D3.5 Report on the implementation status of WGS/RT-PCR for outbreak investigation

Work Package 3: Enhancement & Consolidation of WGS- & PCR-based Methods for Public Health Action

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TABLE OF CONTENTS

1. EXECUTIVE SUMMARY

This deliverable D3.5. "*Report on the implementation status of WGS/RT-PCR for outbreak investigation"* describes the degree of implementation of WGS/qPCR for outbreak investigation but also for pathogen surveillance in each of the four HERA2 member countries. The result of this deliverable is a list of pathogens for which fully functional WGS/qPCR assays are either routinely used or planned to be used in the near future but not yet implemented. Tests for pathogen detection based on WGS/qPCR that have not yet been implemented will coincide with the pathogens indicated in the consortium's national needs survey.

2. BACKGROUND INFORMATION

The key priority of the HERA-2 project is the consolidation, sustainable use, and integration of the improved WGS and RT-PCR infrastructures provided by HERA-1 into routine surveillance and outbreak investigation activities beyond SARS-CoV-2 in the EU. To fulfil this key priority, Work Package 3 "Enhancement & Consolidation of WGS- & PCR-based methods for public health action" aims to increase and consolidate the use of qPCR, but especially WGS-based techniques, for the protection of human health.

3. FRAMEWORK AND OBJECTIVES

The main objective of this deliverable was to assess the current status of WGS/qPCR implementation for pathogen detection for outbreak investigation and surveillance among the consortium.

The strategic framework for the integration of molecular techniques such as qPCR and WGS in multi-country outbreak investigation, as well as in European pathogen surveillance developed by the ECDC between 2019-2021, provides the basis for this deliverable. The strategic framework summarises the capacity of EU countries until 2017 to conduct outbreak investigations and genomic surveillance for a number of pathogens, including *L. monocytogenes*, *S. enterica*, STEC, CRE/CPE, antimicrobial resistant *N. gonorrhoea*, multidrug-resistant *M. tuberculosis*, *N. meningitidis* and influenza viruses using molecular typing. Since this official document was published, there is no information regarding the capacity of the EU countries to detect and type pathogens in the context of outbreaks and surveillance. Our list of pathogens present below gives a hint on the current status on this capacity in the four EU countries represented by the members of this HERA2 consortium.

4. RESULTS

4.1. AGES – Austria

a) WGS short-read sequencing

WGS short-read sequencing using Illumina technologies has been established as a standard tool for surveillance and outbreak investigations for the diverse bacterial species; where AGES leads national reference laboratories (NRLs) or reference centres (NRCs). Currently, all bacterial isolates sent to the respective NRL except *Salmonella sp*. and *Campylobacter sp*., are characterized by WGS using SeqSphere+ software with the respective core genome multilocus sequence typing (cgMLST) schemes. Workflows are automated for the most important pathogens. Automation of the wet lab part, sample management, sample tracking and reporting is in progress.
In addition to cgMLST analysis for surveillance and outbreak investigation, additional information like classical MLST, serotypes, antimicrobial resistance genes, virulence genes, toxin genes, plasmids and mobile genetic elements is extracted from the genomes.

b) WGS long-read sequencing

Five MinIons and one GridIon were purchased within HERA 1 from Oxford Nanopore Technologies (ONT). WGS long read sequencing using ONT has been established for SARS-CoV-2 variant sequencing.
For other viruses and diverse bacterial species ONT protocols and bioinformatic pipelines has been established. Currently, extensive testing to establish ONT as an alternative/ or in addition to Ilumina short read sequencing for surveillance and outbreak investigation is ongoing. For bacterial typing ONT is currently used mainly in combination with Illumina short read sequences to obtain complete or nearly complete genomes for specific applications i.e. plasmid and AMR analysis.

c) qPCR

At our institute we have currently the possibility to detect the following pathogens with qPCR:

- Meningococci
- Pneumococci
- *Haemophilus influenzae*
- *Listeria sp.*
- *Streptococcus B*
- *Staphylococcus aureus*
- Norovirus
- Rotavirus
- Sapovirus
- EHEC/STEC
- *Salmonella sp.*
- *C. botulinum*
- *Campylobacter sp.*
- SARS-CoV-2

- *B. pertussis/B. parapertussis*
- *L. pneumophila*
- Enterovirus
- *C. diphtheriae*
- *V. cholerae*
- *M. tuberculosis*
- *N. gonorrhoea*
- *F. tularensis*
- *B. melitensis*
- *B. anthracis*
- *C. difficile*
- *C. burnetti*
- RSV
- Influenza A and B
- *B. pseudomellei*
- *C. trachomatis*
- Commercial panels only used for specific diagnosis: Biofire Respiratory panel (Pneumonia panel plus), Biofire Gastrointestinal Panel, Biofire Joint Infection Panel (including carbapenemases)

4.2. NNK - Hungary

a) WGS long-read sequencing

Implemented is the detection of the following pathogens:

- SARS-CoV-2
- zoonotic bacterial pathogens (eg. *Francisella sp*.)
- Mpox
- *Legionella*
  Long-read sequencing is used as complementary method to compare resistance or virulence plasmids in outbreaks caused by multidrug-resistant bacterial pathogens (in particular *Klebsiella pneumoniae*).

b) WGS short-read sequencing

We already implemented short read sequencing using Illumina methods. For library preparation, we prefer Illumina DNA prep and Nextera XT kits. We have Illumina MiSeq (n=2), Illumina Nextseq550 (n=1) and Illumina ISeq (n=1) to identify pathogens and investigate outbreaks:

- *Campylobacter sp.*
- *Salmonella sp.*
- Mpox
- SARS-CoV-2
- *Listeria monocytogenes*
- multidrug-resistant bacterial pathogens: vancomycin-resistant *Enterococcus faecium or E. faecalis*,

- methicillin resistant *Staphylococcus aureus*
- multidrug-resistant *Enterobacterales*
- multidrug-resistant *Acinetobacter baumannii*
- molecular surveillance of carbapenemase-producing *Enterobacterales* isolates, *Neisseria meningitidis, Streptococcus pyogenes.*

We plan to integrate sequencing assays, such as the Illumina Surveillance panel or other commercially available Illumina-related NGS kit. One of our main goals is to use metagenome sequencing from clinical samples to improve the diagnosis of diseases of unknown aetiology (Disease-X).

c) qPCR

We have implemented several qPCR methods, including qPCR methods for different types of human pathogenic microorganisms.

- Adenovirus
- Enterovirus
- Influenza A + typing
- Influenza B + typing
- RSV + typing
- SARS-CoV-2 + typing
- WNV
- Usutu virus
- Dengue
- *Salmonella sp.*
- *Campylobacter sp.*
- *E. coli*
- *B. cereus* sensu stricto
- *Borrelia burgdorferi*
- *Bordetella pertussis*
- *Bartonella sp.*
- *Chlamydia pneumoniae*
- *Chlamydia trachomatis*
- *Legionella pneumophila*
- *Leptospira spp.*
- *Mycoplasma genitalium*
- *Mycoplasma pneumoniae*
- *Neisseria gonorrhoeae*
- Ureaplasma spp.
- *Vibrio cholerae*
- *Vibrio spp.*
- *Brucella spp.*
- *Burkholderia spp.*
- *Anaplasma sp.*
- *Yersinia spp.*
- 16S
- Acanthamoeba spp.
- Leishmania sp.
- *Toxoplasma gondii*
- *Trichomonas vaginalis*

- Plasmodium
- Babesia

During the project, we do not plan to implement new qPCR methods.

4.3. EODY – Greece

a) WGS long-read sequencing

Not implemented yet, but we want to implement it.

b) WGS short-read sequencing

Implemented on Illumina NextSeq 2000 and MinION are:

For outbreak investigation:

- SARS-CoV-2, West Nile Virus

For surveillance:

- SARS-CoV-2, West Nile Virus

Until now, the National Reference Lab for Meningitis perform WGS in all *N.meningitidis* isolates in collaboration with the French reference laboratory at Pasteur Institute in Paris. In addition, the Reference lab for *Salmonella/Shigella/Listeria/VTEC* has been sending DNA from clinical *Listeria monocytogenes* isolates to ECDC for WGS.

c) qPCR

Outbreak: For outbreak investigation, the detection of the following pathogens is implemented in the central and 2 regional Public Health labs:

- SARS-CoV-2
- Influenza A & B
- RSV
- Norovirus
- Adenovirus
- West Nile virus
- Mpox
- *Legionella*
- *Diphtheria*
- *Salmonella*
- *Campylobacter*
- *STEC*
- *Listeria*
- Syndromic molecular POC (The BioFire Filmarray GI and Respiratory panels)

Surveillance: For surveillance, the detection of the following pathogens is implemented in the network of Public Health laboratories:

- SARS-CoV-2
- Influenza A & B
- RSV
- *Campylobacter spp*
- *Legionella*
- Carbapenem resistance genes in Carbapenem and/or Colistin Resistant Enterobacterales, Carbapenem Resistant *Acinetobacter baumannii*, Carbapenem Resistant *Pseudomonas aeruginosa*)

On a national level, qPCR protocols are in use for other pathogens in the respective Reference laboratories, for both outbreak and surveillance purposes, with which the National Public Health Organization collaborates under specific contracts:

- Influenza A and B
- Avian influenza
- Mumps virus
- Measles virus
- Rubella virus
- Polio virus
- Chikungunya virus
- Dengue virus
- VHF
- Yellow fever
- Zika virus
- TBE virus
- Variola virus
- HIV
- Hepatitis B, C, D viruses
- *Vibrio cholerae*
- *Bacillus anthracis*
- *Yersinia pestis*
- *Mycobacteriun tuberculosis* complex
- *Neisseria gonorrhoeae*
- *Candida auris*

4.4. CIPH - Croatia

a) WGS long-read sequencing

It is not implemented but we plan to implement it.

b) WGS short-read sequencing

Implemented are on illumina Nextseq 550 and Miniseq platforms the following pathogens:

For outbreak investigation:

- SARS-CoV-2
- Influenza
- *Salmonella spp*
- *Legionella pneumophyla*
- *Campylobacter spp*
- *E.coli*
- *Streptococcus pyogenes*

For surveillance:

- SARS-CoV-2
- Influenza

c) qPCR

Implemented are the following pathogens for outbreak investigation and surveillance:

- Adenovirus
- Enterovirus
- Influenza A + typing
- Influenza B + typing
- HPV + HR typing
- RSV + typing
- SARS-CoV-2 + typing

Only for outbreak investigation:

- Norovirus
- WNV
- TBE
- M-pox
- Orthopox
- CMV
- EBV
- HSV-1, 2
- Norovirus
- Rhinoviruses
- Parainfluenza 1-4
- HMPV
- Bocavirus
- Parechoviruses
- Dengue virus
- HAV
- Mumps virus
- Morbillivirus
- Rubella virus
- Zika virus
- *Bordetella pertussis*
- *Borrelia burgdorferi*
- *Chlamydia pneumoniae*
- *Chlamydia trachomatis*

- *Legionella pneumophila*
- *Leptospira spp*
- *Mycoplasma genitalium*
- *Mycoplasma pneumoniae*
- *Neisseria gonorrhoeae*
- *Ureaplasma spp*
- *Acanthamoeba spp*
- *Leishmania*
- *Toxoplasma gondii*
- *Trichomonas vaginalis*