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The 28th EURL-*Salmonella* workshop

22 and 23 May 2023, Online

RIVM report 2023-0341
K.A. Mooijman

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K.A. Mooijman (author), RIVM

Contact:
K.A. Mooijman
Centre for Zoonoses and Environmental Microbiology (Z&O)
Kirsten.mooijman@rivm.nl

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Synopsis

The 28th EURL-*Salmonella* workshop

22 and 23 May 2023, online

In May 2023, the EU Reference Laboratory (EURL) for *Salmonella* organised the workshop for the European National Reference Laboratories (NRLs) for the 28th year. The aim of the workshop is to exchange information between the EURL and the NRLs. The workshop was held online for the fourth time.

Among other things, the results of four ring trials were presented. The EURL had organised three of these ring trials to monitor the quality of the NRLs. The NRLs scored well for the detection of *Salmonella* in hygiene swabs and in flaxseed. In the ring trial for *Salmonella* typing, the NRLs also achieved a good performance.

The aim of the fourth ring trial was to determine the quality of a method for identification of a *Salmonella* type. This method uses DNA techniques and will be published as an International Standard method (ISO). The ring trial results are summarised in an annex to this new ISO and will give information on the application and quality of the ISO method.

Another presentation provided information on the developments in the area of international and European standardisation of microbiological methods. Several standard methods lack quality information, which is also the case for the ISO culture method for detection of *Salmonella* in food products and other product types. An ISO working group will investigate how to assess this quality information.

The NRL-*Salmonella* from Germany presented a study on the application of Whole Genome Sequencing (WGS). WGS is a DNA technique that enables very precise typing of micro-organisms. The NRL showed that this relatively new technique met the quality standards of the laboratory.

Every year, some NRLs-*Salmonella* give a presentation on how they fulfil their statutory duties. This year, these were the NRLs-*Salmonella* from Bosnia and Herzegovina, Germany, Lithuania and Luxembourg.

The EURL-*Salmonella* forms part of the National Institute for Public Health and the Environment (RIVM) and organises this workshop every year. One important task of the EURL-*Salmonella* is to monitor the performance of the European NRLs for this bacterium.

Keywords: EURL-*Salmonella*, NRL-*Salmonella*, *Salmonella*, workshop 2023

Publiekssamenvatting

De 28e EURL-*Salmonella* workshop

22 en 23 mei 2023, online

In mei 2023 organiseerde het Europese Referentie Laboratorium (EURL) voor *Salmonella* voor het 28e jaar de workshop voor de Europese Nationale Referentie Laboratoria (NRL's). Het doel is om informatie uit te wisselen tussen het EURL en de NRL's. De workshop is voor de vierde keer online gehouden.

Onder andere zijn de resultaten van vier ringonderzoeken gepresenteerd. Het EURL organiseerde drie van deze ringonderzoeken om de kwaliteit van de NRL's te controleren. De NRL's waren goed in staat om *Salmonella* te vinden in hygiënesponsjes en in lijnzaad. Ook in het ringonderzoek voor typering van *Salmonella* haalden de NRL's goede resultaten.

Het vierde ringonderzoek had als doel om de kwaliteit te bepalen van een methode om een type *Salmonella* aan te tonen. Deze methode gebruikt DNA-technieken en zal gepubliceerd worden als een Internationale Standaard methode (ISO). Een samenvatting van de ringonderzoekresultaten is als bijlage in deze nieuwe ISO opgenomen. Hierin staat informatie over het gebruik en de kwaliteit van de ISO-methode.

Ook is informatie gepresenteerd over andere ontwikkelingen op het gebied van Internationale en Europese standaardisatie van microbiologische methoden. In veel van deze methoden ontbreken kwaliteitsgegevens. Dit is ook zo bij de ISO kweekmethode om *Salmonella* in onder andere levensmiddelen aan te tonen. Een ISO werkgroep zoekt uit hoe deze kwaliteitsgegevens kunnen worden bepaald.

Het NRL-*Salmonella* uit Duitsland presenteerde een studie voor het gebruik van Whole Genome Sequencing (WGS). Dit is een DNA-techniek waarmee micro-organismen heel precies kunnen worden getypeerd. Het NRL liet zien dat deze relatief nieuwe techniek voldeed aan de kwaliteitsnormen van het laboratorium.

Elk jaar presenteren een aantal NRL's-*Salmonella* hoe zij hun wettelijke taken invullen. Dit jaar waren dat de NRL's van Bosnië en Herzegovina, Duitsland, Litouwen en Luxemburg.

Het EURL voor *Salmonella* is onderdeel van het RIVM en organiseert deze workshop elk jaar. Een belangrijke taak van het EURL-*Salmonella* is de kwaliteit controleren van de nationale referentielaboratoria voor deze bacterie in Europa.

Kernwoorden: EURL-*Salmonella*, NRL-*Salmonella*, *Salmonella*, workshop 2023

Contents

Summary

On 22 and 23 May 2023, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organised its annual workshop. Due to budget constraints, the workshop was organised as a virtual meeting for the fourth time.
Participants in the workshop were representatives of the National Reference Laboratories (NRLs) for *Salmonella* from the 27 European Union (EU) Member States, three European Free Trade Association (EFTA) countries, and six (potential) EU candidate countries. Also present were representatives of the European Commission Directorate-General for Health and Food Safety (EC DG SANTE) and of the European Food Safety Authority (EFSA). Thanks to the fact that this workshop was organised as a virtual meeting, it was possible to host more participants compared to a physical workshop. In total, 94 participants registered.

During the workshop, presentations were given on several topics:

- The EFSA representative gave a presentation on EU monitoring of *Salmonella* and of salmonellosis foodborne outbreaks in 2021.
- Representatives of the EURL-*Salmonella* presented the results of the Proficiency Tests (PTs) organised in 2022 and 2023, namely the PT on detection of *Salmonella* in hygiene swabs (September/October 2022), the PT on detection of *Salmonella* in seeds (March 2023) and the PT on *Salmonella* typing (November 2022).
- A representative of the EURL-*Salmonella* presented the results of the Interlaboratory Study (ILS) for validation of draft ISO/DTS 6579-4 (2022) on identification of monophasic *Salmonella* Typhimurium.
- Additionally, a presentation was given on ongoing activities in ISO and CEN for standardisation of microbiological methods.
- A representative of the NRL-*Salmonella* from Germany gave a presentation on a *Salmonella* cgMLST validation study for accreditation in Germany.
- Representatives of the NRLs-*Salmonella* from Bosnia and Herzegovina, Germany, Lithuania and Luxembourg illustrated how they are fulfilling their statutory tasks and duties.

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

The workshop presentations are available on the EURL-*Salmonella* website: https://www.eurlsalmonella.eu/workshop-2023.

1 Introduction

This report includes the abstracts of the presentations given at the 2023 EURL-*Salmonella* workshop as well as summaries of the discussions that followed the presentations. The full presentations are available on the EURL-*Salmonella* website (provided the author has given permission for publication):
https://www.eurlsalmonella.eu/workshop-2023

The layout of the report is consistent with that of the workshop programme.
Chapters 2 and 3 include the abstracts of the presentations given on the first and the second day of the workshop, respectively.
The workshop is evaluated in Chapter 4; the evaluation form template can be found in Annex 3.
The list of participants is presented in Annex 1.
The workshop programme is incorporated in Annex 2.

2 Monday 22 May 2023: Day 1 of the workshop

2.1 Opening and introduction

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

Kirsten Mooijman, head of the European Union Reference Laboratory (EURL) for *Salmonella*, opened the 28th workshop of the EURL-*Salmonella*, welcoming all participants to this fourth virtual EURL-*Salmonella* workshop.
In total, 94 participants had registered for this workshop, including representatives of the National Reference Laboratories (NRLs) for *Salmonella* from the 27 EU Member States (MS), six (potential) EU candidate countries, and three member countries of the European Free Trade Association (EFTA). Additionally, representatives of the European Commission Directorate-General for Health and Food Safety (EC DG SANTE) and of the European Food Safety Authority (EFSA) attended.
The evaluation of the online workshops organised in 2020, 2021 and 2022 was presented, showing high (positive) scores for all questions raised.
The workshop started after the presentation of the programme and the general information. The workshop programme can be found in Annex 2.

2.2 EU monitoring of *Salmonella* and of salmonellosis foodborne outbreaks in 2021

Frank Boelaert, EFSA, Parma, Italy

The One Health Zoonoses 2021 report of the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) presents the results of zoonoses monitoring and surveillance activities carried out in 2021 in 27 Member States (MSs), the United Kingdom (Northern Ireland) and nine non-MSs (EFSA and ECDC, 2022). Salmonellosis was the second most commonly reported foodborne gastrointestinal infection in humans after campylobacteriosis, and was a major cause of foodborne outbreaks in EU MSs and non-MS countries. The number of confirmed cases of human salmonellosis was 60 050. Cases of salmonellosis increased in comparison with 2020 but decreased in comparison with previous years. The overall trend for salmonellosis in 2017–2021 did not show any statistically significant increase or decrease. In 2021, data collection and analysis at the EU level were still impacted by the COVID-19 pandemic and the control measures adopted in the MSs, including partial or total lockdowns. *Salmonella* samples from carcases of various animal species were more frequently positive when performed by the competent authorities than when own checks were conducted. Sixteen MSs and the United Kingdom (Northern Ireland) achieved all the established targets in poultry populations for reduction in *Salmonella* prevalence for the relevant serovars. The number of MSs that did not meet the reduction targets was seven for laying hens, five for breeding *Gallus gallus*, three for broilers, one for breeding turkeys and one for fattening turkeys. A significant increase in the estimated breeding turkey flock prevalence of *Salmonella* was noted in 2021 compared with 2016, when the estimated

prevalence reached the lowest value seen in the entire study period (2010–2021). Flock prevalence trends for target *Salmonella* serovars have, in contrast, been fairly stable over the last few years for all poultry populations. Overall, MSs reported more foodborne outbreaks and cases in 2021 than in 2020. *S*. Enteritidis remained the most frequently reported causative agent for foodborne outbreaks. *Salmonella* in 'eggs and egg products' and in 'mixed foods' were the agent/food pairs of most concern. Outbreaks linked to 'vegetables and juices and products thereof' rose considerably compared with previous years. With the report, EFSA has also published two new interactive communication tools on *Salmonella*: the EFSA story map and the dashboard (links to the relevant webpages are provided at the end of this abstract).

Since September 2021, a cross-border outbreak of *Salmonella* Mbandaka ST413 had been ongoing in the European Union/European Economic Area (EU/EEA) countries, Israel, and the UK (ECDC and EFSA, 2022). By 8 November 2022, 196 cases had been reported in the Czech Republic (n=5), Estonia (n=3), Finland (n=89), France (n=10), Germany (n=2), Ireland (n=1), the Netherlands (n=1), the United Kingdom (n=81), and Israel (n=4), according to the European case definition. Nineteen cases were hospitalised, and five cases had septicaemia. One case in the UK died. Based on case interviews from Finland and the UK, ready-to-eat (RTE) chicken products and/or fresh chicken meat were the likely vehicles of infection. Fifteen cases in Finland reported consumption of six RTE products from three brands. All 15 cases had consumed at least one RTE chicken product. Epidemiological data and microbiological evidence from whole genome sequencing of human isolates indicated there were several active sources through different food distribution chains, with a likely common source higher up in the chicken supply chain.

Also during 2021, a persistent cross-border outbreak of *Salmonella* Virchow ST16 had been ongoing, since June 2017, in five EU/EEA countries, the United Kingdom (UK), and the United States (US) (ECDC and EFSA, 2023). A total of 210 cases have been reported from the following countries: Denmark (2), France (111), Germany (26), Ireland (4), the Netherlands (34), the UK (32), and the US (1). Among the interviewed cases (55), hospitalisation rates ranged from 16,7% (2/12) in the UK, to 29,4% (5/17) and 38,5% (10/26) in France and Germany, respectively. No deaths were reported. A majority of cases have been linked to local restaurants serving kebab meat. The number of confirmed cases represented only a small proportion of all infections in the EU/EEA, partly due to the varying sequencing capacities of countries. The comparison of the representative outbreak strains to the available genome profiles of *S*. Virchow ST16 from non-human isolates revealed that most of the matching isolates belonged to broiler meat and broiler-related environments, thereby supporting the hypothesis of chicken meat as a vehicle of infections. The available information from case interviews, traceback investigations, and whole genome sequencing (WGS) cluster analysis showed that kebab meat products containing contaminated chicken meat are the likely vehicles of infections. The information also made clear that the clone has at least been circulating in the EU poultry meat production chain in France, Germany and the Netherlands. In the absence of batch numbers of the contaminated

kebab products and related *Salmonella* testing information, the source(s) of the infections could not be established.

The links to the relevant EFSA webpages are as follows.
EFSA foodborne outbreaks (FBO) story map:
https://storymaps.arcgis.com/stories/13979918ca8948399180651d3b7ce3e1
EFSA FBO dashboard:
https://www.efsa.europa.eu/en/microstrategy/salmonella-dashboard#

Discussion

Q: Concerning the outbreak of *Salmonella* Virchow: were the additives to the kebab implicated?
A: EFSA did not receive information on the additives.

Discussion on the use of alternative methods for official controls
The NRL-*Salmonella* from Ireland highlighted the problems caused by different interpretations of Article 34(2) of Regulation EU 2017/625 (Official Controls Regulation, OCR; EC, 2017) by the EC and by the official control laboratories. According to the EC, Article 34(2) prescribes that the first method of choice for official controls is the EN ISO method and that alternative, validated, methods can only be used in the absence of EN ISO methods or in the absence of EURL-recommended methods. The NRL-*Salmonella* from Ireland indicated that, up to the adoption of the OCR, they followed Regulation EC 2073/2005 (EC, 2005) for the controls performed by Food Business Operators as well as for the official controls performed by Competent Authorities, allowing the use of alternative validated methods. As a result, alternative validated methods are widely used by official control laboratories in Ireland and, quite likely, also by other Member States. However, according to the EC, the OCR does not allow the use of alternative methods for the official control of *Salmonella*, since EN ISO 6579-1:2017/A1:2020 is the method to be used in the absence of EU provisions on the analytical method for official controls on *Salmonella*. Discussions on the interpretation of Regulation EC 2073/2005 at the adoption of the OCR, has resulted in a sharp reduction in the number of official control data in Ireland and probably in other Member States as well. The NRL(s) asked DG SANTE for a possible solution of this problem, as many validated alternative methods have shown to perform equally well as the EN ISO reference method, but generally give a faster result. Scientifically, there is no reason to not allow the use of validated alternative methods for the official controls of *Salmonella*.
After the workshop, EURL-*Salmonella* was informed by DG SANTE that, as this concerns a legal issue, it can only be solved through a legal act. The intention of DG SANTE is to propose a sustainable and legally robust solution as soon as possible.

2.3 Results Interlaboratory Study validation of ISO/TS 6579-4 Identification of monophasic *Salmonella* Typhimurium

Robin Diddens, EURL-Salmonella*, Bilthoven, the Netherlands*

ISO/TS 6579-4 for the identification of monophasic *Salmonella* Typhimurium is under development and describes three PCR methods: a

probe-based multiplex real-time PCR (PCR method 1), an agarose gel-based multiplex PCR (PCR method 2) and an agarose gel-based single target PCR (PCR method 3). These three methods are applicable for:

- the differentiation between monophasic *Salmonella* Typhimurium and the monophasic variant of another *Salmonella* non-Typhimurium serovar that has the same antigenic formula (*S.* Agama, *S.* Farsta, *S.* Gloucester, *S*. Lagos, *S.* Tsevie and *S.* Tumodi);
- identification of the isolate under analysis as being either monophasic *Salmonella* Typhimurium or (biphasic) *Salmonella* Typhimurium.

A validation study was performed to determine the performance characteristics of each PCR method and existed of two parts: (i) a method(s) evaluation study and (ii) an interlaboratory study (ILS).

The ILS was organised by the EURL-*Salmonella* in May/June 2022. For this ILS, each participant had to test a total of 25 strains and a positive control strain with one, two or all three PCR method(s) as described in (3rd) draft ISO/DTS 6579-4:2022. The strains consisted of target strains (inclusivity) and non-target strains (exclusivity). Upon request participants also received PCR materials related to the PCR method(s) (e.g. primers, probes, internal amplification control, etc.). Alternatively, the participants used their own materials, next to their own (real-time) thermal cyclers.

The ILS for PCR method 1 involved 26 participants from 18 different countries. Six participants were excluded from further analysis for technical reasons. Five participants indicated a higher C_t value and a lower RFU (relative fluorescence units) signal for certain samples compared to the positive (process) control (being (biphasic) *Salmonella* Typhimurium). After checking the raw data of each participant, the results of these five participants were re-interpreted by setting a higher threshold, based on the participants' results of the positive control. As a result of these interpretation problems, additional information has been added to the draft ISO standard for better guidance of the users. After re-interpretation, one inclusivity deviation and one exclusivity deviation remained.

The ILS for PCR method 2 involved 18 participants from 14 different countries. Data from one participant was excluded from further analysis for technical reasons. As in the method evaluation study, the monophasic *Salmonella* Typhimurium strain that gave inconsistent results between the three PCR methods and slide agglutination, showed similar results in the ILS. All participants tested this strain as (biphasic) *Salmonella* Typhimurium with PCR method 2 and as monophasic *Salmonella* Typhimurium with the two other PCR methods and with slide agglutination. This resulted in 17 inclusivity deviations. Additionally, one exclusivity deviation was found.

The ILS for PCR method 3 involved 13 participants from 11 different countries. Data from one participant was excluded from further analysis for technical reasons. In total, 10 inclusivity deviations were found. These were caused by the presence of less intense bands on the gels for

one of the targets analysed by some of the participants and thus considered as positive. This influenced the interpretation of ten PCR results of six samples from three different participants, and the results were reported as (biphasic) *Salmonella* Typhimurium instead of monophasic *Salmonella* Typhimurium. In addition, one exclusivity result was found. Twenty results (concerning three participants) were indicated as missing values, because one of the target sequences was negative in combination with a negative Internal Amplification Control. Due to lack of materials, the samples were not re-analysed and no 'official' result could be assigned to these samples.

The exclusivity deviations in all three PCR methods were from one participant, who applied all three PCR methods. This deviation may have been caused by cross-contamination during the culturing or preparation of the cell suspension, because the same incorrect result for this sample was found in all three PCR methods.

Discussion
Q: The inconsistent monophasic *Salmonella* Typhimurium was deliberately used? Which PCR method typed this strain correctly?
A: It is difficult to say what is the true/correct result as this depends on what method is used as reference method. At least, PCR-1 and PCR-3 gave the same results as slide agglutination.
Q: Why didn't you include other monophasic *Salmonella* serovars? Wouldn't that be the main reason to do PCR to differentiate?
A: It would indeed be interesting to test these other monophasic *Salmonella* serovars with the three PCR assays. However, we did not have these strains, nor did we receive them from other laboratories after making a call for isolates.
Q: *S*. Typhimurium 4,[5],12:-:- and *S.* Typhimurium 4,[5],12:-:1,2 will still pass under the radar?
A: These strains are not part of the scope of ISO/TS 6579-4 as the scope is on variants of *Salmonella* Typhimurium lacking or not expressing the second H-phase.
Q: If you use slide agglutination as reference method, how could you find a monophasic strain of another serovar?
A: Slide agglutination was only used as a reference for determining the performance characteristics. Without a reference, inclusivity and exclusivity cannot be calculated.

2.4 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 of potential interest to the NRLs-*Salmonella*. The relevant groups in ISO and CEN are:

- ISO/TC34/SC9: International Organization for Standardization, Technical Committee 34 on Food Products, Sub-committee 9 – Microbiology of the food chain;
- CEN/TC463: European Committee for Standardization, Technical Committee 463 – Microbiology of the food chain.

This year's annual meeting of both groups was organised as a hybrid meeting in Stockholm, Sweden from 26 to 30 June 2023.

ISO/TC34/SC9 WG9 Detection of *Salmonella*

At the annual meeting of ISO/TC34/SC9 and CEN/TC463 in June 2022, it was agreed to re-activate WG9.
In August/September 2022, a call for members and a convenor of WG9 took place. Kirsten Mooijman of EURL-*Salmonella* was (re-) appointed convenor, and the Royal Dutch Standardisation organisation NEN continued to act as the secretariat.
The aims of the re-activated WG9 are:

- To complete the performance characteristics of EN ISO 6579-1:2017 (Detection of *Salmonella*) for all relevant product categories (broad range of food, animal feed, environmental samples and samples from the primary production stage).
- To consider the comments on EN ISO 6579-1:2017 from the systematic review of 2022.

The completion of the performance characteristics is necessary, since for verification of methods following EN ISO 16140-3:2021, the (EN ISO) reference methods need to be validated (transition period until 31-12-2027). If a reference method is not validated for relevant product categories (e.g. animal feed, or not for a broad range of food), the user has to perform an (in-house) validation instead of a verification, which is more labour-intensive.

At the first (online) meeting of WG 9 (March 2023) it was discussed how to generate missing performance characteristics and the next steps. The following was discussed/agreed on:

- The replies to the systematic review of 2022.
- To start with a literature review (especially AFNOR and MicroVal validations) of validation studies. For this purpose an Excel template was sent to the WG 9 members in April 2023.
- To contact some organisations (e.g., Nestlé) for possible validation data of larger test portions.
- For now, to confirm EN ISO 6579-1:2017 (and its amendment) and to start its revision once the additional validation data for EN ISO 6579-1 have been obtained.
- To plan a second WG 9 meeting in the second half of 2023 and discuss available/missing data.

ISO/TC34/SC9 WG10 Typing of *Salmonella*

In WG10, ISO/TS 6579-4 is being developed, entitled: 'Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 4: Identification of monophasic *Salmonella* Typhimurium (1,4,[5],12:i:-) by polymerase chain reaction (PCR)'. The preparatory work for this document started in CEN in 2015. In 2020, the activity was moved to ISO and the first (online) meeting of ISO-WG10 relating to this document was organised in November 2020. Since then, several draft versions of the ISO document have been prepared and distributed for comments. For the Interlaboratory study (ILS) organised in May/June 2022, the 3rd draft ISO/DTS 6579-4 (2022) was used. Results of the ILS were discussed in WG10 and summarised

as performance characteristics in the following draft version(s) of ISO/DTS 6579-4. In May 2023, the 6th draft version of ISO/DTS 6579-4 was sent to ISO, CEN and NRLs-*Salmonella* for further comments.

The validation of draft ISO/DTS 6579-4 was performed in accordance with ISO/DIS 17468:2022. For confirmation and typing methods, the validation study is based on EN ISO 16140-6:2019, describing that the performance characteristics to be determined are inclusivity and exclusivity.
The validation study existed of two parts: method(s) evaluation study and interlaboratory study (ILS).
The method(s) evaluation study for the draft ISO document was performed by the NRL-*Salmonella* in Germany and by the EURL-*Salmonella* in 2018 and 2019. The ILS was organised in May/June 2022 (see 2.3). The results of both parts of the validation study are summarised in Annex E of (draft) ISO/(D)TS 6579-4.

The next steps for this document are:

- Considering the possible comments from the CEN/TC 463 and ISO/TC 34/SC 9 members and from NRLs-*Salmonella* on the 6th draft ISO/DTS 6579-4 and incorporating those where needed.
- Submission of final draft ISO/DTS 6579-4 to ISO/Central Secretariat for the start of the ISO/DTS ballot (last voting step) probably before the end of 2023.
- Considering possible comments to draft ISO/DTS 6579-4 and incorporate those where needed. Preparation of final ISO/TS 6579-4 early 2024.
- Publication of ISO/TS 6579-4 in 2024.

Note by the author: at the annual meeting of ISO/TC34/SC9 in June 2023, it was decided that publishing the document as a full ISO was preferred to its publication as a Technical Specification (TS). As this will involve additional commenting and voting steps (DIS and FDIS), the planning as described above may no longer be valid.

Other subjects

EURL-*Salmonella* is also convenor of an ISO Ad hoc group (AHG A) that is preparing and/or updating a guidance document for drafting ISO/CEN standards for microbiology of the food chain. This is an internal document to help convenors and project leaders of ISO/TC34/SC9 and CEN/TC463 to draft ISO/CEN documents in a harmonised way. Edition 1 of this guidance document was published in 2018, edition 2 in 2020 and edition 3 in August 2022. If needed, an updated version will be drafted in the coming year(s).
When drafting edition 3 of the guidance document, ISO AHG A identified that definitions of some general terms are missing, e.g. food chain, products intended for human consumption, products for feeding animals, and primary production stage. To draft these definitions, ISO AHG E was raised by the end of 2022. During two meetings (in January and April 2023), these definitions were drafted and discussed. In March 2023, the proposed definitions were forwarded to the secretariat of SC9, and it was suggested to include the definitions in (draft) ISO 7218. From mid-

April until the end of May 2023, the definitions were open for comments to the members of SC9.

EURL-*Salmonella* is a member and the project leader of a subgroup of ISO/TC34/SC9-WG3 ('Method validation'). WG3 is employing several activities in the field of method validation:

- AHG C validation status of ISOs. For verification of methods in accordance with EN ISO 16140-3:2021, the (EN ISO) reference methods will have to be validated as from 2027. AHG C started its activities in 2020 and made an inventory on the presence of performance characteristics in EN ISO documents on microbiology of the food chain. As a result, the AHG prepared a table of methods needing (additional) validation studies, in order of importance. Additionally, AHG C is drafting guidelines for ISO/CEN working groups for generation and use of LOD_{50} data from validation studies of, for example, MicroVal and AFNOR.
- Development of Amendment 1 of EN ISO 16140-2:2016, entitled 'Revision of the qualitative method comparison study data evaluation, revision of RLOD calculations in the interlaboratory study, revision of the calculation and interpretation of the relative trueness study, and inclusion of commercial sterility testing protocol of UHT milk'. The DIS voting for this document is expected in the summer of 2023.
- Revision of EN ISO 16140-1:2016 ('Method validation – Vocabulary') and of EN ISO 16140-2:2016 ('Validation of alternative (proprietary) methods'). The official revision of both documents will start following publication of ISO 16140-2/Amd1 (2024), but the preparatory work already started in April 2023.
- Development of Amendment 1 of EN ISO 16140-4:2020, entitled: 'Validation of a larger test portion size for qualitative methods'. The approach in this document is to compare the level of detection (LOD) of the original test portion size with the LOD of the larger test portion size. The DIS voting for this document is expected in the summer of 2023.
- Development of ISO 16140-7 'Protocol for the validation of identification methods of microorganisms'. This document specifies the general principle and the technical protocol for the validation of identification methods of microorganisms for microbiology in the food chain, in case no reference method is available. When a reference confirmation or typing method is available, the alternative method shall be validated in accordance with EN ISO 16140-6:2019. The DIS voting started in May 2023 and will last until July 2023.
- Revision of EN ISO 17468:2016 'Technical requirements and guidance on establishment or revision of a standardized reference method'. This document is revised to include information of EN ISO 16140-4:2020 (in-house validation), EN ISO 16140-6:2019 (validation of confirmation and typing methods) and EN ISO 11133:2014 (performance testing of culture media); to explain the impact of minor and major changes of a revised EN ISO document; and to extend the content to situations where it is not possible to compare a new EN ISO method to a former reference method. The DIS voting took place from September until December 2022, with a positive

outcome. The FDIS voting is expected by the second half of 2023.
- Together with WG2 ('Statistics'), WG3 forms a joint sub-group that reviews evaluation/validation protocols for ISO standards. This sub-group advises other ISO working groups on drafting protocols for performing validation studies.

Other ISO working groups working on general subjects are:
- Joint working group JWG5, revising EN ISO 11133:2014 ('performance testing of culture media'). This JWG published two amendments for EN ISO 11133, in 2018 and in 2020. Additionally, a table with all control strains for performance testing of culture media and reagents from published standards from microbiology of the food chain and from water microbiology is published on the website of ISO/TC34/SC9 (https://committee.iso.org/home/tc34sc9). JWG5 started the revision of the full EN ISO 11133 document in March 2023.
- WG7 is revising EN ISO 7218:2007 ('General requirements and guidance for microbiological examinations'). The DIS voting took place in 2022 and 92 pages of comments were received. It took some time before all these comments were addressed. Therefore, the FDIS voting may take place only by the second half of 2023.

Discussion
Q: Referring to LOD_{50}: shall we lower the lab's LOD_{50} according to the information in the annexes of the ISO reference method for certain matrices?
A: For the verification of a validated method by a laboratory, EN ISO 16140-3:2021 should be followed. This ISO document gives information on the acceptability limits that the laboratory results should adhere to. For a qualitative method, the $eLOD_{50}$ determined in the verification study should be $\leq 4xLOD_{50}$ for the relevant food category determined in the validation study (and listed in the annex of the ISO reference method).

2.5 Results EURL-*Salmonella* Proficiency Test PPS-FOOD 2022 – Detection of *Salmonella* in hygiene swabs

Irene Pol-Hofstad, EURL-Salmonella*, Bilthoven, the Netherlands*

In September 2022, the combined EURL-*Salmonella* Proficiency Test (PT) on the detection of *Salmonella* in samples from the primary production stage (PPS) and Food was organised. Participation was mandatory for the NRLs-*Salmonella* of all European Union (EU) Member States (MSs) that are responsible for the detection of *Salmonella* in PPS samples and for all NRLs responsible for detection of *Salmonella* in food samples. A total of 68 NRLs-*Salmonella* participated in this study:
34 NRLs PPS and 34 NRLs Food from the 27 EU MSs, 11 NRLs from third European countries (EU candidate MSs or potential EU candidate MSs and members of the European Free Trade Association (EFTA)), and one NRL from a non-European country.

In this study, the samples under analysis were hygiene swabs, artificially contaminated at the EURL-*Salmonella* laboratory with two

different mixtures of background flora, and a diluted culture of *Salmonella* Enteritidis and/or *Salmonella* Infantis.
Each NRL-*Salmonella* had to analyse the following set of blindly coded samples:

- 4 hygiene swab samples with a high level of *S.* Infantis (30 cfu/sample) and *S.* Enteritidis (8 cfu/sample), in combination with a mixture of *Enterobacter cloacae* and *Citrobacter freundii* (10^6 cfu/sample).
- 6 hygiene swab samples with a low level of *S.* Infantis (8 cfu/sample), in combination with a mixture of *Enterobacter cloacae* and *Citrobacter freundii* (10^6 cfu/sample).
- 4 negative hygiene swab samples (no *Salmonella* added):
  - 2 samples: *E. cloacae* and *C. freundii* (10^6 cfu/sample);
  - 2 samples*: E. cloacae* and *C. youngae* (10^6 cfu/sample).
- 1 procedure control (hygiene swab samples with sterile peptone saline solution only).
- 1 positive control sample (laboratories' own *Salmonella* control strain).

The samples were prepared at EURL-*Salmonella* laboratory and stored at 5 °C for approximately one week until the day of transport. On Monday, 26 September 2022, the hygiene swab samples were packaged and sent to the NRLs-*Salmonella*. The NRLs were asked to store the samples at 5 °C on arrival until the start of the analysis on Monday, 3 October 2022.

All laboratories used the prescribed method EN ISO 6579-1:2017 (/A1:2020) to test the samples. Only two participating laboratories were not (yet) accredited for this method. Two laboratories reported to be NRL-*Salmonella* for samples from the primary production stage but used Muller-Kauffmann tetrathionate-novobiocin (MKTTn) broth and Rappaport-Vassiliadis soya (RVS) broth instead of modified semi-solid Rappaport-Vassiliadis (MSRV) agar for the selective enrichment. The use of these broths is not in line with the prescribed method in EN ISO 6579-1:2017 for analysing PPS samples.
Twenty laboratories also reported results for a second method. These laboratories all found identical results using the alternative method compared to the results found with EN ISO 6579-1:2017(/A1:2020).

All 68 participating laboratories analysed both the procedure control and their own positive control sample correctly.
Almost all laboratories detected *Salmonella* in the hygiene swab samples contaminated with a low level of *Salmonella* Infantis (8 cfu/sample). One laboratory tested one of the six samples negative for *Salmonella*. These results are still within the criteria for good performance, which permit three negative samples. The sensitivity rate was 99,8% for these samples.
All laboratories detected *Salmonella* in all four high-level samples contaminated with a combination of *Salmonella* Infantis (30 cfu/sample) and *Salmonella* Enteritidis (8 cfu/sample). The sensitivity rate was 100% for these samples.
All 4 negative samples were scored correctly as negative by 43 laboratories. However, 25 laboratories detected *Salmonella* in one or two of the four negative samples. Serotyping of these 'false-positive'

isolates showed that this strain was *Salmonella* Enteritidis. Additional subtyping using Whole Genome Sequencing (WGS) revealed that the 'false-positive' isolate had a WGS pattern that was identical to the *Salmonella* Enteritidis strain used in this PT to artificially contaminate the positive samples. Since almost 10% of the total number of negative samples were tested as positive for *Salmonella*, the EURL-*Salmonella* decided not to evaluate the results of the negative samples.

Overall, the laboratories scored well in this PT, analysing the positive samples with an accuracy of 99,9%. All 68 laboratories fulfilled the criteria of good performance.

More details can be found in the report on this PT (Pol-Hofstad and Mooijman, 2023).

Discussion
Q: Are you planning to organise a PT on *Salmonella* detection in Live Bivalve Molluscs any time soon (perhaps in 2024)?
A: Yes, we are considering organising such a PT in 2024.

2.6 Results EURL-*Salmonella* Proficiency Test Typing 2022 – serotyping and cluster analysis

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

In November 2022, the EURL-*Salmonella* organised the 27th Proficiency Test (PT) on typing of *Salmonella*. A total of 34 laboratories participated in this PT, consisting of an obligatory serotyping part and an optional part on cluster analysis. Participants included 27 NRLs-*Salmonella* from the 27 EU MSs and seven NRLs from third countries (EU candidate or potential EU candidate MSs, EFTA countries, and the United Kingdom). The main objective of this PT was to evaluate the performance of the NRLs for serotyping of *Salmonella*.
A total of 20 obligatory *Salmonella* strains plus one additional *Salmonella* strain from an uncommon type were selected for the serotyping study by the EURL-*Salmonella*. The strains had to be typed with the method routinely used by 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 e-mailed to the participants in February 2023. The O-antigens were typed completely correct by 31 out of the 34 participants (91%). This corresponds to nearly 100% of the total number of strains. The H-antigens were typed completely correct by 27 out of the 34 participants (79%), corresponding to 98% of the total number of strains. As a result, 25 participants (74%) reported all serovar names correctly, which corresponds to 98% of all strains evaluated. A completely correct identification was obtained for ten *Salmonella* serovars: Singapore (S1), Agona (S4), Enteritidis (S5), Kenya (S6), Hadar (S9), Hull (S10), Virchow (S12), Hato (S13), Mishmarhaemek (S17), and Infantis (S20). Strain S8 was characterised with antigenic formula 4,5,12:i:e,n,x, and in accordance with Supplement 2008-2010 (no. 48) to the White-Kauffmann-Le Minor scheme, this new variant of the previously

described serovar Farsta (4,12:i:e,n,x) is now recognised with the updated antigenic formula: 4,[5],12:i:e,n,x (Issenhuth-Jeanjean et al., 2014). All but five participants tried to serotype optional strain S21, a *Salmonella enterica* subsp. *diarizonae* (IIIb). A few laboratories did not have access to all required antisera to finalise this (47:k:z_{35}).
Overall, the NRLs' performance in the 2022 PT on Serotyping was very good. Two EU Member State NRLs did not meet the level of good performance at the first stage of the PT, and a follow-up study for these laboratories was organised. Both NRLs correctly serotyped the ten additional strains in this follow-up study.

The individual laboratory results of the cluster analysis part were emailed to the participants just before the Workshop, and an overview on the general outcome was presented during the Workshop.
A total of 20 NRLs participated in the optional cluster analysis; all performed WGS analysis, and 5 participants also performed MLVA analysis.
Six 'wet' strains (22SCA01 – 22SCA06, shipped in transport tubes to the participants) and six 'dry' strains (22SCA11 – 22SCA16, raw sequence data available on a sftp server) were pre-tested and selected for inclusion in the 2022 PT. As in the year before, the PT on Cluster Analysis 2022 was mimicking an outbreak situation, with *Salmonella* Enteritidis ST11, MLVA type 3-10-6-3-1 as the reference strain (22SCA-REF). For this particular 2022 PT situation, the cgMLST-based cluster definition (WGS) was set at a maximum of six allelic differences from the reference sequence. For MLVA, the cluster definition was set at no loci with a different number of repeats.
Participants were asked to analyse the six 'wet' *Salmonella* strains (MLVA/WGS) and the six 'dry' *Salmonella* strains (WGS only), and to report for each strain whether a clustering match with the reference strain was found or not.
In accordance with the 2022 PT Typing cluster definition, MLVA-based results were expected to indicate strains 22SCA04 (reference strain) and 22SCA06 (technical duplicate of the reference strain) to be a clustering match with the reference outbreak strain. Four participants reported the MLVA-based cluster analysis results completely as expected. The fifth participant reported the allelic profiles in a deviating format, and therefore appropriate evaluation of their results was not possible.
Regarding WGS-based results, 'dry' strain 22SCA13 was expected not to pass the participants' Quality Control (QC), because the data files of this strain also contained numerous *E. coli* reads. The 2022 PT Typing Protocol indicated to exclude strains from the cluster analysis if the data did not pass the QC. 17 out of the 20 participants reported excluding strain 22SCA13 from cluster analysis.
In accordance with the given (cgMLST-based) cluster definition, WGS-based results were expected to indicate the wet strains 22SCA04 (reference strain), 22SCA06 (technical duplicate of the reference strain), 22SCA01 (clustering with the reference strain), 22SCA05 (technical duplicate of strain 22SCA01) and the dry strain 22SCA15 (PT 2021 raw data of strain 22SCA01) to be a clustering match with the provided reference outbreak strain 22SCA-REF data. 19 out of the 26 submissions (five participants with multiple submissions) reported the WGS-based cluster analysis results completely as expected. Deviations concerned a potential misunderstanding of the way of reporting clusters, a swap in

correctly naming strains, and not excluding the contaminated strain 22SCA13 from the cluster analysis.

More details can be found in the interim summary reports of the 2022 PT Typing (Jacobs-Reitsma et al., 2023a, 2023b).

Discussion
Q: Do you know when we can expect a new supplement to the White Kauffmann le Minor scheme? And how will we be updated about the publication of a new supplement so that we can inform our national laboratories?
A: We still do not know when a new supplement will be published. We regularly ask the WHO reference laboratory in Paris, and we generally hear that they are working on it. When a new supplement is published, we will make sure to inform the NRLs-*Salmonella* (by email, Newsletter, publication on our website).

2.7 Preliminary results EURL-*Salmonella* Proficiency Test FOOD-FEED 2023 – Detection of *Salmonella* in seeds

Robin Diddens, EURL-Salmonella*, Bilthoven, the Netherlands*

In March 2023, the EURL-*Salmonella* organised a PT on the detection of *Salmonella* in food and feed for the NRLs-*Salmonella*. The matrix under analysis was flaxseed. NRLs-*Salmonella* that analyse *Salmonella* in food samples and NRLs-*Salmonella* that analyse animal feed products were invited to participate in this PT. NRLs-*Salmonella* that perform the analysis of food and feed samples in one and the same laboratory could request two different laboratory codes with two (similar) sets of samples. In this way, the laboratory could perform the analyses separately as NRL-*Salmonella* food and as NRL-*Salmonella* feed. However, these latter NRLs-*Salmonella* could also choose to analyse only one set of samples under one laboratory code. Obtaining two different laboratory codes gives the laboratory the opportunity to receive two separate performance reports.

In total, 51 laboratory codes were generated for this EURL-*Salmonella* PT. The participants in this PT were NRLs from 27 EU MSs and nine NRLs-*Salmonella* from seven different third countries (EU candidate MSs, EFTA countries, and United Kingdom).
The most important objective was to test the performance of the participating laboratories' detection of *Salmonella* in the artificially contaminated flaxseed samples. The prescribed method for detecting *Salmonella* spp. was EN ISO 6579-1:2017(/A1:2020). The participants were asked to report *Salmonella* 'detected' or 'not detected' for each sample (following confirmation).

Prior to the start of the PT, pre-tests were conducted to ensure that the samples were fit for use. Flaxseed samples, artificially contaminated with different concentrations of *Salmonella* Typhimurium (STm), were tested for their stability at different storage temperatures (5 °C and 10 °C). Additionally, the concentration of the natural background flora (aerobic count and number of *Enterobacteriaceae*) in the flaxseed was measured.

For the pre-tests, flaxseed samples were artificially contaminated with 7 cfu STm/25 g or with 11 cfu STm/25 g. The flaxseed samples artificially contaminated with 11 cfu STm/25 g were stable at 5 °C and at 10 °C during the storage period of 13 days. The samples artificially contaminated with 7 cfu STm/25 g were less stable. After 13 days, only three out of six samples were still positive for *Salmonella* when stored at 5 °C or 10 °C. On the basis of these results, the aim was to inoculate the low-level flaxseed samples for the PT with approximately 9 cfu STm/25 g.
The number of aerobic bacteria and *Enterobacteriaceae* in the flaxseed samples remained relatively stable when stored at 5 °C and at 10 °C. The concentration of both florae remained approximately 10^7 cfu/g when stored at both temperatures for up to three weeks.

Each laboratory received 14 samples of 25 g flaxseed each. These samples consisted of four negative samples (no *Salmonella* added), six samples with a low level of STm (inoculum 9 cfu/sample) and four samples with a high level of STm (inoculum 52 cfu/sample). The PT samples were artificially contaminated with a diluted culture of *Salmonella* Typhimurium at the EURL-*Salmonella* laboratory. In addition, each participating laboratory had to test two control samples: a procedure control (Buffered Peptone Water only) and a positive control with *Salmonella*.

Fifty laboratories fulfilled the criteria of good performance for the EURL-*Salmonella* Proficiency Test for the detection of *Salmonella* in flaxseed samples.
One laboratory scored a 'Good performance*', with an additional explanation. The laboratory made an administrative error when reporting the result of a negative sample. The laboratory communicated this error with the EURL-*Salmonella* after the reporting deadline of the PT, but before the intended results were shared with the laboratories. An explanation was given as to how this error came to light, and raw data confirmed the correct result.

The accuracy rate of all control samples was 100%. The sensitivity rates of the flaxseed samples artificially contaminated with *Salmonella* Typhimurium was 96%. The accuracy rate of all flaxseed samples for all participating laboratories was 97%. The specificity rate of the negative flaxseed samples was 100%.

The NRLs-*Salmonella* were given the opportunity to analyse the samples with a second detection method as well, if this method was (routinely) used in their laboratories. The results obtained with the second method were not used for assessing the performance of the NRL-*Salmonella*. Nineteen participants also used a second detection method (real-time PCR, VIDAS and PCR) for analysing the samples. The results of the second detection methods were all similar to the reported results that the laboratories obtained with EN ISO 6579-1:2017(/A1:2020) by the laboratories.

More details can be found in the interim summary report on this PT (Diddens and Mooijman, 2023).

3 Tuesday 23 May 2023: Day 2 of the workshop

3.1 A *Salmonella* cgMLST validation study for accreditation in Germany

*Marina Lamparter, NRL-*Salmonella*, Berlin, Germany*

Introduction

Whole genome sequencing (WGS) is now established as a key tool for surveillance and outbreak investigation in public health. The German National Reference Laboratory (NRL) for *Salmonella* receives 3000-4000 strains from food, feed, animal and environmental sources per year. Starting in 2018, WGS was implemented as a routine molecular testing tool, resulting in >7000 *Salmonella* spp. sequences being stored in the database (DB) today. This sequence data represents a geographical cross section of *Salmonella* isolates from the various non-human sources belonging to serovars with the highest human health relevance in Germany. This sequence collection is increasingly requested for genome comparison analyses with human outbreak sequences by means of bioinformatics tools to support trace-back studies and source identification. In this context, the core-genome multi-locus-sequence-typing (cgMLST) analysis method became the gold standard at the NRL for *Salmonella*. In order to offer high-quality results and working under a strict quality assurance system (EN ISO/IEC 17025:2017), general WGS wet- and dry-lab workflows are validated for accreditation, mainly by the responsible 'National study centre for sequencing in risk assessment (4NSZ)' at BfR. Evaluation and validation of different cgMLST workflows for *Salmonella* cluster detection were performed by the NRL for *Salmonella* in collaboration with 4NSZ.

Material & Methods

The cgMLST validation study was divided into a general method part and a more detailed genus-specific (e.g. *Salmonella enterica*) part. The *Salmonella*-specific part is further elaborated here. Test data sets for the cgMLST analysis workflow validation as well as performance and acceptance criteria were defined.

For the *Salmonella* cgMLST analysis, harmonised QC-checked and assembled sequence data was used. The data was previously generated through a standardised wet-lab protocol and processed by the in-house AQUAMIS pipeline with both workflows already accredited under guidance of 4NSZ.

Two cgMLST pipelines were assessed within the validation study: 1) The in-house developed and published ChewieSnake cgMLST pipeline was used with an adapted Enterobase (3000 loci) and the EFSA (3255 loci) scheme for *Salmonella enterica*. 2) The commercial RIDOM SeqSphere+ programme was used with the included *Salmonella enterica* Enterobase (3002 loci) scheme. For both, a maximum threshold of 5% missing cgMLST loci was set.

To test repeatability, reproducibility and robustness, a test dataset of 21 shovill-assembled sequences was analysed with all three cgMLST schemes, and results were compared. The data set was generated from four different *Salmonella enterica* strains, belonging to four different

serovars (*S*. Enteritidis, *S*. Infantis, *S*. Typhimurium and monophasic *S*. Typhimurium), from various matrix categories (food, feed, environment, animal) and prepared in biological replicates or under varying wet-lab conditions (e.g. different Illumina machines or volumes of reagents). To test trueness, data from the 2020 EURL-*Salmonella* Proficiency Test (PT) was re-analysed (Enterobase scheme) and checked for correct cluster detection. In addition, results of cgMLST analyses from the NRL-*Salmonella* were compared with cgMLST analyses previously performed at the EURL-*Salmonella* as part of the analysis of international outbreaks (linked to UI-367, UI-644 and UI-716) caused by different serovars (*S*. Enteritidis, *S*. Amsterdam, *S*. Havana, *S*. Mbandaka, *S*. Orion, *S*. Senftenberg and *S*. Tennessee), using fastq sequences submitted by the NRL-*Salmonella*.

Results
All samples showed more than 95% valid cgMLST targets. Allelic difference (AD) results were displayed in distance matrices. Observed AD of identical DNA samples within repeatability testing were 0-2 AD. The reproducibility testing showed 0-3 AD and robustness testing 0-2 AD. Comparison of RIDOM-based analysis and ChewieSnake-based analysis yielded AD of maximum 3. Testing trueness with PT data, clusters (threshold ≤ 6 AD) were correctly identified with the three different approaches. Maximum difference compared to EURL results observed for isolates with AD <10 was 1. When comparing the international outbreak data, allelic distances were similar, but not identical. Again, within common cluster thresholds of (10 AD), distances of 0-3 alleles were observed between applied workflows. Generally, greater variance in allelic distances was not compared.

Conclusion
The methods applied are suitable for the purpose. Our results were comparable or identical within cluster detection-relevant ranges. However, observed differences of 3 AD might play a role if cluster thresholds are strictly applied, for instance within outbreak analysis. Occurrence of higher allelic differences based on the use of different approaches, i.e. in different laboratories, underlines that strict thresholds can lead to misinterpretations, especially for *Salmonella* spp.. Harmonised bioinformatics workflows providing consistent results for all samples in a cluster (e.g. EFSA's WGS system), regardless of where the data were generated, are a valuable benefit. However, even here, the impact of wet-lab effects on the emergence of AD cannot be prevented. Thus, and in general, interpretation of the cgMLST results must take place in the context of metadata.

Discussion
Q: Is your pipeline publicly available?
A: Yes, several BfR pipelines can be found on git-lab/bfr_bioinformatics.
Q: Regarding 'robustness' you've mentioned that, among other variable parameters, you have used expired cartridges/kits. Is this a policy generally considered while one tests robustness?
A: Our core unit gave us advice on performing the study, including the use of expired consumables. When testing robustness, you test the 'extreme' situations, which may include expired materials or materials close to the expiration date.

Q: Did you check the antimicrobial resistance profiles of the strains identified with the different approaches, or just the distance matrix?
A: Generally, we checked the distance matrix only. Going deeper into the profiles would have taken a lot of time and was not part of the validation study for this pipeline.

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

*Alexandra Schoos, Catarina Martins and Catherine Ragimbeau, NRL-*Salmonella*, Luxembourg*

In Luxembourg, three laboratories work in close collaboration and share the NRL activities for *Salmonella*:
(i) the Food surveillance service (at Laboratoire National de Santé - LNS); (ii) the State veterinary Medicine Laboratory (LMVE, located at the same site as LNS); and (iii) the Epidemiology and Microbial Genomics service (Epigem at LNS).

The food microbiology laboratory service is the largest official control laboratory for food and feed in Luxembourg. Not only microbiological analyses are performed for the national official food monitoring, but also detection of genetically modified organisms, pesticides, mycotoxins, food contact material, etc (in total, National Reference Laboratory for 14 domains).
LMVE is part of the new Luxembourg Veterinary and Food Administration (ALVA) in charge of controls in the food chain. Their role in monitoring is more strongly associated with food animal production.
When *Salmonella* is detected in one of the microbiological laboratories, the isolates are sent to Epigem laboratory for sequencing by NGS. Integrated Surveillance by WGS has been implemented in Luxembourg since 2013, in order to detect human clusters and make the link with food or animals.

During the *Salmonella* crisis of the Kinder chocolate products, the food microbiological department in the LNS received approximately 30 samples. None of the samples was positive, despite two human cases that were reported in Luxembourg. Genomics analysis of the outbreak strain revealed that, in addition to its multi-drug resistant (MDR) profile, five heavy metals were also detected in the genome including: arsenic, copper, silver, gold and mercury. Finally, one gene conferring reduced susceptibility to quaternary ammoniums compounds (QAC) captivated the minds of the scientific community. A retrospective study was then conducted in Luxembourg, focused on the frequency of the qacL gene (enhancing biocide tolerance) in *Salmonella* Typhimurium/ monophasic *S*. Typhimurium isolated between 2017 and 2022. A collection of 200 genomes were screened and only 4,5% harboured this specific gene. On average, the related strains were also MDR (ranging from 5 to 7 antibiotic classes) and possessed genes for resistance to 5 heavy metals as well.

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

Tatjana Kutyriova , NRL-Salmonell*a, Vilnius, Lithuania*

The Lithuanian National Reference Laboratory for the control of *Salmonella* and other specified foodborne zoonotic agents is part of the National Food and Veterinary Risk Assessment Institute (NFVRAI). The NFVRAI was established in 2008 following the reorganisation of the National Veterinary Laboratory (established in 1945) and the Lithuanian State Inspection on Veterinary Preparations. NFVRAI (main institution located in Vilnius) with 4 branches (Kaunas, Klaipėda, Šauliai and Telšiai) is the subordinate body of the State Food and Veterinary Service of the Republic of Lithuania. The NFVRAI mission is to ensure monitoring and control of contagious animal diseases, zoonoses and animal welfare, to eliminate outbreaks of diseases; to apply all necessary biological measures for prevention of introduction of contagious diseases and zoonoses into the territory of Lithuania and the EU; to ensure food safety and control at all stages of food handling according to the principle 'from stable/field to table', to safeguard the interests of consumers; to ensure that the food that is supplied on the market or is intended for export complies with safety, labelling and other mandatory requirements established by European and Lithuanian legislation. The National Food and Veterinary Risk Assessment Institute carries out an assessment of risk factors and provides scientific and technical assistance to risk management in food safety and veterinary fields, cooperating closely with the European Food Safety Authority (EFSA). EFSA is an independent organisation that provides scientific advice on all direct or indirect impact on food safety issues, as well as animal health and welfare, and plant protection. EFSA collects and analyses scientific data, determines and controls risk factors and provides scientific assistance to the European Commission, the European Parliament and the Member States. Experts of the Lithuanian Institute participate in the EFSA's expert working groups, exchange scientific information, provide research data, participate in EFSA's working programmes and joint international projects. All NFVRAI laboratories have been accredited according to ISO/IEC 17025 since May 2015 (assessed by the Lithuanian National Accreditation Bureau (LA)). The laboratory uses various techniques, including microbiological culture, biochemical confirmation, MALDI-TOF MS, serotyping, antimicrobial resistance testing, PCR method for detection of *Salmonella* spp. in food, animal feed and primary production stage samples. Detection methods for *Salmonella* spp. are EN ISO 6579-1:2017, EN ISO 6579-1:2017/A1:2020 and *Salmonella* spp. serotyping method – CEN ISO/TR 6579-3:2014. The antimicrobial susceptibility testing is performed according to (in-house) SOP B.36. This SOP has been drafted in accordance with protocols developed by the EURL-AR. All previously mentioned laboratory methods, except for MALDI-TOF MS, are accredited according to EN ISO/IEC 17025.

The main official programmes of the NRL-*Salmonella* are:

- *Salmonella* tests to assess the criteria of hygiene processes, in primary processing facility (poultry and pig slaughterhouses);

- The programme for monitoring and importing animal feed and feed materials (samples of animal feed taken in accordance with the official animal feed sampling programme);
- National control programmes for *Salmonella* in poultry farms (breeding flocks, broilers, laying hens, turkeys);
- Samples of pig cecum taken under the AMR monitoring programme.

In 2022 and 2021, *Salmonella* was not detected in any of the 55 and 52 breeding flocks of *Gallus gallus* respectively. In 2020, S*almonella enterica* was detected in 2 flocks (3,28%) out of the 61 breeding flocks of *Gallus gallus*: *Salmonella* Infantis and *Salmonella* Mbandaka. In 2022, 139 broiler flocks were examined before slaughter. *Salmonella* Mbandaka was detected in 1 flock (0,71%). In 2021, *Salmonella enterica* was detected in 4 flocks (2,72%): *Salmonella* Enteritidis (3 units) and *Salmonella* Mbandaka (1 unit). In 2020, *Salmonella enterica* was detected in 3 flocks (2,68%) out of the 121 broiler flocks (*Gallus gallus*) before slaughter. *Salmonella* Enteritidis was detected in two units and *Salmonella* spp. in one unit (no serovar name due to auto-agglutination).

Discussion

Q: What kind of differentiating tests are you using to differentiate wild strains from vaccine strains, and are you accredited for these tests?
A: We use the culture test supplied by the vaccine supplier. We are not accredited for this test.

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

Istvan Szabo , NRL-Salmonella*, Berlin, Germany*

The main task of the NRL for *Salmonella* is the identification and analysis of *Salmonella* isolates originating from feed, food and primary production, using serological and molecular biological methods. The NRL deals with the epidemiological surveys on the occurrence of various *Salmonella* serovars and participates in the implementation of national and European monitoring programs, as well as in the control programmes for *Salmonella* in poultry. The NRL is also strongly requested to support analysis for the elucidation of infection chains and food-borne disease outbreaks. For this purpose and other epidemiological studies, several molecular biological methods have been established. However, the high resolution and standardisability of Next-Generation Sequencing (NGS) methods enables identification of food-related disease outbreaks much faster and more targeted than before. For this reason, the NRL is increasingly relying on NGS methods in particular. In the network of the World Organization for Animal Health, the NRL for *Salmonella* acts as the OIE - reference laboratory for salmonellosis. In addition, aspects of the transmission of *Salmonella* via food, feed, food-producing animals to humans are investigated at the NRL. This also includes the research and monitoring of resistance mechanisms and the spread of resistance determinants in *Salmonella*. Another research focus is the development of new methods for the

detection and typing of *Salmonella*. Here too, the use of NGS methods is playing an increasingly important role.

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

Amira Koro-Spahic, NRL-Salmonella*, Sarajevo, Bosnia and Herzegovina*

The Veterinary Faculty in Sarajevo, Bosnia and Herzegovina, was established in 1949. As an organisational unit of the University of Sarajevo, it conducts higher education activities by organising and implementing teaching, research, and educational processes. The Veterinary Institute, which operates under the Faculty, focuses on scientific research, scientific teaching, and professional operational work in the field of veterinary activities. These activities are governed by laws on veterinary medicine, higher education, and the Statute of the University of Sarajevo.
The Veterinary Institute's scientific research in veterinary, biomedical, and biotechnical sciences contributes to expanding scientific knowledge and achievements, with the aims of protecting animal and human health and well-being, ensuring food safety, and environmental protection. The Institute carries out its professional and operational activities under the authorisation of competent authorities, contributing to public interest services and assisting individuals involved in the production and trade of animals, their products, and animal feed. The Institute's laboratories comply with the BSL-2 safety level requirements, and since 2017, they have implemented a quality assurance system based on EN ISO/IEC 17025. Previously, the Laboratory for the diagnosis of bacterial diseases of poultry was part of the Poultry Center at the Veterinary Faculty in Sarajevo. Since 2021, as part of the University of Sarajevo's internal organisation and systematisation, the laboratory has continued its long-term work in the Veterinary Institute's Laboratories - Veterinary Faculty, now known as the Laboratory for Bacteriology and Mycology (BPK). BPK has successfully met the requirements for continuous health surveillance and health control of poultry and birds through laboratory and field monitoring of infectious poultry diseases' occurrence, spread, elimination, and eradication. The laboratory's activities primarily involve monitoring *Salmonella* in commercial and backyard flocks, collaborating with the EURL, participating in training courses, cooperating with the competent authorities of Bosnia and Herzegovina (Ministry of Agriculture, Water Management and Forestry of the Federation of Bosnia and Herzegovina as well as the Veterinary Office of Bosnia and Herzegovina), testing official samples, and coordinating official laboratory activities.
Bosnia and Herzegovina has the following control programmes for *Salmonella*:

- Control programme for *Salmonella* in broiler flocks (*Gallus gallus*);
- Control programme for *Salmonella* in laying hens flocks (*Gallus gallus*);
- Control programme for *Salmonella* in breeding flocks (*Gallus gallus*).

Discussion
Q: Do you organise PTs for the national official laboratories?
A: The last time we organised a PT (for approximately 40 laboratories) was in 2013.

3.6 Work programme EURL-*Salmonella* second half 2023 - first half 2024, concluding remarks workshop 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 second half of 2023 and for early 2024.

Due to late adoption of the SMP (Single Market Programme Regulation) Work Programme for 2023-2024 by the European Commission (February 2023), the call for the EURLs to submit their work programmes for 2023-2024 was sent late. In January 2023, EURL-*Salmonella* sent its draft work programme 2023-2024 to the desk officer at DG SANTE for feedback. On 28 April, HaDEA organised an information session about the new procedure for submitting proposals, and only by 2 May, the formal call to submit a proposal was sent to the EURLs, with a deadline of 15 June next. HaDEA will evaluate the proposals until approximately November 2023 and the signature of the grant agreement is expected only by January-February 2024.
The template for the work programme still follows Regulation EU No 625/2017 (EC, 2017), Article 94(2) and is subdivided into four main activities. Each activity is further divided into one or more work packages (previously called sub-activities).

Activity 1 To ensure availability and use of high-quality methods and to ensure high-quality performance by NRLs

Work package 1.1 Analytical methods
Objectives:
- to standardise methods (ISO and CEN);
- to keep track of developments in (alternative) methods;
- to provide NRLs with information on developments regarding relevant (standardised/new) analytical methods.

This activity includes activities for ISO and CEN, further described in clause 2.4 of this report.

Work package 1.2 joint EURLs working group on NGS
Objectives:
- to promote the use of NGS across the EURL networks;
- to build capacity for producing and using NGS data within the EU;
- to ensure liaison between the work of the EURLs and the work of EFSA and ECDC on NGS.

The working group consists of eight biological EURLs, and nine activities have been defined in relation to NGS. For each activity, guidance documents are prepared and published on the EURLs' websites. Other EURLs provide links to the documents published by colleague EURLs (see also https://www.eurlsalmonella.eu/publications/analytical-methods - Next Generation Sequencing (NGS)). In cooperation with EFSA, the

working group will organise the second Science meets policy conference on the use of NGS in Parma, Italy (hybrid meeting) on 5 and 6 September 2023. Additionally, the working group organises joint EURL trainings on NGS for the EURL/NRL networks. The next training is organised on the premises of EURL-*Salmonella* in Bilthoven in June 2023 (see also work package 2.2).

Work package 1.3 Proficiency Tests (PTs)

Objectives:

Evaluation of the performance of the NRLs-*Salmonella* for detection and typing of *Salmonella* by means of interlaboratory comparisons (Proficiency Tests).

Organisation of 3 PTs per year:

1. PT on detection of *Salmonella* in food or animal feed samples, including Live Bivalve Molluscs, generally organised in March of each year. For 2023, the matrix under analysis is flaxseed and both NRLs Food and NRLs Feed should participate. For 2024, a PT for Live Bivalve Molluscs may be considered.
2. PT on detection of *Salmonella* in primary production stage samples (PPS), generally organised in September of each year. For 2023, the matrix under analysis will be chicken faeces. The matrix for the 2024-PT is not yet known.
3. Typing of *Salmonella* (serotyping, molecular typing), generally organised in November of each year. This PT will include serotyping of *Salmonella* (obligatory) and an optional part on cluster analysis (using WGS).

Activity 2 To provide scientific and technical assistance to NRLs

Work package 2.1 Workshop
Objective:
To exchange information on the activities of the NRLs-*Salmonella* and the EURL-*Salmonella* and on (new) developments in the relevant work field. The next workshop is expected to take place by the end of May 2024, and hopefully, it will be possible to organise this workshop as a physical meeting again.

Work package 2.2 Training courses
Objective:
To train NRLs-*Salmonella* in a specific work field. The following trainings are foreseen:

- Joint EURL training on NGS. In 2023, this training will be organised in June in Bilthoven, the Netherlands. The location for the 2024 training is not yet known.
- Individual trainings of NRLs on request of an NRL or on the advice of the EURL (for instance, in case of unsatisfactory performance in PTs).

Work package 2.3 Scientific advice and support of NRLs
Objectives:

- to provide scientific and technical assistance to the NRLs-*Salmonella* for the relevant work field;
- to perform confirmatory testing and/or typing (samples/isolates) for NRLs-*Salmonella* when needed;
- to perform WGS analysis of isolates of NRLs-*Salmonella* for outbreak investigations;
- to maintain the EURL-*Salmonella* website and keep the information up to date (https://www.eurlsalmonella.eu);
- to inform NRLs on the activities of the EURL and other parties in the relevant work field, as well as on developments in this field;
- to publish four newsletters per year, through the website.

Activity 3 To provide scientific and technical assistance to the European Commission and other organisations

Work package 3.1 Scientific advice and support of EC and other organisations
Objectives:

- to provide scientific and technical assistance to EC DG SANTE in the relevant work field;
- to provide assistance to DG SANTE, EFSA, and (NRLs of) Member States in the event of (international) *Salmonella* outbreaks;
- to collaborate with EFSA and ECDC in the relevant work fields;
- to cooperate with other biological EURLs.

Description:

- ad hoc scientific and technical assistance of DG SANTE;
- Member of 'EFSA-ECDC Advisory Board on the management and sharing of molecular typing data of isolates from human, food, feed, animal, and the related environment for public health purposes'.
- Assistance of DG SANTE, EFSA, NRLs and ECDC in case of outbreaks, e.g. consultation of NRL network for specific information, (sub)typing of suspect isolates (WGS), analysis of data.

Activity 4 Reagents and reference collections

Work package 4.1 Reference strains and reference materials
Objective:
To supply information on available culture collections and suppliers of microbiological reference materials. Setting up a reference collection of WGS data.

Description:

- Reference to culture collections and reference materials on the EURL-*Salmonella* website;
- Maintenance of the in-house culture collection, for use in PTs; for testing, validation, verification of methods; to be provided to NRLs for specific tests;
- Providing sets of reference strains of *S.* Enteritidis and *S.* Typhimurium for MLVA typing;

- Publication of a reference collection of genomes obtained from Proficiency Tests;
- Providing a link to the White-Kauffmann-Le Minor (WKLM) scheme and keeping contact with the WHO reference centre.

General Discussion

Q: Would it be possible to coordinate the activities of various EURLs, so that PTs or workshops of different EURLs do not overlap in time?
A: We will do our utmost to avoid overlap with activities of other EURLs. However, sometimes this is almost unavoidable as the eight biological EURLs all have to organise several PTs and workshops each year, also trying to avoid periods of national and public holidays.
Q: Which PTs on detection of *Salmonella* in primary production stage samples do other NRLs use for the performance testing of their national laboratories network? Do some NRLs still use APHA/VETQAS (UK)? In our country, we are having serious problems with customs clearance of the parcels from UK.
A: Some NRLs do use the VETQAS PTs, and a few are also facing problems with receiving the samples. Unfortunately, it is not always clear why some laboratories occasionally face these problems.
Q: Does any NRL have experiences with the use of the IR Biotyper?
A: One NRL-*Salmonella* tried it for a short time and tested approximately 200 strains with the aim to verify if the method would be applicable to serotyping. The method worked well for testing or differentiation of serogroups, but not for serotyping. Another NRL indicated that the method did not always show the same results for the same strain.
Q: Since the beginning of this year, the use of live vaccines is authorised in France. The competent authority has asked the NRL to assess the capacity of laboratories from the network to differentiate vaccine strains from wild strains. How is the situation in other European countries concerning vaccination? Do laboratories need an accreditation for this differentiation step? Do NRLs organise PTs to assess the differentiation capacity of laboratories from their network? What tests are commonly used by laboratories to differentiate this kind of strains?
A.1: For the differentiation of *Salmonella* Enteritidis vaccine strains from wild strains, we use an assay developed in our laboratory. We validated this method and have an accreditation for performing it. For *S.* Typhimurium we do not yet have such an assay. Some laboratories in the country use the test advised by the vaccine supplier (biochemical or PCR tests). In case of problems, these laboratories send the strains to the NRL for performing the differentiation test.
A.2: In our country, some official laboratories are accredited for differentiation of *S.* Enteritidis vaccine strains from wild strains, and as an NRL we provide reference strains for quality control. In 2022, the NRL organised a PT for the official laboratories regarding the differentiation test.
A.3: In our country, only one official laboratory is performing the differentiation test, and this laboratory is accredited for this activity. No PTs are being organised for this activity by the NRL.
A.4: In our NRL we use real-time PCR for differentiation of *S.* Enteritidis vaccine strains from wild strains and we are accredited for this activity.

For the differentiation of *S.* Typhimurium vaccine strains from wild strains, we test for antimicrobial resistance.
A.5: As an NRL, we organise PTs for the national official laboratories for the differentiation of *S.* Enteritidis vaccine strains from wild strains. The laboratories can use the various commercial kits that are available for the differentiation tests.

4 Evaluation of the workshop

4.1 Introduction

At the end of the workshop, the participants were sent a link to an evaluation form, asking them for their opinion by answering nine questions (see Annex 3). For several questions, participants were asked to give a score from 1 to 5. The scores represent: very poor (1), poor (2), fair (3), good (4) and very good (5). In addition, it was possible to add comments. Two questions were 'open' questions, in which the participants were asked to give their opinion.

While the evaluation form was sent to all participants, the staff members of the EURL-*Salmonella* were excluded from the evaluation, making a total of 87. In total, 49 participants completed the evaluation form, a response rate of 56%.

In section 4.2, the scores for 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 4.1 shows that the majority of respondents scored the information given in advance of the workshop as very good (score 5).

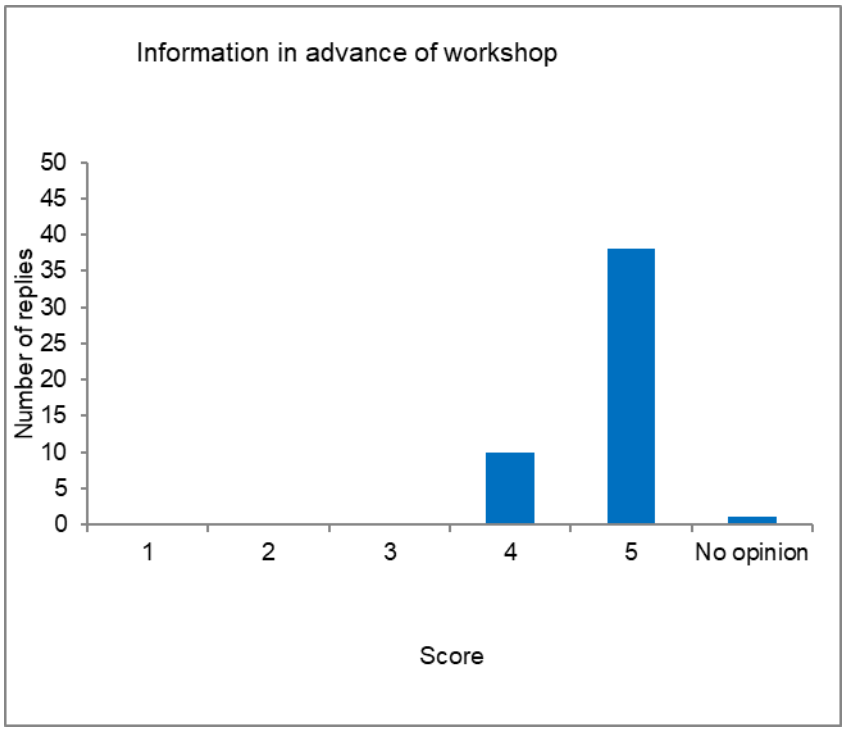

Figure 4.1 Scores given to question 1 'Opinion on information given in advance of the workshop'

2. What is your opinion on the ease of logging into the meeting?
Nearly all participants found it easy to log into the online meeting (see Figure 4.2).

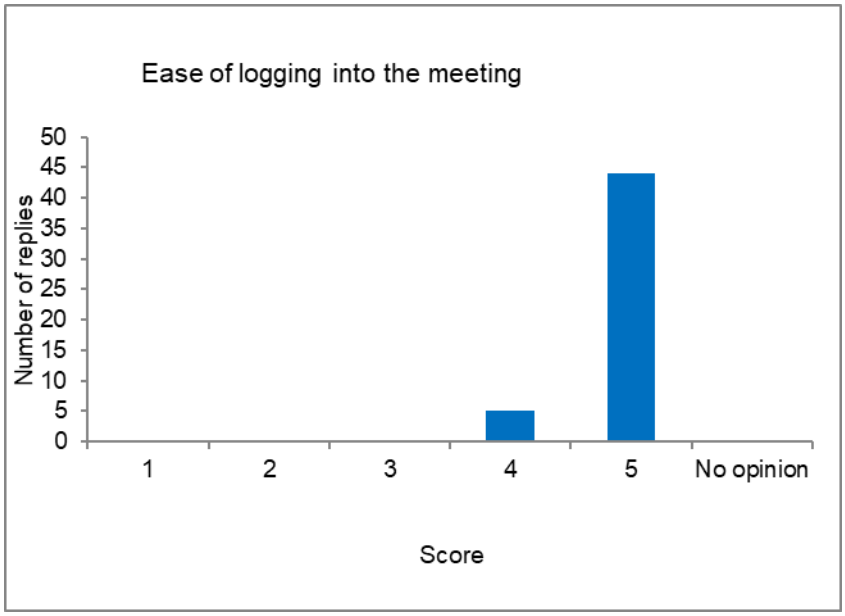

Figure 4.2 Scores given to question 2 'Opinion on the ease of logging into the meeting'

3. Did you face any technical problems during the meeting?
One respondent reported a technical problem during the meeting (see Figure 4.3), as the respondent was logged off for some minutes.

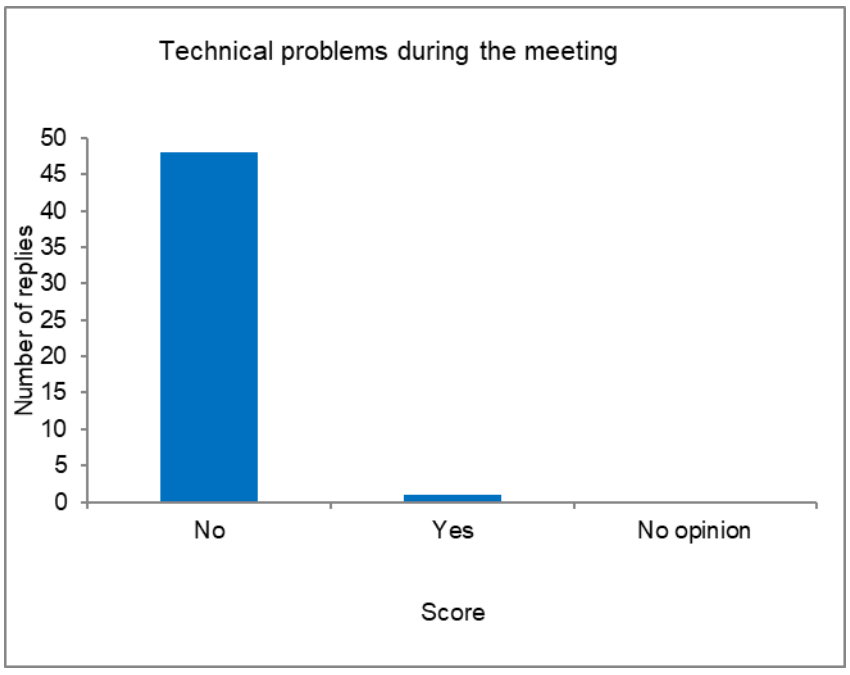

Figure 4.3 Replies given to question 3 'Did you face any technical problems during the meeting?'

4. What is your opinion on the length of the meeting and the number of breaks?
41 out of the 49 respondents considered the length of the meeting to be fine (Figure 4.4a) and 47 respondents considered the number of breaks to be fine (Figure 4.4b). The following remarks were made: 'The length of the meeting and the number of breaks were fine, but it is quite complicated to stay online at the end of the afternoon in the event of a delay in the agenda'; 'The time schedule was okay'.

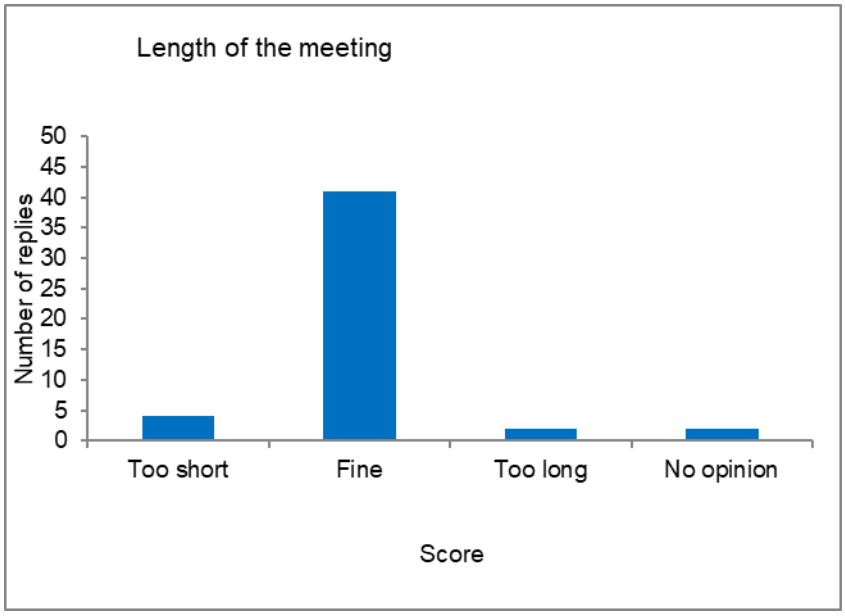

Figure 4.4a Replies given to question 4a 'What is your opinion on the length of the meeting?'

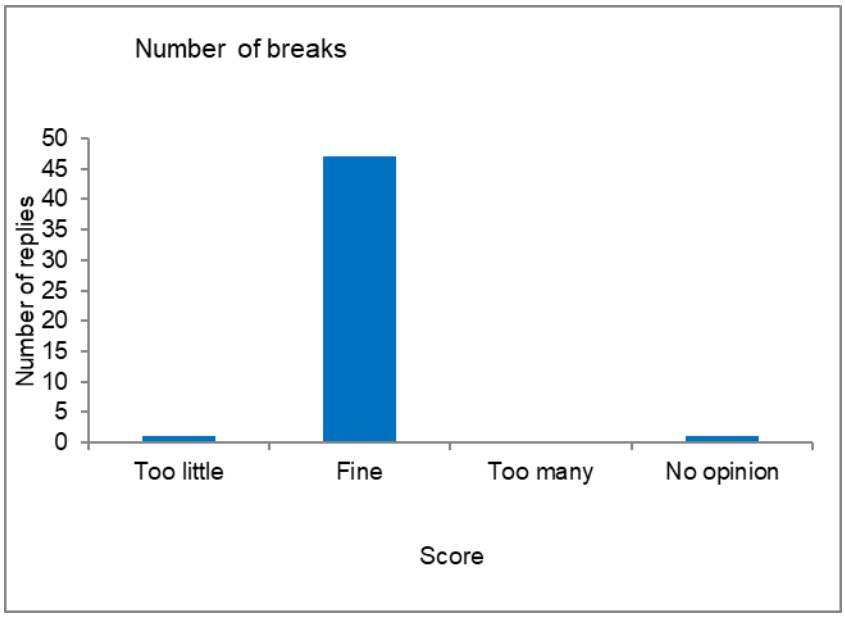

Figure 4.4b Replies given to question 4b 'What is your opinion on the number of breaks?'

5. Were you satisfied with the options for raising questions during the meeting (chat function; discussion time at the end of a presentation and at the end of a session)?
47 out of the 49 respondents were satisfied with the options for raising questions, two respondents were not satisfied and five had no opinion (Figure 4.5). The following remarks were made: 'I was satisfied with the options for raising questions, but I always prefer to speak live in front of you and colleagues'; 'Lengthy discussions should be brought to a close when they don't lead anywhere. The discussion about approved methods for different matrices in official samples was indeed important for all of us, but after a while there was no progression, and too long time passed'.

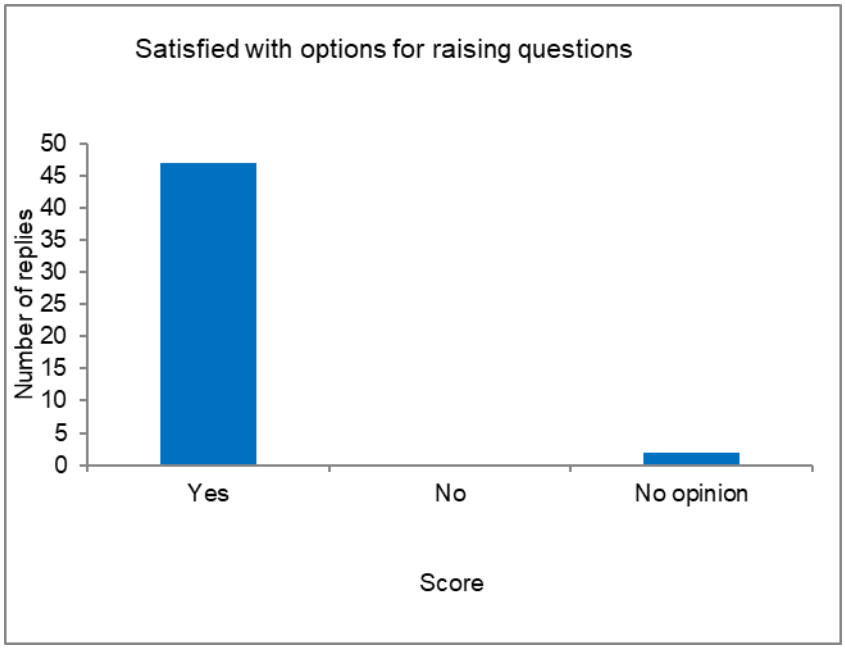

Figure 4.5 Scores given to question 5 'Were you satisfied with the options for raising questions during the meeting?'

6. What is your opinion on the scientific programme of the workshop?
The majority of respondents were satisfied with the workshop's scientific programme; the majority of the scores were good (score 4) to very good (score 5), see Figure 4.6. Only one remark was made: 'Unfortunately I attended only one day'.

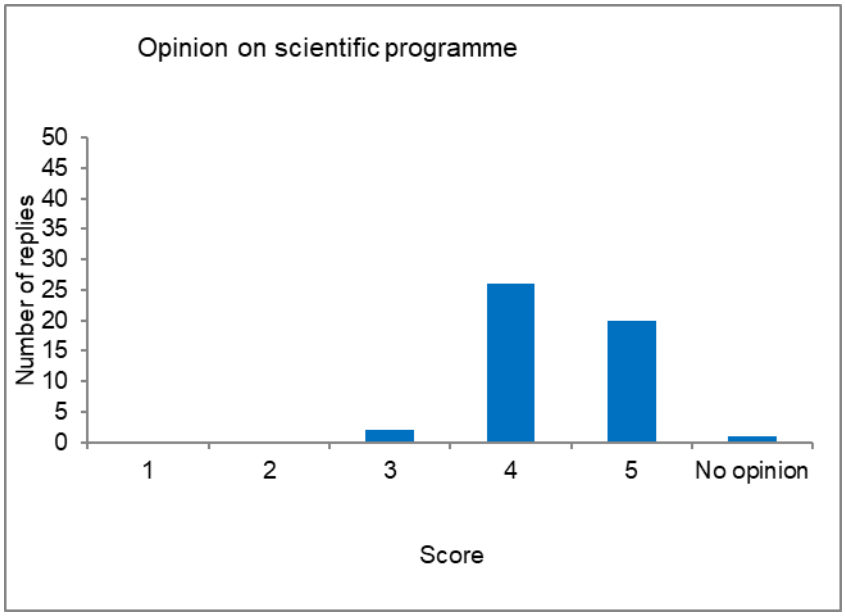

Figure 4.6 Scores given to question 6 'What is your opinion on the scientific programme of the workshop?'

7. Are there specific presentations you want to comment on, or did you miss information on certain subjects?
This was an 'open' question and several responded 'no'. Remarks given were:

- 'It would be great if more presentations could be given about research that has been performed about *Salmonella*.'
- 'I missed some presentations on Tuesday due to cross time scheduled workshops planned on the same period (EURL-AR workshop and EURL-*Salmonella* workshop).'
- 'Quantification of *Salmonella.*'
- 'Control strategies for some emerging *Salmonella* serovars.'
- 'Thank you for the discussion session, and for your help with our question about the method of differentiation between vaccine and field strains.'
- 'I miss some practical laboratory work, e.g. for serotyping.'
- 'No, only that it is extremely important to manage the time; and for the presenters to respect the time.'
- 'Presentations on WGS were most interesting to me.'
- 'The EFSA presentation went too fast and was not so clear. It would be great if more time could be foreseen for this presentation.'

8. What is your general opinion of the workshop?
Almost all respondents indicated that the workshop as a whole had been good (score 4) or very good (score 5). One respondent rated the workshop as a whole as 'fair' (score 3), see Figure 4.7. Remarks given were: 'We thank the EURL team for offering us the opportunity to participate in the annual workshop organised by EURL-*Salmonella*'; 'Good distribution of presentations with "hot" topics'.

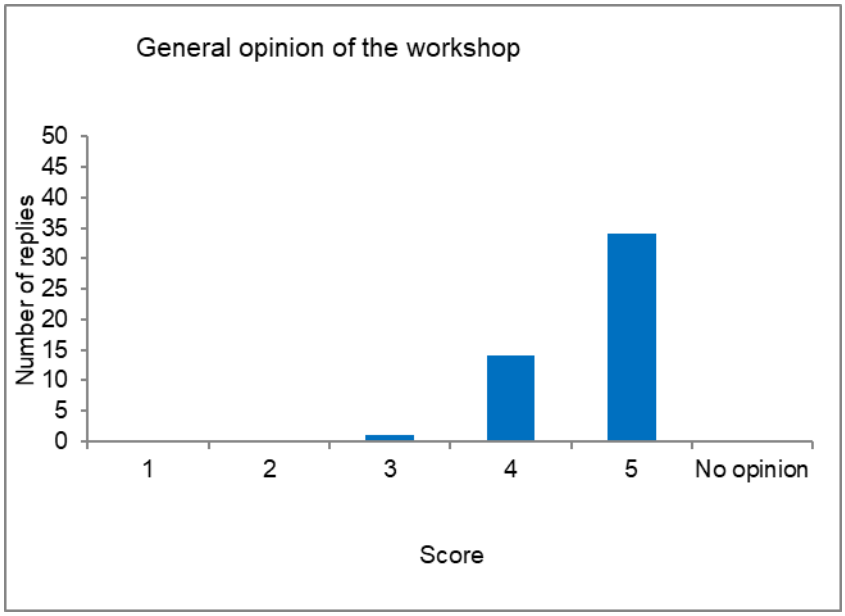

Figure 4.7 Scores given to question 8 'What is your general opinion of the workshop?'

9. Do you have any remarks or suggestions which we can use for future workshops?
This was another 'open' question and the following responses were received:

- 'For defining the dates of the workshop, it would be good to check if other EURLs have also scheduled something on the same day (the same thing the other way round for the other EURLs) (4).'
- 'Thank you for organising this very interesting workshop.'
- 'The availability of online participation is appreciated since it is more likely that one or more officers can attend and obtain first-hand information through the presentations and discussions in such workshops, whereas otherwise due to travel restrictions or other commitments, no one may be in a position to attend. Keep up to the good work.'
- 'It will be great to have the option of the online meeting for next workshops, for transport purposes and also for more people to be able to join the workshop. Additionally, it would be very helpful to have the workshops in cities where the access for all participants is easy (direct or with few transmit flights without the need of train or bus).'
- 'I hope that physical meetings will return soon in order to strengthen relationships among experts (2).'
- 'For a physical meeting, it would be nice to have some predefined discussion points, perhaps as parallel sessions in smaller groups where you sign up beforehand.'
- 'I prefer physical meetings, because we have the possibility to discuss more. The current time for questions and discussions is not enough.'
- 'Not everybody has expertise on all the different topics (going from primary production to food, all the different methods for detection and serotyping, NGS, organisation of PTs), so it would

be nice if more time was spent on explaining different topics, going from basic to more complex/specialised.'

4.3 Discussion and conclusions of the evaluation

For the fourth time, EURL-*Salmonella* organised this workshop as a virtual meeting. This time, it was due to budgetary constraints and not because of the pandemic.
It was frequently remarked that the advantage of a virtual meeting is the fact that more/other people can participate. At former virtual workshops, it was regularly remarked that a disadvantage of such meetings is the lack of discussions/interactions compared to physical meetings. However, at this year's workshop, there was a discussion about the use of alternative methods for official control of *Salmonella*. Although this discussion was considered interesting, some participants considered it too lengthy. It is a challenge to find the right balance between an interesting discussion and the time needed for such a discussion.
In general, the participants were satisfied with the organisation, the technical aspects, and the scientific programme of this fourth online EURL-*Salmonella* workshop.

Acknowledgements

The author is very grateful to work with a great team of highly experienced colleagues. After so many years of organising workshops, each colleague has shown their own professional speciality in the organisation, resulting in the fact that the workshop went very smoothly. Still, this great help should not be taken for granted and therefore, the author would like to thank the following persons in particular.
Arieke Docters van Leeuwen, for at first helping to find a nice location for a physical meeting and then smoothly switching to the organisation of an online meeting.
Robin Diddens, for his 'youthful' knowledge of computers and software and for his valuable help for a smooth sharing of the presentations.
Wilma Jacobs-Reitsma, for her valuable creative ideas for and help with presentations, slide shows for the breaks, group pictures and managing the information on the EURL-*Salmonella* website.
Irene Pol-Hofstad, for her positive vibe in the EURL-*Salmonella* activities and in the organisation of the workshop, and for seeing opportunities rather than problems.

Of course, also all other colleagues are thanked who made valuable contributions to the workshop by giving interesting presentations and/or input in the discussions. Without their input the workshop could not have been a success.

Thank you all so much!

List of abbreviations

A	Answer
AD	allelic difference
AHG	Ad hoc group
AMR	antimicrobial resistance
BPW	Buffered peptone water
BSL	Biosafety level
CEN	European Committee for Standardization
CEN/TC463	European Committee for Standardization, Technical committee 463 - Microbiology of the food chain
cfu	colony forming units
cgMLST	core genome multi-locus sequence typing
DG SANTE	Directorate-General for Health and Food Safety
DIS	Draft International Standard
DTS	Draft International Technical Specification
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
EU	European Union
EURL	European Union Reference Laboratory
FBO	Foodborne outbreak
FDIS	Final Draft International Standard
HaDEA	European Health and Digital Executive Agency
IEC	International Electrotechnical Commission
ILS	Interlaboratory study
ISO	International Organization for Standardization
ISO/TC34/SC9	International Organization for Standardization, Technical Committee 34 on Food Products, Sub-committee 9 – Microbiology of the food chain
JWG	Joint working group
LOD	Level of detection
MALDI-TOF MS	Matrix assisted laser desorption/ionisation time-of-flight mass spectrometry
MDR	multi-drug resistant
MKTTn	Muller-Kauffmann tetrathionate-novobiocin broth
MLVA	Multi-locus variable number of tandem repeats analysis
MS	Member State
MSRV	Modified semi-solid Rappaport-Vassiliadis
NGS	Next Generation Sequencing
NRL	National Reference Laboratory
OIE	Office International des Epizooties (World Organisation for Animal Health)
OCR	Official Control Regulation (Reg EU 2017/625)
PCR	Polymerase Chain Reaction
PPS	Primary production stage
PT	Proficiency Test
Q	Question
RIVM	National Institute for Public Health and the Environment
RVS	Rappaport-Vassiliadis broth with soya

SC	Sub Committee
SMP	Single Market Programme Regulation
STm	*Salmonella* Typhimurium
TC	Technical Committee
TS	Technical Specification
WG	Working group
WGS	Whole Genome Sequencing
WHO	World Health Organization
WKLM scheme	White-Kauffmann-Le Minor scheme

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Annex 1 Participants

EC DG SANTE	Kris de Smet
European Food Safety Authority (EFSA)	Frank Boelaert
EURL-*Salmonella* (and NRL-*Salmonella* the Netherlands)	Robin Diddens Angela van Hoek Wilma Jacobs-Reitsma Kirsten Mooijman Irene Pol-Hofstad Maaike van den Beld

National Reference Laboratories for *Salmonella*

ALBANIA	Renis Maçi
AUSTRIA	Sonja Axmann Christian Kornschober Thomas Pölzler Kornelia Vötsch
BELGIUM	Cristina Garcia Graells Inge van Damme (Northern Ireland representative) Marie Polet
BOSNIA AND HERZEGOVINA	Amira Koro-Spahic
BULGARIA	Gergana Mateva Mihail Milanov
CROATIA	Andrea Humski Gordan Kompes Borka Simpraga
CYPRUS	Eleni Papageorgiou
CZECH REPUBLIC	Tomás Cerný
DENMARK	Lise Bonnichsen
ESTONIA	Maiu Kuningas Epp Moks
FINLAND	Tiina Autio Satu Hakola Henry Kuronen
FRANCE	Laetitia Bonifait Pascal Garry Frédérique Moury Amandine Thepault
GERMANY	Jennie Fischer Marina Lamparter Istvan Szabo
GREECE	Dimitrios Katsaros Nikki Mouttotou Aphrodite Smpiraki Eleni Valkanou
HUNGARY	Henriett Demenyne Boros Sára Kostyák

	Viktória Rácz
	Zsuzsanna Sreterne Lancz
ICELAND	Pall Steinthorsson
IRELAND	William Byrne
ITALY	Lisa Barco
	Giulia Cento
	Veronica Cibin
	Clara Tassinato
KOSOVO	Besart Jashari
LATVIA	Jelena Avsejenko
	Jekaterina Mogiluka
	Madara Streikisa
LITHUANIA	Tatjana Kutyriova
LUXEMBOURG	Dominique Claude
	Catarina Martins
	Catherine Ragimbeau
	Alexandra Schoos
MALTA	Gertrude Gatt Lanzon
	David Sammut
	Renato Zerafa
NETHERLANDS	See above
NORWAY	Bjarne Bergsjø
	Julia Storesund
	Bjørn Tore Lunestad
POLAND	Elzbieta Mackiw
	Weronika Koza
	Dominika Pastuszka
	Ewelina Skrzypiec
	Kinga Wieczorek
	Dominika Wojdat
PORTUGAL	Maria José Barahona
	Patricia Themudo
REPUBLIC OF MALDOVA	Tatiana Bejenari
	Margarita Dociu
	Oxana Groza
REPUBLIC OF NORTH MACEDONIA	Dean Jankuloski
ROMANIA	Mihaela Costea
	Maria Ionescu
	Carmen Manea
SERBIA	Dalibor Todorovic
SLOVAK REPUBLIC	Miriam Kantikova
	Zuzana Kubicova
	Andrea Mojzisova
SLOVENIA	Jasna Micunovic
	Tina Pirs
SPAIN	Cristina de Frutos
	Irene Suárez Fernández
	Iria Uhía
SWEDEN	Jenny Eriksson
	Erik Eriksson
SWITZERLAND	Jule Horlbog
	Sonja Kittl

Annex 2 Programme 28th EURL-*Salmonella* workshop; 22 and 23 May 2023 - Online

Monday 22 May 2023

13:00 - 13:30	Opening and introduction	Kirsten Mooijman EURL-*Salmonella*
13:30 - 14:00	EU monitoring of *Salmonella* and of salmonellosis foodborne outbreaks, in 2021	Frank Boelaert EFSA
14:00 - 14:15	*Break*	
14:15 - 14:45	Results Interlaboratory Study validation of ISO/TS 6579-4 Identification of monophasic *Salmonella* Typhimurium	Robin Diddens EURL-*Salmonella*
14:45 - 15:15	Update on activities in ISO and CEN	Kirsten Mooijman EURL-*Salmonella*
15:15 - 15:45	Results EURL-*Salmonella* Proficiency Test PPS-FOOD 2022 - Detection of *Salmonella* in hygiene swabs	Irene Pol-Hofstad EURL-*Salmonella*
15:45 – 16:00	*Break*	
16:00 – 16:30	Results EURL-*Salmonella* Proficiency Test Typing 2022 – serotyping and cluster analysis	Wilma Jacobs-Reitsma EURL-*Salmonella*
16:30 - 17:00	Preliminary results EURL-*Salmonella* Proficiency Test FOOD-FEED 2023 - Detection of *Salmonella* in seeds	Robin Diddens EURL-*Salmonella*

---- End of day 1 ----

Tuesday 23 May 2023

09:00 - 09:30	A *Salmonella* cgMLST validation study for accreditation in Germany	Marina Lamparter BfR Germany
09:30 - 11:25	Activities NRLs to fulfil tasks and duties	
09:30 - 09:55	NRL-*Salmonella* Luxembourg	Catarina Martins
09:55 - 10:20	NRL-*Salmonella* Lithuania	Tatjana Kutyriova
10:20 – 10:35	*Break*	
10:35 - 11:00	NRL-*Salmonella* Germany	Istvan Szabo
11:00 - 11:25	NRL-*Salmonella* Bosnia and Herzegovina	Amira Koro-Spahic
11:25 - 12:00	Work programme EURL-*Salmonella* second half 2023, first half 2024 Concluding remarks workshop and closure	*Kirsten Mooijman* EURL-*Salmonella*

---------- End of workshop----------

Annex 3 Workshop evaluation form

Evaluation of the 28th EURL-*Salmonella* workshop,
22 and 23 May 2023 - online

We would highly appreciate if you could give us your opinion on the 28th EURL-*Salmonella* workshop, organised as online meeting on 22 and 23 May 2023. Thank you very much in advance for completing the questionnaire by 9 June 2023 at the latest.

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

1 (Very poor)	2 (poor)	3 (fair)	4 (good)	5 (very good)	No opinion

Remarks:_____

2. What is your opinion on the ease of logging into the meeting?

1 (Very poor)	2 (poor)	3 (fair)	4 (good)	5 (very good)	No opinion

Remarks:_____

3. Did you face any technical problems during the meeting?
   - ☐ No
   - ☐ Yes, I encountered the following problems: _____

Remarks:_____

4. What is your opinion on the length of the meeting and the number of breaks?
   a. Length meeting:
      - ☐ Too short
      - ☐ Fine
      - ☐ Too long
   b. Number of breaks:
      - ☐ Too little
      - ☐ Fine
      - ☐ Too many

Remarks:_____

5. Were you satisfied with the options for raising questions during the meeting (chat function; discussion time at the end of a presentation and at the end of a session)?
   - ☐ Yes
   - ☐ No opinion
   - ☐ No, but I have a suggestion for improvement _____

Remarks:_____

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

1 (Very poor)	2 (poor)	3 (fair)	4 (good)	5 (very good)	No opinion

Remarks:_____

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

8. What is your general opinion of the workshop?

1 (Very poor)	2 (poor)	3 (fair)	4 (good)	5 (very good)	No opinion

Remarks:_____

9. Do you have any remarks or suggestions that we can use for future workshops?

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