Foreword

The WG has been established by the European Commission with the aim to promote the use of NGS across the EURLs' networks, build NGS capacity within the EU and ensure liaison with the work of the EURLs and the work of EFSA and ECDC on the NGS mandate sent by the Commission. The WG includes all the EURLs operating in the field of the microbiological contamination of food and feed and this document represents a deliverable of the WG and is meant to be diffused to all the respective networks of NRLs.

Survey on the use of NGS across the NRLs networks

1. Introduction

As the first action by the Inter-EURLs Working Group on Next Generation Sequencing, a survey was conducted across the NRLs in order to acquire knowledge on the level of adoption and the status of the capacity towards the use of such methodologies across the NRLs networks, in order to define the activities of the WG and to target the actions of the EURLs on the actual needs of the NRLs, by considering specific needs in the different networks to be addressed through combined and harmonised actions by the EURLs in the WG.

2. Methods

The survey was administered by each EURL to the respective network of NRLs in March/April 2018 and the participation by the NRLs was left as voluntary. The EURLs that took part in this survey were EURLs for *Escherichia coli*, *Listeria monocytogenes*, Coagulase Positive *Staphylococci* (CPS), *Salmonella*, *Campylobacter*, Parasites and Antimicrobial Resistance (AMR). The EURL for Food borne viruses, also part of the Inter EURLs WG, decided not to participate in the common survey, because the NRLs network still had to be properly established at the time of survey administration.
The survey was composed of a total of 20 questions, including questions about the adoption and the purpose of NGS in the NRL (Q1-Q3), methodological questions about wet-lab protocols (Q4-Q10) and dry-lab procedures on bioinformatics analysis (Q11-Q14), questions about participation in proficiency tests on NGS (Q15-Q16) and in specific trainings (Q17-Q19) and about experience in benchmarking of NGS protocols (Q20). Finally, comments could be added as free text by replying to question Q21. The complete text of the questions composing the survey is attached to this document as **Annex 1**.
During November 2018 a secondary follow-up survey was also administered to the NRLs that replied not to have access to the technology, yet. This action was meant to investigate the reasons of the lack of adoption of NGS methodologies across the EU, aiming to find solutions at the EU level to address this limitation, if possible. This survey was composed of six questions investigating the plans for NGS implementation in the next future, the main hindrances at present and the opinion on the application which would benefit the most of such a technological advancement in the NRL's activities. The complete text of the questions composing the follow-up survey is attached to this document as **Annex 2**.
In May-June 2019 the EURL for Foodborne viruses administered to its NRLs network a survey on the adoption of NGS methods inspired and modified from the one administered by the rest of the Inter EURLs WG, by adapting the questions to the study of viruses. The most relevant questions for the benefit of the WG are included in a dedicated section of the results.

3. Results

A total of 178 NRLs replied to the survey, including 20 NRLs for *E. coli*, 32 NRLs for *Campylobacter*, 23 NRLs for *Salmonella*, 20 NRLs for AMR, 13 NRLs for Parasites, 35 NRLs for *L. monocytogenes* and 35 NRLs for Coagulase Positive *Staphylococci* (CPS). The detailed list of participants is appended to this document as **Annex 3**.

3.1 Adoption and purpose of NGS use in the NRLs (Questions Q1-Q3)

The first section of the survey was meant to investigate the level of adoption of NGS methodologies in the work of the NRLs either through in house or outsource facilities and the main purposes for their use, if mainly concerning research or also diagnosis, outbreak investigation and surveillance activities.

Question 1: "In your role of NRL, which of the following NGS activities do you perform?" (multiple choice)

Among the networks surveyed, the majority of the NRLs replied to have the possibility perform Whole Genome Sequencing (WGS) either in house or in outsourcing for what concerns the *E. coli* (VTEC), *Salmonella* and AMR networks, while those having this possibility were half of the replying NRLs for the networks for *Campylobacter*, Parasites and *L. monocytogenes* and eight out of 35 NRLs for CPS (Figure 1). The use of metagenomics was instead confined to a few laboratories in all the networks. The bioinformatics analysis of the NGS data produced was performed in house by the majority of the NRLs for all the networks (Figure 1).

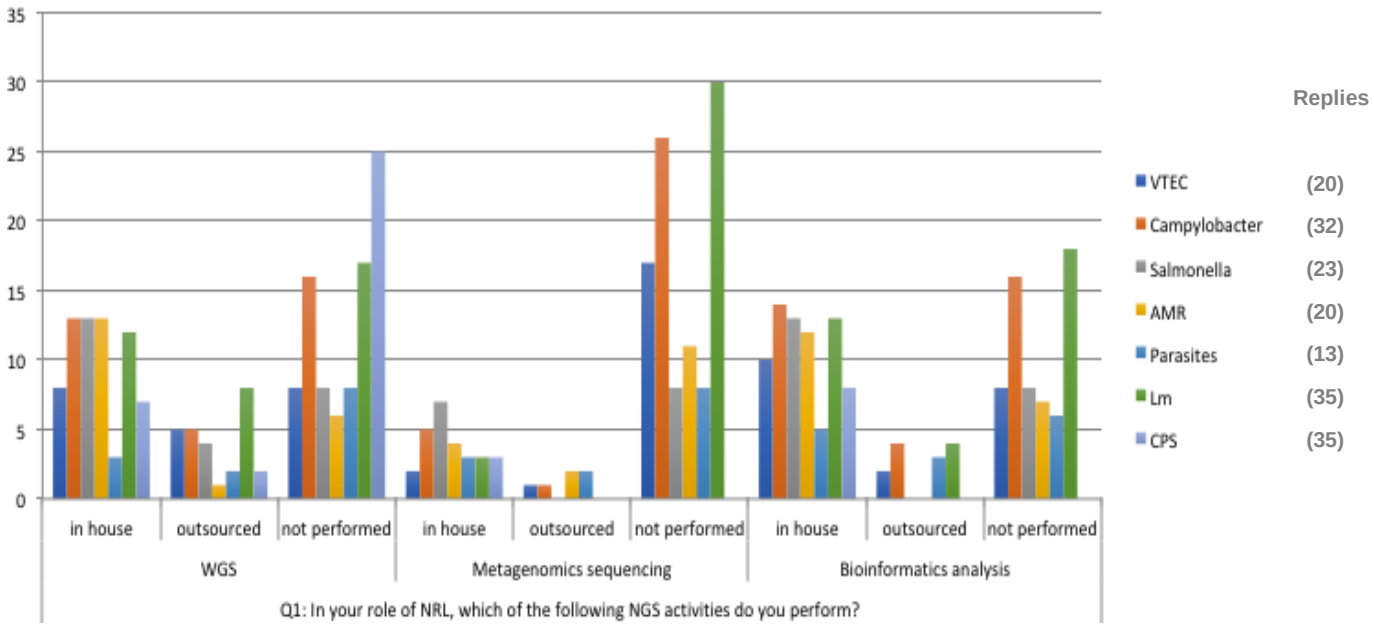

Figure 1. Replies to Question 1

Question 2: "If you answered positively to at least one of the options in Q1, what is the purpose of using NGS in your NRL?" (multiple choice)

Many NRLs replied that research projects still represent the main field of application of NGS in their activities. Nevertheless, monitoring and surveillance activities as well as outbreak investigation already benefit from the application of such methodology in the activities of the majority of the NRLs in all the networks surveyed.

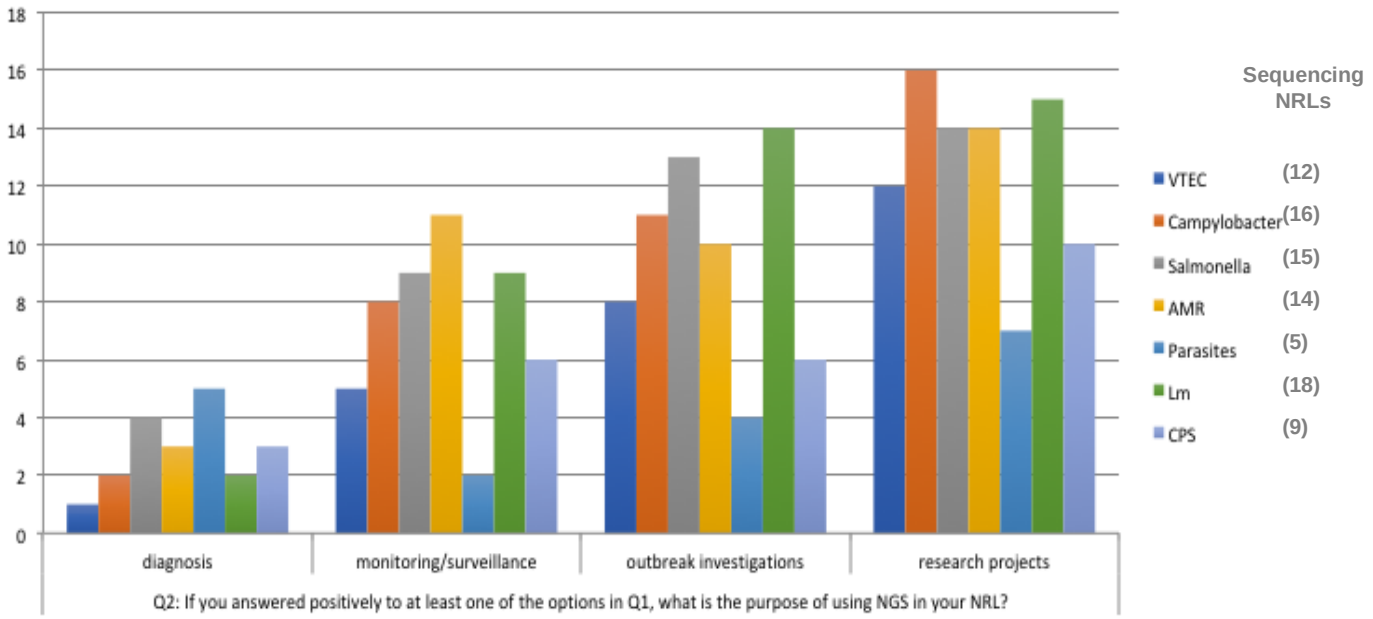

Figure 2. Replies to Question 2

Question 3: "If you replied 'No NGS activity' to Q1, please specify why" (multiple choice)

In all the networks, the main hindrance in adopting NGS was identified in the lack of capacity, either in performing the sequencing or in analysing the results. Only a few NRLs declared to have no plans to replace current methodologies with NGS and particularly these represented the majority of the NRLs not applying NGS for the network on Parasites.

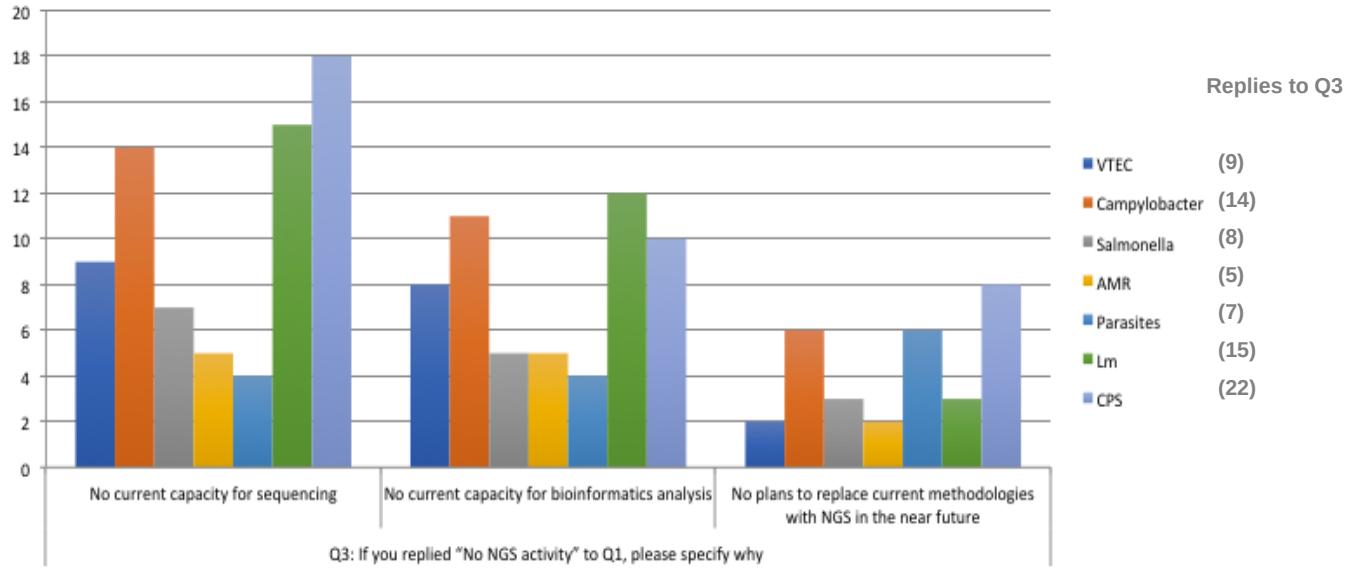

Figure 3. Replies to Question 3

3.2 Methods applied for wet-lab procedures in NGS protocols (Questions Q4-Q10)

The second section of the survey was focused on the laboratory protocols in use in the steps of NGS involving wet-lab procedures, including all the steps from the choice of the isolates, to nucleic acid extraction, quality control and concentration estimation to the library preparation and evaluation and finally the choice of the NGS platform.

Question 4: "Do you sequence a selection of isolates/matrices?" (single choice)

The majority of the replying NRLs replied not to sequence all the available isolates, but to apply a selection, with the network of NRLs for AMR appearing in contrast with the others, with only two laboratories out of the 16 using NRLs applying a selection. The possibility to comment on the criteria for the selection allowed to identify outbreak investigation as the main trigger for selecting isolated for NGS, followed by research projects. The adoption of preliminary screening based on other typing methodologies was also reported (e.g. selection of strains with different PFGE profiles or serotypes).

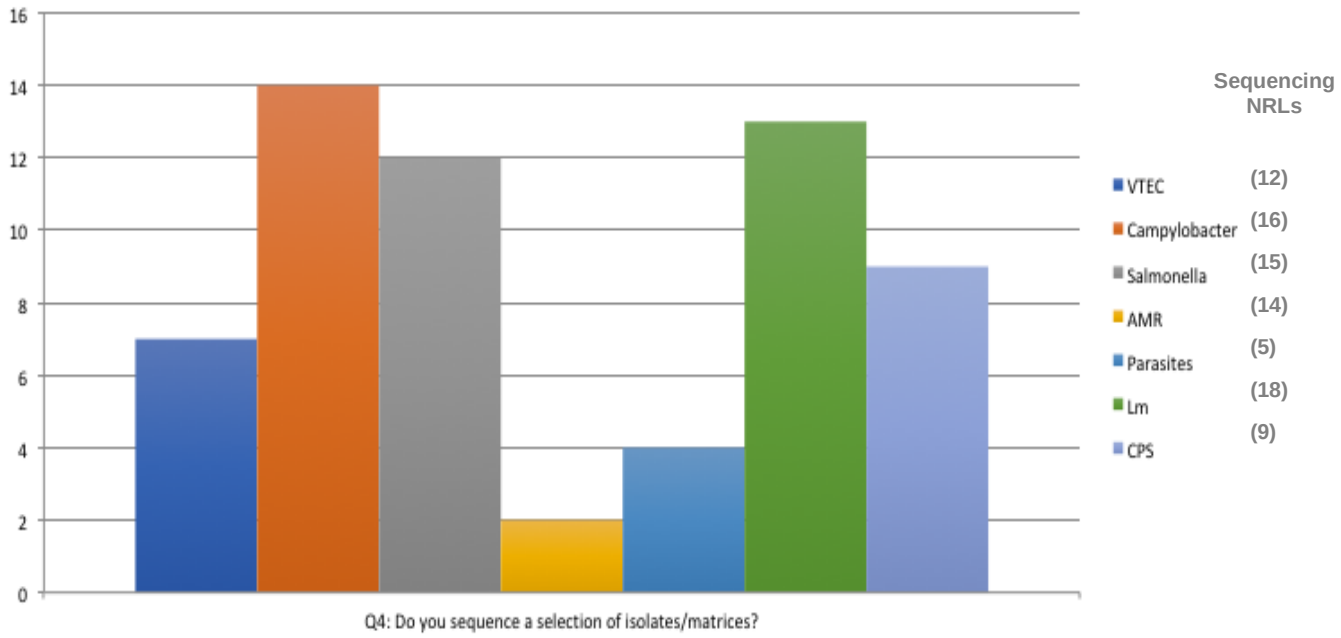

Figure 4. Replies to Question 4: positive replies are shown in the histograms.

Question 5: "What concept do you use to extract DNA/RNA for sequencing?"

General agreement on the use of spin-column based methods for nucleic acid extraction was observed. Magnetic beads-based strategies were also reported by many NRLs, especially in Campylobacter NRLs network, in which they appeared as the most applied methods.

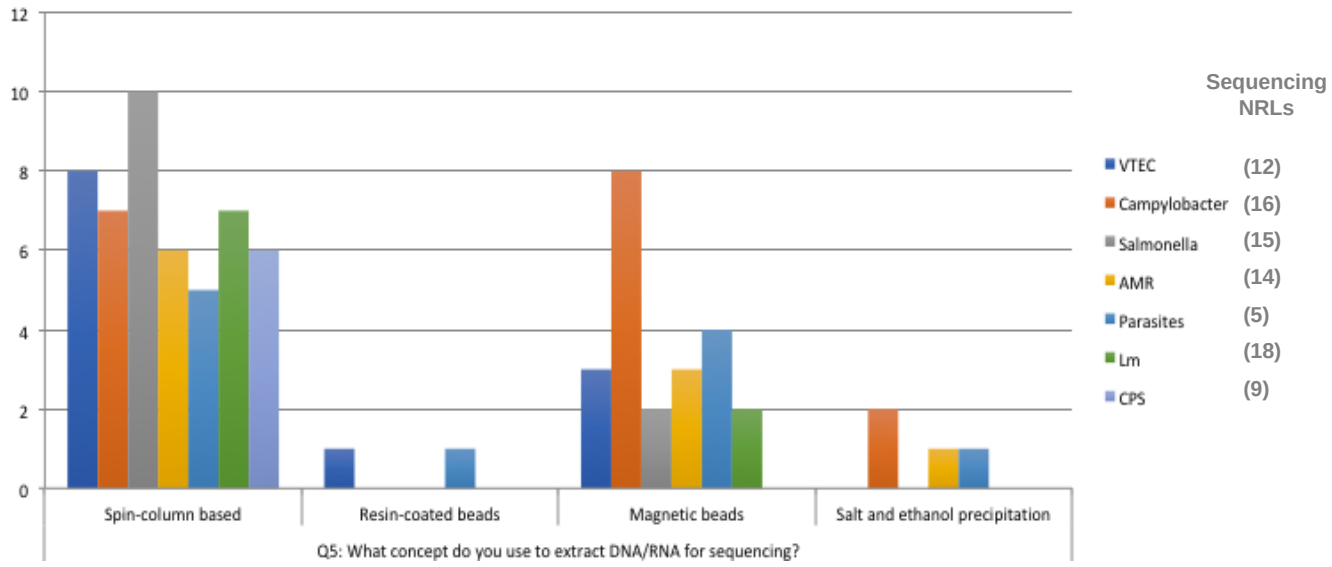

Figure 5. Replies to Question 5

Question 6: "How do you assess the quality of the extracted DNA/RNA before sequencing?" (multiple choice)

The most widely adopted method to check the quality of the extracted nucleic acids was the use of Nanodrop instrument, even if agarose gel electrophoresis still resulted to be used by many NRLs. Only a few laboratories declared not to perform this step. Among the other mentioned methods, spectrophotometers and instruments for fragment analysis were mentioned.

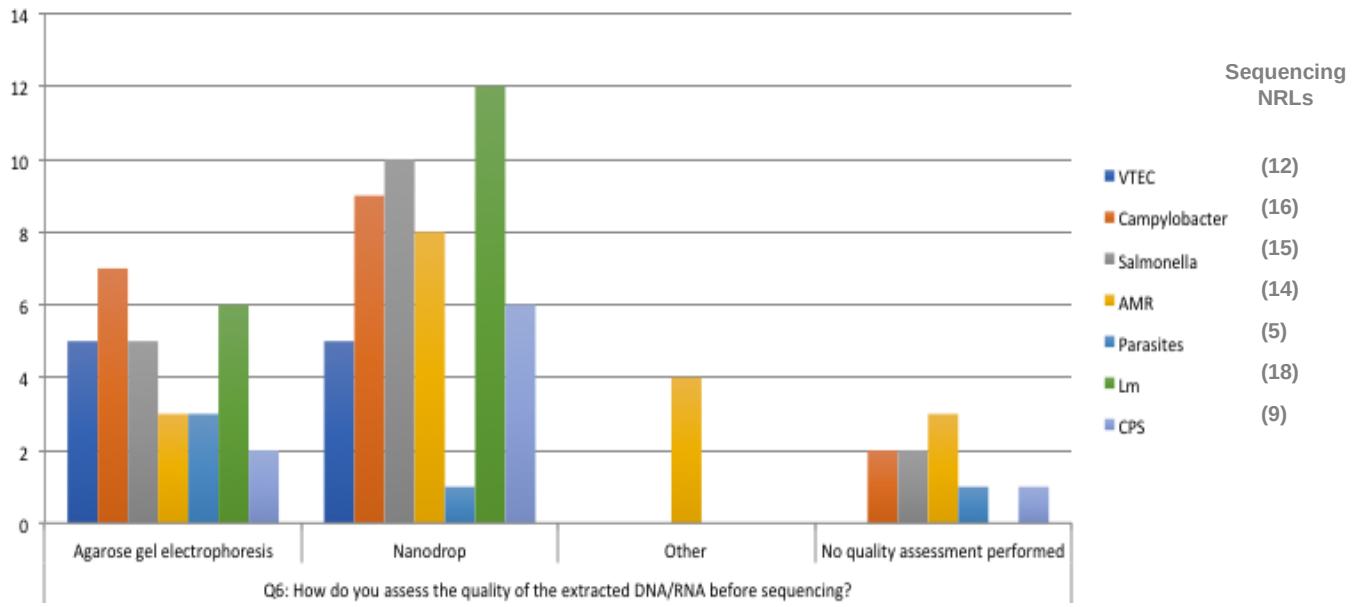

Figure 6. Replies to Question 6

Question 7: "How do you estimate the concentration of the extracted DNA/RNA before sequencing?" (multiple choice)

Nucleic acids quantification is a recommended step in all the protocols for NGS and in this respect a general agreement could be observed in the replies by the NRLs of all the networks in the use of Qubit fluorimeter for this purpose, followed by Nanodrop. Tape station and fluorimetric methods other than Qubit (e.g. Quantifluor) were also mentioned among the other instruments used.

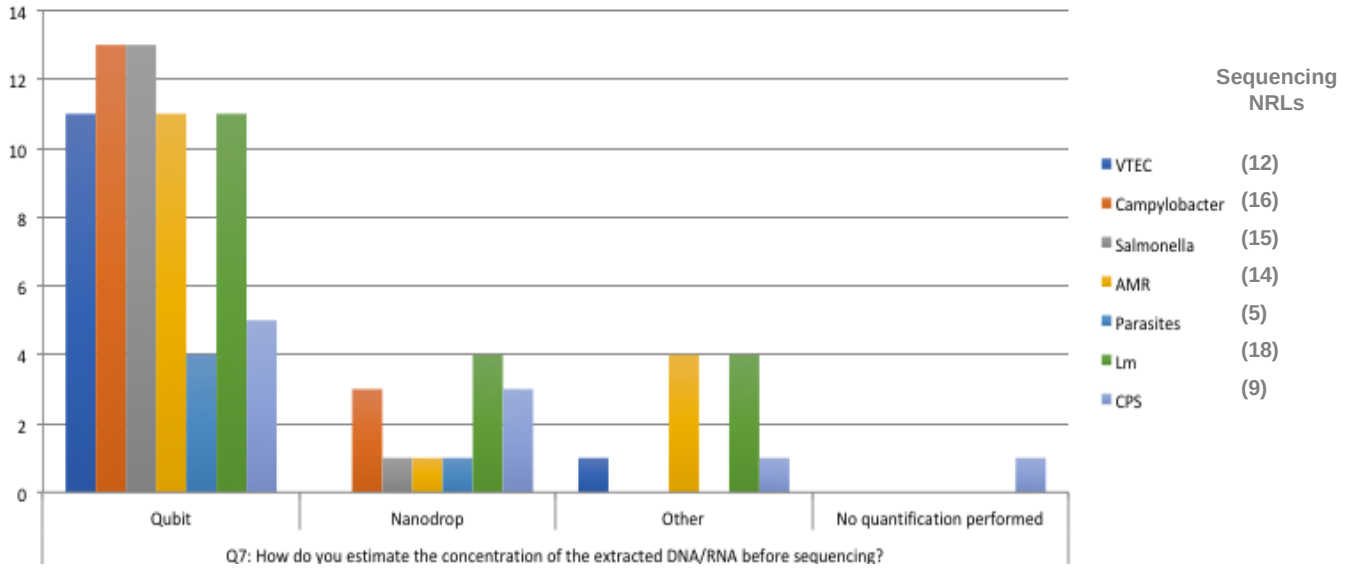

Figure 7. Replies to Question 7

Question 8: "Which protocol do you use to prepare the library for sequencing?" (multiple choice)

The library preparation kits reflect the platform used. The wide majority of the NRLs replied to use library preparation kits specific for Illumina sequencing, with only a few exceptions consisting in Thermo Fisher kits specific for Ion Torrent sequencing. Only one NRL declared to use a library preparation kit of an alternative brand to that suggested by the manufacturer of the NGS platform. A few NRLs commented they outsourced library preparation together with sequencing.

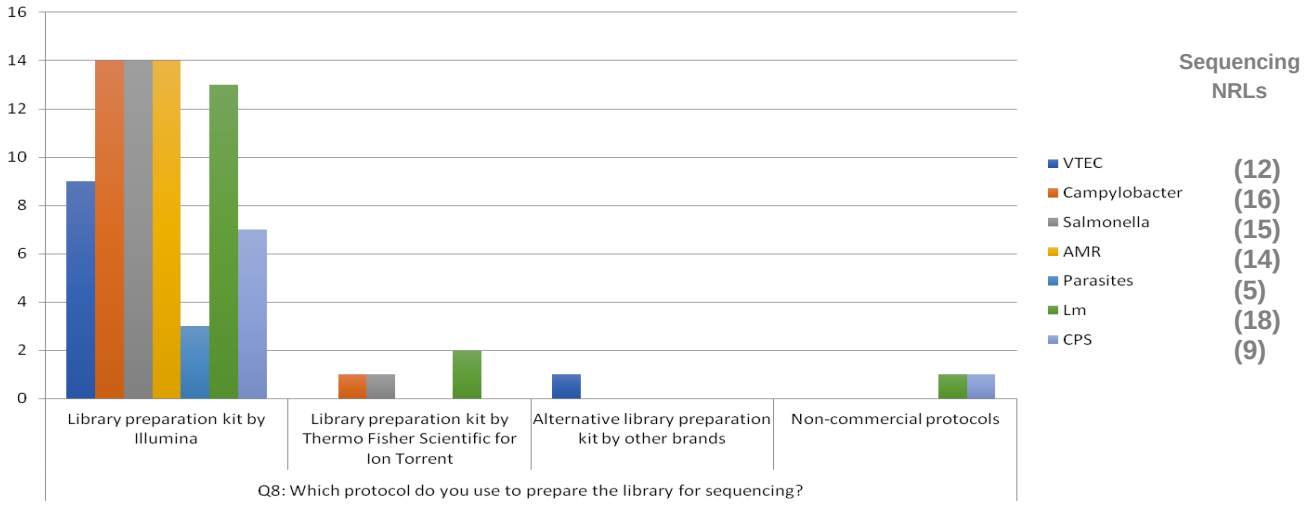

Figure 8. Replies to Question 8

Question 9: "How do you evaluate the quality/concentration of the prepared library before sequencing?" (multiple choice)

Bioanalyser and Qubit instruments resulted the most used instruments for evaluating the prepared libraries in all the networks.

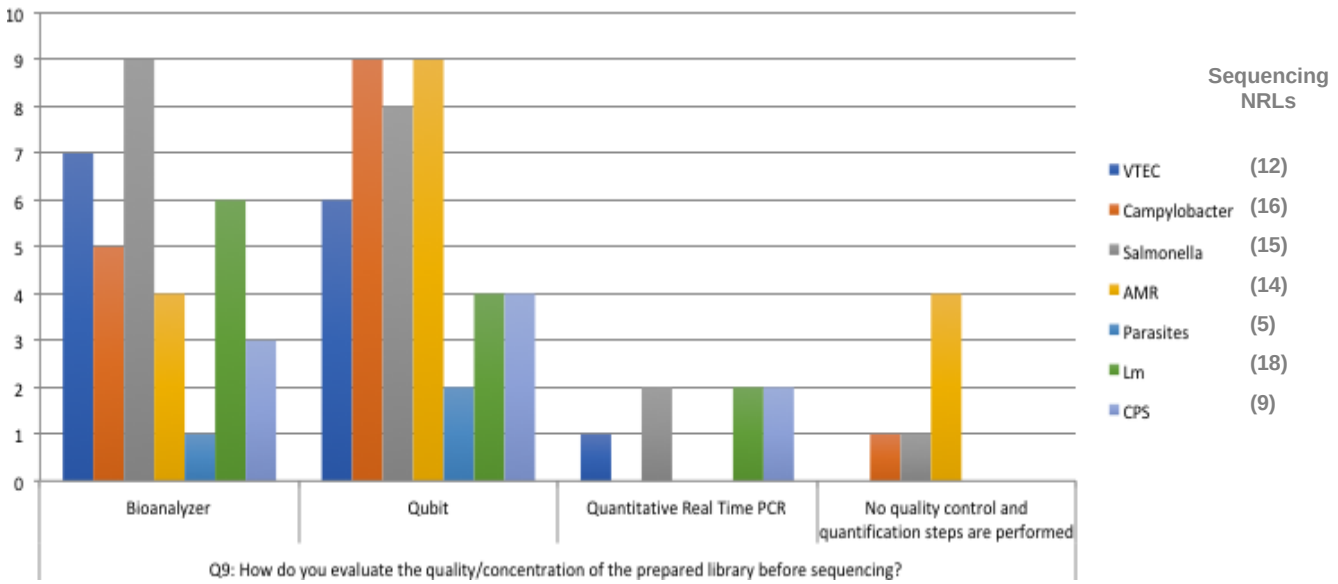

Figure 9. Replies to Question 9

Question 10: "Please specify which NGS platforms you use and/or are used by the lab/company to which the activity is outsourced" (multiple choice)

Illumina MiSeq platform was indicated to be by far the most adopted NGS platform across all the networks of NRLs, followed by other platforms from the same brand. Despite this, Ion Torrent platforms was also used by a few NRLs across the networks. Additionally, long-reads sequencers such as PacBio and MinION were also reported among those in use.

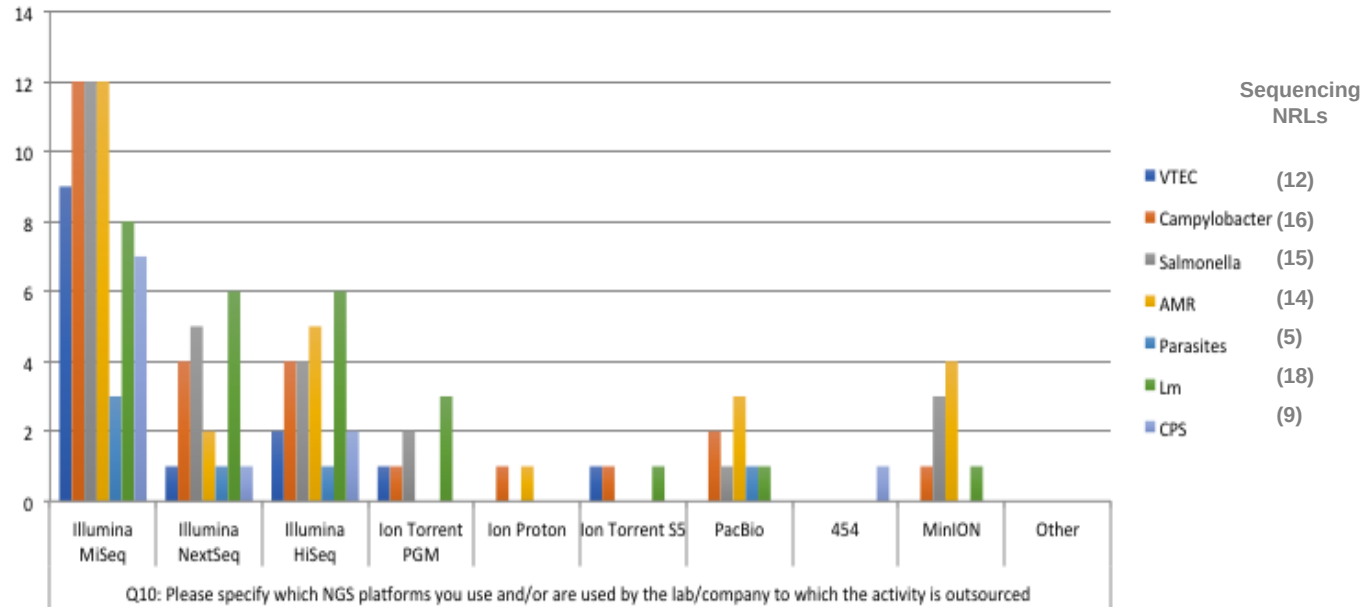

Figure 10. Replies to Question 10

3.3 Methods applied for bioinformatic analysis (Questions Q11-Q14)

Question 11: "What parameters do you use to evaluate the quality of sequence data (reads and/or contigs)?" (multiple choice)

The wide majority (about 88%) of all the NRLs that replied to perform NGS declared to evaluate the coverage as useful parameter for checking the quality of the sequencing data. The other two most used parameters for quality check were the total number and the N50 of the assembled contigs. Almost all laboratories declared to use all the proposed parameters for quality check. Among the additional quality checks mentioned as "other" the most commonly cited was the percentage of the loci of cMLST schemes detected and the taxonomic classification using Kraken.

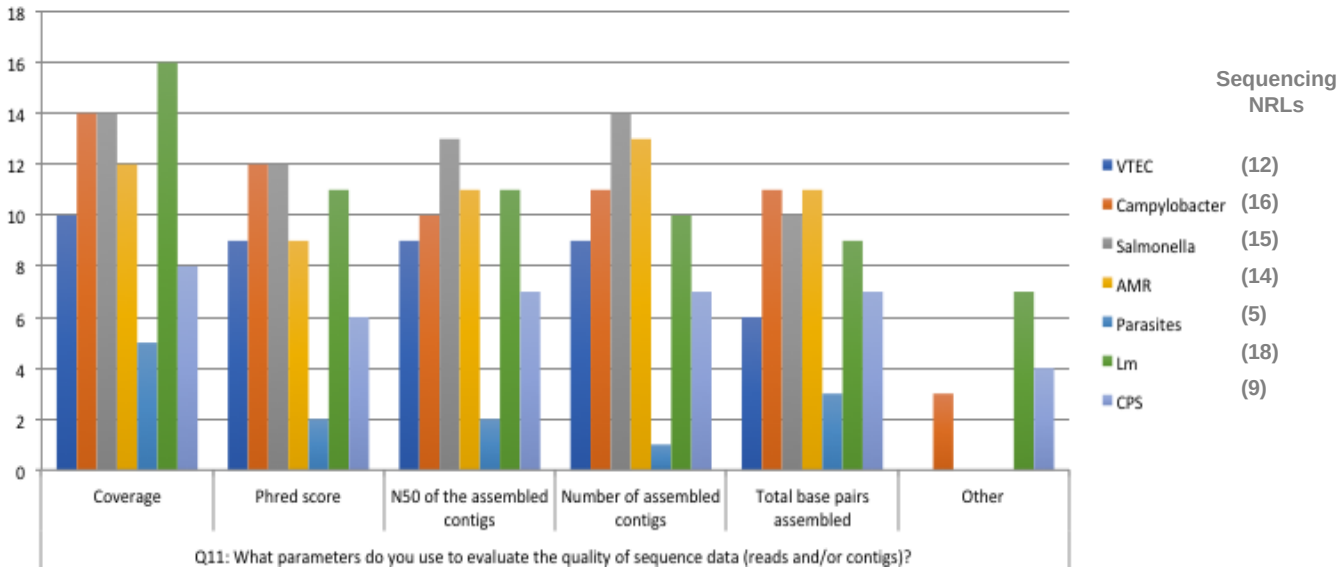

Figure 11. Replies to Question 11

Question 12: "Where is NGS data analysis performed? Which approach do you use?" (multiple choice)

Only a few NRLs declared to outsource the NGS data analysis, while the wide majority performs it in house. In all the networks, all the proposed approaches are in use, with the exception of online servers in the AMR network. It is interesting to note that command-line tools and even in house-developed pipelines were reported to be used by many NRLs, highlighting the existence of informatics and bioinformatics expertise in almost half of the those declaring to have access to NGS across all the networks.

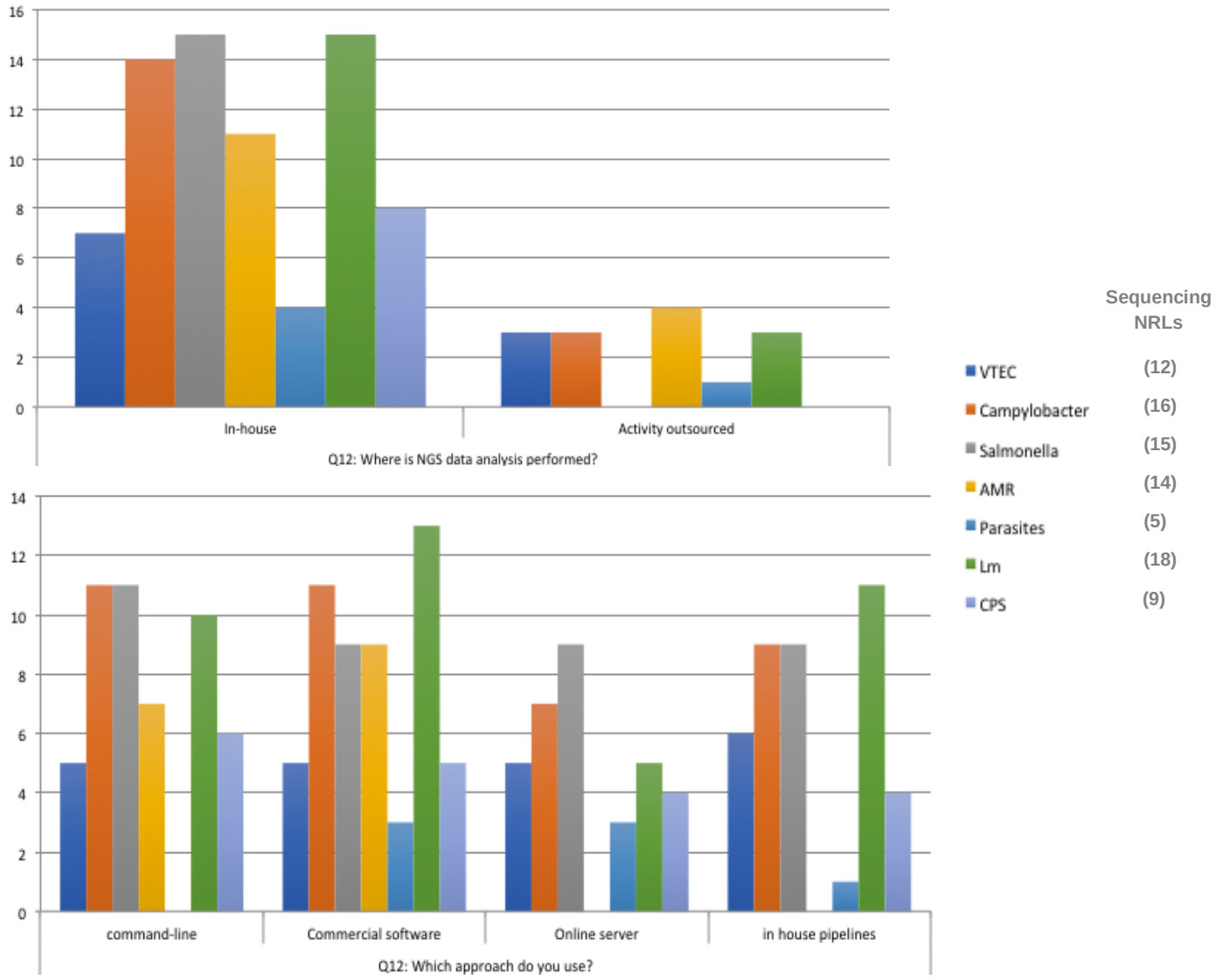

Figure 12. Replies to Question 12

Question 13: "Do you perform cluster analysis?" (single choice)

The majority (82%) of the total NLRs performing NGS declared to perform cluster analysis. The NRLs for AMR and for Parasites showed a lower level of adoption of such strategies.

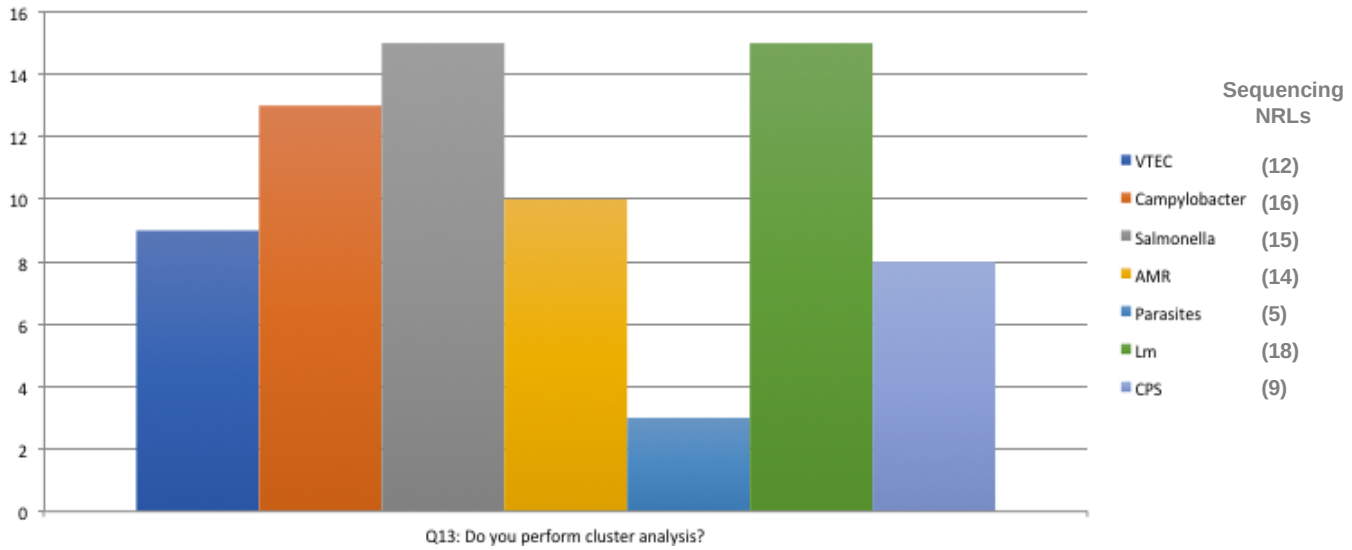

Figure 13. Replies to Question 13. Positive replies are shown in the histograms.

Question 14: "If you answered yes to Q13: How do you perform cluster analysis?" (multiple choice)

All the proposed methods for cluster analysis were adopted by all the networks, with the only exception of cgMLST in NRLs for CPS. cgMLST and SNPs analysis resulted the most widely used methods across all the networks (Figure 14A). When asked to provide details about the bioinformatics solutions used for cluster analysis, in house pipelines appeared as the most used approach for SNPs analysis, while commercial softwares resulted the most spread strategy for wgMLST and cgMLST (Figure 14B).

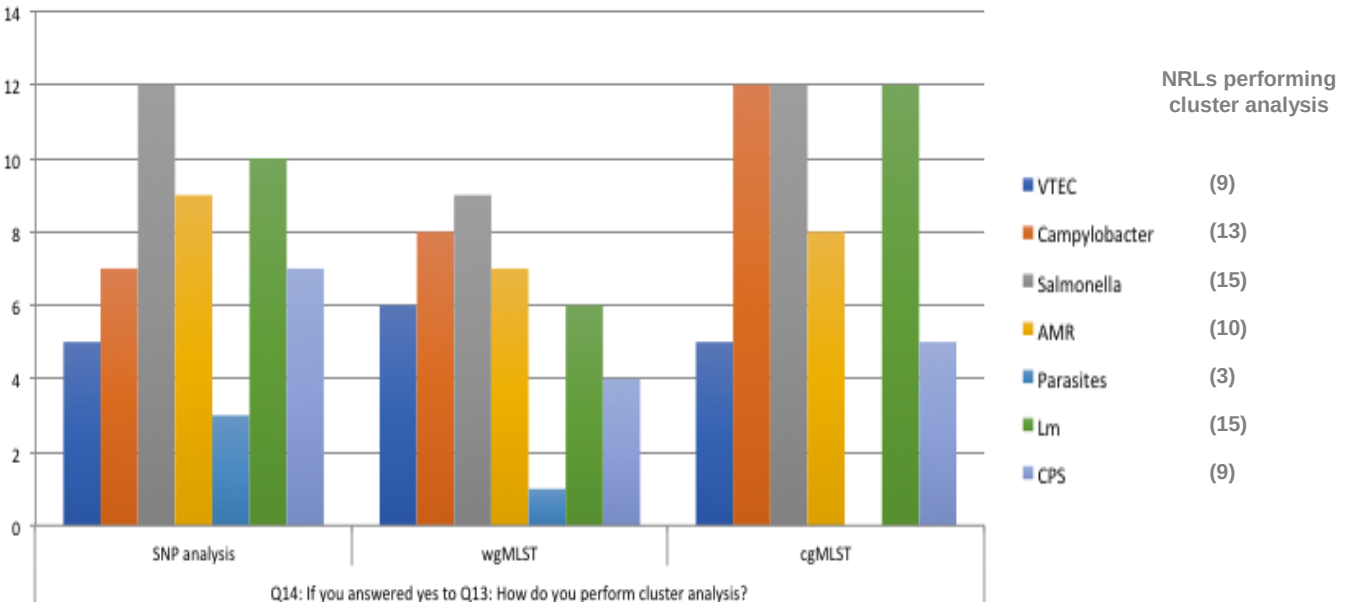

Figure 14A. Replies to Question 14

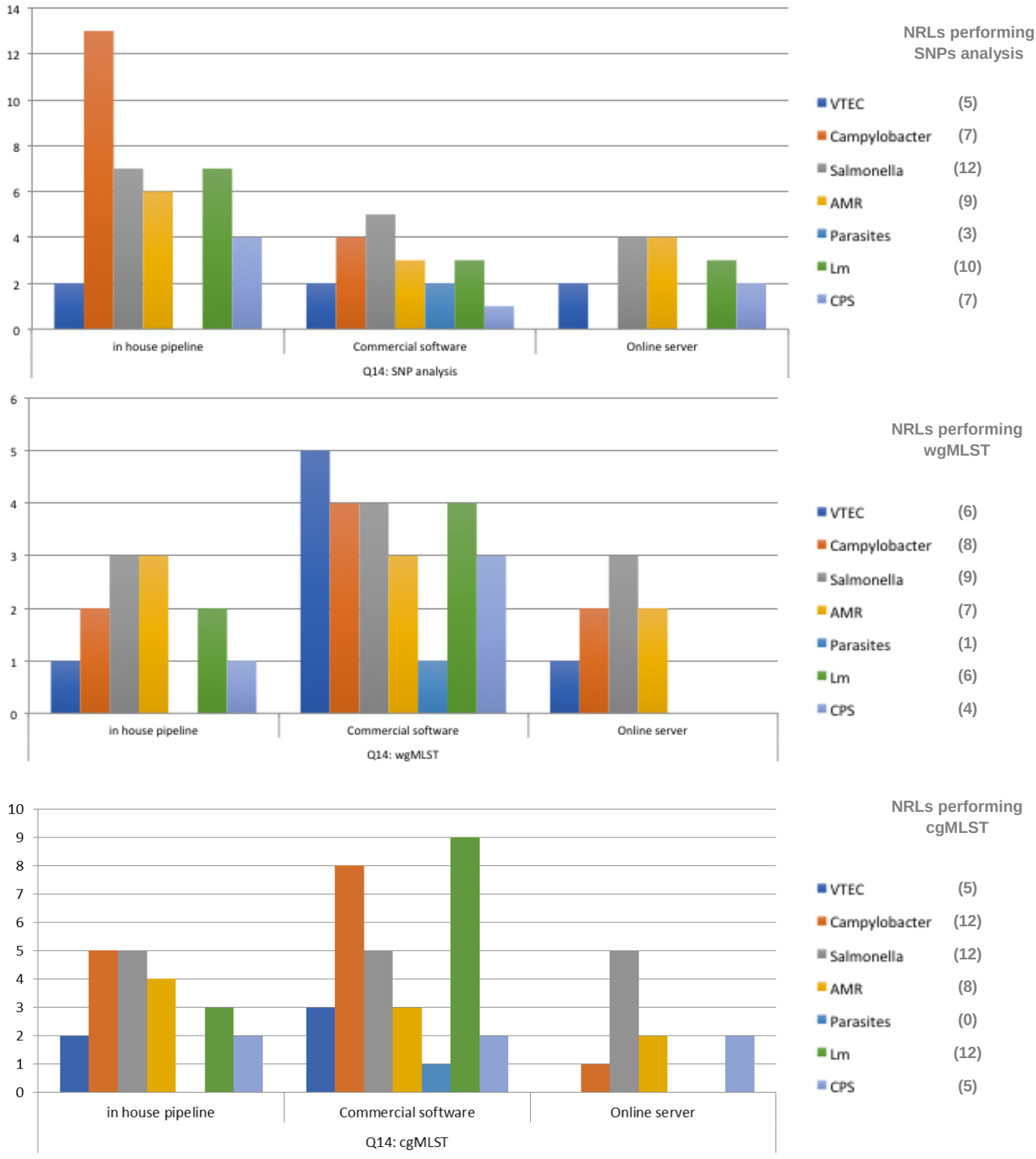

Figure 14B. Details given on the approaches used for cluster analysis (Question 14)

3.4 Participation in proficiency tests, trainings and benchmarking experiments on NGS (Q15-Q20)

Question 15: "Have you ever participated in NGS-based proficiency schemes? If Yes, please indicate for which analyses the proficiency was focused on" (multiple choice)

All the networks but that on Parasites had some experience in participation in proficiency tests on the use of NGS, with more than half of the NRLs that have access NGS in each network replying positively. These PTs appeared to focus on all the three proposed topics, from quality check to target genes identification and cluster analysis. The number of replies about details on PTs given by NRLs for AMR highlighted that the participation in PTs was higher than what declared when answering the first part of the question (at least 11 NRLs for AMR took part in PT schemes).

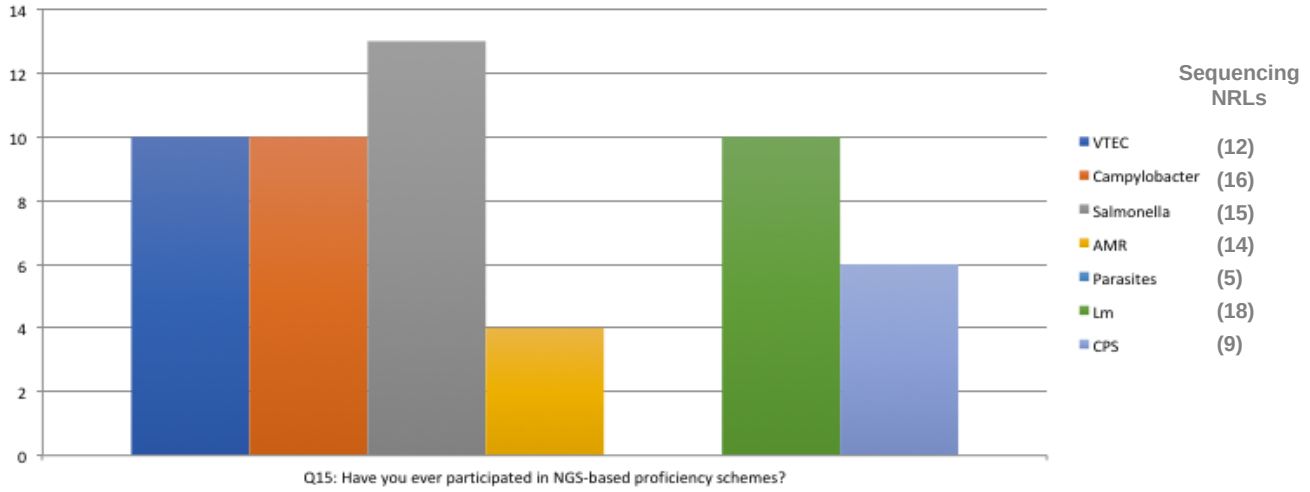

Figure 15A. Replies to Question 15. Positive replies are shown in the histograms.

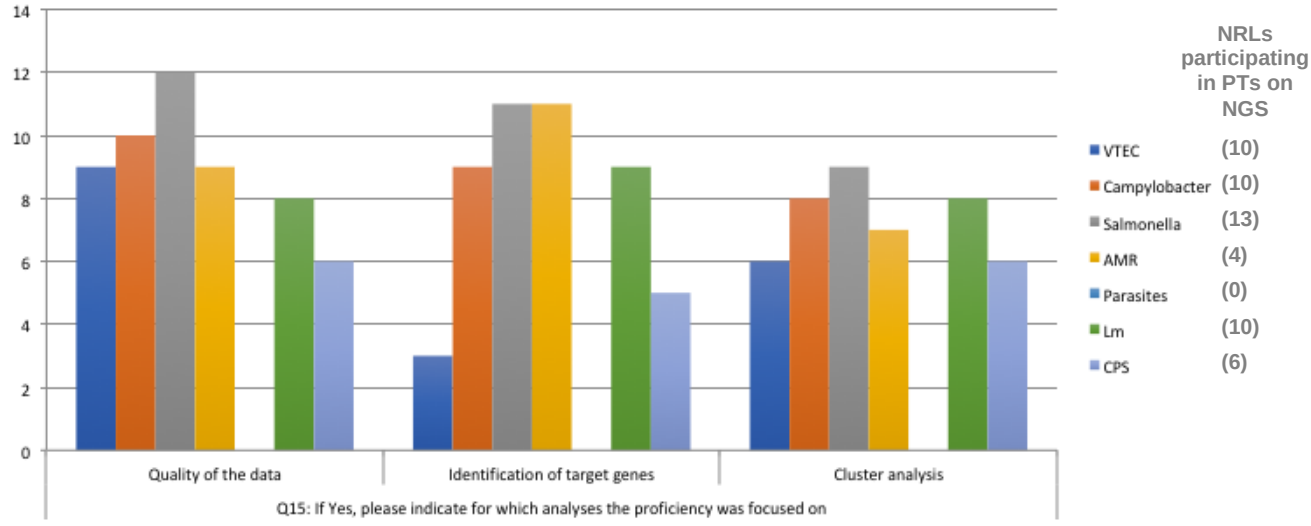

Figure 15B. Details given on the topics of the NGS proficiency tests in which the NRLs participated (Question 15)

Question 16: "Are you interested in participating in Proficiency Tests organised by the EURL?" (multiple choice) If Yes, for what activities?" (multiple choice)

All the networks declared interest in participating in PTs organised by the respective EURLs. The fraction of interested NRLs with respect to those replying to the survey was lower in the networks for Parasites and CPS, in which the level of adoption of NGS technologies resulted to be lower than the others at the time of the survey. The topics of interest for the PTs were very differently distributed in the different networks (e.g. data production for AMR and data analysis for *L. monocytogenes*).

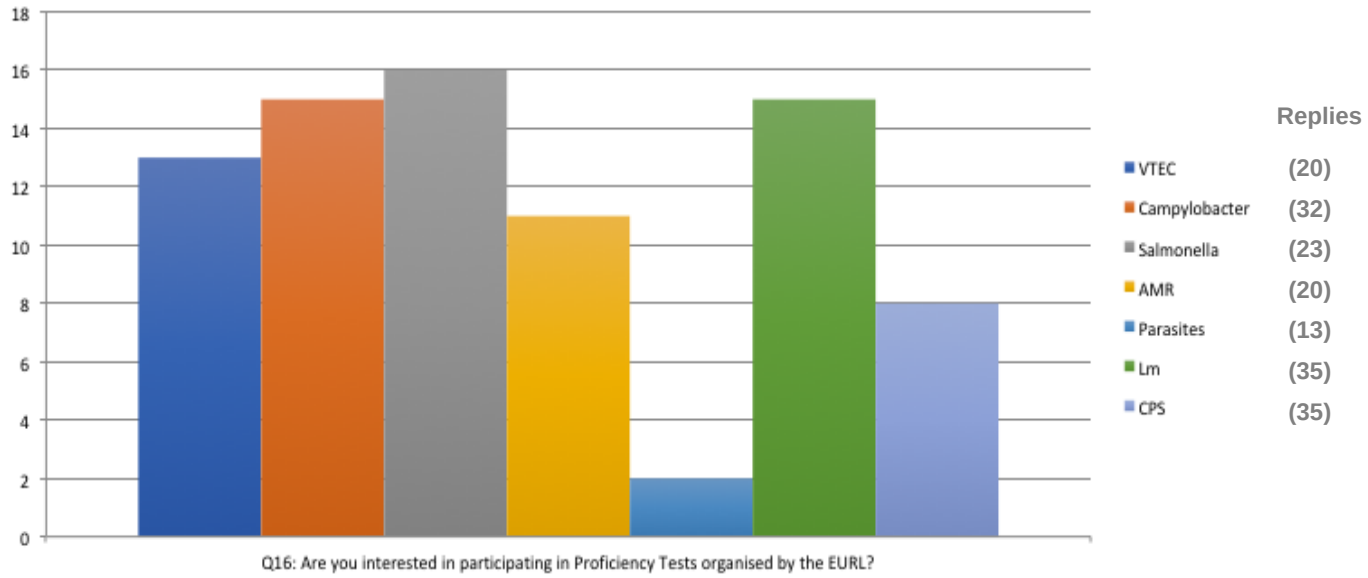

Figure 16A. Replies to Question 16. Positive replies are shown in the histograms.

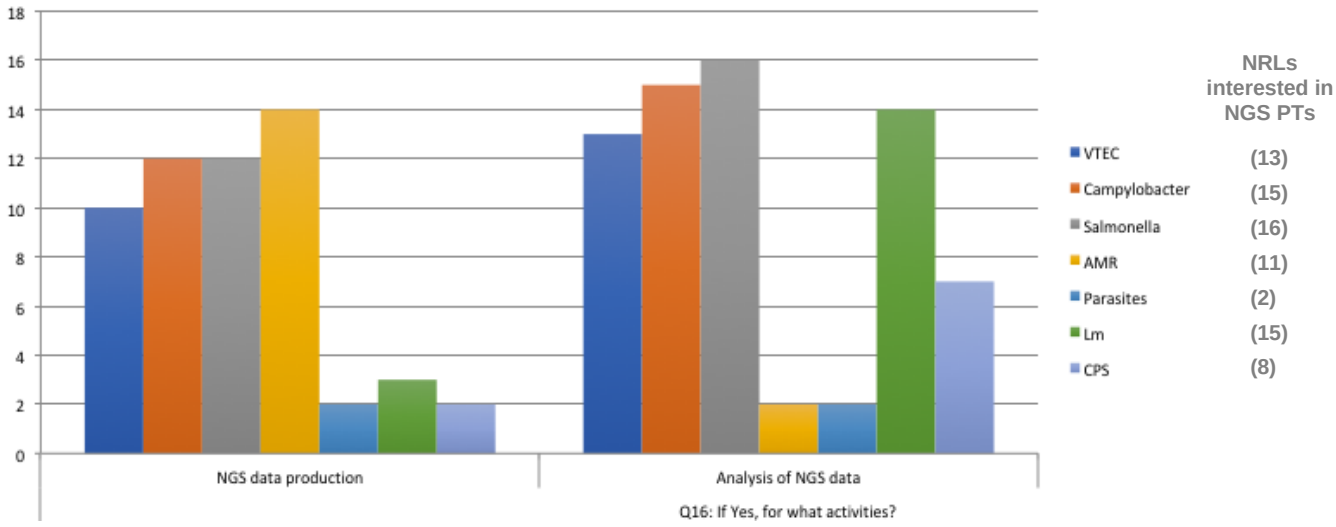

Figure 16B. Details given on the topics of the NGS proficiency tests in which the NRLs would be interested to participate (Question 16)

Question 17: "Has your NRL staff ever received training on NGS? If Yes: please specify the training sessions (multiple choice); In which form?; Please give information on the training provider"

Many NRLs in all the networks declared to have received training on NGS, either in the wet or dry lab parts, with the exception of NRLs for Parasites. All the forms of training were experienced by all the networks, with lower numbers for e-learning trainings. Many NRLs received training from the respective EURL or from other EURLs (being NRLs for several pathogens). Among other training providers, Illumina and Ion Torrent courses were mentioned by several NRLs, together with trainings organised in the framework of INNUENDO, ENGAGE and COMPARE projects. No information on training providers was received for Campylobacter network.

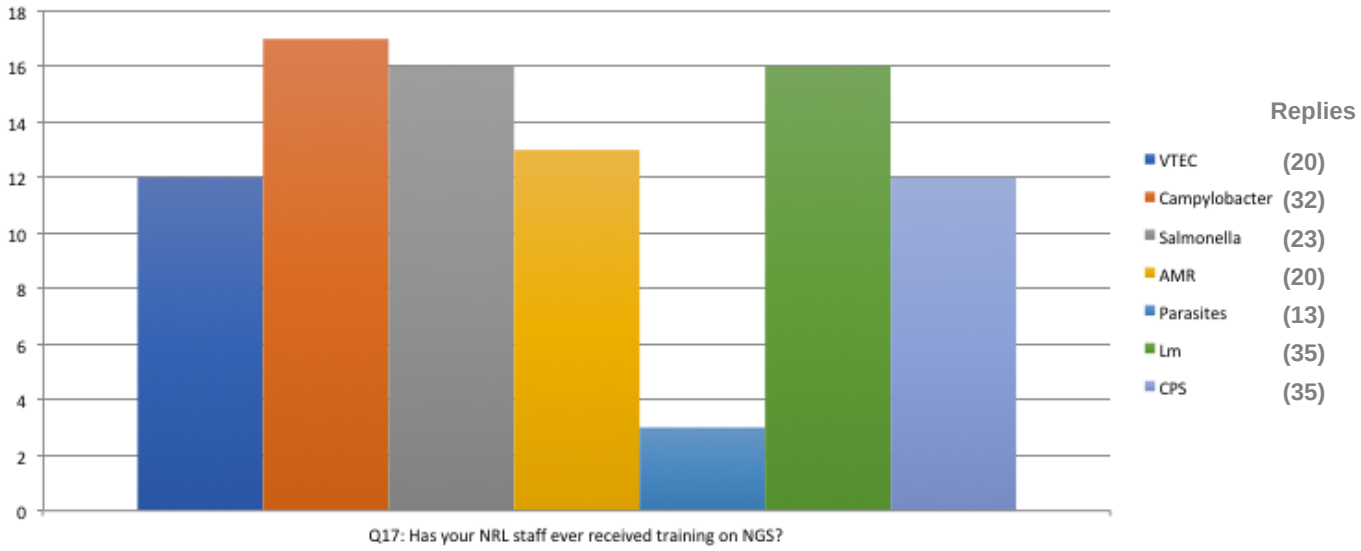

Figure 17A. Replies to Question 17. Positive replies are shown in the histograms.

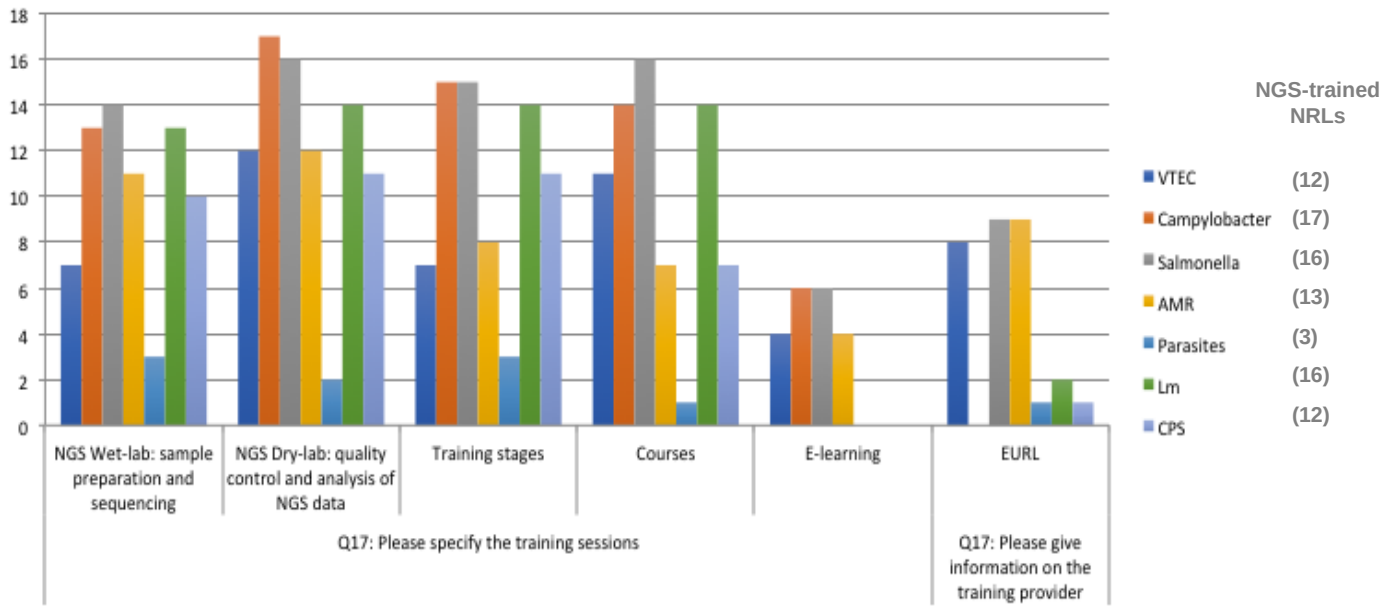

Figure 17B. Details given on the form and providers of received NGS training (Question 17)

Question 18: "Would you be interested in participating in training on NGS organized by the EURL? If Yes, please specify the part of NGS analysis which you interest for the training" (multiple choice)

All the networks declared interest in participating in NGS training organised by the EURLs (no replies were available for AMR network). When asked on the topics of interest for the trainings, interest was registered in either the wet or dry-lab procedures, with slight preference for dry-lab part in all the networks.

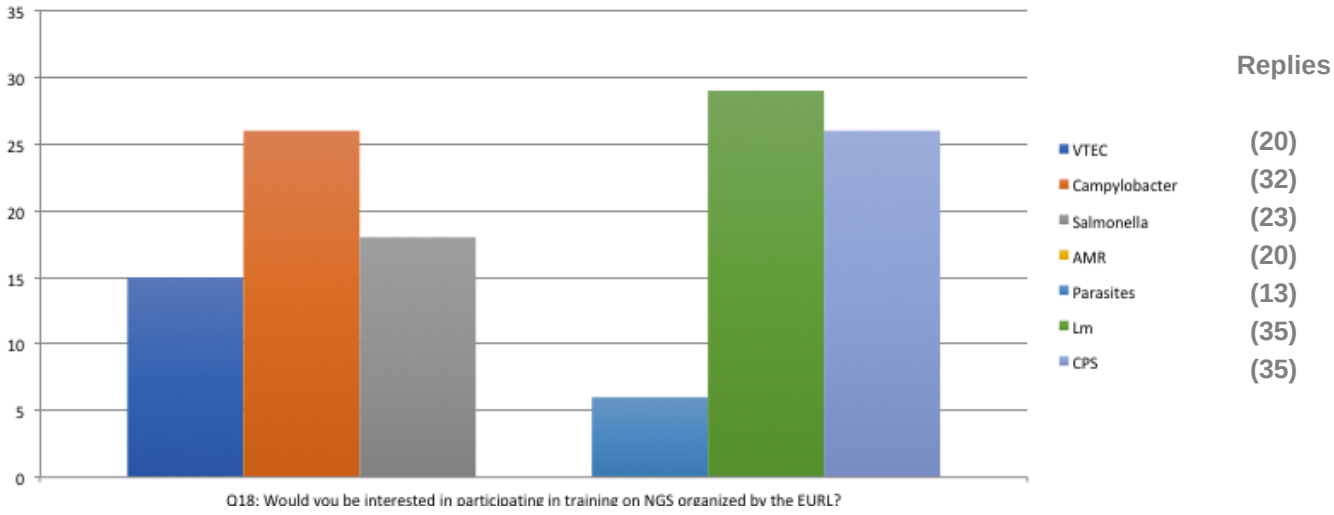

Figure 18A. Replies to Question 18. Positive replies are shown in the histograms.

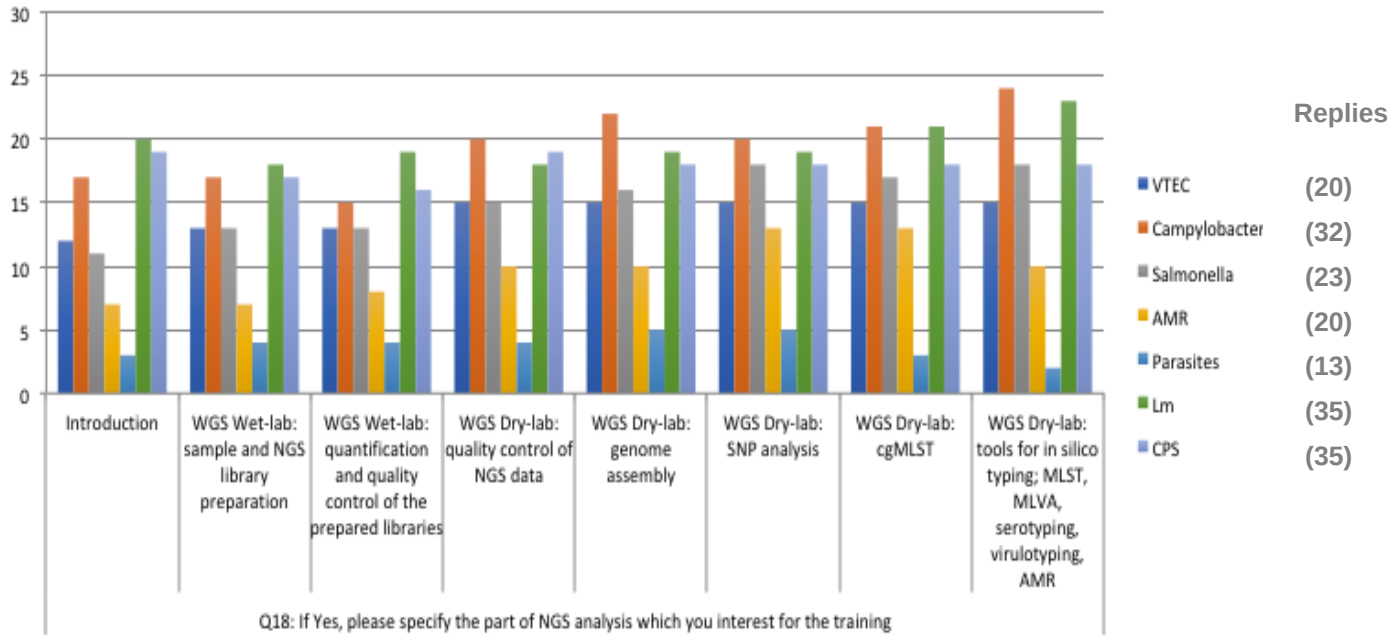

Figure 18B. Details given on the topics of interest for future NGS trainings (Question 18)

Question 19: "Which approach would you prefer for training on NGS data analysis?" (multiple choice)

The training at the EURLs was by far the preferred approach. Nevertheless, interest was also high for the E-learning modules in all the networks.

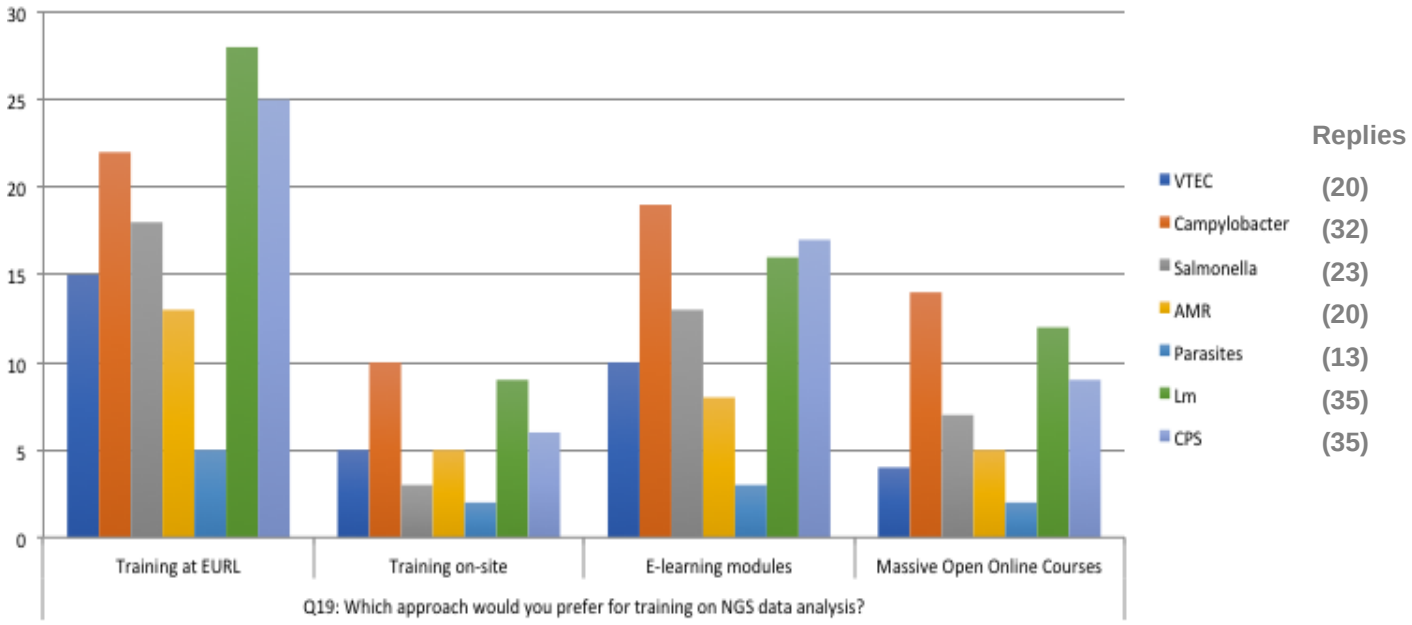

Figure 19. Replies to Question 19

Question 20: "Have you performed benchmarking to compare protocols? If yes, please specify the methodology" (multiple choice)

Apart from the network of NRLs for Parasites, all the others showed some level of experience on benchmarking of NGS protocols, with AMR NRLs having more experience on the wet-lab part and all the others more on the dry-lab analysis.

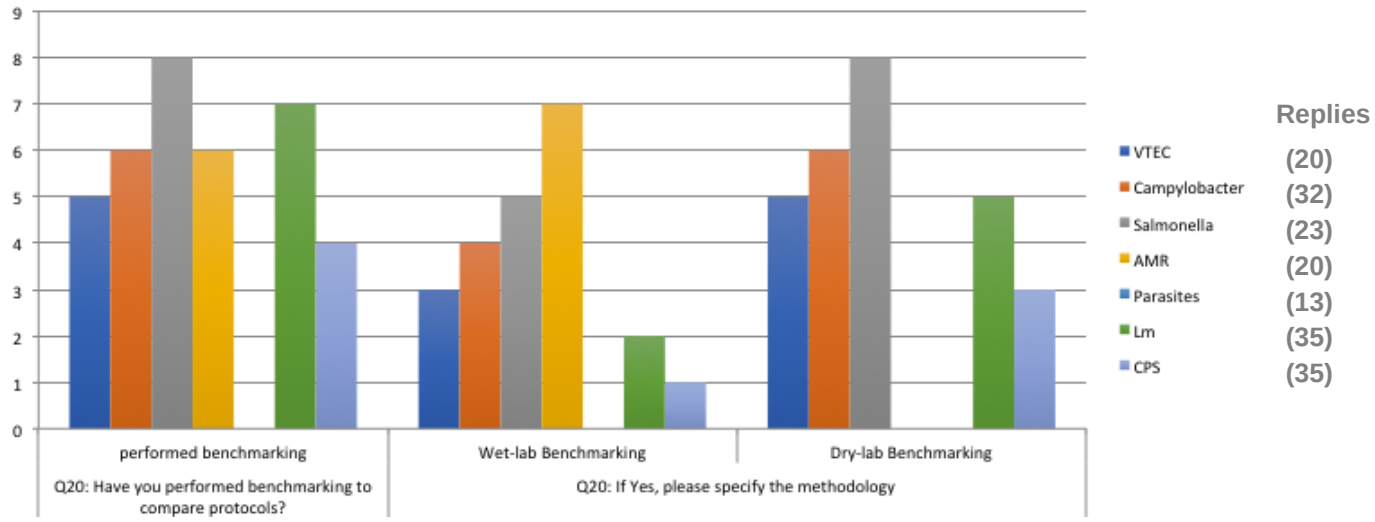

Figure 20. Replies to Question 20

3.5 Additional comments (Question 21)

Free text comments were collected as the final question of the survey. This section was used by several NRLs to stress the interest in NGS despite lack of funding and lack of time and personnel to dedicate in this activity. This aspect was thus better investigated in the follow up survey, illustrated below.
Additionally, interest in harmonization with public health microbiology laboratories in the clinical sector was reported.

3.6 Follow-up survey - Questionnaire on the application of Next Generation Sequencing technology, including WGS of bacterial/viral/parasites and metagenomics by the EU NRLs

The follow up survey was compiled by 34 NRLs across the networks. The results are reported in Tables 1-5. The majority of them replied to have plans for the implementation of NGS. The main hindrance resulted to be the lack of funding and the lack of expertise, but six NRLs replied to believe that NGS was out of the scope of their NRL tasks. Some NRLs commented to take advantage of collaboration with other Institutes for performing NGS. The majority of NRLs declared to expect NGS funding from National grants in the future, recognising monitoring, surveillance and outbreak investigation as the activities which would benefit the most from the adoption of NGS.

Table 1. Do you have plans for setting up NGS activities at your NRL?

ANSWER CHOICES	RESPONSES	
No, currently we have no plans to setup NGS activities at our NRL	33.33%	11
Yes, we have (unspecific) plans to setup NGS activities at our NRL	48.48%	16
Yes, we have specific plans to setup NGS activities at our NRL (please specify, e.g. when will the plans be effective?)	18.18%	6
Total Respondents: 33		

Table 2. Please indicate the main reason(s) for not having plans to setup NGS at your NRL

ANSWER CHOICES	RESPONSES	
NGS is currently out of scope of the tasks for our NRL	25.00%	6
We would like to setup NGS, but currently no financial resources have been allocated for it	62.50%	15
We would like to setup NGS, but currently employees with relevant expertise within the field of WGS are not available to take the task	45.83%	11
Other reason (please add a comment)	25.00%	6
Total Respondents: 24		

Table 3. For setting up NGS at your institute/organization from where do you envision that the funding will be provided? [please select all that apply]

ANSWER CHOICES	RESPONSES	
National grants (basic funding)	50.00%	16
National/international research grants	40.63%	13
Other source of funding (please add comment)	31.25%	10
Total Respondents: 32		

Table 4. In your opinion, which application would benefit the most from the use of NGS in your NRL? [Please select all that apply]

ANSWER CHOICES	RESPONSES	
For diagnosis	30.30%	10
For monitoring/surveillance	72.73%	24
For outbreak investigations	72.73%	24
Other (please specify):	12.12%	4
Total Respondents: 33		

Table 5. At your NRL, has an analysis/assessment been made to identify the critical milestones during the process towards the implementation of NGS?

ANSWER CHOICES	RESPONSES	
Yes	24.24%	8
No	75.76%	25
TOTAL		33

3.7 Adoption of NGS in the NRLs for Foodborne Viruses, June 2019

A total of 21 NRLs replied to an *ad hoc* survey administered by EURL for Foodborne Viruses, including questions on the adoption of NGS methods specific for the analysis of viral genomes. The results of the topics useful for the benefit of the activities of the Inter EURLs Working Group of NGS are briefly illustrated in this paragraph.

Nine NRLs declared to have access to NGS, mainly thanks to the availability of in house platforms. The rest of the NRLs declared that the reason not to use NGS consisted in the lack of dedicated funding and expertise and to the absence of a specific regulation requiring the adoption of such technology. The labs having access to NGS declared to use it either for monitoring, outbreak investigation and research project. Similarly to what observed for the other NRLs networks, spin columns and magnetic beads-based methods were those preferred for nucleic acid extraction, spectrofluorometers and fluorimeters for the quality check and concentration calculation, and fragment-analysis was used for the control of the libraries. Illumina platforms resulted by far the most widely adopted, with only one laboratory mentioning the access also to an Ion Torrent platform and a few having also access to MinIon Nanopore sequencers.

The majority of the NRLs reported to perform bioinformatics analysis in house and declared to use all the mentioned parameters for quality check of the data (Phred value, coverage, N50, number of assembled contigs and total basepairs assembled).

An interest in participating in Proficiency Tests organised by the EURL was reported by 14 out of the 21 replying NRLs, while interest in participating in trainings was declared by all of them, either in the wet-lab protocols or in the data analysis, preferentially if organised at the EURL site, followed by e-learning courses. Eight NRLs declared to have already participated in trainings and only one to have experience in benchmarking of NGS wet-lab procedures.

4. Concluding remarks

- Half of the NRLs (88/178) reported not to have access to NGS technology at the time of this survey.
- The level of adoption of the methods was not homogeneous across all the networks. Among the reasons to be considered for this, there is the intrinsic difficulty in the development of NGS methods for pathogens-specific issues, from the isolation and growth of the pathogen to the bioinformatics data analysis.


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Foodborne Viruses

- The vast majority of the NRLs having access to NGS, reported not to use it for routine analysis on all the isolates, yet, but to apply a selection to the isolates to be processed.
- A good harmonization of the use of a few strategies for nucleic acids extraction, quality and quantity check and for library control was detected across all the networks. Optimization of the wet-lab procedures has already allowed some NRLs to bypass some quality/quantity control steps.
- Illumina technology seemed to be the most widely adopted, even if Ion Torrent is also in use in several NRLs. The harmonization of data analysis among at least these two different technologies should be considered to be able to compare data at the EU level.
- Besides the wide use of commercial softwares and online servers, many NRLs declared to use command line and in house-developed pipelines for data analysis, suggesting that a good bioinformatics capacity is already present in several NRLs.
- The lack of availability of online servers exposing wgMLST and cgMLST could have played a role in the spread of use of commercial softwares more in some networks than in others. Nevertheless the knowledge and adoption of these approaches appeared quite spread across the NRLs.
- A great interest in participating in future PTs on NGS organised by the EURLs was registered in all the networks, especially in those about NGS data production
- Interest was declared by almost all the NRLs in training activities on the application of NGS organised by the EURLs, with preference given to trainings at EURLs premises or through E-learning courses
- Limited funding and lack of specific expertise were detected as the main hindrances for the NRLs still not having access to NGS. Nevertheless lack of perception of the importance of the adoption of this technology in the scope of NRLs activities was also registered. The activities of the EURLs, particularly those performed in the framework of the present Inter EURLs Working Group, will be targeted to grow the expertise in this field across the NRLs and will aim at growing the awareness of the relevance of adopting this methodology. In addition, the limited perception of its importance will be discussed with all the relevant bodies including the NRLs and the competent authorities during the MedVetNet-funded workshop organised by this working group and entitled “Science meets Policy conference: Modern technologies to enable response to crises: Next Generation Sequencing to tackle food-borne diseases in the EU”, which is scheduled for September 2020, with the final goal of promoting the diffusion of NGS technology for the monitoring and control of microbial food-borne hazards in EU.

Annex 1

Questionnaire on the support expected from the EURL on the application of Next Generation Sequencing technology, including WGS of bacterial/viral/parasites and metagenomics by the EU NRLs

Introduction

A working group (WG) has been established by DG SANTE with the aim to promote the use of Next Generation Sequencing (NGS) across the EURLs' networks, to build capacity towards the use of this analytical technology within the EU and ensure liaison with the work of EFSA and ECDC on the Whole Genome Sequencing (WGS) mandate sent by the Commission. The WG includes the following EURLs and also involves the Commission and the agencies EFSA and ECDC as observers.

1. EURL *E. coli* (coordinator)
2. EURL *Listeria monocytogenes*
3. EURL Coagulase Positive Staphylococci (CPS)
4. EURL *Salmonella*
5. EURL *Campylobacter*
6. EURL Parasites
7. EURL Antimicrobial Resistance (AMR)
8. EURL Food borne viruses

Observers: SANTE G4, EFSA, ECDC

As a first action, it has been decided to administer the following questionnaire to the NRL networks in order to get an updated view of the NRL capacity of NGS to define the activities of the WG and to target the actions on the actual needs of the NRLs.

Definitions:

Next Generation Sequencing (NGS): the high throughput sequencing technology that can be applied to different kind of samples, including whole genomes of isolates or metagenomes from complex matrices.

Whole Genome Sequencing (WGS): the process of sequencing of the genomic content of an isolated strain.

Metagenomics: the process of sequencing the DNA extracted from complex matrices. The term includes the two main strategies currently adopted, consisting in the shotgun sequencing of the whole DNA extracted or in the amplification and sequencing of species-specific target regions (i.e. 16S rDNA).

MLST: Multi Locus Sequence Typing

cgMLST: Multi Locus Sequence Typing of genomes based on a panel of core genes of a given species

wgMLST: Multi Locus Sequence Typing of genomes based the whole gene content of a given species

Laboratory name:

Please specify the country for which you are appointed NRL:

Contact person(s) For this enquiry (not necessarily the contact person of NRL):

Email address:

Additional information (free text):

Q1.In your role of NRL, which of the following NGS activities do you perform? *(multiple choice)*

☐ WGS	in house ☐	outsourced ☐
☐ Metagenomics sequencing	in house ☐	outsourced ☐
☐ Bioinformatics analysis	in house ☐	outsourced ☐
☐ No NGS/WGS activities		

Q2.If you answered positively to at least one of the options in Q1, what is the purpose of using NGS in your NRL? *(multiple choice)*

- ☐ For diagnosis
- ☐ For monitoring/surveillance
- ☐ For outbreak investigations
- ☐ For research projects

Q3.If you replied "No NGS activity" to Q1, please specify why: *(multiple choice)*

- ☐ no current capacity for sequencing
- ☐ no current capacity for bioinformatics analysis
- ☐ no plans to replace current methodologies with NGS in the near future
- ☐ Other, please specify_____

Section on NGS capacity. To reply if you selected at least one of the first three options in Q1

Q4.Do you sequence a selection of isolates/matrices? *(single choice)*

- ☐ No, all isolates/matrices are subjected to sequencing
- ☐ Yes, a selection is applied: please specify_____

Q5. What concept do you use to extract DNA/RNA for sequencing? *(single choice)*

- ☐ Spin-column based
- ☐ Resin-coated beads
- ☐ Magnetic beads
- ☐ Salt and ethanol precipitation
- ☐ Other, please specify: _____

Q6. How do you assess the quality of the extracted DNA/RNA before sequencing? *(multiple choice)*

- ☐ Agarose gel electrophoresis
- ☐ Nanodrop
- ☐ Other, please specify _____
- ☐ No quality assessment is performed

Q7. How do you estimate the concentration of the extracted DNA/RNA before sequencing? *(multiple choice)*

- ☐ Qubit
- ☐ Nanodrop
- ☐ Other, specify _____
- ☐ No quantification is performed

Q8. Which protocol do you use to prepare the library for sequencing? *(multiple choice)*

- ☐ Library preparation kit by Illumina
- ☐ Library preparation kit by Thermo Fisher Scientific for Ion Torrent
- ☐ Alternative library preparation kit by other brands
- ☐ Non-commercial protocols, please specify: _____

Q9. How do you evaluate the quality/concentration of the prepared library before sequencing? *(multiple choice)*

- ☐ Bioanalyzer
- ☐ Qubit
- ☐ Quantitative Real Time PCR
- ☐ Other, please specify: _____
- ☐ No quality control and quantification steps are performed

Q10. Please specify which NGS **platforms** you use and/or are used by the lab/company to which the activity is outsourced *(multiple choice)*:

- ☐ Illumina MiSeq
- ☐ Illumina NextSeq
- ☐ Illumina HiSeq
- ☐ Ion Torrent PGM
- ☐ Ion Proton
- ☐ Ion Torrent S5
- ☐ PacBio
- ☐ 454
- ☐ MinION
- ☐ Other, please specify _____

Q11. What parameters do you use to evaluate the quality of sequence data (reads and/or contigs)? *(multiple choice)*

- ☐ Coverage
- ☐ Phred score
- ☐ N50 of the assembled contigs
- ☐ Number of assembled contigs
- ☐ Total base pairs assembled
- ☐ Other, please specify _____

Q12. Where is NGS data analysis performed?

- ☐ In-house:
  - Which approach do you use? *(multiple choice)*
    - ☐ Tools operated through command-line
    - ☐ Commercially available software with user-friendly interface
    - ☐ Online Public servers
  - Do you use in house developed pipelines? *(single choice)*
    - ☐ Yes
    - ☐ No
- ☐ Activity outsourced

Q13. Do you perform cluster analysis? *(single choice)*

- ☐ Yes
- ☐ No

Q14. If you answered yes to Q13: How do you perform cluster analysis? *(multiple choice)*

- ☐ SNP analysis:
  - ☐ In house developed pipeline.
  - ☐ Tool part of a commercially available software.
    Please specify:_____
  - ☐ Tool part of an online server.
    Please specify:_____
- ☐ wgMLST. *(multiple choice)*
  - ☐ In house developed pipeline
  - ☐ Tool part of a commercially available software.
    Please specify:_____
  - ☐ Tool part of an online server.
    Please specify:_____
- ☐ cgMLST. *(multiple choice)*
  - ☐ In house developed pipeline
  - ☐ Tool part of a commercially available software.
    Please specify:_____
  - ☐ Tool part of an online server.
    Please specify:_____
- ☐ Other, please specify _____

Q15. Have you ever participated in NGS-based proficiency schemes?

- ☐ No
- ☐ Yes

  If Yes, please indicate for which analyses the proficiency was focused on: *(multiple choice)*
  - ☐ Quality of the data
  - ☐ Identification of target genes (i.e. virulence genes, resistance genes)
  - ☐ Cluster analysis
  - ☐ Other, please specify: _____

Q16. Are you interested in participating in Proficiency Tests organised by the EURL? *(multiple choice)*

- ☐ No
- ☐ Yes

  If Yes, for what activities? *(multiple choice)*
  - ☐ NGS data production
  - ☐ Analysis of NGS data

☐ Other, please specify: ……

Q17. Has your NRL staff ever received training on NGS?

☐ No
☐ Yes

If Yes: *(multiple choice)*

- Please specify the training sessions:
  - ☐ NGS Wet-lab: sample preparation and sequencing
  - ☐ NGS Dry-lab: quality control and analysis of NGS data
- In which form?
  - ☐ Training stages
  - ☐ Courses
  - ☐ E-learning
- Please give information on the training provider:
  - ☐ EURL
  - ☐ Other, please specify: _____

Q18. Would you be interested in participating in training on NGS organized by the EURL? *(single choice)*

☐ No
☐ Yes

If Yes, please specify the part of NGS analysis which you interest for the training: *(multiple choice)*

☐ Introduction to NGS
☐ WGS Wet-lab: sample and NGS library preparation
☐ WGS Wet-lab: quantification and quality control of the prepared libraries
☐ WGS Dry-lab: quality control of NGS data
☐ WGS Dry-lab: genome assembly
☐ WGS Dry-lab: SNP analysis
☐ WGS Dry-lab: cgMLST
☐ WGS Dry-lab: tools for *in silico* typing; MLST, MLVA, serotyping, virulotyping, AMR

Q19. Which approach would you prefer for training on NGS data analysis? *(multiple choice)*

☐ training at EURL
☐ training on-site
☐ E-learning modules
☐ Massive Open Online Courses

Q20. Have you performed benchmarking to compare protocols*:*

☐ Yes
☐ No

If yes please specify the methodology: *(multiple choice)*

☐ Wet-lab Benchmarking

☐ Dry-lab Benchmarking

Q21. Additional comments:

_____

_____

_____

_____

Annex 2.

Follow-up survey - Questionnaire on the application of Next Generation Sequencing technology, including WGS of bacterial/viral/parasites and metagenomics by the EU NRLs

Follow-up on EURL Antimicrobial Resistance, WG NGS Survey
Questionnaire on the application of Next Generation Sequencing technology, including WGS of bacterial/viral/parasites and metagenomics by the EU NRLs

Introduction

The current survey aims to follow-up on the questionnaire from earlier this year related to the laboratories that reported no NGS activities are performed in their laboratory.
We have recorded that your NRL reported that no NGS activities are not applied at your laboratory and therefore ask you to respond to a few more clarifying questions to help the WG for NGS to see a fuller picture of this field.

Below you find the introduction from the first questionnaire that was circulated earlier this year:
A working group (WG) has been established by DG SANTE with the aim to promote the use of Next Generation Sequencing (NGS) across the EURLs' networks, to build capacity towards the use of this analytical technology within the EU and ensure liaison with the work of EFSA and ECDC on the Whole Genome Sequencing (WGS) mandate sent by the Commission. The WG includes the following EURLs and also involves the Commission and the agencies EFSA and ECDC as observers.

1. ***EURL E. coli (coordinator)***
2. ***EURL Listeria monocytogenes***
3. ***EURL Coagulase Positive Staphylococci (CPS)***
4. ***EURL Salmonella***
5. ***EURL Campylobacter***
6. ***EURL Parasites***
7. ***EURL Antimicrobial Resistance (AMR)***
8. ***EURL Food borne viruses***

Observers: SANTE G4, EFSA, ECDC

As a first action, it has been decided to administer the following questionnaire to the NRL networks in order to get an updated view of the NRL capacity of NGS to define the activities of the WG and to target the actions on the actual needs of the NRLs.

Definitions:

Next Generation Sequencing (NGS): the high throughput sequencing technology that can be applied to different kind of samples, including whole genomes of isolates or metagenomes from complex matrices.

Whole Genome Sequencing (WGS): the process of sequencing of the genomic content of an isolated strain.

Metagenomics: the process of sequencing the DNA extracted from complex matrices. The term

includes the two main strategies currently adopted, consisting in the shotgun sequencing of the whole DNA extracted or in the amplification and sequencing of species-specific target regions (i.e. 16S rDNA).

MLST: Multi Locus Sequence Typing

cgMLST: Multi Locus Sequence Typing of genomes based on a panel of core genes of a given species

wgMLST: Multi Locus Sequence Typing of genomes based the whole gene content of a given species

1. Please enter the following information

Laboratory name:

The country for which you are appointed NRL:

Area of NRL:

Contact person(s) for this enquiry (not necessarily the contact person of the NRL):

Email address on contact person(s):

Additional information:

2. Do you have plans for setting up NGS activities at your NRL?

- [ ] No, currently we have no plans to setup NGS activities at our NRL
- [ ] Yes, we have (unspecific) plans to setup NGS activities at our NRL
- [ ] Yes, we have specific plans to setup NGS activities at our NRL (please specify, e.g. when will the plans be effective?)

3. Please indicate the main reason(s) for not having plans to setup NGS at your NRL? [please select all that apply]

- [ ] NGS is currently out of scope of the tasks for our NRL
- [ ] We would like to setup NGS, but currently no financial resources have been allocated for it
- [ ] We would like to setup NGS, but currently employees with relevant expertise within the field of WGS are not available to take the task
- [ ] Other reason (please add a comment)

4. For setting up NGS at your institute/organization from where do you envision that the funding will be provided? [please select all that apply]

- [ ] National grants (basic funding)
- [ ] National/international research grants
- [ ] Other source of funding (please add comment)

5. In your opinion, which application would benefit the most from the use of NGS in your NRL? [Please select all that apply]

- [ ] For diagnosis
- [ ] For monitoring/surveillance
- [ ] For outbreak investigations
- [ ] Other (please specify):

6. At your NRL, has an analysis/assessment been made to identify the critical milestones during the process towards the implementation of NGS?

- ○ Yes
- ○ No

7. What is the largest barrier to setting up NGS at your NRL (please indicate max 5 key words)

8. Additional comments

Annex 3.

List of participants

EURL network	Laboratory name	Country
Escherichia coli (VTEC)	National Reference Laboratory for Escherichia coli including Verotoxin producing E. coli	Austria
	Foodborne Pathogens	Belgium
	Laboratory for Food Microbiology, CVI Zagreb, Croatia	Croatia
	LABORATORY FOR THE CONTROL OF FOOD OF ANIMAL ORIGIN (LCFAO) CYPRUS VETERINARY SERVICES	Cyprus
	Veterinary and Food laboratory	Estonia
	Finnish Food Safety Authority Evira	Finland
	NRL E. coli Germany	Germany
	Veterinary Public Health Regulatory Laboratory (VPHRL), Department of Agriculture, Food and the Marine (DAFM)	Ireland
	Istituto Superiore di Sanità, Department of Food Safety, Nutrition and Veterinary Public Health	Italy
	National Veterinary Research Institute, Pulawy, Poland - NRL for VTEC	Poland
	INIAV-Instituto Nacional de Investigação Agrária e Veterinária	Portugal
	INIAV - National Institute for Agrarian and Veterinary Research	Portugal
	INSTITUTE FOR HYGIENE AND VETERINARY PUBLIC HEALTH	Romania
	National Reference Centre of Environmental Microbiology, Public Health Authority of the Slovak Republic	Slovakia
	State Veterinary and Food Institute, VFI in Dolny Kubin	Slovakia
	Centro Nacional de Alimentación-AECOSAN	Spain
	Laboratorio Central de Veterinaria	Spain
	National Food Agency in Sweden, Biology division	Sweden
	RIVM	The Netherlands
	Public Health England	United Kingdom
Campylobacter	FSVI- Food Microbiology	Albania
	NRL Campylobacter, NRL Antimicrobial Resistance	Austria
	foodborne pathogens	Belgium
	NRL Salmonella, campylobacter, staphylococci and antimicrobial resistance, NCFS, NDRVMI	Bulgaria
	Laboratory for Food Microbiology	Croatia
	Laboratory for the Control of Food of animal origin (LCFAO), Cyprus Veterinary Services	Cyprus
	State Veterinary Institute Olomouc (NRLCampylobacter)	Czech Republic
	Technical University of Denmark, National Food Institute	Denmark
	Veterinary and Food Laboratory	Estonia
	Finnish Food Safety Authority Evira	Finland
	Anses	France
	NRL for Campylobacter	Germany
	National Food Chain Safety Office, Food and Feed Safety Directorate	Hungary

	Institute for Experimental Pathology at Keldur, University of Iceland	Iceland
	CVRL, Department of Agriculture, Food and the Marine	Ireland
	Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale"	Italy
	Institute of Food safety, Animal Health and Environment "BIOR"	Latvia
	Laboratoire National de Sante	Luxembourg
	Malta Public Health Laboratory	Malta
	National Veterinary Research Institute, Pulawy, Poland - NRL for Campylobacter	Poland
	Instituto Nacional de Investigação Agrária e Veterinária, I. P.	Portugal
	Bacteriology Laboratory of the Instituto Nacional de Investigação Agrária e Veterinária	Portugal
	Hygiene and Veterinary Public Health Institute	Romania
	Institute of Meat Hygiene and Technology	Serbia
	State Veterinary and Food Institute, VFI in Dolny Kubin	Slovakia
	Veterinary Faculty, Institute of Microbiology and Parasitology / National Veterinary Institute, Ljubljana, Slovenia)	Slovenia
	Laboratorio Central de Veterinaria	Spain
	Centro Nacional de Alimentación.AECOSAN	Spain
	National Food Agency Sweden	Sweden
	ZOBA, Institute of Veterinary Bacteriology	Switzerland
	Wageningen Bioveterinary Research (WBVR)	The Netherlands
	Public Health England	United Kingdom
Salmonella	Foodborne Pathogens, Sciensano	Belgium
	Laboratory for Food Microbiology, CVI, Croatia	Croatia
	Laboratory for the Control of Food of Animal Origin (LCFAO), Cyprus Veterinary Services	Cyprus
	State Veterinary Institute Prague	Czech Republic
	The DTU and the DVFA laboratory.	Denmark
	Finnish Food Safety Authority Evira	Finland
	Finnish Food Safety Authority Evira	Finland
	NRL-Salmonella	Germany
	CVRL, Department of Agriculture, Food and the Marine	Ireland
	Italian National Reference Laboratory for Salmonella	Italy
	Institute of Food Safety, Animal Health and Environment "BIOR"	Latvia
	Laboratoire National de Santé	Luxembourgh
	National Veterinary Research Institute	Poland
	INIAV- Instituto Nacional de Investigação Agrária e Veterinária	Portugal
	Hygiene and Veterinary Public Health Institute	Romania
	State Veterinary and Food Institute Bratislava	Slovakia
	Veterinary Faculty / National Veterinary Institute, Gerbiceva 60, SI-1000 Ljubljana, Slovenia	Slovenia
	Centro Nacional de Alimentación-AECOSAN	Spain
	Laboratorio Central de Veterinaria	Spain
	ZOBA, Institute of Veterinary Bacteriology	Switzerland
	National Institute for Public Health and the Environment (RIVM)	the Netherlands

	Public Health England	United Kingdom
	Animal and Plant health Agency (APHA)	United Kingdom
AMR	NRL AMR Food pathogens and food producing animals	Belgium
	The DVFA laboratory (the wet-lab part of NRL on WGS)	Denmark
	GenEpi	Denmark
	Finnish Food Safety Authority Evira/Microbiology Research Unit/Antibiotics Section	Finland
	Veterinary Laboratory of Chalkis	Greece
	The Institute for Experimental Pathology, University of Iceland, at Keldur	Iceland
	Central Veterinary Research Laboratory	Ireland
	General Diag1stic Department, National Reference Laboratory for Antimicrobial Resistance	Italy
	Institute of Food Safety, Animal Health and Environmnet "BIOR"	Latvia
	National Food and Veterinary Risk Assessment Institute	Lithuania
	National Veterinary Research Institute	Poland
	Instituto Nacional de Investigação Agrária e Veterinária (INIAV)	Portugal
	Institute 1 and Animal Health	ROMANIA
	State Veterinary and Food Institute, VFI in Dolny Kubin	Slovakia
	CENTRO NACIONAL DE ALIMENTACION-AECOSAN	Spain
	National Veterinary Institute (SVA)	Sweden
	ZOBA, Institute of Veterinary Bacteriology	Switzerland
	Wageningen Bioveterinary Research	the Netherlands
	Public Health England	United Kingdom
	Animal and Plant Health Agency	United Kingdom
Parasites	Croatian Veterinary Institute, Department Vinkovci, Laboratory for diagnostic-NRL for parasites	Croatia
	Laboratoty for the control of food of animal origin (LCFAO)	Cyprus
	State Veterinary Institute Olomouc	Czech Republic
	Finnish Food Safety Authority Evira, National Reference Laboratory for Parasites	Finland
	Anses Nancy laboratory for Rabies and wildlife	France
	Friedrich-Loeffler-Institut NRL for Echinococcosis	Germany
	Parasitology-Parasitic Diseases, Entomology and Bee Health	Greece
	National Food Chain Safety Office Veterinary Diagnostics	Hungary
	Foodborne and Neglected Parasites Unit, Department of Infecious Diseases	Italy
	Italian National Reference Center of Toxoplasmosis	Italy
	Instituto Nacional de Investigação Agrária e Veterinária	Portugal
	Centro Nacional de Alimentación-AECOSAN	Spain
	National Veterinary Institute	Sweden
Listeria monocytogenes	Sciensano	Belgium
	Laboratory for Food Microbiology, CVI Zagreb	Croatia
	Croatian National Institute of Public Health	Croatia
	State General Laboratory, Food Microbiology Laboratory	Cyprus
	LABORATORY FOR THE CONTROL OF FOOD OF ANIMAL ORIGIN (LCFAO) CYPRUS VETERINARY SERVICES	Cyprus

	NRL for Lm, State Veterinary institute Jihlava	Czech republic
	DTU, National Food Institute	Denmark
	Veterinary and Food Laboratory	Estonia
	Finnish Food Safety Authority Evira	Finland
	ANSES	France
	Federal Institute for Risk Assessment, NRL for Listeria monocytogenes	Germany
	'Departement of Food Hygiene of Athens	Greece
	National Food Chain safety Office	Hungary
	Dairy Science Laboratory DAFM	Ireland
	Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise	Italy
	Institute of Food Safety, Animal Health and Environment BIOR	Latvia
	National Food and Veterinary Risk Assessment Institute	Lithuania
	Laboratoire National de Santé	Luxembourg
	Laboratoire de Médecine Vétérinaire de l'Etat	Luxembourg
	Malta Public Health Laboratory	Malta
	Institute of Marine Research	Norway
	Norwegian Veterinary Institute	Norway
	National Institute of Public Health – National Institute of Hygiene (NIPH – NIH)	Poland
	Instituto Nacional de Investigação Agrária e Veterinária, I.P.	Portugal
	Faculty of veterinary medicine-Skopje, Food institute	Republic of Macedonia
	Hygiene and Veterinay Public Health Institute	Romania
	State Veterinary and Food Institute, VFI in Dolny Kubin	Slovakia
	National Reference Center of Environmental Microbiology, PHA of the Slovak Republic, Bratislava	Slovakia
	Veterinary Faculty / National Veterinary Institute, Gerbiceva 60, SI-1000 Ljubljana, Slovenia	Slovenia
	Centro Nacional de Alimentación -AECOSAN	Spain
	National food agency, Sweden	Sweden
	Agroscope	Switzerland
	RIVM	The Netherlands
	Netherlands Food and Consumer Product Safety Authority (NVWA)	The Netherlands
	Public Health England	United Kingdom
Coagulase Positive *Staphylococci* (CPS)	AGES Graz	Austria
	Foodborne Pathogens	Belgium
	NRL Salmonella, campylobacter, staphylococci and antimicrobial resistance	Bulgaria
	Laboratory for Food Microbiology CVI Zagreb	Croatia
	Croatian National Institute of Public Health	Croatia
	State General Laboratory, Food Microbiology Laboratory	Cyprus
	Laboratory for the Control of Food of Animal Origin (LCFAO), Cyprus Veterinary Services	Cyprus
	State veterinary institute Olomouc	Czech republic
	Veterinary and Food Laboratory	Estonia

	Finnish Food Safety Authority Evira	Finland
	Anses	France
	German Federal Institute for Risk Assessment, NRL Staph	Germany
	'Departement of Food Hygiene of Athens	Greece
	National Food Chain safety Office	Hungary
	Dairy Sceince Laboratory	Ireland
	IT-NRL	Italia
	Institute of Food Safety, Animal Health and Environment "BIOR"	Latvia
	National Food and Veterinary Risk Assessment Institute	Lithuania
	Malta Public Health Laboratory	Malta
	Norwegian Veterinary Institute	Norway
	National Institute of Public Health – National Institute of Hygiene (NIPH – NIH)	Poland
	National Veterinary Research Institute, Poland - NRL for Staphylococci	Poland
	Instituto Nacional de Investigação Veterinária, I.P.	Portugal
	Faculty of veterinary medicine-Skopje, Food institute	Republic of Macedonia
	Institute for Hygiene and Veterinary Public Health	Romania
	National Reference Center of Environmental Microbiology, PHA of the Slovak Republic	Slovakia
	State Veterinary and Food Institute, VFI in Dolny Kubin	Slovakia
	National Veterinary Institute, Unit for food safety, Ljubljana	Slovenia
	Centro Nacional Alimentación-AECOSAN	Spain
	National Food Agency	Sweden
	Agroscope	Switzerland
	LABOR SPIEZ	Switzerland
	Nvwa	The Netherlands
	RIVM	The Netherlands
	Public Health England	United Kingdom
Foodborne Viruses	Sciensano	Belgium
	NRL for Listeria monocytogenes, E. coli and contaminants in bivalve	Bulgaria
	State Veterinary Institute Jihlava	Czech Republic
	National Food Institute, Technical University of Denmark	Denmark
	Finnish Food Authority	Finland
	Ifremer, laboratoire de Microbiologie	France
	Service commun des laboratoires 205, rue de la croix verte 34196 Montpellier Cedex 5	France
	German Federal Institute of Risk assessment (BfR)	Germany
	Department of Food Hygiene of Athens{NRL Greece}	Greece
	Marine Institute	Ireland
	Dairy Science Laboratory, Backweston Campus, Celbridge, Co. Kildare	Ireland
	Istituto Superiore di Sanità	Italy
	Institute of Food safety, Animal Health and Environment "BIOR", NRL-Latvia	Latvia

	Norwegian University of Life Sciences	Norway
	Department of Food and Environmental Virology, National Veterinary Research Institute	Poland
	Institute of Meat Hygiene and Technology	Serbia
	State veterinary and food institute - VFI in Dolny Kubin	Slovakia
	Centro Nacional de Alimentación	Spain
	National Food Agency	Sweden
	Federal Food Safety and Veterinary Office FSVO	Switzerland
	Centre for Environment, Fisheries & Aquaculture Science	United Kingdom