D.1.1 – Report – Mapping of existing and proposals for new PT schemes

Workpackage WP1-T1

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MAPPING OF EXISTING AND PROPOSALS FOR NEW PT SCHEMES

Mapping of existing and proposals for new Proficiency Tests / External Quality Assurance schemes

1. Introduction

1.1. Background

The objective of EJP-CARE is to enhance collaboration between the public health, food and animal health sectors across the European Union (EU), in order to increase joint preparedness, in relation to bacterial zoonotic (especially foodborne) infections as mentioned in the priority topics. The project will be setting standards for quality assurance testing that will strengthen already existing systems for proficiency testing, reference material and quality/availability of demographic data.
In the EU, networks exist of European and national reference labs for various microorganisms. EC DG-SANTE funds the European reference laboratories serving the food and veterinary sector , whereas the European Centre for Disease Prevention and Control (ECDC) supports the established network of laboratories within the European Food- and Waterborne Diseases and Zoonoses Network (FWD-Net). ECDC does not currently have reference laboratories but conduct different reference laboratory activities, e.g. different quality assurance schemes, using subcontractors. The “non-human” European Reference Laboratories (EURLs) and the “human” ECDC FWD network of laboratories are coordinating activities at different levels due to the individual specific terms of references. One of the objectives of these networks is to ensure harmonization throughout the EU in the capacity for detection and characterization of food borne microorganisms. Collaboration between veterinary, food, and public health laboratories and the need for exchange of data is increasing, and therefore calls for systems that can ensure the comparability of results. To facilitate the comparability of results, a One Health system of quality assurance testing, preferably in the form of Proficiency Tests (PTs) or External Quality Assurance schemes (EQAs) (see Intermezzo), to be used cross sectorial would help.

1.2. Approach

Before microorganisms can be identified/characterized, they have to be detected and isolated. Although the approaches used for the analysis of samples in the different sectors are different, the end point is to detect and isolate microorganisms. For detection and isolation of pathogenic microorganisms in samples from the food chain mostly validated internationally standardized (CEN/ISO) methods are used, thus ensuring harmonization within the food sector. For the animal health sector, the World Organization for Animal Health (OIE) has issued reference methods, but for the public health sector there are currently no reference methods in place at the EU level.

Most important common feature for all three sectors is the result: one or more strains to be characterized. At the stage of identification/characterization differences between the domains no longer exist. This is schematically depicted in Figure 1.

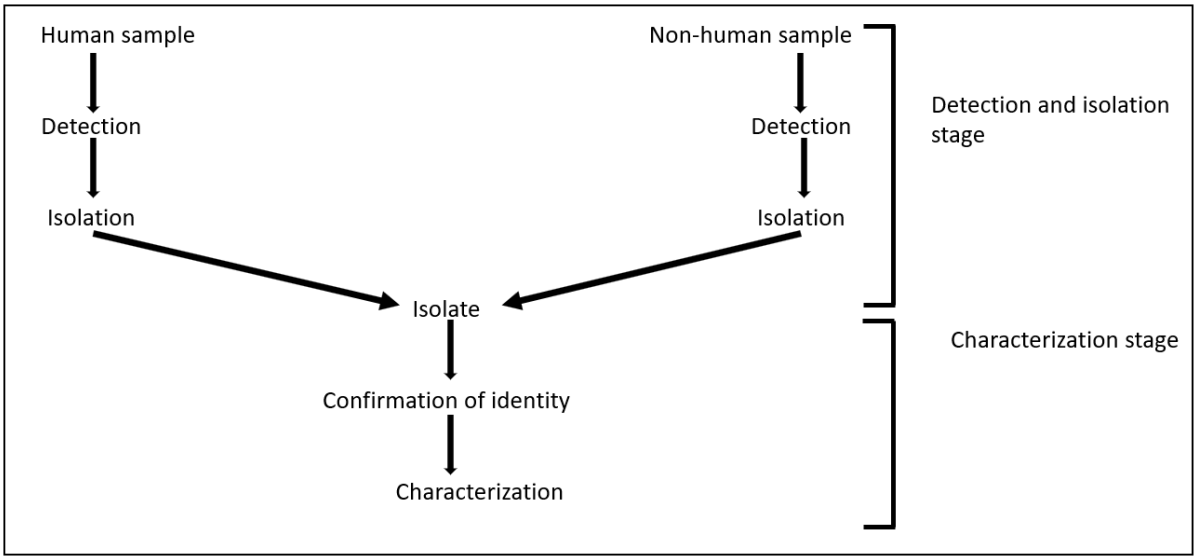

Figure 1. Detection and characterization of microorganisms from human and non-human samples.

For decades, phenotypic characteristics have been used for identification/characterization of microorganisms. In the recent years the focus in identification/characterization methods has shifted towards molecular techniques, such as Multi Locus Sequence Typing (MLST), Pulsed Field Gel Electrophoresis (PFGE) and Multi Locus Variable number of tandem repeats Analysis (MLVA). Nowadays, laboratories switch more and more to Next Generation (NGS)/Whole Genome Sequencing (WGS) techniques, as these approach have rapidly expanded the possibilities for discriminating or confirming identity of strains.

Standardization and quality control of earlier molecular techniques (MLST, PFGE, MLVA etc.) is/was quite well organized by the ECDC network and the EURLs. Standardization and quality control of NGS/WGS techniques is being developed, but is as yet still far from ideal. Reasons for this may be the rapid changing range of machinery/platforms used for NGS/WGS, and the improvement in *in-silico* techniques that can be carried out for characterizing microorganisms. Many steps are involved from an isolate to a full identification/characterization of the isolate. Also, in case of comparison of isolates, many steps are involved to conclude that the characteristics of strains, isolated in different sectors, are identical or not. Since NGS/WGS techniques are rapidly developing, it makes sense to rather evaluate results of analyses than to evaluate methods that are being used. Questions that could be raised are: can you identify a clonal cluster, which generic criteria did you use to call the cluster: MLST schemes, SNPs etc., and how did you define which bacteria should be included in the cluster. Similar questions could be raised for detection of virulence or toxins, and antimicrobial resistance (AMR) gene detection

For quality assurance schemes throughout both sectors there is a general need for OneHealth PT/EQA-schemes and reference materials directed towards NGS/WGS techniques throughout the EU.

1.3. Aim of the work

Proficiency testing (PT) schemes or External Quality Assurance (EQA) schemes is an integrated part of quality assurance management for laboratories within the field of human and veterinary medical microbiology, and in microbiology of the food chain. The aim of this Work Package of EJP-CARE is to develop guidance for proficiency testing schemes that can be used cross-sectorial. This will be done by mapping the currently available PTs/EQAs and assess the usefulness of these schemes in a cross-sectorial context and by developing proposals for new PT/EQA schemes that can accommodate unmet and new needs.

The rapid emergence of WGS methodologies for characterization of pathogens including analytical approaches that can predict virulence factors, antimicrobial resistance, clonal relationship, and identify food- and water borne outbreaks is an area where there is a need for new cross-sectorial PT/EQA schemes. Likewise, in the area of molecular based methodologies, including WGS, for culture-free detection/identification of pathogens in complex sample material new PT/EQA schemes are needed.

In order to facilitate the development of the new PT/EQA proposals this Work Package (WP) will include a number of well-designed pilot PTs/EQAs that will be performed among the WP participants. The WP will be concluded by developing a final guidance document giving specific recommendations in relation to which PTs/EQAs that should be prioritized and containing specific guidance on how to perform the proposed cross-sectorial PTs/EQAs.

The WP will be divided into three parts (tasks):

- Mapping of existing quality assurance schemes and proposals for new PT/EQA schemes
- Pilot trials (organized as sub-tasks) and documentation of outcome
- Development of guidance document and proposals for Standard Operating Procedures (SOPs) where appropriate with suggestions for design of future cross-sectorial PT schemes

Task 1 of this WP 1 aims at preparing an inventory of existing PT/EQA schemes, to discern the possible gaps, and to propose new PTs/EQAs for cross-sectorial use.

For this the focus will be on PT/EQA schemes for identification/characterization: what is currently available, what is planned and what is necessary in the near future.

Since in this EJP-CARE project the focus is on cross-sectorial quality assurance, the inventory for Task 1 is set, as explained before, at the lower part of Figure 1, the characterization of isolates.

2. Intermezzo

For testing quality assurance of laboratory data several scheme types are available. The different testing systems are shown below.

Quality assurance testing

Scheme	Utility	Organisation	Strengths	Limitations
Internal quality assurance (IQA)	Routine inter assay assessment of test performance	Internal provider	Cost effective Routine test performance evaluation	Data limited to in house. Not blind
External quality assurance (EQA)	Routine external inter assay assessment of test performance	Internal (e.g. different labs same institute) or external provider	External/ internal assessment of test performance. Routine test performance evaluation	Target values may be known May or may not be blind
Ring trial (RT)	Inter laboratory assessment often for development of new tests or unusual samples	External provider (e.g. EU-RL)	Provides comparison between external laboratories. Blind samples may be assessed.	Not compliant with ISO standard Often a one-off distribution
Proficiency testing (PT)	On-going periodic assessment of laboratory inter assay test performance	External provider	Run to an ISO standard (e.g. 17043). Enables comparison of data between participants.	Most costly option. Limited number of distributions per year

Copied from Nick Coldham, Animal and Plant Health Agency UK (APHA)

For the task at hand, the most important types of test schemes are External Quality Assurance (EQA) and Proficiency Testing (PT)

3. Availability of PT-schemes for (molecular) characterization:

In the EU, various organizations are responsible for the different sectors (public health, food and animal health). Each of these organizations funds or organizes PTs/EQAs.
Next to these EU organizations there is a number of commercial PT/EQA providers for food-borne and zoonotic microorganisms.
In the following sections, PTs organized for (molecular) characterization, and organized by one of the above mentioned organizations are listed. For this overview, only PTs are mentioned that somehow deal with characterization of microorganisms.

1) PTs/EQAs organized by SSI (Denmark) funded by ECDC (1)
Laboratories for which PTs/EQAs are intended: 'public health reference' laboratories of ECDC- Food- and Waterborne Diseases and Zoonoses (FWD) network

Scheme	Frequency	Name	Analysis	Isolates for WGS	Downloaded sequence
EQA	1/year	Typing of Salmonella	Cluster analysis	10	< 5
EQA	1/year	Listeria	serotyping/cluster analysis	10 + 10	< 5
EQA	1/year	Typing of STEC	Serotyping/virulence Cluster analysis	20	5
EQA	1/year	AST	AMR WGS-based	8 Salmonella 5 Campylobacter	
ECDC	2018	Listeria	assembly of raw reads By pipeline of choice	15 sets	dry work

2) PTs/EQAs organized by the European Reference Laboratories
Laboratories for which PTs/EQAs are intended: 'non-human' National Reference Laboratories (NRLs) of network of relevant EURL

a) EURL-*Listeria*
- Has organized in 2017: PT on MLST typing
- Has organized in 2018 PT on WGS cluster analysis by SNP or cg-MLST
- Will organize in 2019 and 2020 PT on typing, method free of choice (molecular serotyping, PFGE, MLLST, WGS). Participants will indicate which method they applied.

b) EURL-*E. coli* (2,3)
- Since 2008, has organized approximately once a year PTs for identification and typing of pathogenic *E. coli*, mainly STEC, focusing on serotyping and virulence genes identification.
- In 2010 and 2012 has organized joint studies with the WHO Collaborating Centre for Reference and Research on Escherichia and Klebsiella, Statens Serum Institute, Copenhagen (SSI), in charge for the EQA program for the ECDC network of the medical NRLs for VTEC referring to the ECDC Food- and Waterborne Diseases and Zoonoses Surveillance Program. The aim of such a liaison was the harmonization of the typing methods used by both the NRL networks, to make the respective monitoring programs and databases compatible for comparison of data referring to human and non-human isolates of VTEC. The two studies mainly targeted virulotyping and serotyping of pathogenic *E. coli*. (2, 3). Despite these studies were performed with the cross-sectorial

approach, no joint report was produced and results were compared among the networks at the annual EURL workshop.
- Six PTs on molecular typing through PFGE were conducted since 2012.
- Has organized in 2017 PT on DNA-extraction and NGS (in house methods collaborating labs (4). Output: raw (Fastq files).
  EURL used in-house pipeline for quality check/trimming/assembly/assembly statistics/MLST/sero- and virulotyping/phylogenetic analysis.
  Aim 1: evaluation of quality parameters of the sequences and their effect on characterization of STEC by WGS.
  Aim 2: evaluate interlab and platform variability in SNPs and allelic differences.
- Has performed in 2018 (5) and in 2019 PTs on identification and typing of STECs and other types of E. coli. Method free of choice. If performed WGS results could be reported instead of conventional methods. A similar approach will be used in 2020 and 2021.
  Aims: 10 correct identification of strains, and 2) correct identification of cluster of strains based on WGS, included from 2019.

c) EURL-*Campylobacter*
- In 2019 and in 2020 a (voluntary) PT on subtyping *C. jejuni* by sending out pre-extracted DNA to sequence by any method.
  Method: report MLST-types and do cluster analysis/submit raw data/assemblies/cluster images (in case WGS is performed)
  Aim: identify differences in methods and the extent of deviations in results.
- In 2020 organized a PT to assess performance of DNA-extraction and WGS. Submission of lyophilized *C. jejuni* isolates and DNA extracts. Evaluation against reference genome and quality parameters.

d) EURL-*Salmonella*
- Organizes annually a PT on serotyping of *Salmonella*.
- 2013-2018: included also PFGE typing in the PTs on serotyping of *Salmonella*.
- 2019 (and onward) included also cluster analysis (molecular method free of choice) in the PTs on serotyping of *Salmonella*.

e) EURL-Antimicrobial Resistance
- In 2020, the EURL AR has planned to organize a genomic PT assessing genomic metrics, characterization and typing of bacterial cultures and prediction of phenotypic AMR based on cultures and pre-extracted DNA of PH relevant bacterial species including E.coli, *Salmonella*, *Campylobacter*.
  Aim: evaluate consistency and robustness of labs to perform DNA-extraction/library preparation/WGS. Own choice of protocols/software/sequencing platforms, assessed by coverage/reads/size compared to closed ref genome/phred score/insert size/sequencing depth/phylogeny/prediction of MLST/AMR genes.

f) EURL-Parasites
- No plans for PTs on molecular characterization in the coming years due to lack of NGS activities in most of the networks NRLs.

Things to bear in mind (upon recommendations of the WG NGS EURLs)
- Identify synergies spanning more than one EURL network and coordinate with ECDC
- Make submission of data and sequences as simple as possible for both supplier and participant

3) European PT/EQA Providers as mentioned on website of Eptis from the Bundesamt für Materialforschung and -prüfung (6)

Initial search criteria: Microbiology, Food + Drink, identification

NB 1. All PTs/EQAs mentioned in this section are wet PTs/EQAs

NB 2. Most PTs/EQAs in this group were for detection and/or enumeration only. The PTs/EQAs mentioned here also bear an identification component. Although identification is mostly carried out with traditional microbiological methods, the use of molecular methods is not ruled out or excluded.

LGC Standards UK	- confirmation and identification of microorganisms - panels of 5 cultures of single (bacterial) species/group - mainly meant for traditional methods
Public Health England	- Shiga Toxin producing *E. coli* (STEC) scheme - Detection of virulence genes in top 6 O-types
Public Health England	- *Staphylococcus aureus* enterotoxin Food Scheme - detection and/or identification enterotoxins - various methods
LGC Standards UK	- STEC scheme - two matrices (powdered beef, milk powder) - detection of STEC - identification of O-type
APHA[1] Vetqas (UK)	- various matrices - various microorganisms - isolation and identification
Swedish Food Agency	- a panel of lyophilized cultures - detection and/or enumeration of indicator organisms or food-borne pathogens

4) Various PTs/EQAs coordinated by DTU, Gen-Epi

a) Identification (characterization) of *Salmonella*, *E. coli*, and *S. aureus* (2014), pilot PT
Bacterial strains and corresponding DNA as investigation material for identification/characterization (wet and dry work)

b) Identification (characterization) of *Salmonella*, *E. coli*, and *S. aureus* (2015)
Use of raw sequence files for identification/characterization (only dry work)

c) Production of lab results with good quality by WGS (2016). Carried out under auspices of EFSA (ENGAGE).
Bacterial strains of *Campylobacter*, *Listeria*, and *Klebsiella* plus their DNA (wet work)

d) Assessment of differences between labs in analysis of WGS data from Campylobacter (2016)
Analysis of three datasets with current protocol used in each lab (dry work)

e) As d) with datasets for *Salmonella*, *E. coli*, and *S. aureus* (2017)
(dry work)

f) As c). (2017). Carried out under auspices of EFSA (ENGAGE).

5) Miscellaneous PTs

a) 2015 - the Food and Drug Administration (FDA) – PT for foodborne pathogen surveillance.
Culture isolates from *Salmonella* Heidelberg and *S.* Montevideo, *E. coli*, *Listeria monocytogenes*, *Shigella sonnei*, *Campylobacter coli* and *C. jejuni*

b) National Veterinary Institute (SVA) - PT of detection of *Salmonella* in a matrix of animal feces (wet work)

[1] Animal and Plant Health Agency

4. Summary

The rapid emergence of whole genome DNA sequencing (WGS) methodologies for characterization of pathogens including analytical approaches that can predict virulence factors, antimicrobial resistance, clonal relationship, and identify food- and water borne warrant the need for new cross-sectorial quality assurance schemes to be used in the veterinary, food and public health sectors. Likewise, in the area of molecular based methodologies, including WGS, for culture-free detection/identification of pathogens in complex sample material new quality assurance schemes are needed.

This inventory on existing PT/EQA schemes, carried out within the framework of Task 1 of Work Package 1 of this EJP-CARE project, shows that in Europe many PT/EQA-schemes for food borne pathogens are available. Most of these schemes, however, focus on isolation, detection and enumeration, and not on detailed characterization/(sub)typing of isolates.

Even though ECDC, several EURLs, and other institutes provide PT/EQA schemes meant to evaluate molecular methods for characterization/(sub)typing of isolates, most of these PTs/EQAs are either intended for the public health laboratories (ECDC) or for the non-public health laboratories (EURLs). No indication was found that PTs/EQAs currently exist that are intended for laboratories from each of the veterinary, food or public health sector as meant in the OneHealth principle.

5. Conclusion

Various molecular methods for comparing microorganisms isolated from different samples (human and non-human), are increasingly used to (sub)type the isolates. Nowadays, WGS/NGS has become the method of choice to perform this (sub)typing. To be able to compare the outcomes of the (sub)typing performed by the different laboratories, for example within the framework of outbreak investigation, it is important to know that the typing techniques are performed at equal quality in the different laboratories. For this reason quality Proficiency Tests/External Quality Assurance schemes, in which the different organizations participate, are invaluable. Only then isolates can be compared making it possible to identify possible sources of an outbreaks. Better comparison of results also leads to improved collaboration between the different sectors, and more harmonization of methodologies.
In addition to participation in quality assurance schemes, also the regular use of (appropriate) reference materials is important to show the quality of the laboratories.

Up until now, several organizations (ECDC, EURLs) have started to conduct PTs/EQAs in the field of WGS/NGS as shown in this inventory. However, these PTs/EQAs are mainly intended for each different network of laboratories, like network of public health FWD laboratories (ECDC), network of NRLs-*E. coli* (EURL-*E. coli*), network of NRLs-*Salmonella* (EURL-*Salmonella*), etc. In the EURLs working group on NGS it was already concluded that cross-sectorial PTs/EQAs for the various EURL networks is highly recommended. But, also from the One Health point of view, cross-sectorial PTs/EQAs for human and non-human laboratories are recommended, in order to be able to compare the performance of the different laboratories when (sub)typing isolates.

From the inventory described in this report can be concluded that none of the existing PTs/EQAs cover all the different sectors. Therefore, new PTs/EQAs have to be developed covering all three sectors involved in One Health. The focus must be on new molecular, preferably WGS-based, methodologies, that are being used cross-sectorial. The areas covered will include both wet and dry laboratory procedures, and include detection of pathogens in primary materials and methods used for characterization of isolates.

Pilot PTs/EQAs organized among the CARE participants will support the development of the new PT/EQA schemes. A further, and novel component, is to develop a PT/EQA scheme where the proficiency in the speed of response to an outbreak is assessed. The outcome will be a guidance document giving specific recommendations in relation to which PTs/EQAs should be prioritized and containing specific guidance on how to perform the proposed cross-sectorial Pts/EQAs.

Designing cross-sectorial PTs/EQAs and carrying out pilot-PTs/EQAs can be done within the set-up of EJP-CARE. For the long term, and continuity of such PTs/EQAs many things still have to be arranged. Like, who will be the lead institute for long term existing PTs/EQAs, where do the strains/DNA come from, and how is ownership of materials and data arranged.
For PTs/EQAs aimed at investigating outbreaks with food-borne pathogens, metadata have to be available as well. Therefore, also thought has to be given to the ownership of metadata, privacy-issues when exchanging metadata between various laboratories.
These questions are not to be answered within the framework of Task 1 of Work package 1 of EJP-CARE, but must certainly be taken into account before the end of this project.

6. References

1. https://www.ecdc.europa.eu/en/all-topics-z/microbiology/external-quality-assessments-eqa
2. http://old.iss.it/binary/vtec/cont/PT6 Report.pdf
3. http://old.iss.it/binary/vtec/cont/PT10_Report.pdf
4. http://old.iss.it/binary/vtec/cont/Report_PT_WGS1_Rev2.pdf
5. http://old.iss.it/binary/vtec/cont/Report_PT23.pdf
6. http://www.eptis.org/index.htm