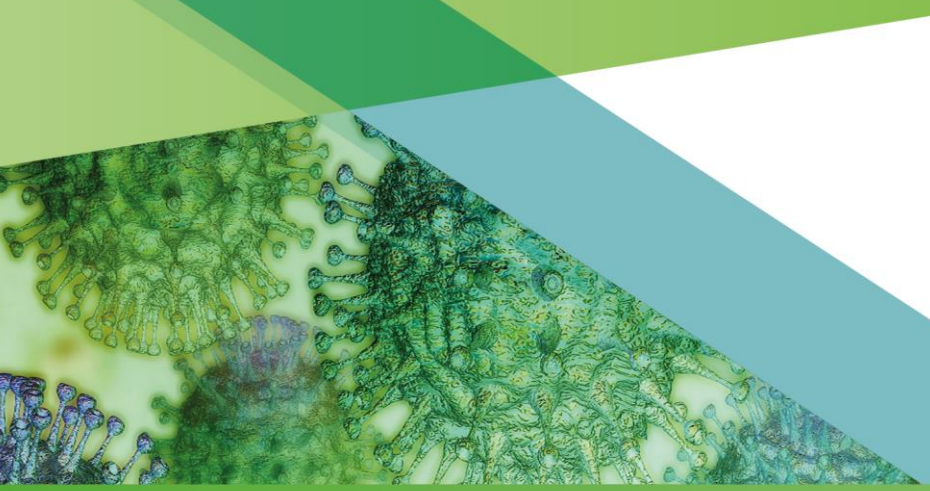


TECHNICAL REPORT

Antiviral resistance monitoring capability and capacity for SARS-CoV-2 and influenza within the EU/EEA

15 December 2022

Key messages

- More than half of EU/EEA countries perform antiviral resistance testing for influenza, and three countries perform antiviral resistance testing for SARS-CoV-2.
- Most of the countries are interested in extending their antiviral resistance testing, either to influenza or SARS-CoV-2 or to both.
- Antiviral resistance monitoring of new virus variants could improve the appropriate clinical use of antiviral drugs.
- For SARS-CoV-2, most countries rely on genotypic analyses for antiviral resistance testing, with very few countries performing both genotypic and phenotypic analyses.
- For influenza, most countries perform both genotypic and phenotypic analyses.
- Whole Genome Sequencing is becoming the most commonly used approach for antiviral resistance analysis, allowing rapid identification, monitoring and assessment of mutations creating antiviral resistant variants.

Background and methods

A questionnaire (see Annex 1) using the EU Survey Tool was sent out to 30 EU/EEA countries on 31 May 2022 The recipients included ECDC Operational Contact Points (OCP) for COVID-19 for Microbiology, ECDC National Focal Points (NFP) for Viral Respiratory Diseases and ECDC National Coordinators (NC).

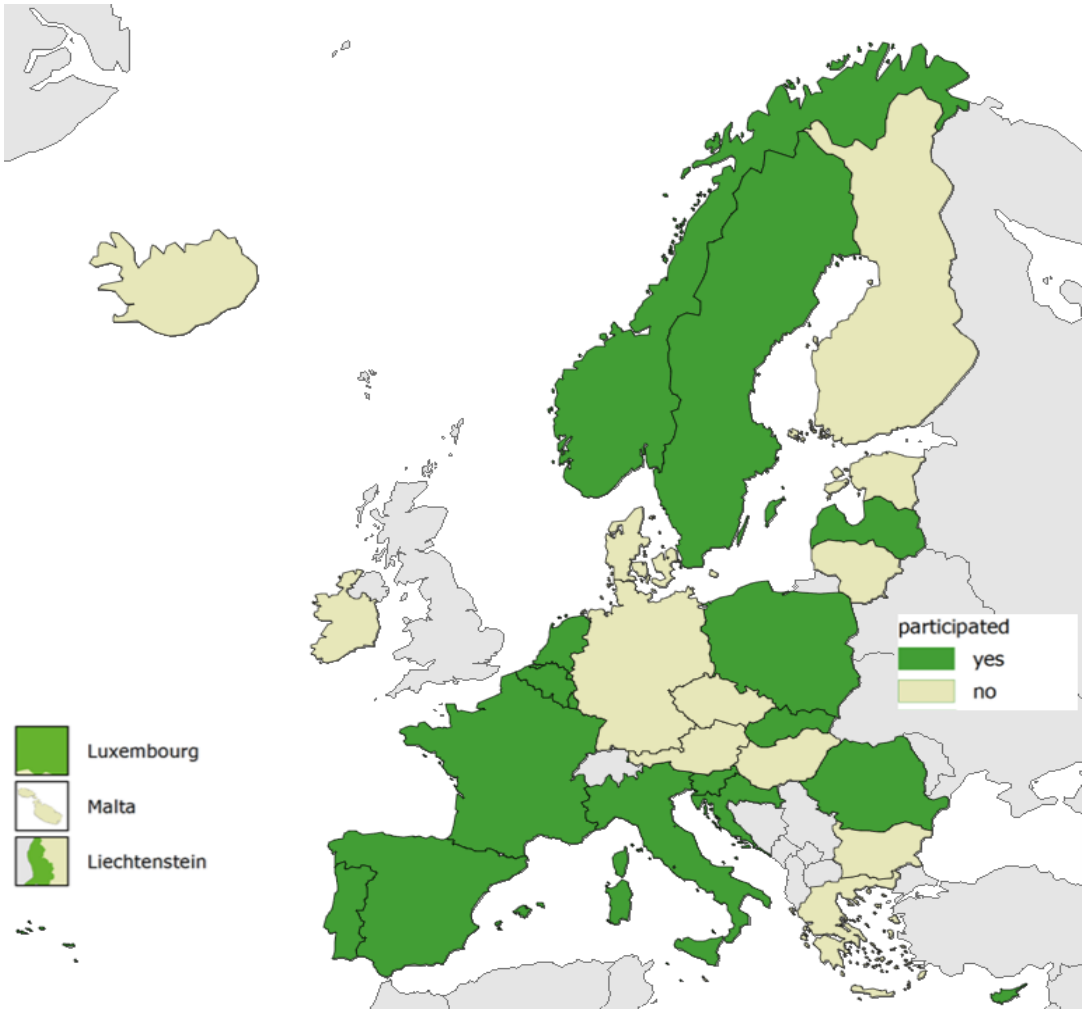
Results

A total of 17 EU/EEA Member States replied to the survey which consisted of 19 questions (Figure 1). One country completed the full questionnaire, another 12 countries responded to more than two thirds of the questions and four countries responded to less than two thirds of the questions.

Among those responding on behalf of the 17 countries, three were ECDC National Focal Points for Viral Respiratory Diseases, ten were ECDC Operational Focal Points (OCP) for COVID-19 Microbiology and one was ECDC OCP for influenza epidemiology. One responding institution was registered as ECDC OCP for influenza microbiology, one was registered as a microbiologist at the Unit for Laboratory Surveillance of Viral Pathogens and Vaccine-Preventable Diseases and one as National Microbiology Focal Point (NMFP).

Suggested citation: Antiviral resistance monitoring capability and capacity for SARS-CoV-2 and influenza within the EU/EEA. Stockholm: ECDC; 2022.

Figure 1. Countries (n=17) that took part in the survey (green), EU/EEA, June 2022



Countries that were invited but did not provide responses are depicted in light green. Countries outside the EU/EEA region are shown in grey.

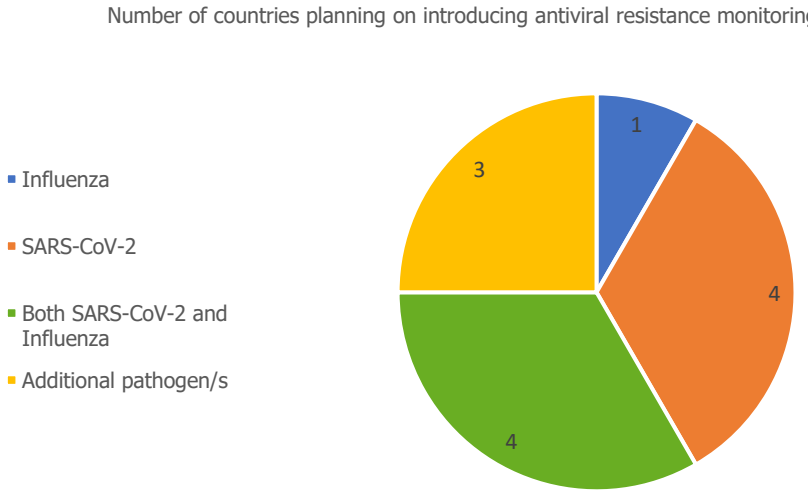
Antiviral resistance testing on influenza and SARS-CoV-2 surveillance specimens

Here, the aim was to understand if the countries are at all performing antiviral resistance testing on either influenza or SARS-CoV-2 surveillance specimens or on both. At this stage, no further details on specimen type or selection criteria were subject of the questions posed. Ten out of 17 countries responded that they perform antiviral resistance testing for influenza, while three countries said they perform antiviral resistance testing for SARS-CoV-2. Four and 11 countries responded that are not performing antiviral resistance testing for influenza and SARS-CoV-2, respectively. Three countries plan to introduce antiviral resistance testing for influenza or SARS-CoV-2 but have not started yet. Three of the countries that do perform antiviral resistance testing for influenza, replied that they refer their specimens for testing to the Worldwide Influenza Centre at the Francis Crick Institute (WHO Collaborating Centre) or to their National Reference Centre (NRC).

Implementation of antiviral resistance monitoring for influenza and SARS-CoV-2

Seven countries do not currently perform antiviral resistance testing for influenza, and 14 countries do not currently test for antiviral resistance for SARS-CoV-2. Among these countries, one country plans to implement influenza antiviral resistance monitoring and four countries instead plan to focus on the introduction of SARS-CoV-2 antiviral resistance monitoring. Another four countries responded that their plans comprise implementation of both influenza and SARS-CoV-2 antiviral resistance monitoring (Figure 2). Three countries that reported plans on introducing antiviral resistance monitoring for additional pathogens mentioned the intention to implement antiviral resistance testing for Respiratory Syncytial Virus (RSV).

Figure 2. Implementation of antiviral resistance monitoring for influenza and SARS-CoV-2 (multiple choice)

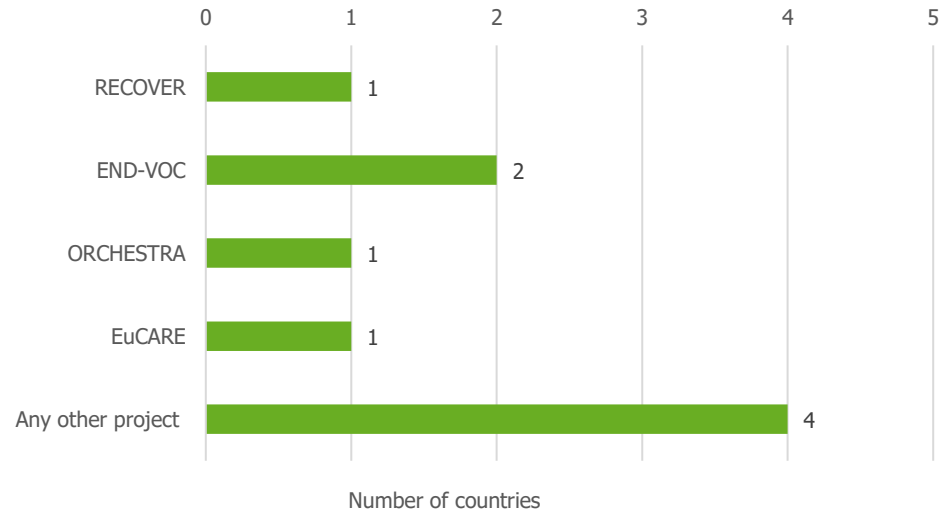


Participation of countries in clinical or research-funded projects

Countries were asked if their institution is part of any clinical- or research-funded projects (Figure 3) to understand better which specimens are collected, if any cohorts have been set up or followed, and if any antiviral resistance testing is performed within these projects.

Eight out of 17 countries responded to this question, with one country reporting participation in the RECOVER project (Rapid European COVID-19 Emergency Response research, https://www.recover-europe.eu/), one in ORCHESTRA (https://orchestra-cohort.eu/) and one in EuCARE (European Cohorts of patients and schools to Advance Response to Epidemics, https://eucareresearch.eu/). Two countries responded that they are part of the END-VOC project (ENDing COVID-19 Variants Of concern through Cohort studies, https://www.isglobal.org/-/endvoc-project). From four countries that confirmed their participation in other projects that are not listed here, three of them stated that they are part of a HERA-Incubator project (https://health.ec.europa.eu/publications/hera-incubator-anticipating-together-threat-covid-19-variants_en) and one country confirmed participation in the DISCOVERY project (https://eu-response.eu/the-project/).

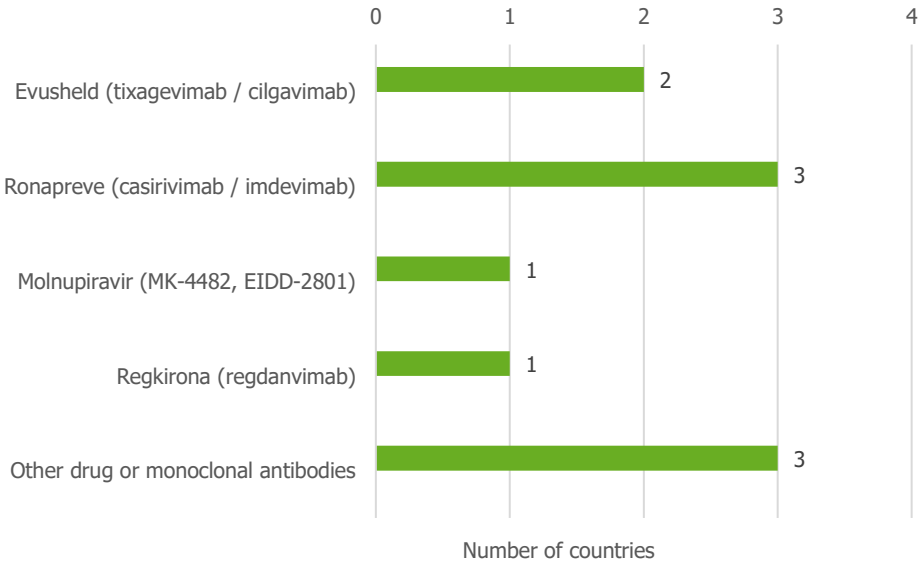
Figure 3. Participation of countries in clinical or research-funded projects (multiple choice)



Monitoring of SARS-CoV-2-specific drugs or monoclonal antibodies

Countries reported on the SARS-CoV-2-specific drugs or monoclonal antibodies that they monitored for resistance (Figure 4). Two countries reported monitoring Evusheld, which is a combination of two monoclonal antibodies tixagevimab and cilgavimab, and three countries monitored Ronapreve, which is a combination of the casirivimab and imdevimab monoclonal antibodies. One country is monitoring the SARS-CoV-2 specific drug Molnupiravir (MK-4482, EIDD-2801) and one the monoclonal antibody Regkirona (regdanvimab). All SARS-CoV-2-specific drugs or monoclonal antibodies reported to be monitored for resistance were approved for use by the European Medical Agency (EMA) at the time of the survey. Among the three countries that reported monitoring other drugs or monoclonal antibodies, one country said that bamlanivimab (LY-CoV555) and sotrovimab (Xevudy) are the monoclonal antibodies being monitored, while one country monitored only sotrovimab (Xevudy). One country reported that their National Reference Centre (NRC) has previously monitored Paxlovid (PF-07321332/ritonavir), however this SARS-CoV-2 specific drug was not usually administered in their country.

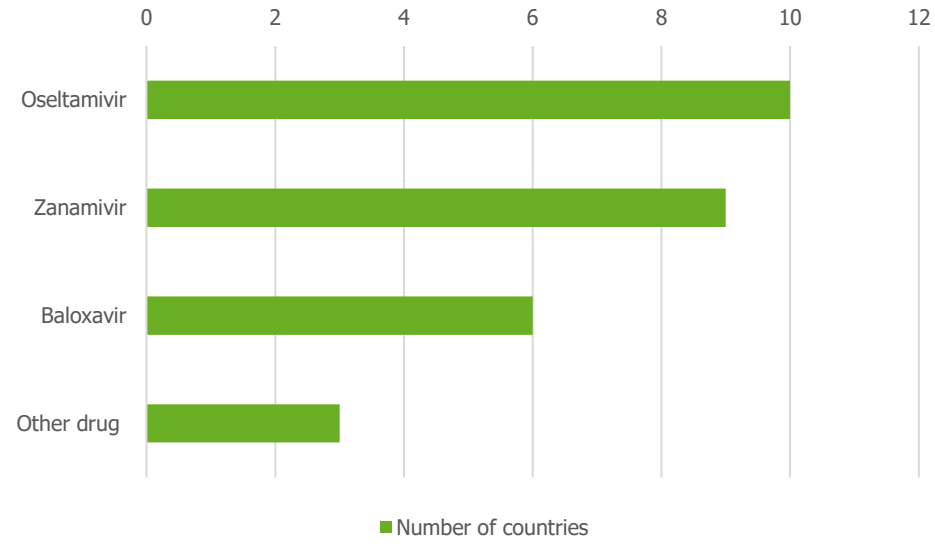
Figure 4. Monitoring of SARS-CoV-2-specific drugs or monoclonal antibodies (multiple choice)



Monitoring of influenza specific drugs

Oseltamivir and zanamivir were the most commonly monitored drugs against influenza as stated by the responding 17 countries (ten and nine countries, respectively) (Figure 5). Six countries reported monitoring baloxavir, while three countries responded that they are monitoring for other influenza-specific drugs. Furthermore, six out of 17 countries responded that they monitor three influenza specific drugs in parallel, namely oseltamivir, zanamivir and baloxavir. Among the three countries, which answered 'other' influenza specific drug, two of them stated that they monitor amantadine and one country reported monitoring for laninamivir and peramivir.

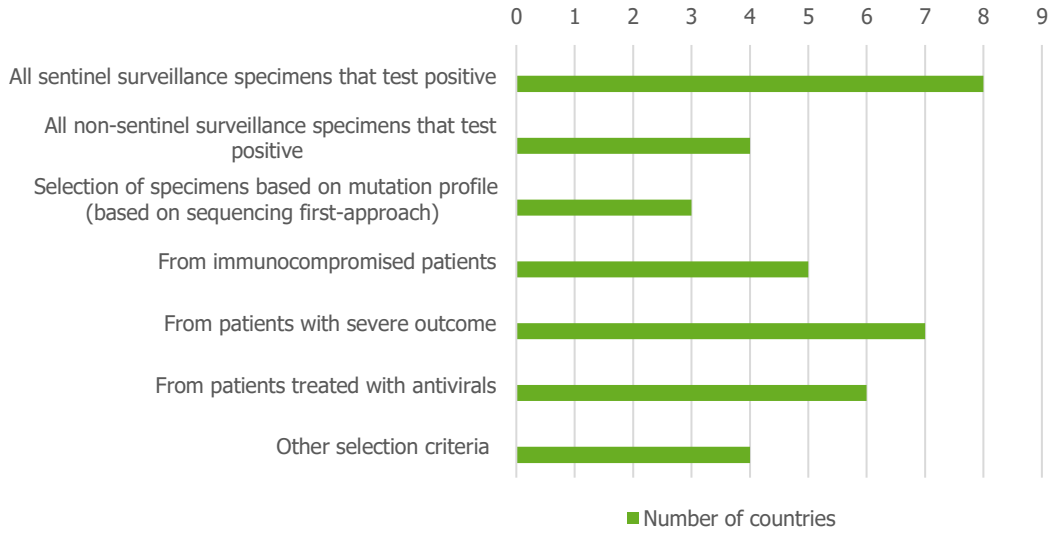
Figure 5. Monitoring for influenza specific drugs (multiple choice)



Influenza and SARS-CoV-2 specimen collection criteria for antiviral resistance testing

Countries were asked to report on specimen selection criteria for influenza and SARS-CoV-2 antivirals resistance testing (Figure 6). Eight countries collect all sentinel surveillance specimens that tested positive. Seven countries reported collection of specimens from patients with severe outcome. Six countries collect specimen from patients treated with antivirals, from a not further defined subset of patients. Five countries stated that they collect specimens from immunocompromised patients, while three countries reported basing their selection criteria on the mutational profile of the viruses (based on sequencing first-approach). Four countries said that they collect all non-sentinel surveillance specimens that tested positive. Another four countries selected 'other selection criteria', among which they reported collecting a representative selection of a specific subtype/lineage (depending on the season) with a Ct value ≤30, a proportion of sentinel and non-sentinel surveillance specimens or random selection of clinical specimens. Most responding countries claimed multiple specimen selection criteria for antiviral resistance testing.

Figure 6. Influenza and SARS-CoV-2 specimen collection criteria for antiviral resistance testing (multiple choice)

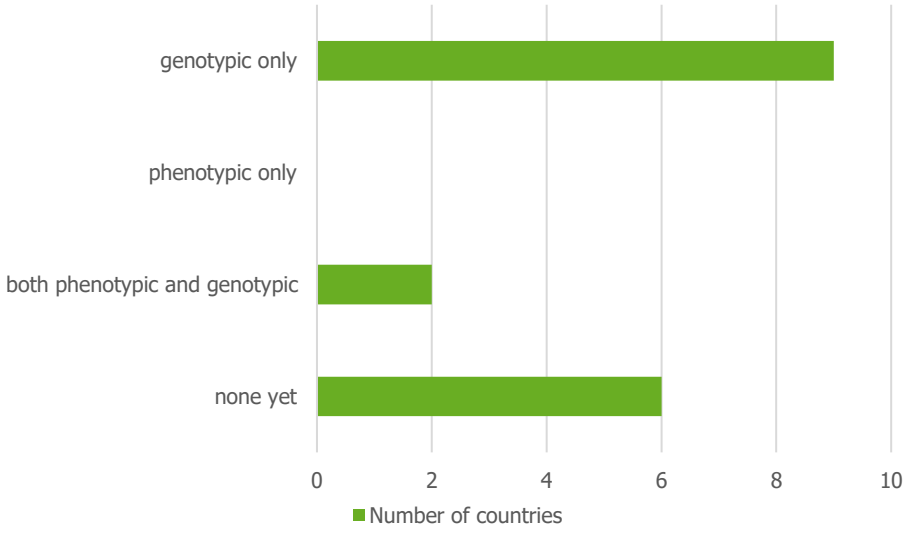


Analysis of antiviral resistance monitoring for SARS-CoV-2 and influenza

The countries were asked about the kind of analyses they perform to determine antiviral resistance to SARS-CoV-2 and influenza viruses, respectively. A set of complementing questions aimed at understanding how often such analyses are performed were asked.

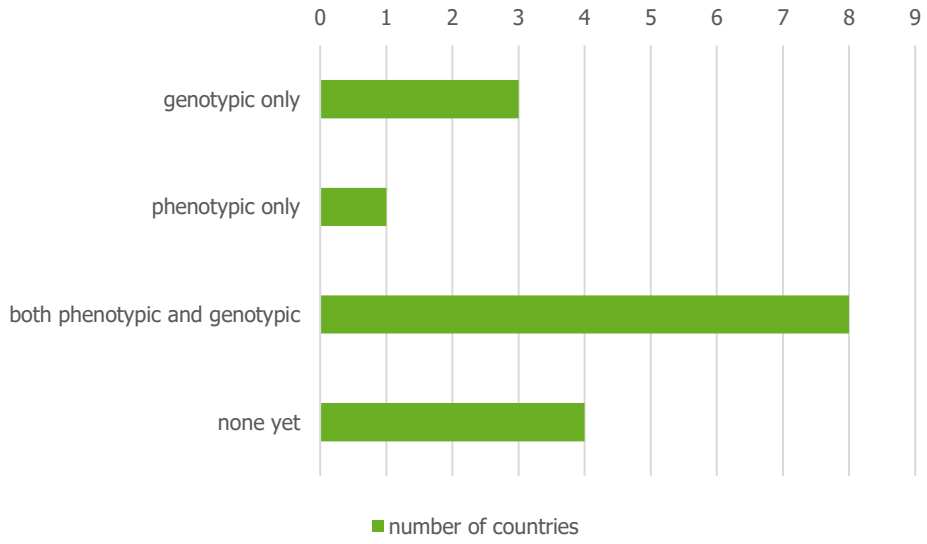
For SARS-CoV-2, nine countries reported that they perform genotypic analyses only, while two countries perform both genotypic and phenotypic analyses. None of the responding countries relied on phenotypic analysis only. About one third of the responding countries (six countries) have not performed any such analyses on SARS-CoV-2 yet. One country has the possibility to perform whole genome sequencing (WGS) and virus isolation, but none of these tools are being used for antiviral resistance testing. The above-mentioned analyses are not performed on a regular basis in any of the responding countries, but rather irregularly. It is important to note that some countries carry out SARS-CoV-2 genotypic analysis but lack the bioinformatic support and capability for analysis of antiviral resistance monitoring.

Figure 7. Number of countries in the EU/EEA performing either genotypic, phenotypic, or both antiviral resistance analyses for SARS-CoV-2, or none of the above



For influenza viruses, the situation looked different as most countries carry out either genotypic or the phenotypic antiviral resistance analyses: three countries perform genotypic analyses only, and one country focuses on the phenotypic analysis of influenza viruses. Eight countries perform both analyses and only four countries reported not performing any antiviral resistance monitoring for influenza viruses at all. Of these twelve countries, two countries analyse specimens on a weekly basis, while ten countries test specimens irregularly. One country confirmed that they send their specimens to the WHO Collaborating Centre (WHO CC) in London for analysis as they have no capacity to analyse the specimens.

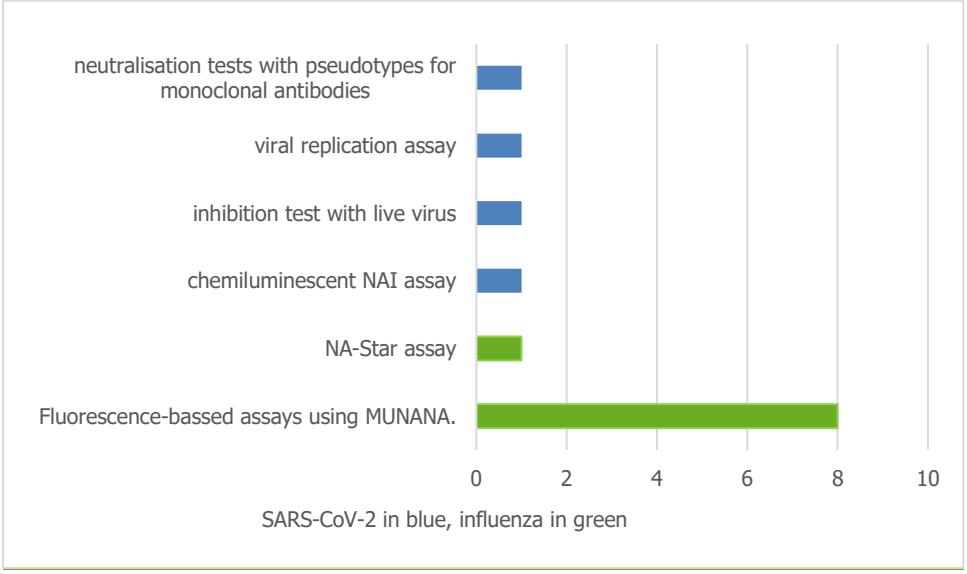
Figure 8. Number of countries in the EU/EEA performing either genotypic, phenotypic, or both antiviral resistance analyses for influenza viruses, or none of the above



Phenotypic antiviral resistance testing

Countries were asked about the phenotypic methods used to analyse antiviral resistance of SARS-CoV-2 and influenza. The results are shown in Figure 9. Eight countries reported that they use fluorescent-based MUNANA assays (using 2′-(4-Methylumbelliferyl)-α-D-N-acetylneuraminic acid (MUNANA) substrate) and one also used NA-Star assay (using the commercial Applied Biosystems NA-Star® Influenza Neuraminidase Inhibitor Resistance Detection Kit) for phenotypic testing for influenza viruses. One country reported using neutralisation tests with pseudotype viruses for monoclonal antibodies, one country uses viral replication assays, and one country employs inhibition tests (plaque forming units, PFU, or 50% Tissue Culture Infectious Dose, TCID50) with live virus.

Figure 9. Phenotypic methods used for influenza (green) or SARS-CoV-2 (blue) antiviral resistance testing by reporting laboratories (multiple choice)

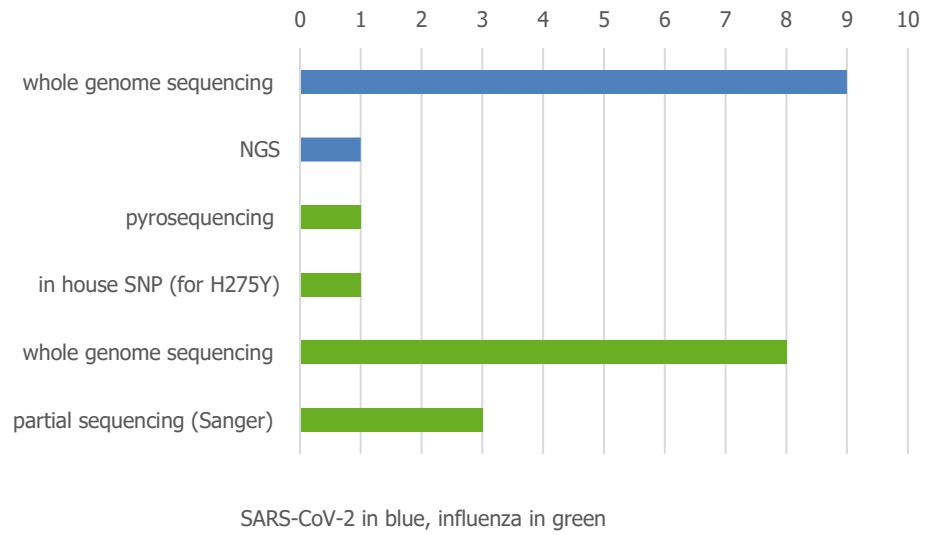


Genotypic antiviral resistance testing

The countries were asked about the genotypic methods used to analyse antiviral resistance of SARS-CoV-2 and influenza. Ten countries described the type of analysis performed on influenza, and eleven countries described their analyses of SARS-CoV-2. The results are shown in Figure 10.

The only method reported to be used for genetic analysis of SARS-CoV-2 was Whole Genome Sequencing (WGS). Nine countries reported that they use WGS for influenza, while three countries perform partial gene sequencing using Sanger sequencing (neuraminidase, NA, and/or polymerase, PA). One country reported using pyrosequencing and one country reported using an in-house single nucleotide polymorphism (SNP) assay for detecting H275Y in NA. Multiple choices could be made in this question and several countries used two or more methods for the analysis of influenza viruses.

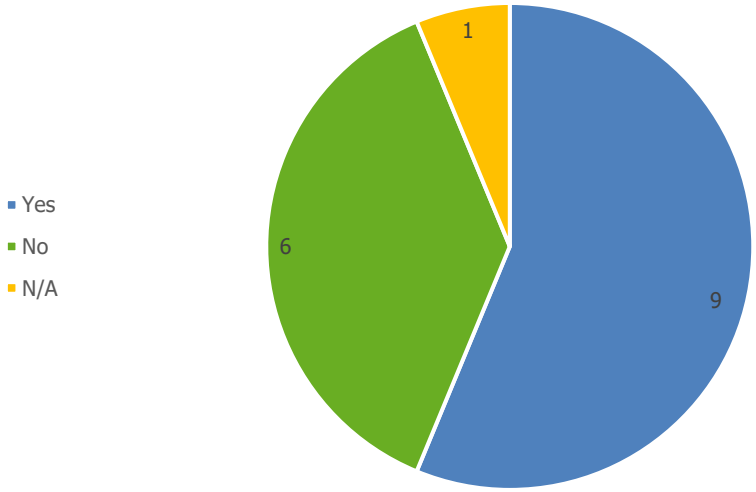
Figure 10. Genotypic methods used for influenza (green) or SARS-CoV-2 (blue) antiviral resistance testing by reporting laboratories (multiple choice)



Reporting of influenza antiviral drug resistance data to The European Surveillance System

All countries were asked if they report influenza antiviral resistance data to The European Surveillance System, TESSy (Figure 11). Nine countries report their antiviral resistance data to TESSy, while six did not report influenza antiviral resistance drug data. One country responded 'not applicable' to this question and one country did not submit an answer.

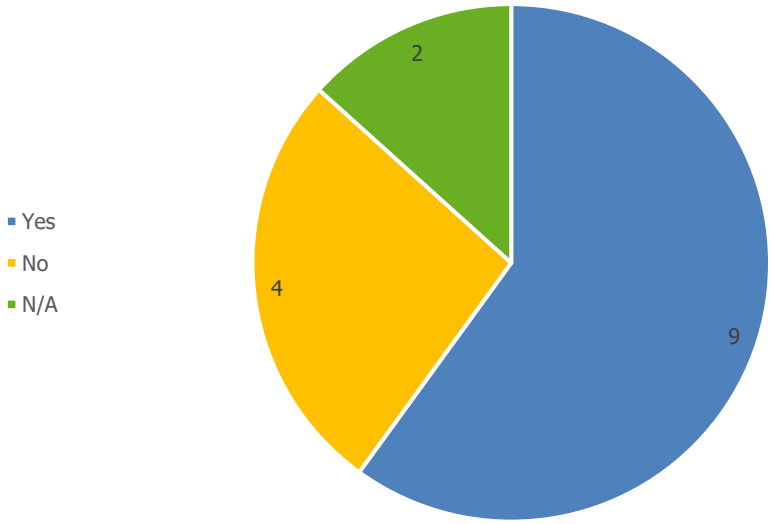
Figure 11. Number of countries of the EU/EEA reporting influenza antiviral drug resistance data to TESSy



Reporting of influenza antiviral drug resistance data to The European Surveillance System in a virus-based record type

Nine countries said they report influenza antiviral drug resistance data to TESSy in a virus-based record type, while four countries did not report antiviral drug resistance data to TESSy (Figure 12). The answer from two countries was 'not applicable' and one country did not submit an answer.

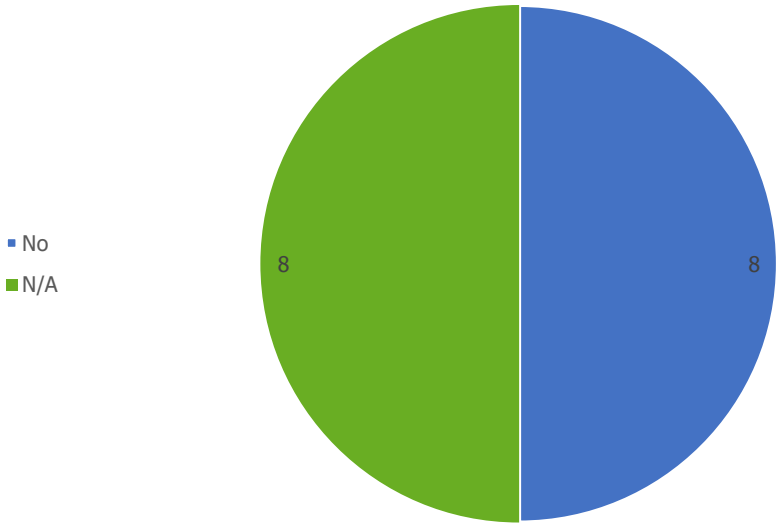
Figure 12. Reporting of antiviral drug resistance data to TESSy in a virus-based record type



Reporting of SARS-CoV-2 neutralisation data and/or antiviral drug resistance data to EpiPulse events

From the 17 countries that answered the survey, eight countries confirmed that they do not report SARS-CoV-2 neutralisation data or antiviral resistance data to EpiPulse, while eight countries answered 'not applicable' to this question (Figure 13). One country did not answer.

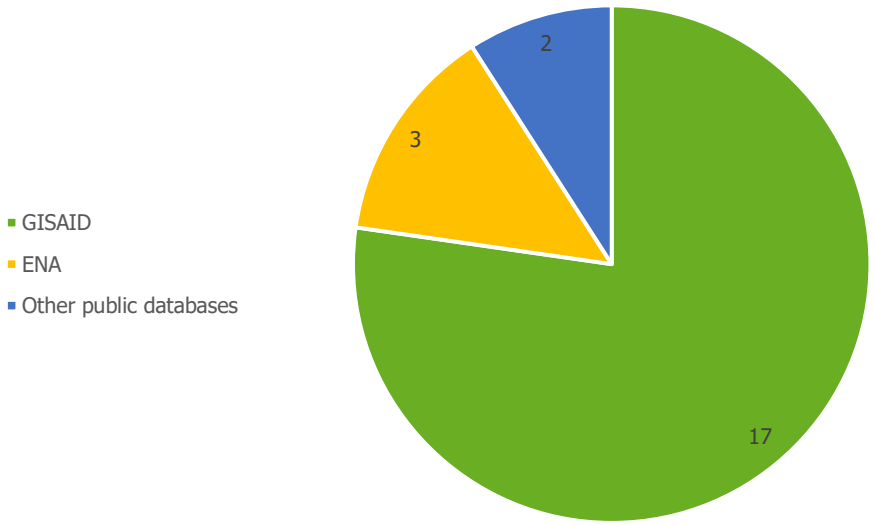
Figure 13. Reporting of SARS-CoV-2 neutralisation data and/or antiviral drug resistance data to EpiPulse events



Reporting of influenza and SARS-CoV-2 sequence data to publicly available databases

When asked if countries were submitting sequence data to any publicly available database, all responding countries (n=17) confirmed that they did so. More specifically, all 17 countries were reporting to GISAID. Among the countries that report to GISAID (Global Initiative on Sharing All Influenza Data), three countries additionally submitted their sequence data to ENA (European Nucleotide Archive). Another two countries answered that they reported sequence data to other public databases, as shown in Figure 14. Countries enlisted national sequencing databases as additional public database which they were reporting to. It is important to note that countries could make multiple choices and five countries stated that they reported to more than one database.

Figure 14. Reporting of influenza and SARS-CoV-2 sequence data to publicly available databases (multiple choice)



Conclusions

This survey assessed the current capability and capacity of influenza and SARS-CoV-2 antiviral resistance testing in EU/EEA countries (Annex 1). All EU/EEA countries were asked to respond to the 19 questions and describe the situation of their country.

As of 14 June 2022, more than half of the countries (ten of 17) performed antiviral resistance testing for influenza and three countries performed antiviral resistance testing for SARS-CoV-2. The objectives of the implementation of antiviral resistance monitoring included the identification of mutations that may alter the antigenic properties of the virus affecting the effectiveness of certain mAb treatments, or the determination of their effect on viral drug susceptibility. Moreover, antiviral resistance monitoring of new variants for mAb-based antiviral treatments could improve the proper clinical use of some mAbs and the decisions on whether the use of certain treatments should be discontinued or different combinations of mAbs should be used. It is important to note that most of the countries (n=12) are planning to or are interested in implementing antiviral resistance testing either for influenza or SARS-CoV-2, or for both. Three countries are planning to extend the analysis to RSV.

Most countries reported monitoring the following SARS-CoV-2 monoclonal antibodies for antiviral resistance: Evusheld, Ronapreve and Regkirona. Regarding influenza, most countries reported monitoring well-known antiviral drugs such as oseltamivir and zanamivir.

As no specific sampling strategy guidance for antiviral resistance monitoring is currently available, countries are applying different specimen collection criteria. ECDC published a document introducing Operational considerations for respiratory virus surveillance in Europe - July 2022 (europa.eu), which contains guidance on virological testing.

According to this document, a subset of available and technically suitable specimens testing positive for influenza viruses and/or SARS-CoV-2 from targeted surveillance and sentinel surveillance should be sequenced and genotypic antiviral characterisation should be carried out. Based on the genotypic results, further antigenic characterisation and phenotypic testing for antiviral drug or monoclonal antibody resistance should be carried out on a subset of specimens from the targeted surveillance and sentinel surveillance.

All sentinel specimens positive for influenza viruses or SARS-CoV-2 should be sequenced, and a subset shared for further virus characterisation and antiviral/therapy resistance testing at National Influenza Centres (NIC), SARS-CoV-2 reference laboratories, and/or WHO reference laboratories. For non-sentinel samples, the document suggests that a subset is shared for further virus characterisation and antiviral/therapy resistance testing at National Influenza Centres (NIC), SARS-CoV-2 reference laboratories, and/or WHO reference laboratories.

Also, a representative selection of all available specimens should be sent to the NICs, SARS-CoV2 reference laboratories and/or WHO reference laboratories to confirm the virus characterisation results and to perform additional antigenic characterisation and testing for antiviral drug or monoclonal antibody resistance.

The majority of countries collect either all sentinel surveillance specimens that test positive for influenza or SARS-CoV-2, specimens from patients with severe outcomes, or specimens from patients treated with antivirals. Few countries base their specimen selection criteria on the mutational profile of the viruses (based on sequencing first-approach) or collect specimens from immunocompromised patients.

Looking at the methods used for antiviral resistance testing, for SARS-CoV-2, most of the countries rely on genotypic analysis, while only two countries perform both genotypic and phenotypic analyses. For influenza, on the other hand, most countries perform both genotypic and phenotypic analyses. For phenotypic antiviral resistance testing for influenza, most countries use fluorescent-based MUNANA assays, whereas for genotypic antiviral resistance testing, the most common method for both influenza and SARS-CoV-2 is WGS. Although phenotypic tests remain the only way to confirm antiviral resistance of the virus, WGS is becoming the most commonly used approach, allowing rapid identification, monitoring and assessment of mutations creating antiviral resistant variants.

About half of participating countries confirmed that they report influenza antiviral resistance data in a virus-based record type to (TESSy). Indeed, in both 2021 and 2022, nine countries have reported such data to TESSy. However, none of the responding countries reported SARS-CoV-2 neutralisation data and/or antiviral drug resistance data to EpiPulse events yet. ECDC has been promoting the use of EpiPulse events for monitoring and for effective and timely sharing of information regarding new SARS-CoV-2 variants. EpiPulse events specifically for antiviral resistance monitoring have, however, not been used so far. EpiPulse as an online portal enables users from Member States assigned by National Coordinators to share data from antiviral resistance monitoring and information regarding ongoing investigation related to identification of mutations conferring resistance to drugs or mAbs. On the other hand, all participating countries share their sequence data on GISAID, while few upload the obtained genetic data to ENA. The sharing of sequence data through GISAID or other sequence databases is important for public health assessment, improvement of diagnostics and the development of candidate vaccines.

In order to facilitate the exchange of scientific knowledge and best practices, ECDC has established a virus characterisation working group in January 2021, specifically focusing on discussing advancements in virus characterisation approaches and efforts. Importantly, the well-established information exchange channels, such as the regularly organised ECOVID-LabNet meetings continue to take place. Activities within the ECOVID-LabNet network are expected to continue and to be expanded towards training (through study visits and working groups), development of guidance and sharing of laboratory protocols.

ECDC has been preparing a literature review on currently available data on SARS-CoV-2 therapeutic monoclonal antibodies, mutations altering the antigenic characteristics of the virus and methods and standards used for the analysis.

Limitations of the study

Only 17 out of 30 EU/EEA countries responded to the survey. Only one country completed the entire questionnaire and four countries responded to less than two thirds of the questions.

It is important to point out that the results here represent a snapshot at a particular moment in time. National strategies, including testing strategies and methods, are continuing to be discussed and are constantly evolving. Also, sub-national variations may have not been captured.

Contributors (in alphabetical order)

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- Christos Karagiannis – Nicosia General Hospital, Cyprus
- Mihaela Lazar – Cantacuzino National Military-Medical Institute for Research and Development, Romania
- Adam Meijer – National Institute for Public Health and The Environment, Netherlands
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Annex 1. Questionnaire

Question 1. Do you perform antiviral resistance testing on Influenza surveillance specimens?

- Yes
- No
- We are planning to but have not started yet.

Question 2. Do you perform antiviral resistance testing on SARS-CoV-2 surveillance specimens?

- Yes
- No
- We are planning to but have not started yet.

Question 3. If you are currently planning to implement antiviral resistance monitoring, which pathogen are you planning to focus on?

- SARS-CoV-2Influenza
- Both SARS-CoV-2 and influenza
- Additional pathogens – please specify in the comment box

Question 4. Is your institution part of any of the below listed clinical- or research-funded projects?

- RECOVER
- ORCHESTRA
- END-VOC
- EuCARE
- VERDI
- COVICIS
- UnCoVer
- ReCoDID
- Any other project – please specify in the comment box

Question 5. Which SARS-CoV-2-specific drugs or monoclonal antibodies are you monitoring for?

- Evusheld (tixagevimab / cilgavimab)
- Regkirona (regdanvimab)
- Ronapreve (casirivimab / imdevimab)
- Paxlovid (PF-07321332 / ritonavir)
- Molnupiravir (MK-4482, EIDD-2801)
- Any other drug or monoclonal antibody - please specify in the comment box

Question 6. Which Influenza-specific drugs are you monitoring for?

- Oseltamivir
- Zanamivir
- Baloxavir
- Any other drug

Question 7. How are the specimens collected?

- all sentinel surveillance specimens that test positive
- all non-sentinel surveillance specimens that test positive
- selection of samples based on mutation profile (based on sequencing first-approach)
- from immunocompromised patients
- samples from LTCFs
- from patients with severe outcome
- from patients treated with antivirals
- other selection criteria - please specify in the comment box.

Question 8. Which kind of analysis are you performing on SARS-CoV-2?

- Genotypic only
- Phenotypic only
- Both phenotypic and genotypic
- None yet

Question 9. How often are you analysing samples for antiviral resistance monitoring for SARS-CoV-2?

- weekly
- monthly
- irregularly (e.g. in batches)
- not at al

Question 10. Which kind of analysis are you performing on Influenza?

- genotypic only
- phenotypic only
- both phenotypic and genotypic
- none yet

Question 11. How often are you analysing samples for antiviral resistance monitoring for influenza?

- weekly
- monthly
- irregularly (e.g. in batches)
- not at all

Question 12. Which methods are you using to analyse antiviral resistance phenotypically for influenza?

- Comments (free text)

Question 13. Which methods are you using to analyse antiviral resistance phenotypically for SARS-CoV-2?

- Comments (free text)

Question 14. Which methods are you using to analyse antiviral resistance genotypically for influenza?

- SNP assays
- Sanger sequencing
- WGS

Question 15. Which methods are you using to analyse antiviral resistance genotypically for SARS-CoV-2?

- SNP assays
- Sanger sequencing
- WGS

Question 16. Are you reporting influenza antiviral drug resistance data to TESSy (INFLANTIVIR virus-based record type)?

- Yes
- No
- N/A

Question 17. Will you be able to report antiviral drug resistance data to TESSy in a virus-based record type?

- Yes
- No

- N/A

Question 18. Are you reporting SARS-CoV-2 neutralisation data and/or antiviral drug resistance data to the relevant EpiPulse events?

- Yes
- No
- N/A

Question 19. Are you reporting influenza and SARS-CoV-2 sequence data to:

- GISAID
- ENA
- Other public databases - please specify in the comment box

References

1. Commission E. COMMUNICATION FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT, THE EUROPEAN COUNCIL AND THE COUNCIL - A united front to beat COVID-19. 2021.
2. (ECDC) ECfDPaC. Risk related to the spread of new SARS-CoV-2 variants of concern in the EU/EEA – first update 2021 Available from: https://www.ecdc.europa.eu/sites/default/files/documents/COVID-19-risk-related-to-spread-of-new-SARS-CoV-2-variants-EU-EEA-first-update.pdf.