Update from the Inter EURL working group on NGS

INTER EURLs WORKING GROUP ON NGS

AIM

to promote the use of WGS across the EURLs' networks, build WGS capacity within the EU and ensure liaison with the work of the EURLs and the work of EFSA and ECDC on the WGS mandate sent by the Commission

from
November 2017

Meeting twice a year
(12 meetings done)

Inter EURLs WG webpages

- **EURL *E. coli*:**

  https://www.iss.it/en/web/guest/-/inter-european-union-reference-laboratories-working-group-on-next-generation-sequencing-1

- EURL *Salmonella*

- EURL *Campylobacter*

- EURL AMR

- EURL *Listeria*

- EURL Coagulase Positive *Staphylococci*

- EURL Parasites

- EURL Foodborne Viruses

Direct link!

⭐ Overview of conducted and planned PTs – curated by EURL Antimicrobial Resistance

⭐ Reference Whole Genome Sequencing collection – curated by EURL *Salmonella*

Guidance document for WGS-laboratory procedures – curated by EURL Parasites

⭐ Bioinformatics tools for basic analysis of Next Generation Sequencing data – curated by EURL VTEC

Guidance document for Whole Genome Sequencing - cluster analysis – curated by EURL *Campylobacter*

Guidance document for NGS-Benchmarking – curated by EURL *Listeria*

⭐ Inventory of training supports – curated by EURL Coagulase Positive *Staphylococci*

Survey on the use of NGS across the NRLs networks – curated by EURL VTEC

⭐ Supporting document for preparing high quality DNA for Whole Genome Sequencing – curated by EURL VTEC

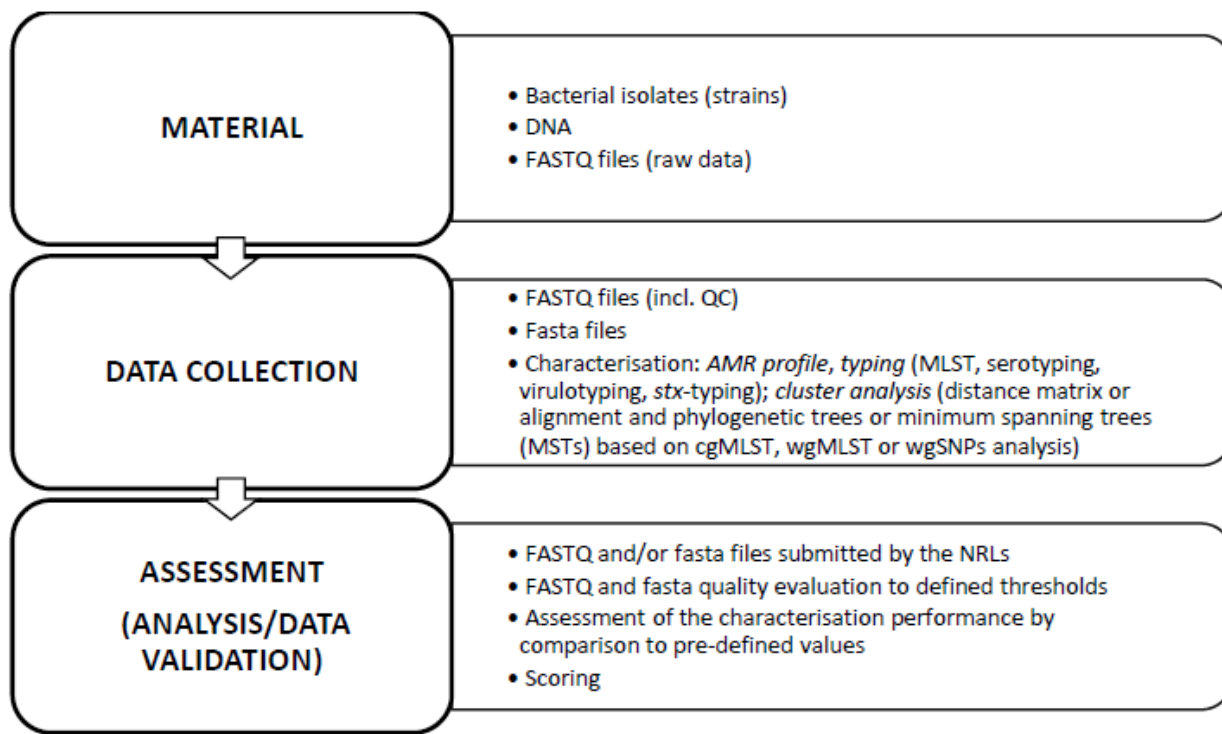# Overview of Conducted and Planned Proficiency Tests

curated by EURL AMR

Updated version

Approaches and

strategies for NGS PTs in

use at EURLs for:

- AMR
- *Campylobacter*
- *E. coli*
- *L. monocytogenes*
- *Salmonella*

MATERIAL
- Bacterial isolates (strains)
- DNA
- FASTQ files (raw data)

DATA COLLECTION
- FASTQ files (incl. QC)
- Fasta files
- Characterisation: *AMR profile, typing* (MLST, serotyping, virulotyping, *stx*-typing); *cluster analysis* (distance matrix or alignment and phylogenetic trees or minimum spanning trees (MSTs) based on cgMLST, wgMLST or wgSNPs analysis)

ASSESSMENT (ANALYSIS/DATA VALIDATION)
- FASTQ and/or fasta files submitted by the NRLs
- FASTQ and fasta quality evaluation to defined thresholds
- Assessment of the characterisation performance by comparison to pre-defined values
- Scoring

Updated reference WGS collection

curated by EURL *Salmonella*

Including:

- *E. coli*

- *Salmonella*

- *Campylobacter*

- *Listeria monocytogenes*

- AMR relevant strains

e.g. E. coli: Multiple sequences of the same six **O26 STEC strains** obtained during the 1st PT on WGS (anonymzed)

E. coli sequences available upon direct request to:

crl.vtec@iss.it

Bioinformatics tools for basic analysis of NGS data

curated by EURL *E. coli*

List of **tools routinely used** by the majority of NRLs and by EURLs for NGS data analysis

Brief description of analytical steps for basic NGS analysis:

- Quality check
- Trimming

- Assembly
- Seven genes MLST

- Virulotyping
- Serotype identification
- Inference on AMR

Open source, commercial software, webservers

EFSA One Health WGS System and analytical pipeline:
not only accessible for the officially appointed users through the EFSA One Health WGS System Portal, but **also available for download and setup through a dedicated reposito**ry (linked in the document)

Supporting document for DNA preparation and quality check

curated by EURL *E. coli*

Following a request made by EFSA in the framework of the Joint EFSA-ECDC Steering Committing on Molecular Typing Data collection

AIM:

supporting the laboratories in the first steps of the NGS protocol, starting from the experiences on *E. coli, Listeria* and *Salmonella*, representing the first three pathogens for which WGS data collection has started

Suggestions for:

DNA extraction (isolated colonies; control of genetic features; plasmids; DNA manipulation; elution buffers)
DNA quality check
DNA concentration

Inventory of training supports

curated by EURL CPS

Updated with links to new resources

Training programs available within EURLs

Guidance for training organization:

- Wet-lab: DNA extraction; Quality control of DNA; Library preparation; Sequencing; Quality control of raw data and assembly.

- Dry-lab: Annotation; Cluster analysis; Gene detection

2nd joint training – June 20th-21st 2023

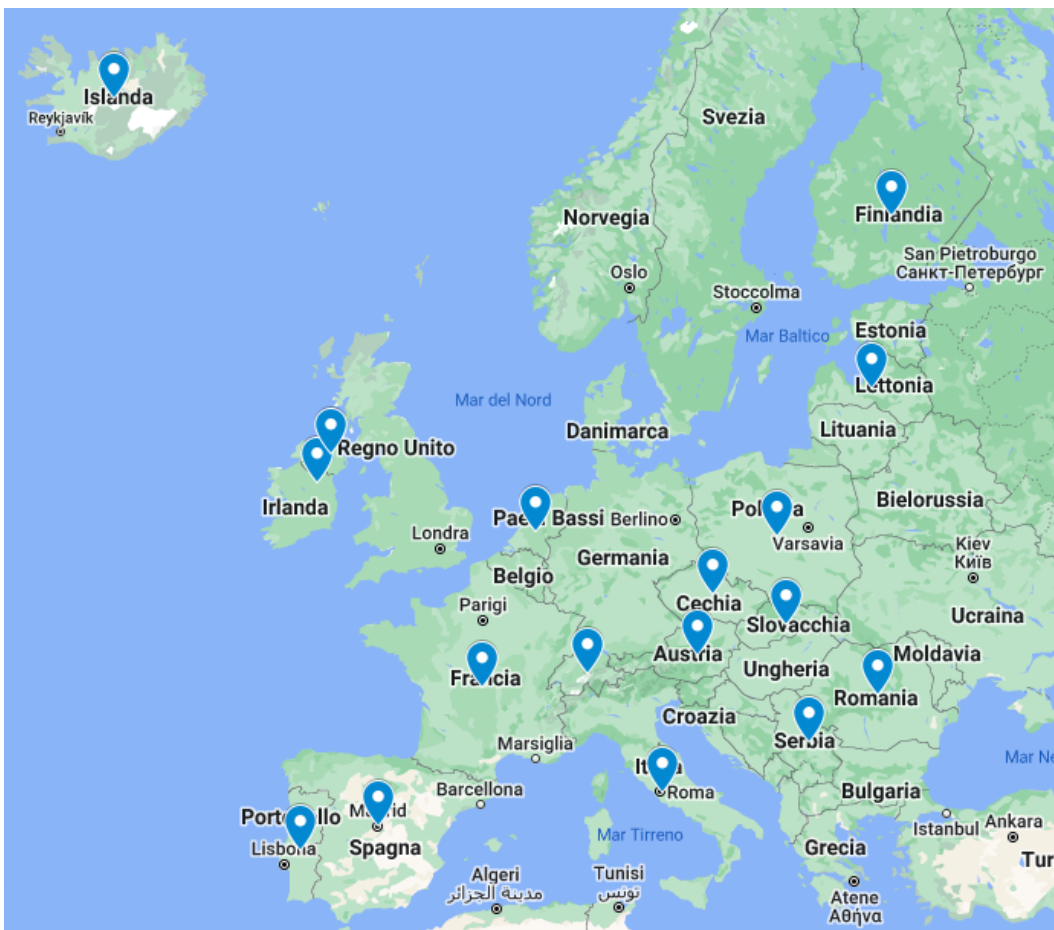## Introduction to Bioinformatics for genomic data mining

TUESDAY 20 JUNE 2023

9.15	**Registration (for entering the premises of RIVM)**
9.45	Welcome and general overview on the joint training activities (Wilma Jacobs, EURL-*Salmonella*)
10.00	Introduction to WGS
	1. Sequencing platforms & output data (20') (Adrien Asséré, EURL-*Listeria monocytogenes*)
	2. Verification of the integrity of the raw data files (like md5sum) (20') (Angela van Hoek, EURL-*Salmonella*)
	3. Bioinformatics analysis of NGS data: approaches and opportunities (command-line tools, commercial software, webservers) (20') (Joakim Skarin, EURL-Viruses)
11.00	**Coffee break**
11.20	Introduction on quality check and trimming (Valeria Michelacci, EURL-VTEC)
11.40	**Hands-on exercises – Quality check and trimming** Quality check and trimming using different tools and platforms. Results interpretation. *FastQC and Positional and Quality Trimming on ARIES (Valeria Michelacci, EURL-VTEC)* *Quality check and trimming with Seqsphere (Bo Segerman, EURL-Campylobacter)*
12.45	**Lunch break**
13.45	Introduction on assembly and assembly statistics (Lauge Holm Sørensen, EURL-AMR)
14.00	**Hands-on exercises – Assembly statistics** Assembly statistics using different tools and platforms. Results interpretation. *Hands-on SPAdes and Quast from ARIES (Federica Gigliucci, EURL-VTEC)* *Demo: Seqsphere Assembly Quality Check (Angela van Hoek, EURL-Salmonella)*
15.00	From BAM to BCF and beyond, making sense of aligned data (Paolo Vatta, EURL-Parasites)
15.30	**Hands-on exercises – Mapping approach** Demonstration of mapping through different platforms. *Hands-on E. coli virulotyping using a mapping approach (Federica Gigliucci, EURL-VTEC)* *Demo: Mapping with Seqsphere (Déborah Merda, EURL-Listeria monocytogenes)*

WEDNESDAY 21 JUNE 2023

8.45	**Registration (for entering the premises of RIVM)**
9.15	Introduction to gene detection using BLAST approach (Marina Cavaiuolo, EURL-CPS)
9.30	**Hands-on exercises – Search of genetic features on contigs** Identification of virulence and AMR genes using different tools and platforms. Results interpretation. Demonstration of genes identification on contigs through different platforms. *Hands-on ResFinder on CGE webserver (Lauge Holm Sørensen, EURL-AMR)* *Demo: Salmonella virulotyping with Seqsphere (Angela van Hoek, EURL-Salmonella)*
10.15	Parasites WGS: opportunities and challenges (Simone Cacciò, EURL-Parasites)
10.45	**Coffee break**
11.00	Introduction to genome comparisons: gene-by-gene VS SNPs (Guidance document for cluster analysis) (Bo Segerman, EURL-*Campylobacter*)
11.30	Demonstration of gene-by-gene approach through different platforms: ARIES (EURL-VTEC) Seqsphere (Angela van Hoek, EURL-*Salmonella*) Starflow (Déborah Merda, EURL-*Listeria monocytogenes* and Marina Cavaiuolo, EURL-CPS)
12.15	**Hands-on exercises – Visualisation of clustering data** Demonstration via Grapetree (Déborah Merda, EURL-*Listeria monocytogenes*)
12:45	**Lunch break**
13.45	Update on the EFSA OneHealth WGS database (Mirko Rossi, EFSA)
14:15	Information on activities of inter EURLs Working Group on NGS and guidance documents released (Valeria Michelacci, EURL-VTEC)
14:45	Wrap up (Wilma Jacobs-Reitsma, EURL-*Salmonella*)
15.00	**Closure**

Istituto Superiore di Sanità, Dept. Food Safety, Nutrition and Veterinary Public Health
European Union and National Reference Laboratory for *E. coli*, Rome, Italy

EU-RL VTEC

Participants – geographic distribution

24 participants from different NRLs
4 at own costs
2 supported by EURL VTEC

17 different countries
(13 Member States + 2 EFTA/EEA + Northern Ireland + 1 Candidate Country)

Many participants came from labs appointed as NRLs for multiple pathogens

Position paper published

- Adoption of NGS across NRLs

- Harmonization of procedures

- Proficiency tests

- Benchmarking guidelines

- Training and capacity building

MICROBIAL GENOMICS

European Union Reference Laboratories support the National food, feed and veterinary Reference Laboratories with rolling out whole genome sequencing in Europe

Valeria Michelacci[1,*], Adrien Asséré[2], Simone Cacciò[3], Marina Cavaiuolo[4], Kirsten Mooijman[5], Stefano Morabito[1], Susanne Karlsmose Pedersen[6], Maroua Sayeb[2], Bo Segerman[7], Magnus Simonsson[8], Hanna Skarin[7], Rosangela Tozzoli[1], Angela van Hoek[5] and Rene Sjøgren Hendriksen[6]

Abstract
The Inter European Union Reference Laboratories (EURLs) Working Group on Next Generation Sequencing (NGS) involves eight EURLs for microbiological food and feed hazards and has been working since 2017 to promote the adoption of NGS by the National Reference Laboratories (NRLs) in the European Union. This work illustrates the results of the first 5 years of activity. By working together, the EURLs involved have released guidance documents for assisting NRLs in all the steps of NGS, helping the transition from classical molecular methods towards whole genome sequencing while ensuring harmonization, with the final aim of improving preparedness in the use of NGS to characterize microbial hazards and trace the sources of infection.

Published on July 25th 2023

**Istituto Superiore di Sanità, Dept. Food Safety, Nutrition and Veterinary Public Health
European Union and National Reference Laboratory for *E. coli*, Rome, Italy**

EU-RL VTEC

Webinar dedicated to NGS PTs

Held on September 29th 2023 through Microsoft Teams

Proficiency Tests on Next Generation Sequencing: approaches in use at the European Union Reference Laboratories

Friday September 29th 2023, 10 am - 12 am

Online through Microsoft Teams

Organised by the Inter EURLs WG on NGS, involving:
EURL-AMR
EURL-*Campylobacter*
EURL-Coagulase Positive Staphylococci (CPS)
EURL-Foodborne viruses
EURL-*Listeria monocytogenes*
EURL-Parasites
EURL-*Salmonella*
EURL-VTEC

Presentations will be published on EURL *E. coli* website and mirrored in all the websites of the other EURLs

Webinar on NGS PTs participants - Geography

178 participants
29 Countries

22 EU Member States
3 EEA+ EFTA
2 EU Candidates
2 Extra EU

1 EC
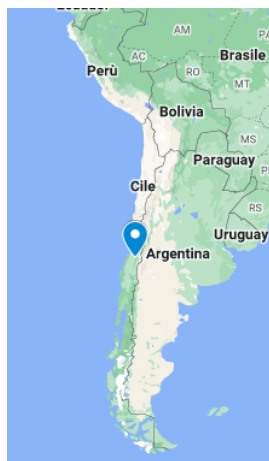
Webinar on NGS PTs participants - Networks

EURLs networks
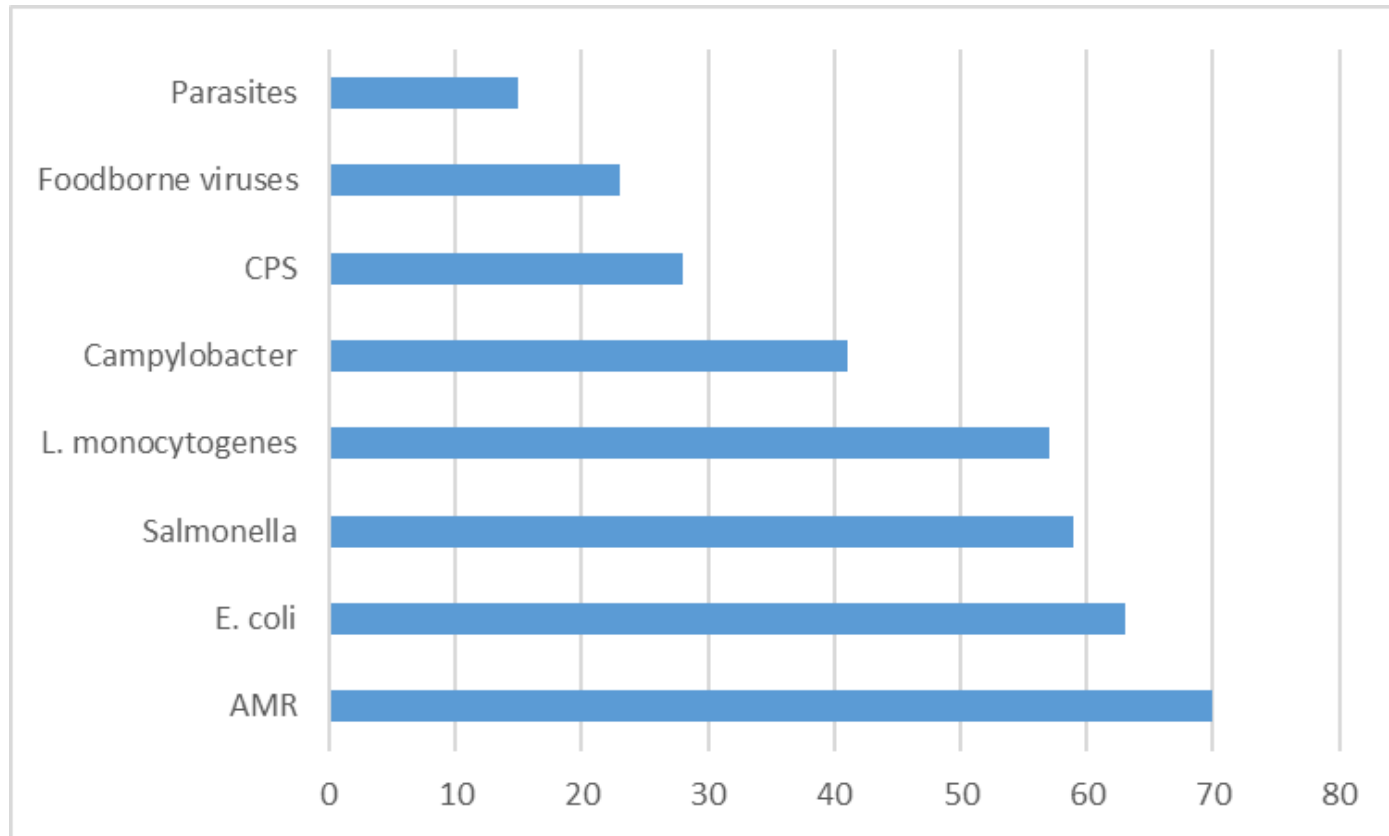
Many participants from labs appointed as NRLs for multiple pathogens

"Science Meets Policy" conference

- First edition: virtual conference, September 2020: over 500 participants from 49 countries worldwide

- Second edition: **"EU initiatives towards the large-scale use of NGS to tackle foodborne threats"** organized by EFSA and the Inter EURLs WG on NGS EFSA, Parma, Italy and online, September 5th-6th 2023

  Recorded videos and presentations available at:

  https://www.efsa.europa.eu/en/events/science-meets-policy-conference-using-next-generation-sequencing-tackle-foodborne-threats

REGISTRANTS
- 844 registrants whose:
  - 155 in-person registrants;
  - 689 online registrants;

PARTICIPANTS
- In-person attendees: 102; No-show rate: 34.19%

- Online attendees:
- Day one peak viewers: 257;
- Day 2 peak viewers: 177;

98% of Survey participants declared that their event experience was **excellent (51%) or good (44%)**
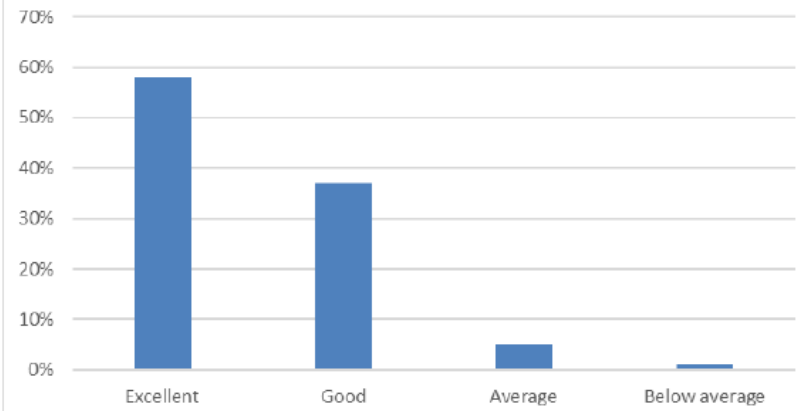
FORMAT AND TOOL

how would you rate your event experience?

HOW DID YOU ATTEND THE MEETING?

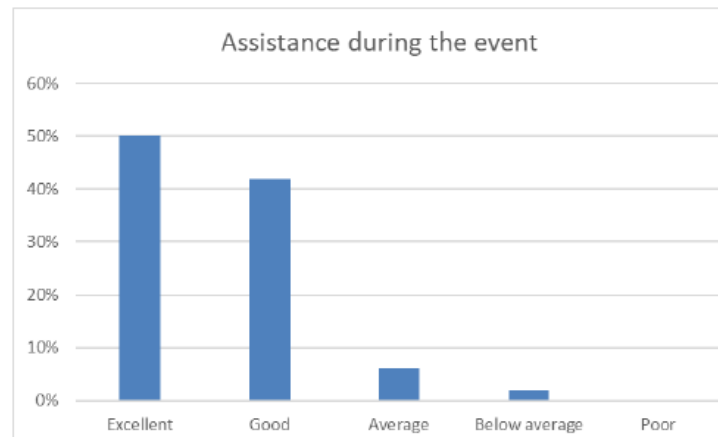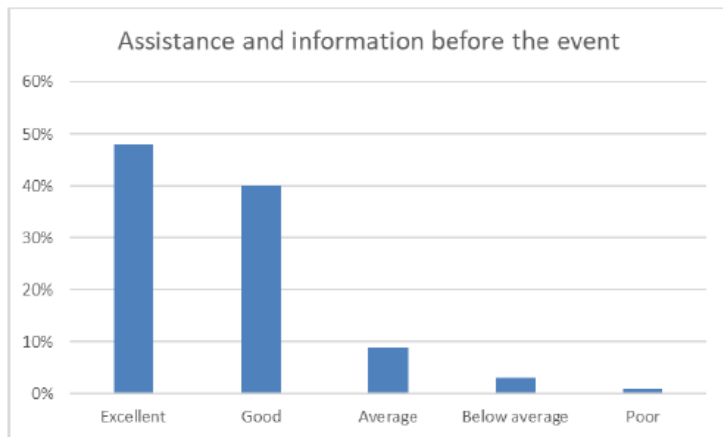■ In-person ■ Online

24%

76%

Audio quality

Video quality

5
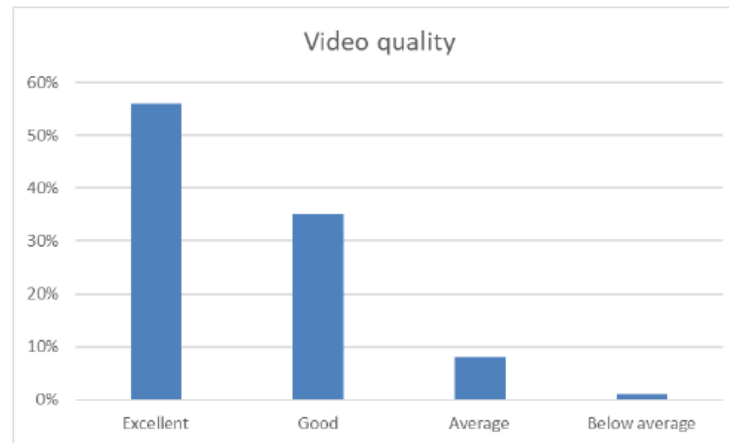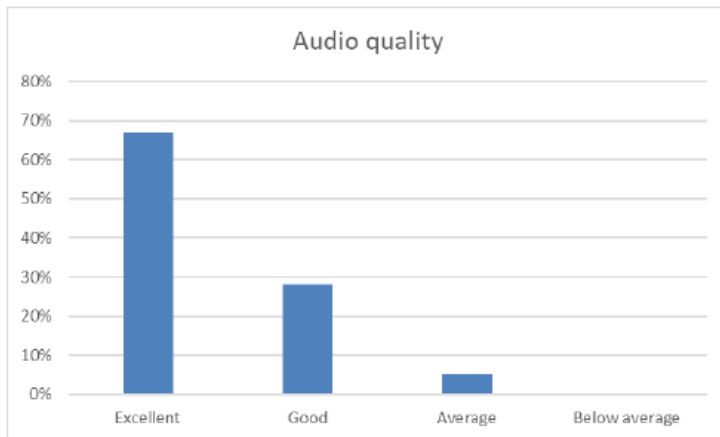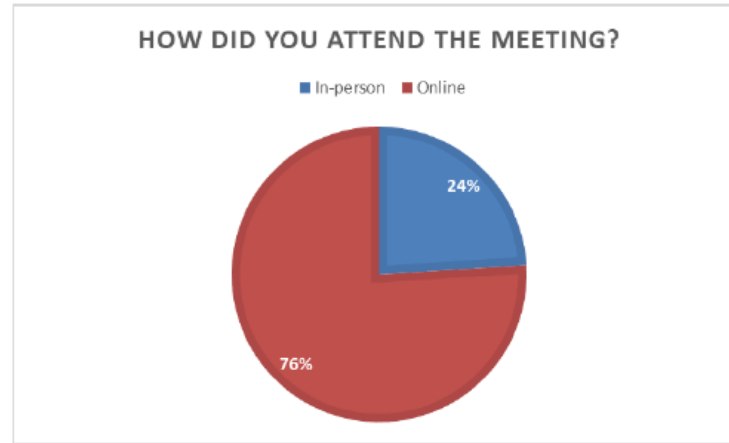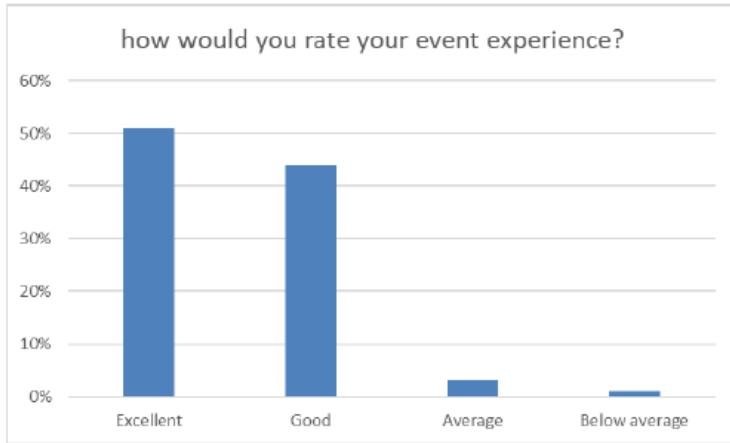
TOPICS AND AGENDA

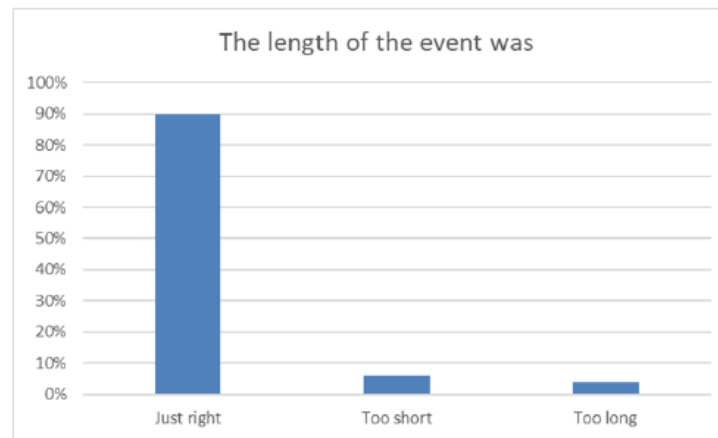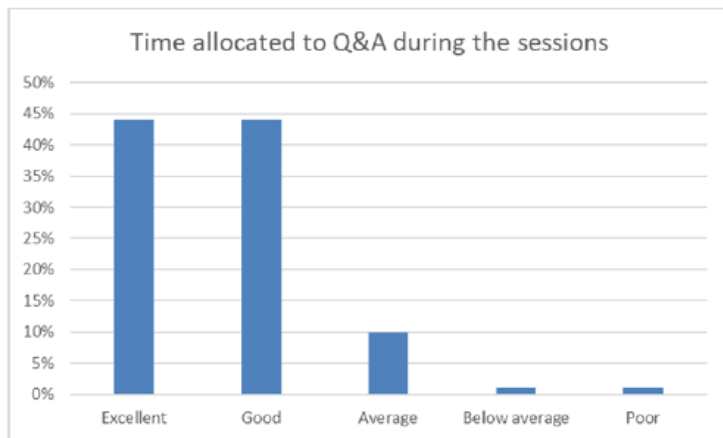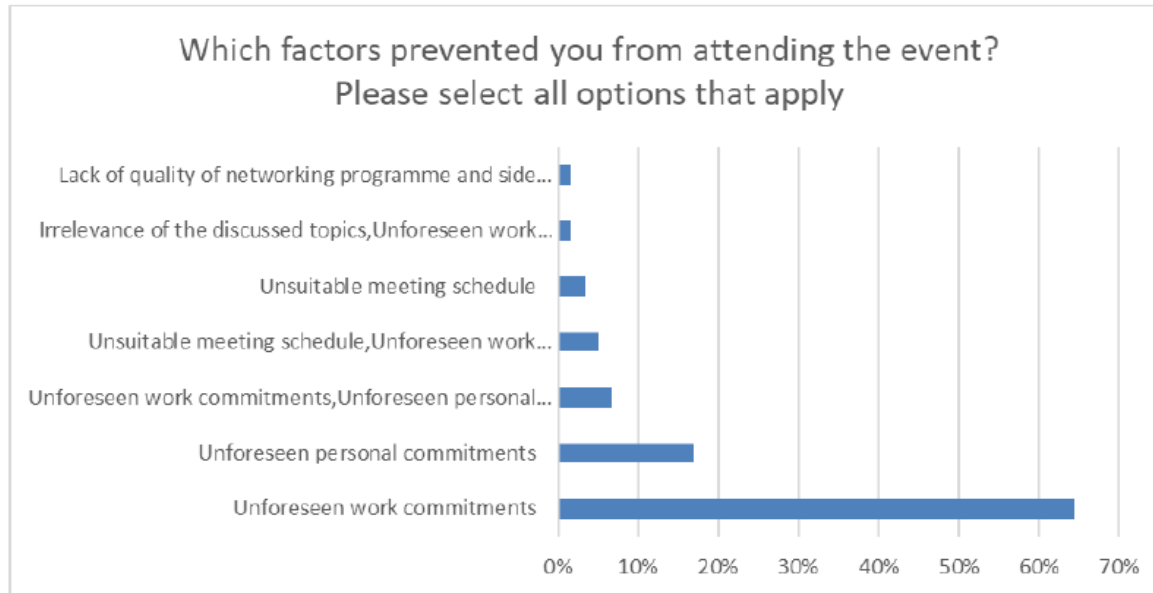Overall content of the agenda
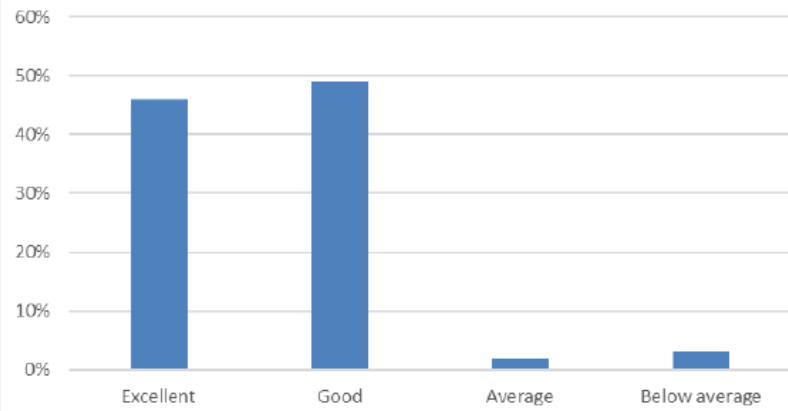
Relevance of the topics addressed

LEVEL OF SATISFACTION REGARDING THE EVENT

9

LEVEL OF SATISFACTION

Count of Meeting rooms and venue facilities

Total

Meeting rooms and venue facilities ▾

Catering services

33%   38%

29%

■ Excellent   ■ Good   ■ Average

Which factors prevented you from attending the event?
Please select all options that apply

11

Questions?

Suggestions?