most Probable Number
MS	Member State
MSRV	Modified Semi-solid Rappaport Vassiliadis
NRL	National Reference Laboratory
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PPS	Primary Production Stage
PT	Proficiency Test
Q	Question
RASFF	Rapid Alert System for Food and Feed
RIVM	National Institute for Public Health and the Environment
RVS	Rappaport Vassiliadis broth with Soya
SC	Sub Committee
SOP	Standard Operating Procedure
STEC	Shiga toxin-producing *Escherichia coli*
TAG	Technical Advisory Group
TC	Technical Committee
TESSy	European Surveillance System
TS	Technical Specification
UK	United Kingdom
USA	United States of America
WG	Working Group
WGS	Whole Genome Sequencing

Appendix 1 Participants

European Food Safety Authority (EFSA)	Valentina Rizzi
EC DG-Sante	Pamina Mika Suzuki
EURL – *Salmonella*	Kirsten Mooijman Angelina Kuijpers Irene Pol-Hofstad
Guest speaker (the Netherlands)	Paul in 't Veld (NVWA, Utrecht)

National Reference Laboratories for *Salmonella*

AUSTRIA	Heimo Lassnig
BELGIUM	Florence Crombé
BOSNIA HERZEGOVINA	-
BULGARIA	Gergana Mateva
CROATIA	Gordan Kompes Borka Simpraga
CYPRUS	Konstantinos Arsenoglou
CZECH REPUBLIC	Tomas Cerny
DENMARK	Søren Aabo
ESTONIA	Age Kärssin
FINLAND	Henry Kuronen
FRANCE	Laetitia Bonifait David Albert
FYROM	Mirko Prodanov
GERMANY	Istvan Szabo
GREECE	Aphrodite Smpiraki
HUNGARY	Sara Kostyak
ICELAND	Franklin Georgsson
IRELAND	William Byrne
ITALY	Veronica Cibin
LATVIA	Madara Streikisa
LITHUANIA	Aista Darata Brazdilyte
LUXEMBOURG	Gilbert Moris
MALTA	-
NETHERLANDS	Wilma Jacobs-Reitsma Anjo Verbruggen
NORTHERN IRELAND	Angela Lahuerta Marin
NORWAY	Bjarne Bergsjø
POLAND	Magdalena Skarzynska Kinga Wieczorek Lukasz Maka
PORTUGAL	Patricia Themudo
ROMANIA	Luminata Monica Vanghele
SERBIA	Jasna Kureljusic
SLOVAK REPUBLIC	Lubos Mikula
SLOVENIA	Tina Pirs

SPAIN	Maria Cristina de Frutos Escobar
SWEDEN	Lennart Melin
SWITZERLAND	Gudrun Overesch
TURKEY	-
UNITED KINGDOM	Kathie Grant
	Heather Aird
	Doris Mueller-Dobles

Appendix 2 Workshop Programme

Programme of the 22nd EURL-*Salmonella* workshop
29 and 30 May 2017, Zaandam, the Netherlands

General information

Place of accommodation and Meeting venue:

Inntel hotel Zaandam
Provincialeweg 102, 1506 MD Zaandam
Tel: +31 (0)75 631 1711
http://www.inntelhotelsamsterdamzaandam.nl/

Information for those giving a presentation:
Presentations: Send your presentation to Kirsten Mooijman (kirsten.mooijman@rivm.nl), preferably one week before the workshop.

Abstract: For the preparation of the report of the workshop it is necessary to also receive an abstract of your presentation (approximately 0.5-1 page). Please hand this to Kirsten during the workshop or send it to Kirsten.mooijman@rivm.nl **preferably before 1 June 2017**

Sunday 28 May 2017
Dinner information: For participants for whom the costs of travel and stay are paid from the EURL-*Salmonella* budget, the EURL will also cover the expenses of a dinner on Sunday 28 May, with a maximum of € 30 per person. You can enjoy dinner at the Inntel hotel in Zaandam and ask to have the costs added to the invoice of your room. Alternatively, you can have dinner at another location, for which we will need a receipt in order to reimburse you for this meal.

Monday 29 May 2017
08:15 - 09:00Registration

Morning chair: Wilma Jacobs

09:00 - 09:30	Opening and introduction	Kirsten Mooijman, EURL-*Salmonella*
09:30 - 10:00	*Salmonella* monitoring data and food-borne outbreaks for 2015 in the European Union	Valentina Rizzi, EFSA
10:00 - 10:30	Results 8th interlaboratory comparison study on detection of *Salmonella* in minced chicken meat (2016)	Angelina Kuijpers, EURL-*Salmonella*
10:30 - 11:00	*Coffee/tea*	
11:00 - 11:30	Preliminary results 20th interlaboratory comparison study on detection of *Salmonella* in chicken faeces (2017)	Irene Pol, EURL-*Salmonella*
11:30 – 12:00	Results 21st interlaboratory comparison study on typing of *Salmonella* (2016) – serotyping and PFGE	Wilma Jacobs, EURL-*Salmonella*
12:00 – 13:30	*Lunch*	

Afternoon chair: Kirsten Mooijman

13:30 - 14:15	Update on the joint EFSA/ECDC molecular typing database and preliminary results of the survey on the use of WGS for typing *Salmonella*	Valentina Rizzi, EFSA
14:15 - 14:45	*Salmonella* Enteritidis outbreak related to Polish eggs	Pamina Mika Suzuki, DG-Sante
14:45 - 15:15	Outbreak of a new serotype *Salmonella enterica* subsp. *enterica* with the antigenic formula 11:z_{41}:e,n,z_{15}, in Greece: 2016-2017	Aphrodite Smpiraki NRL-*Salmonella* Greece
15:15 – 15:45	*Coffee/tea*	
15:45 - 16:15	Salmonellosis or *Salmonella* infection – high nasal colonization rates of *Salmonella enterica* subspecies *diarizonae* 61:k:1,5,(7) in Swiss sheep herds	Gudrun Overesch NRL-*Salmonella* Switzerland
16:15 - 16:45	Update on activities in ISO and CEN	Kirsten Mooijman, EURL-*Salmonella*
16:45 - 17:15	Validation of alternative microbiological methods – the ISO 16140 series	Paul in 't Veld, Food Authority, The Netherlands
19:00 -	*Dinner at hotel Inntel*	

Tuesday 30 May 2017

Morning chair: Kirsten Mooijman

09:00 - 10:40	Activities NRLs to fulfill tasks and duties, and information on national *Salmonella* control programmes	
09:00 – 09:20	NRL-*Salmonella* the Netherlands	Kirsten Mooijman
09:20 – 09:40	NRL-*Salmonella* Serbia	Jasna Kureljusic
09:40 – 10:00	NRL-*Salmonella* Bulgaria	Gergana Mateva
10:00 – 10:20	NRL-*Salmonella* Cyprus	Konstantinos Arsenoglou
10:20 – 10:40	NRL-*Salmonella* Romania	Monica Vanghele
10:40 - 11:15	*Coffee/tea*	
11:15 - 11:45	New official control regulation (revision of Regulation 882/2004)	Pamina Mika Suzuki, DG-Sante
11:45 – 12:30	Work programme EURL-*Salmonella* second half 2017, first half 2018 Discussion on general items Closure	Kirsten Mooijman, EURL-*Salmonella*
12:30 – 14:00	*Lunch*	

---------- End workshop----------

Appendix 3 Workshop evaluation form

Evaluation of the 22nd EURL-*Salmonella* workshop
29 and 30 May 2017, Zaandam, the Netherlands

We would highly appreciate if you could give us your opinion on the 22nd EURL-*Salmonella* workshop, organised in Zaandam, the Netherlands on 29 and 30 May 2017. 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, where 1 is the lowest score and 5 is the highest score representing the following:
1 = very poor; 2 = poor; 3 = fair; 4 = good; 5 = excellent

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 the booking of the tickets by the EURL-*Salmonella* (if relevant)?

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

Remarks:_____

3. 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:_____

4. What is your opinion of the hotel room?

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

Remarks:_____

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

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

Remarks:_____

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

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

Remarks:_____

7. 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:_____

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

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

Remarks:_____

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

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

Remarks:_____

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

11. What is your general opinion of the workshop?

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

Remarks:_____

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

Thank you very much!

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