



D3.3 Interim report on the implementation status on the proposed molecular protocols and WGS for sentinel surveillance

**WP3 Enhancement & Consolidation of WGS- & PCR-based
methods for public health actions**

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1. EXECUTIVE SUMMARY

This document describes the implementation status of the proposed molecular protocols and WGS for sentinel surveillance. Its main objective is to describe the current implementation of sentinel surveillance by the consortium partners for respiratory and/or gastrointestinal infections.

The main advantages of sentinel systems, compared to the classical population-based epidemiology, is lower cost and higher value of the data, consequently the operation of sentinel systems is recommended by international Public Health authorities.

All partner countries are currently operating sentinel systems and partner Institutions are participating in the surveillance activities, although differences are observed depending on the organization of the health system and the specific country needs. Molecular detection methods and, for most pathogens, NGS based approaches (WGS or metagenomics) are currently used by all consortium members for diagnosis and molecular epidemiology.

Knowledge and experience exchange between the HERA 2 partners, may expand the use of modern molecular tools towards more sensitive pathogen detection and high-resolution typing, thus increasing the efficacy of each country's surveillance activities.

2. BACKGROUND INFORMATION

"The systematic ongoing collection, collation and analysis of data for public health purposes and the timely dissemination of public health information for assessment and public health response as necessary" is the definition of public health surveillance given by the IHR 2005 (1). The most basic tool to monitor public health issues inside a community is population-based epidemiological surveillance. This is the systematic collection of data regarding health-related issues. Nevertheless, there are sporadic problems associated with health data reporting, that hamper the undisputable value of the tool.

A sentinel surveillance system consists of a relatively small number of health facilities that collect data on cases that are enrolled based on a specific case definition for the disease under monitoring. Laboratory confirmation or detection of the responsible pathogen may also be necessary depending on the disease being monitored. Although the data collected are limited compared to population-based surveillance, they are usually of higher value and the cost is lower. For these reasons sentinel surveillance systems are recommended by the ECDC, usually along with classical surveillance approaches for monitoring of certain diseases in EU/EEA countries.

Due to the non-pharmaceutical measures taken during the COVID-19 pandemic, respiratory disease burden may be increased (2). Respiratory tract infections constitute almost 1/3 of healthcare-associated infections (HAIs) in European countries, as indicated by the third ECDC point prevalence survey (PPS) of healthcare-associated infections and antimicrobial use in European acute care hospitals (3). ECDC, WHO Regional office for Europe and European surveillance networks for COVID-19 and influenza suggest, among others, that the development and operation of sentinel surveillance systems should be the "central surveillance method for acute respiratory infections". Influenza-like illness (ILI) and acute respiratory infection (ARI) case definition can be used for respiratory surveillance. Also, severe acute respiratory infection (SARI) surveillance is considered an important source of epidemiological information (4). It is recommended that laboratory testing of patients' samples, be performed by multiplex PCR assays for simultaneous detection of at least Influenza and SARS-CoV-2, along with genomic monitoring of the detected viruses (5).

According to the third ECDC PPS of healthcare-associated infections and antimicrobial use in European acute care hospitals (3), gastrointestinal infections constitute almost 10% of HAIs in European countries. However, due to climate change, their number is expected to increase. Currently, ECDC does not recommend the operation of sentinel systems for gastrointestinal diseases in the European countries, although each country may establish sentinel systems for specific pathogens according to national needs.

3. METHOD

Information on the sentinel implementation was obtained from a questionnaire filled out by all consortium partners and sent to the authors by email. The questionnaire involved structured questions as well as questions with free-commenting space for providing more information/clarification on the given answers. The questionnaire is presented in the appendix section. Each partner's sentinel organization is presented in alphabetical order in the following section.

4. INTERIM REPORT ON EACH CONSORTIUM PARTNER

a) Austrian Agency for Health and Food Safety GmbH – AGES (Austria)

In Austria, the Austrian Reference Center for Influenza Epidemiology located at the Institute of Medical Microbiology and Hygiene Vienna – AGES, in a Public Health Business Unit, is responsible for recording the epidemiological situation of influenza in Austria based on a clinical and a virological sentinel

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surveillance system as well as laboratory reports of influenza virus detections from a further six virological laboratories.

Among the respiratory pathogens AGES routinely carry out WGS for *N. meningitidis* isolates derived from invasive meningococcal disease (IMD) which is notifiable in Austria.

Regarding GI pathogens a sentinel surveillance system is in place for Norovirus and *Campylobacter*.

For Norovirus, a sentinel monitoring was established in March 2023. Samples are collected in diagnostic laboratories and are sent to the laboratories by courier service. Patients' data are sent to the laboratory by hardcopy along the samples. 1 Public Health, 2 University, and 3 Private laboratories participate in the sentinel system and each laboratory sends 10 samples per month to AGES Reference Center. An in-house multiplex one tube RT-qPCR is used for detection (7). WGS is not performed but norovirus RNA is only sequenced in the two genome sections relevant for typing.

For *Campylobacter*, AGES has established a sentinel surveillance system since 2021 (previously all human isolates had to be sent to AGES). The senders include 8 private laboratories, 15 hospitals, and 3 university laboratories. Samples are collected by general practitioners, health services or hospitals. The patient data are sent together with the sample by means of a hard copy. The samples are transported by courier service. Patients' data are stored in LIMS, the isolate is typed using MALDI-ToF and a subset of the isolates is tested for antimicrobial resistance and subjected to WGS, using the Illumina DNA Prep kit in Illumina sequencers. To date, approx. 1500 isolates have been typed using WGS.

For *Salmonella*, *Shigella*, *Listeria*, and *E. coli*/STEC sentinel system is not established, but all isolates (including human and non-human origin) must be sent to the NRL. For all these isolates, typing with WGS is routinely performed.

b) Croatian Institute of Public Health (Croatia)

Croatia has established an operating national respiratory sentinel surveillance system for SARS-CoV-2, influenza and RSV, whereas a sentinel system for gastrointestinal diseases has not been put in place.

Regarding the sentinel system for respiratory pathogens, samples from patients fulfilling the requirements for ILI are collected by Public Health system's physicians.

Patients with ILI characteristic symptoms are swabbed at Public Health institute's testing site and samples are tested for respiratory pathogens in the respective Public Health microbiology laboratories. In the sentinel system the participating laboratories are two Public Health laboratories.

The collected samples are sent along with the patient's data in hardcopy for processing to the relevant laboratories that are housed at the same collection sites.

In parallel, patient's data are filled and kept in a web-based platform, whereas the results are sent to the relevant epidemiology department by email.

The multiplex "1 copy™ COVID-19/FluA/FluB/RSV qPCR Kit" (Clinomics Inc, Korea) is used for the detection of the pathogens.

WGS is performed for SARS-CoV-2, Influenza and RSV in Illumina platforms. Protocols used for SARS-CoV-2 is accomplished according to published protocol (8). For Influenza, the protocol is adapted from a RT-PCR assay described by Zhou and colleagues (9-11). Protocols used for RSV are previously published (12).

During the project RT-PCR has been used for approximately: 51,000 samples for the detection of respiratory viruses. Regarding WGS, the number of samples processed per pathogen during the project are the following: SARS-CoV-2 5,417, Influenza 171, RSV 57.

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Beyond the sentinel system, 250 samples for the detection of gastrointestinal viruses and 10,500 samples for the detection of respiratory bacteria were also performed, while WGS was performed for 2 adenoviruses, 11 enteroviruses, 5 mumps viruses, 83 *Streptococcus pyogenes* group A, 123 *Campylobacter* spp, 30 *E. coli*, 5 *Enterococcus faecium*, 1 *H. influenzae*, 13 *K. pneumoniae*, 13 *L. pneumophila*, 11 *L. monocytogenes*, 2 *Nocardia* spp., 54 *B. pertussis*, 305 *Salmonella* spp., 2 *Shigella* spp.

c) National Public Health Organization (Greece)

Greece is using a sentinel system for respiratory and not gastrointestinal infections. Samples from patients fulfilling the requirements for ILI and SARI, are collected by Public Health system's physicians (ILI) and hospitals (SARI).

Samples are sent along with the patient's data in hardcopy, to the designated laboratories for RT-qPCR. The Central and 2 Regional Public Health Laboratories (Thessaly and Crete), 1 University laboratory and 2 Research Centers participate in the sentinel system. During the last 4 months POC and/or syndromic testing has been installed in some of the sentinel points (using resources from another program), consequently pathogen detection with RT-qPCR is performed in situ. Positive samples are sent to the laboratories using a courier company.

The "TaqPath™ COVID-19, FluA/B, RSV Combo Kit" and the "Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay" are used for pathogen detection. "Xpert® Xpress CoV-2/Flu/RSV plus Respiratory," and "BIOFIRE® Respiratory 2.1 plus Panel," are used in selected sentinel points during the last 4 months.

WGS is performed for SARS-CoV-2 by the Public Health Laboratories using Illumina sequencers. For sequencing in Illumina platforms (MiSeq and NextSeq2000), the "COVIDSEQ test" kits along with suitable reagent kits for each sequencer were used. Samples were also sent to Eurofins for SARS-CoV-2 WGS in the context of the agreement between Eurofins and ECDC.

Since 1/1/2023, approximately 5,500 ILI, and 1,800 SARI samples have been analyzed for SARS-CoV-2/Influenza/RSV.

d) National Center for Public Health and Pharmacy (Hungary)

Hungary has an operating national sentinel system for respiratory diseases, and not for gastrointestinal diseases. Samples from patients fulfilling the requirements for ILI and SARI, are collected by 150 Public Health system's physicians (ILI) and 24 Hospitals (SARI).

Samples are sent along with the patient's data in hardcopy, to the National Center for Public Health and Pharmacy (National Influenza Centre) or hospital laboratories for RT-qPCR. The transportation is organized by the Regional Government Office and by the hospitals involved.

The CDC Respiratory Panel Kit is used for Influenza and SARS-CoV-2 detection, while an in-house method based on a publication (13) is used for RSV detection.

WGS is performed for Influenza and SARS-CoV-2 from selected samples for which viruses have been successfully isolated. Illumina and Nanopore sequencers were used for WGS. For sequencing in Illumina platforms (MiSeq, NextSeq550 and iSeq 100), the "DNA prep", "NexteraXT" and "COVIDSEQ test" kits along with suitable reagent kits for each sequencer were used. For Nanopore sequencers "Native barcoding kit" and "Rapid barcoding sequencing kit" were used.

Patients' data and test results are sent to the epidemiological department by mail or by a web-based platform, depending on the pathogen.

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Approximately 7,000 respiratory samples were processed by RT-qPCR in the season, while 200 influenza isolates and 3 direct clinical samples were processed for WGS, and metagenomics analysis was performed in 100 respiratory samples and in 100 CSF samples.

5. CONCLUSIONS

All consortium partners have established sentinel systems with variations, depending on the country and the specific needs. Most partners use a multiplex molecular method for simultaneous detection of SARS-CoV-2/Influenza/RSV, as guided by ECDC, and WGS analysis of SARS-CoV-2 or influenza, whereas Austria has long ago operated an independent sentinel system for influenza.

A sentinel system for gastrointestinal infectious diseases is operating in Austria for Norovirus and *Campylobacter* infections. For other enteropathogens such as *Salmonella*, *E. coli*/STEC, and *Listeria* sentinel surveillance is not used but all positive samples are sent to AGES for WGS.

Independently of the sentinel systems, Croatia also performs molecular detection and WGS for a number of respiratory and gastrointestinal pathogens, while Hungary has performed metagenomic analysis in respiratory and CSF samples.

All partners have implemented molecular and WGS protocols for sentinel surveillance resulting in higher efficiency of the surveillance systems.

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7. APPENDIX

Questionnaire

1. Have you established a sentinel system for respiratory pathogens?
 - a) Yes
 - b) No

If yes, please include all pathogens for which you have established a sentinel system:

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.....
.....
.....
.....

2. Have you established a sentinel system for GI pathogens?
 - a) Yes
 - b) No

If yes, please include all pathogens for which you have established a sentinel system:

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.....
.....

3. Concerning the sentinel system for respiratory pathogens, what samples are referred to the lab?
 - a) ILI and SARI samples
 - b) Only ILI samples
 - c) Only SARI samples

4. What are your samples collection points?
 - a) private physicians
 - b) Public Health system's physicians
 - c) hospitals
 - d) other

If other, please specify:

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.....

5. Describe the way the samples are transported to the laboratory
 - a) courier company
 - b) own transport means of the institute
 - c) other

If other, please specify:

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6. Describe the way patient's data are sent to the laboratory
- a) by mail
 - b) hardcopy along the sample
 - c) by email
 - d) web based platform
 - e) other

If other, please specify:

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.....
.....

7. Describe the way patient's data and results are sent to the relevant epidemiology department
- a) by mail
 - b) hardcopy
 - c) by email
 - d) web based platform
 - e) other

If other, please specify:

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.....

8. Describe the RT-qPCR method (or methods) you use (or intent to use) for detection of each pathogen or for a number of pathogens simultaneously detected (multiplex) (e.g. multiplex respiratory panel of company, in house according to the X paper by, etc)

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9. Please specify the number and the type of laboratories that participate in the sentinel system (e.g. 1 Public Health lab, 1 Central lab and 3 private labs, X university labs, etc)

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10. Describe the workflow of samples' referral

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.....

11. Do you perform WGS of the sentinel samples?
- a) Yes
 - b) No

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If yes, please specify the pathogens:

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.....
.....

12. Describe the method you use (or intent to use) for WGS (kit X of the company..., in house method based on X paper by..., etc)

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.....
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13. Which platform do you use for WGS?

- a) Illumina
- b) Nanopore
- c) Ion Torrent
- d) PacBio
- e) Other

If other, please specify:

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.....

14. How many samples have been processed by RT-PCR for pathogen detection so far during the project? (e.g. 1000 for respiratory, 1000 for GI, 100 for GI bacterial, etc)

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15. How many samples per pathogen have been processed for WGS so far during the project? (e.g. 100 flu, 100 *Salmonella*, etc)

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