Guidance for representative and targeted genomic SARS-CoV-2 monitoring

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Key messages

Genomic surveillance of SARS-CoV-2 is essential to detect, monitor and assess virus variants that can result in increased transmissibility, disease severity, or have other adverse effects on public health and social control measures. Obtaining timely and accurate information on the emergence and circulation of variants of concern (VOCs) and variants of interest (VOIs) requires robust surveillance systems, including integrated genome sequencing with a well-defined sampling and sequencing strategy to ensure representativeness and reliability of findings.

The current document offers practical guidance to EU/EEA Member States on implementing genomic SARS-CoV-2 surveillance, including advice on the number of samples that need to be sequenced to achieve various objectives.

For genomic surveillance of SARS-CoV-2, ECDC recommends two complementary sampling approaches:

- representative sampling of SARS-CoV-2 RT-PCR positive cases from existing, population-based surveillance systems;
- targeted sampling of SARS-CoV-2 positive cases occurring in special settings or populations.

Representative sampling and sequencing of SARS-CoV-2 cases for genomic monitoring from routine surveillance

The objective for representative sampling and sequencing of SARS-CoV-2 from cases identified during routine surveillance is to detect and monitor new variants of concern.

- For novel or emerging variant detection, ECDC recommends a detection threshold minimum which is a relative proportion of 2.5%, but ideally 1%, of a particular variant among all variants within one unit of time.
- For monitoring, ECDC recommends sequencing adequate numbers of samples to detect a difference in the relative proportion of a certain variant from one unit of time to the next (e.g. increase from 1% to 3%, or 2.5% to 5%).

Respective sample sizes and options for specimen selection are provided in the guidance.

Targeted sampling and sequencing of SARS-CoV-2 cases from special settings or populations

The following sampling frames are recommended for the respective settings or populations:

- vaccine breakthrough infections and reinfections: comprehensive sampling to detect and characterise variants causing infection in the presence of SARS-CoV-2 antibodies;
- outbreaks and clusters: a representative sample, with a minimum of five specimens per event to investigate virus transmission dynamics; detect novel genetic variants; assess the relatedness of viral strains within epidemiological clusters, and support contact tracing and other public health interventions;
- confirmed cases with travel history in areas where VOCs or VOIs are endemic: to detect potential introductions of variants and slow down their spread, ECDC recommends comprehensive sequencing sampling of all SARS-CoV-2 positive cases with travel history in areas/countries where new VOCs or VOIs are circulating;
- unusual events: a representative sample, with a minimum of five specimens from superspreading events or settings with unusually high transmission; for cases with unusual clinical presentations, ECDC recommends comprehensive sampling to support investigations of virus transmission dynamics and detection of novel genetic variants.

This guidance may need updating when the epidemiological situation changes - for example after extensive vaccination uptake in the population resulting in a significant reduction in case numbers.

Background

Sequence-based SARS-CoV-2 surveillance is essential to detect, monitor and assess virus variants that might result in increased transmissibility and disease severity, or have adverse effects on public health and social control measures. Timely and reliable information on circulating variants is the key to public health decisions concerning the reduction of general transmission and assessment of the implications for vaccination programmes [1]. Although public health and social control measures are currently the same for the different variants, they may need to be maintained, reinforced or reimplemented if certain variants increase in incidence. The SARS-CoV-2 variants of concern (VOCs) that emerged recently were first reported in the United Kingdom (UK), South Africa and Brazil, and subsequently in all EU/EEA countries.

Detection and monitoring of variants will be affected by the level of SARS-CoV-2 circulation in a country. If countries face a wave of infections, increased transmission might drive the emergence of new variants. High or intermediate levels of vaccination coverage and population immunity, together with circulation of the virus in the population may still facilitate variant emergence through selection pressure, even if case numbers are low [2].

The potential for rapid spread due to increased transmissibility, and/or immune escape linked to certain variants demonstrates the need for a strategic approach to genomic monitoring of the SARS-CoV-2 variants circulating in the EU/EEA and globally.

Scope of this document

With the implementation of broad vaccination campaigns across the EU/EEA, the SARS-CoV-2 pandemic response has entered a new phase. Although the currently authorised vaccines have shown very high efficacy and effectiveness in clinical trials and post-marketing studies respectively [3-6], their public health impact could be compromised by the emergence of variants evading the immune response [7]. Timely and accurate information on the emergence and circulation of VOCs requires robust surveillance systems and integrated genome sequencing is an essential component of such systems. These systems would allow for the timely and effective adjustment of public health measures until high vaccination coverage is achieved in the population. Furthermore, even after vaccination programmes have progressed and the majority of the population has received a complete course of vaccination, new VOCs will probably emerge. Their timely detection and characterisation will be essential in order to limit spread and to inform decisions on whether to update vaccines, keep measures in place or reinforce them - in the event of a potential increase in the presence of a certain variant. Therefore, the reliable detection and monitoring of the presence of VOCs and VOIs in the population involves being able to include this information in situation assessments to guide decision-making processes. Such genomic surveillance systems must be integrated into existing population-based surveillance systems, with a well-defined sampling and sequencing strategy to ensure representativeness and reliability of findings. This means that sampling schemes for SARS-CoV-2 should be integrated into both community-based and hospital-based COVID-19 surveillance systems [8]. In addition, performing sequencing on samples collected from outbreaks offers a unique opportunity to rapidly define the main epidemiological and clinical characteristics of VOCs, thus allowing for appropriate risk assessment and management. Such surveillance systems should also be able to detect as yet unknown variants in a timely fashion.

This document offers practical guidance for EU/EEA Member States on implementing genomic SARS-CoV-2 surveillance. It also includes advice on how to estimate the number of sequenced samples needed to achieve various objectives, including the early detection of novel variants.

The guidance is intended to support short and mid-term data collection at EU/EEA level, and it may need updating when the epidemiological situation changes. For example, this might be after extensive vaccination uptake in the population results in a significant reduction in case numbers.

Target audience

Public health authorities in EU/EEA Member States.

Overall objectives for genomic SARS-CoV-2 surveillance

The main objectives of SARS-CoV-2 sequencing are to:

- accurately monitor the prevalence and spread of circulating variants in the EU/EEA countries;
- detect novel or emerging VOCs and VOIs in a timely fashion;
- support epidemiological and genomic virological characterisation of variants.

Definitions

Definition of a 'SARS-CoV-2 variant of interest'

A SARS-CoV-2 isolate is a VOI if it is phenotypically changed compared to a reference isolate or has a genome with mutations that lead to amino acid changes associated with established or suspected phenotypic implications[1];

AND

has been identified as causing community transmission[2]/multiple COVID-19 cases/clusters, or has been detected in multiple countries;

OR

is otherwise assessed to be a VOI by WHO in consultation with the WHO SARS-CoV-2 Virus Evolution Working Group [9].

Working definition of a 'SARS-CoV-2 variant of concern'

A VOI (as defined above) is a VOC if, through a comparative assessment, it has been demonstrated to be associated with:

- increase in transmissibility or detrimental change in COVID-19 epidemiology;
- increase in virulence or change in clinical disease presentation; or
- decrease in effectiveness of public health and social measures or available diagnostics, vaccines and therapeutics;

OR

is assessed to be a VOC by WHO in consultation with the WHO SARS-CoV-2 Virus Evolution Working Group [9].

Samples to be sequenced

Sequencing should be performed on a clinical sample from a laboratory-confirmed COVID-19 case according to the EU case definition [10]. The sample should fall within one of the categories prioritised in this guidance and have been reported to the national public health institutes in the EU/EEA.

Case definition for suspected reinfection

To describe the epidemiology of reinfection in the EU/EEA and to understand the risk of reinfection with VOCs, ECDC developed the following case definition for suspected COVID-19 reinfection, published on 15 March 2021 [11]:

A suspected COVID-19 reinfection case is defined as:

positive RT-PCR or rapid antigen test (RAT) sample ≥60 days following:

- previous positive RT-PCR; or
- previous positive RAT; or
- previous positive serology (anti-spike IgG Ab).

Consideration for vaccine breakthrough infection

A vaccine breakthrough infection can be considered as a fully-vaccinated person with a positive RT-PCR or rapid antigen test (RAT) > 14 days after completing vaccination.

People are considered fully vaccinated for COVID-19 ≥ 2 weeks after they have received the second dose in a two-dose series (Pfizer-BioNTech or Moderna or AstraZeneca), or ≥2 weeks after they have received a single-dose vaccine (Johnson and Johnson (J&J)/Janssen) [12].

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[1] Phenotypical changes include changes in the epidemiology, antigenicity, or virulence or changes that have or potentially have a negative impact on available diagnostics, vaccines, therapeutics or public health and social measures. WHO will provide guidance on amino acid changes with established or suspected phenotypical implications and may be informed via a database on key amino acid changes, or as reported in scientific literature.
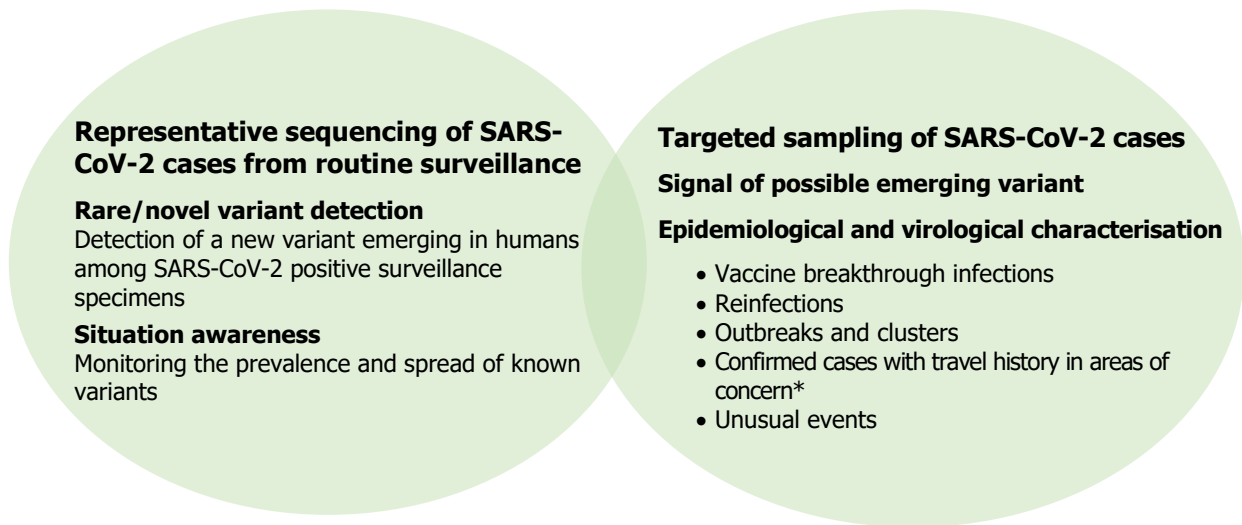
[2] For definitions see WHO public health surveillance for COVID-19: interim guidance. 16 December 2020. Available from: https://www.who.int/publications/i/item/who-2019-nCoV-surveillanceguidance-2020.8

SARS-CoV-2 genomic surveillance approaches

Effective national and EU-level SARS-CoV-2 genomic surveillance is dependent on the existence of population-based SARS-CoV-2 surveillance systems which are also able to identify cases with reinfection and vaccine breakthrough. Furthermore, countries are advised to establish capacity to detect, describe and investigate outbreaks occurring in the community and in special settings. Therefore, genomic surveillance will be based on two complementary sampling schemes:

1. representative (i.e. unbiased) sampling of SARS-CoV-2 RT-PCR positive cases from existing, population-based surveillance systems;
2. targeted sampling of SARS-CoV-2 positive cases occurring in special settings or populations (outbreaks, vaccine breakthrough infections, travellers, etc).

Figure 1. **Complementary SARS-CoV-2 genomic monitoring approaches**

Representative sequencing of SARS-CoV-2 cases from routine surveillance

Rare/novel variant detection
Detection of a new variant emerging in humans among SARS-CoV-2 positive surveillance specimens

Situation awareness
Monitoring the prevalence and spread of known variants

Targeted sampling of SARS-CoV-2 cases

Signal of possible emerging variant

Epidemiological and virological characterisation

- Vaccine breakthrough infections
- Reinfections
- Outbreaks and clusters
- Confirmed cases with travel history in areas of concern*
- Unusual events

Defined as areas where VOCs or VOIs, as yet detected through testing, are circulating in the community.

1. Representative sampling of SARS-CoV-2 cases from routine surveillance

Objectives of representative sampling of SARS-CoV-2 cases for genomic monitoring from routine surveillance

Novel or emerging variant detection

- Identification of introductions of a known new variant not yet circulating in an EU/EEA country.
- Early detection of novel and emerging variant viruses with new genomic composition for further assessment as potential VOIs or VOCs.
- Early spatio-temporal monitoring of newly-identified virus variants to support assessment and public health measures.

Situation awareness

- Monitor the prevalence and spread of circulating variants to guide public health action.
- Monitor the spatio-temporal dynamics of currently circulating variants.
- Monitor virus evolution.
- Support public health assessment of changes in the patterns of transmission and/or disease severity through linkage with detailed case data.

Strategy and sample selection for representative sampling of SARS-CoV-2 cases for genomic monitoring

Sampling strategy is determined by the purpose of the sequencing effort. To generate data that reflect the situation in the country, specimens selected for sequencing should be representative.

Specimens for genome analysis should be selected as being representative of the SARS-CoV-2 cases in the country. Sample collection should be made using methods that ensure the unbiased selection of cases for sequencing. It is important to ensure that sequencing is performed on a sufficient number of cases every week (representative in terms of time), at every level of the healthcare systems (representative in terms of clinical spectrum), and in all regions or other administrative areas of a country (representative in terms of geography). This should ensure representativeness in terms of age, gender and disease severity of cases.

Representative sequencing will include vaccine breakthrough infections, reinfections, cases from outbreaks and among travellers. They should be included in the denominator as they have been selected as part of the representative sampling.

Different sampling methods can be used for the selection process, as described below.

It is important that the sampling strategy is well-documented and taken into consideration during further data analysis, so that bias is minimised and, where present, considered in the analysis and interpretation of data.

Options for specimen selection as part of representative sampling of SARS-CoV-2 cases for genomic monitoring

Sampling bias should be reduced to a minimum when selecting specimens for sequencing. Four options for representative sample selection are described below. Representativeness can be achieved through systematic/consecutive sampling or simple random sampling.

Systematic/consecutive sampling
- Draw up a sampling frame (for instance, all positive samples tested within one week in one laboratory – e.g. 500 samples). Select samples at regular intervals, for example choose one of every fifth or one of every tenth sample to be sequenced, until the sampling frame is reached.
- *Potential bias:* If the samples are grouped so that particular characteristics occur at regular intervals, bias can occur.

Alternative systematic/consecutive sampling
- Draw up a sampling frame (for instance, all positive samples tested within one week in one laboratory - e.g. 500 samples). Select samples in bulk: for 10%, this could be the first 50 positive samples arriving at the laboratory at the beginning of the week in question, or the first 10 positive samples arriving each day on Monday, Tuesday, Wednesday, Thursday and Friday, respectively.
- *Potential bias:* If the samples are grouped so that particular characteristics occur at regular intervals, bias can occur.

Simple random sampling

- Draw up a sampling frame (e.g. all positive samples tested within one week in one laboratory). Choose a random selection of the required number of positive samples by using random number tables or using random numbers generated by a computer program (e.g. Excel).
- *Potential bias:* Bias is limited compared to the previously described methods, but workload is increased. This method also involves waiting until the sampling frame is complete before selecting samples which may have a negative impact on the timeliness of sequencing.

Alternative simple random sampling

- Draw up a sampling frame. Select every sample with a certain probability (if aiming to sequence 10% of confirmed cases, use a random number generator for each sample to include it with 10% probability).
- *Potential bias*: Bias is limited and there is no waiting time involved to complete sampling frames, but workload is increased compared to the first three options. It does not result in exactly 10%, but statistically that is acceptable.

Representativeness of the selected samples depends on the specific practices in the laboratories (for example, how samples are received and processed) and should be validated by comparing the distribution of age, gender, geographical area, time and clinical spectrum for representativeness, and comparing severity among the sequenced cases with that among all cases.

Definitions of thresholds

ECDC recommends using sensitivity thresholds to determine the necessary minimal number of representatively selected samples to be sequenced for genomic monitoring of SARS-CoV-2. In this context, a threshold defines a certain proportion of a variant, or a certain increase in the proportion of a variant which calls for enhanced public health measures (increased contact tracing, further epidemiological and laboratory assessment of the variant, etc). These thresholds can be used to improve the sensitivity and reliability of the surveillance system to detect the presence of a variant and to accurately estimate its prevalence and trends over time.

Objective 1: novel or emerging variant detection

A detection threshold minimum should be used for a relative proportion of 2.5% of a particular variant among all variants over one time unit of sampling. With a growth rate of around 50% per week, which has previously been observed for B.1.1.7 replacing other variants [13], this corresponds to 7−8 weeks forecasting before the variant becomes the dominant one. This forecasting ability allows for potential effective interventions, such as containment measures. If capacities allow, a lower threshold (e.g. 1%) can also be used. In this case, planned interventions may need to be adapted accordingly (e.g. close observation of the epidemiological situation and early characterisation of the virus).

Objective 2: situation awareness

For situation awareness and spread of known VOCs/VOIs, ECDC recommends assessing the trend in the relative proportion of these variants over time. If the relative proportion of a certain variant increases significantly from one time unit for sampling to the other, this threshold should be considered as an alert for enhanced awareness and/or further actions.

Sample size

Sample sizes for different scenarios relating to the recommended thresholds can be found in Tables 1, 2 and 3. Table 1 shows how many samples need to be sequenced to estimate the proportion of a certain circulating variant when it reaches 1%, 2.5% or 5% (Objective 1). The sample size was computed for the estimation of the 95% confidence interval based on the proportions 1%, 2.5% and 5% and with a relative precision of 50%. Table 2 shows how many samples need to be sequenced to have at least 80% power and 5% significance level in detecting a difference in the proportion of a certain variant, from 1% to 2%, 3%, 5% or 10% respectively, within a certain time unit for sampling (Objective 2). Similarly, Table 3 shows how many samples need to be sequenced to have at least 80% power and a 5% confidence level in detecting a difference in the proportion of a certain variant, from 2.5% to 5% or 10% respectively, within a certain time unit for sampling.

In Tables 1, 2 and 3, a finite population correction factor (where the term population corresponds in this case to the total number of positive SARS-CoV-2 cases) was applied to the computed sample size for several ranges of the total number of positive SARS-CoV-2 cases. The proportion of samples to be selected for sequencing is based on the time unit for sampling the overall number of positive SARS-CoV-2 cases.

Details of the formulas used for computing the sample size calculation and the population correction factor are shown in Annex 1.

Table 1. Sample size needed to estimate the 95% confidence interval for the proportion of a certain circulating variant when its proportion reaches 1%, 2.5% or 5% (Objective 1) with a relative precision of 50% based on representative selection of samples (Objective 1)

Number of positive SARS-CoV-2 cases	Sample size* based on the minimum prevalence of a variant to be detected		
	1%	2.5%	5%
> 100 000	1 522	600	292
50 001−100 000	1 500	597	292
25 001−50 000	1 478	593	291
10 001−25 000	1 435	586	289
5 001−10 000	1 321	567	284
2 501−5 000	1 167	536	276
1 001−2 500	947	484	262
501−1 000	604	375	227
< 500	377	273	185

* Green shade marks the recommended minimum threshold.

Examples of how to use Table 1 for Objective 1

a)   If a country has more than 100 000 cases per week (possible time unit for sampling) and wants to detect a new variant at a prevalence of 2.5% with a 95% confidence interval, it would need to sequence 600 samples per week.

b)   If a country has more than 100 000 cases per week (time unit for sampling) and wants to detect a new variant at a lower prevalence (1%), it needs to sequence 1 522 samples per week.

Table 2. Sample size needed to detect a difference in the proportion of a certain variant, from 1% to 2%, 3%, 5% or 10 % respectively, within a certain time unit for sampling (Objective 2)

Number of positive SARS-CoV-2 cases	From 1% to 2%*	From 1% to 3%*	From 1% to 5%*	From 1% to 10%*
> 100 000	1 859	615	227	78
50 001−100 000	1 826	612	227	78
25 001−50 000	1 793	608	226	78
10 001−25 000	1 731	601	225	78
5 001−10 000	1 568	580	222	78
2 501−5 000	1 356	548	218	77
1 001−2 500	1 067	494	209	76
501−1 000	651	381	186	73
< 500	395	276	157	68

* Refers to the detection of a significant difference in the proportions of a certain variant from one time unit for sampling to another.

Green shade marks the recommended minimum threshold.

Table 3. Sample size needed to detect a difference in the proportion of a certain variant from 2.5% to 5% or 10% respectively, within a certain time unit for sampling (Objective 2)

Number of positive SARS-CoV-2 cases	From 2.5% to 5%*	From 2.5% to 10%*
> 100 000	725	129
50 001−100 000	720	129
25 001−50 000	715	129
10 001−25 000	705	129
5 001−10 000	676	128
2 501−5 000	634	126
1 001−2 500	563	123
501−1 000	421	115
< 500	296	103

* Refers to the detection of a significant difference in proportions of a certain variant from one time unit for sampling to another.

Examples of how to use Tables 2 and 3 for Objective 2

a) If a country with 15 000 cases per week (time unit for sampling) would like to detect a significant difference in the proportion of a variant from one week to another (e.g. an increase from a proportion of 1% in one week to a proportion of 3% the next week), it needs to sequence 601 samples (Table 2, row with 10 001–25 000 cases, column 'From 1% to 3%') per week.

b) If a country with 3 000 cases per week (time unit for sampling) would like to detect a significant difference in the proportion of a variant from one week to another (e.g. an increase from a proportion of 2.5% in one week to a proportion of 10% in the next week), it needs to sequence 126 samples (Table 3, row with 2 501–5 000 cases, column 'From 2.5% to 10%') per week.

Considerations for sample size calculations and ways to avoid bias

The underlying assumptions for the sample size calculations are that the chosen samples are representative of the target population according to the criteria mention earlier, so that bias is minimised, and sequencing leads to the timely and accurate identification of VOCs. However, bias might still occur, and it should be taken into consideration when analysing the data.

The following list provides considerations for sampling and ways to avoid bias:

- Diagnostic assay considerations:
    - Pre-screening of samples using specific RT-PCR assays to detect specific SNPs or S-gene target failure. Optimally, specimens for representative genomic monitoring are chosen from among SARS-CoV-2 positive samples, irrespective of whether the samples have been pre-screened using a non-sequencing method. Pre-screening may be needed if the capacity for sequencing is limited and variants are not common. For the detection and monitoring of variants, this strategy is straightforward so long as random selections are made for sequencing, regardless of the outcome of the RT-PCR variant.
    - Pre-screening at local level might also delay whole genome sequencing results. Local positive samples might be screened in batches as soon as sufficient positive samples have been collected (e.g. only once or twice a week). If samples for sequencing are selected after screening this will significantly delay the timeliness of sequencing results.
    - In some situations, an elaborate variant-screening PCR algorithm can consume specimens, so that little or no material is left that can be forwarded for sequencing.
    - If standard diagnostic assays fail to detect certain variants (e.g. negative signal due to primer/probe mismatch) sequencing will also not be conducted. In such cases if the clinical and/or epidemiological information means that a case is highly likely to be a SARS-CoV-2 infection, other targets or primers/probes with modifications for the same target should be used for nucleic acid amplification. It is therefore important to assess the performance of routine diagnostic methods against VOCs.
    - Certain variants may be more or less likely to be detected by RT-PCR (e.g. B.1.616 [14]) because the viral load may be too low in the most common clinical specimens (nasopharyngeal swab). This could result in a lower representation of variants among samples selected for sequencing, leading to an underestimation of the prevalence. This might also lead to an incorrect assessment of severity if most detections are based on more invasive sample collections (which are more likely to be done in hospitals for severe cases).
- Sampling strategy is highly dependent on the overall testing strategy in countries. Sample size calculations are performed based on the proportion of confirmed SARS-CoV-2 infections, which can include not only RT-PCR-confirmed cases, but also rapid antigen-test confirmed cases. Sequencing is usually only done on RT-PCR-confirmed samples which might introduce bias. In addition, rapid antigen tests are often used in specific settings, such as contact tracing for early identification of cases, and detection of milder or asymptomatic cases is more probable. This could also lead to bias if RT-PCR is used more for severe cases, or if a variant might potentially give a negative result in RATs (e.g. if there are genomic changes, especially in the N-gene).
- If countries have situations where they prefer to use RATs instead of RT PCR tests, they might need to consider sequencing samples from cases testing positive, requiring an additional sample for RT PCR and sequencing.
- Certain groups of the population may be over- or underrepresented among the specimens available for sequencing in relation to their true size in the population. This may be the case in certain geographical areas or population groups with specific demographic characteristics (e.g. younger age). Underrepresentation may lead to delayed detection of novel variants and additional bias. In such situations, data need to be interpreted carefully or a stratified sampling approach should be considered.

It is recommended that each country adapts its strategy of sample selection according to regional laboratory structures, practices, sustainability and capacities.

2. Targeted sampling of SARS-CoV-2 cases from special settings or populations

Targeted sampling of SARS-CoV-2 cases from special settings or focused monitoring can include cases from outbreak investigations (occupational settings, long-term care facilities, healthcare institutions, schools, kindergartens, sports activities, etc.), testing of incoming travellers returning from specific destinations, vaccinated people, reinfections, and others. Samples from vaccine breakthrough infections and reinfections will also automatically be part of representative sampling of SARS-CoV-2 cases for genomic monitoring, as random selection is made irrespective of the specific meta-data. Therefore, representative sequencing might provide further information in addition to the targeted sequencing of such cases. However, if possible, sequencing of samples from travellers returning from areas endemic for VOCs should not be included in representative sampling. These travellers do not share the same risk of VOC infection as residents (they represent infection risk for the country they have arrived from) and including them would overestimate the incidence.

Targeted sequencing complements the representative sampling of SARS-CoV-2 cases as new variants can be detected by investigating outbreaks or reinfections/vaccine breakthrough infections that a representative sample might not catch early enough.

Strategies and objectives for targeted sampling of SARS-CoV-2 cases

As stated above, ECDC recommends implementing enhanced targeted genomic monitoring in addition to representative sampling. The objectives and sampling frames for targeted monitoring are listed below.

Vaccine breakthrough infections

Monitoring of vaccine breakthrough infections provides important information regarding viral variants that may have an impact on vaccine effectiveness, duration of immunity and/or sub-optimal immune response [15,16]. SARS-CoV-2 surveillance systems should be set to capture vaccine breakthrough infections in order to prompt an investigation, including sequencing. Rapid detection and reporting of potential vaccine escape variant strains provides an early warning and may help prevent the spread of novel variant types. It is particularly important to be able to characterise breakthrough infections by vaccine product, time since vaccination, severity (e.g. hospitalisation, death), and age group.

Objectives

- Detection and characterisation of SARS-CoV-2 causing infection in the presence of SARS-CoV-2 antibodies, evoked by vaccination, thus informing control measures and vaccine development.

Sampling frame

- With the current vaccine rollout, ECDC recommends comprehensive sampling and subsequent sequencing of all vaccine breakthrough infections. This advice may need to be updated when the epidemiological situation changes - for example after comprehensive vaccine uptake in the population, resulting in a significant reduction in case numbers.

Reinfection

As outlined in a previous ECDC Threat Assessment Brief [17] and technical report on the sequencing of SARS-CoV-2 [16], specimens from previously confirmed COVID-19 cases with suspected reinfection should be investigated and specimens sequenced to confirm the reinfection and provide detailed information concerning the virus. COVID-19 surveillance systems should be set to identify reinfections.

Objectives

- Detection and characterisation of SARS-CoV-2 causing infection in the presence of SARS-CoV-2 antibodies, evoked by a previous infection, thus informing control measures and vaccine development.

Sampling frame

- ECDC recommends comprehensive sampling and subsequent sequencing of all suspected reinfections in accordance with its technical report on the sequencing of SARS-CoV-2 [16]. This advice may need to be updated when the epidemiological situation changes - for example after comprehensive vaccine uptake in the population, resulting in a significant reduction in case numbers and/or increases in the number of reinfections in the presence of SARS-CoV-2 antibodies.

Outbreaks and clusters

Sequencing in the context of outbreaks is important to identify VOCs and their epidemiological and clinical features (secondary attack rates, age, gender, type of exposure, vaccination status, prior infection, use of face masks, setting). In addition, samples from positive cases in clusters with an unusual signature should be sequenced. Sequencing makes it possible to assess the timing and source of virus and the relatedness of cases. This type of genomic surveillance requires that countries have systems in place for the timely detection, reporting and investigation of outbreaks.

Objectives

- Investigating virus transmission dynamics and detection of novel genetic variants.
- Assessing relatedness of viral strains within epidemiological clusters and supporting contact tracing and other public health interventions.

Sampling frame

Representative sample from outbreaks or clusters, with a minimum of five specimens (to be able to assess whether the event is dominated by a single variant).

Confirmed cases with travel history in areas where VOCs or VOIs are endemic

In order to detect the importation and slow down the spread of new VOCs in areas or countries where they are not yet present or are only circulating at very low levels, ECDC recommends sequencing samples from travellers having tested positive for SARS-CoV-2. This is particularly relevant for, but not limited to, those coming from areas where VOCs or VOIs are endemic that are not yet present in the country/area of arrival.

Objective

- Detecting potential variant introductions and slowing down their spread.

Sampling frame

- Comprehensive sequencing of all SARS-CoV-2 positive cases with travel history to areas/countries where new VOCs or VOIs are circulating.

Unusual events

Large outbreaks associated with a specific setting/behaviour/age-group may signal the presence of a VOC with certain characteristics - e.g. increased transmissibility. Therefore, when such events are detected, a subset of cases should be sequenced. Other examples of events that require sequencing can be cases with an unusual clinical presentation, such as severe infections and deaths in younger age groups with no underlying diseases, prolonged infections, a general change in the clinical presentation and cases where zoonotic transmission has been raised as a possibility and cannot be ruled out. This may indicate a change in pathogen virulence or inter-species transmission and should be monitored.

Objectives

- Investigation of virus transmission dynamics and detection of novel genetic variants.

Sampling frame

- Representative sample, with a minimum of five specimens (to be able to assess whether the event is dominated by a single variant) in settings with high transmission, such as superspreading events.
- For cases with unusual clinical presentation (e.g. severe infection of younger age groups with no underlying diseases, prolonged infections, etc.), ECDC recommends comprehensive sampling and subsequent sequencing.

Timeliness

Genomic monitoring in real time is the key to being able to rapidly adjust public health measures and enhance contact tracing before widespread transmission occurs. Sequencing results for both representative and targeted sampling of SARS-CoV-2 cases should be available in a timely manner to allow such interventions to be implemented.

Data providers and data sources

Countries should use existing laboratory networks or establish a laboratory network to coordinate sequencing efforts, data (including meta data) and specimen sharing. In countries where SARS-CoV-2 testing is decentralised, specimen providers might need to be recruited from primary healthcare providers, hospital, and clinical laboratories, academic or commercial laboratories. In order to achieve geographical representativeness of the samples, sampling should cover the SARS-CoV-2 diagnostics in all regions of the country. Those submitting specimens should receive instructions on how to forward samples to be sequenced to the respective sequencing facilities, including instructions to ensure that the samples selected are representative and of good quality.

For optimal sequencing results, the quality of the sample and the viral load are important. The selected specimen should:

- contain enough material for nucleic acid extraction;
- when already extracted, have a low Ct value (it should be noted that the RNA quality may drop and Ct value increase with repeated freeze-thaw cycles of extracted RNA);
- be submitted to the sequencing facility in a timely manner.

The Ct value of samples should be <30, but this depends on the performance of the sequencing facility and it may be possible to process samples with higher Ct values as well. The cold chain should be always maintained during transport and RNA samples should be stored at ≤ 70° C.

Sample submission and compliance may be enhanced by providing:

- clear instructions and submission forms adapted for the sampling laboratory;
- cost-free specimen collection kits and shipping or reimbursement for sampling;
- guidance on optimum sample selection;
- feedback and data to sample submitters, including sequencing results.

The establishment of a network and cross-disciplinary collaboration between national and local public health institutes, laboratories, healthcare providers and universities is essential in order to establish capacity and enable communications that support efficient genomic monitoring of variants.

Data management and analysis

In order to use the genomic surveillance data for public health decisions, both virus sequence data and patient metadata are required. Linking sequence data and patient metadata can be challenging, especially when different organisations are involved in testing, sequencing, and collecting linked case and epidemiological data. An identification system needs to be devised that allows data entry and management for the respective organisations involved. This type of laboratory information management system is critical for reporting results back to sample providers (local and regional laboratories, clinics, and hospitals, etc.) and providing timely data for analysis.

For optimal data analysis to guide public health interventions, a certain minimum set of metadata is required together with the sequence information. A list of metadata which should be collected and reported to The European Surveillance System (TESSy) can be found in ECDC's reporting protocol [18].

Further information on sequence methodologies, analysis and suggested applications at the local and national level can be found in ECDC's Technical Guidance 'Sequencing of SARS-CoV-2: first update' published on 18 January 2021 [19].

Data sharing and reporting

Data sharing and reporting are described in the report 'Methods for the detection and identification of SARS-CoV-2 variants' published by ECDC and WHO's Regional Office for Europe on 3 March 2021 [20] and ECDC's Technical Guidance 'Sequencing of SARS-CoV-2: first update' published on 18 January 2021 [19].

Detections of SARS-CoV-2 should be reported on a weekly basis to The European Surveillance System (TESSy). Detection of novel VOCs or outbreaks of currently circulating VOCs should be reported immediately through the Early Warning and Response System (EWRS), while VOC detections should be reported to TESSy on a weekly basis [18].

In addition, it is recommended that SARS-CoV-2 sequences are submitted to GISAID (https://www.gisaid.org), the European COVID-19 Data Platform (https://www.covid19dataportal.org) or other public databases in a timely manner (ideally within one or two weeks of sample collection). Raw data can also be deposited in the COVID-19 Data Platform. Member States should not upload any information or data that can link back the submitted virus sequence to an individual patient in a national database, such as personal identifiers (e.g. name, date of birth, unique identification code) or granular demographic data (e.g. the patient's age) etc. It is the Member State's responsibility to ensure that sequencing data are submitted in accordance with all pertinent rules and regulations.

It is also the responsibility of reporting Member States to assess whether the virus is a variant included in the variant list, irrespective of the method used for detection/identification. Variables for reporting VOCs have been

implemented within the aggregated (NCOVAGGR) and case-based (NCOV) TESSy record types, where sequence ID numbers (e.g. GISAID identifiers) should be reported as well. ENA/SRA accession numbers pointing to raw sequence data, can also be submitted to TESSy. It is also important to share raw sequencing reads because this allows uniform bioinformatic approaches for consensus genome generation, making it possible to avoid potential errors in the generation of consensus sequences while also enabling further analyses (e.g. minority variants). Any epidemiological data available, including severity and probable country of infection, should be reported if data are submitted using the case-based record type (NCOV).

Please contact tessy@ecdc.europa.eu for assistance with TESSy uploading. Please contact PHE.Support.Microbiology@ecdc.europa.eu if you need assistance with the interpretation/reporting of the sequencing results.

Monitoring of quality

Laboratories should have a quality assurance system in place for the sequencing of SARS-CoV-2 and are encouraged to participate in external quality assessment (EQA) schemes or perform result comparison between laboratories for subsets of samples [21]. ECDC is planning a molecular External Quality Assessment for national COVID-19 reference laboratories in summer 2021. Please contact PHE.Support.Microbiology@ecdc.europa.eu for more information.

Laboratory support

ECDC and WHO's Regional Office for Europe coordinate their support to countries in the WHO European Region. ECDC is supporting the scale-up of sequencing and neutralisation assay capacity in EU/EEA Member States. Please contact PHE.Support.Microbiology@ecdc.europa.eu for more information. If other countries in south-eastern Europe or in Central Asia would like to receive support from WHO's Regional Office for Europe, they should contact eulab@who.int.

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Disclaimer

All data published in this report are correct to the best of our knowledge at the time of publication. Maps and figures published do not represent a statement on the part of ECDC or its partners on the legal or border status of the countries and territories shown.

References

1. Grubaugh ND, Hodcroft EB, Fauver JR, Phelan AL, Cevik M. Public health actions to control new SARS-CoV-2 variants. Cell. 2021 Mar 4;184(5):1127-32. Available at: https://www.ncbi.nlm.nih.gov/pubmed/33581746

2. Williams TC, Burgers WA. SARS-CoV-2 evolution and vaccines: cause for concern? Lancet Respir Med. 2021 Apr;9(4):333-5. Available at: https://www.ncbi.nlm.nih.gov/pubmed/33524316

3. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med. 2020 Dec 31;383(27):2603-15. Available at: https://www.ncbi.nlm.nih.gov/pubmed/33301246

4. Dagan N, Barda N, Kepten E, Miron O, Perchik S, Katz MA, et al. BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting. N Engl J Med. 2021 Apr 15;384(15):1412-23. Available at: https://www.ncbi.nlm.nih.gov/pubmed/33626250

5. Voysey M, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet. 2021 Jan 9;397(10269):99-111. Available at: https://www.ncbi.nlm.nih.gov/pubmed/33306989

6. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. N Engl J Med. 2021 Feb 4;384(5):403-16. Available at: https://www.ncbi.nlm.nih.gov/pubmed/33378609

7. Emary KRW, Golubchik T, Aley PK, Ariani CV, Angus B, Bibi S, et al. Efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 variant of concern 202012/01 (B.1.1.7): an exploratory analysis of a randomised controlled trial. Lancet. 2021 Apr 10;397(10282):1351-62. Available at: https://www.ncbi.nlm.nih.gov/pubmed/33798499

8. World Health Organization. Operational considerations to expedite genomic sequencing component of GISRS surveillance of SARS-CoV-2. Available at: https://www.who.int/publications/i/item/WHO-2019-nCoV-genomic-sequencing-GISRS-2021.1

9. World Health Organization. COVID-19 Weekly Epidemiological Update - 25 February 2021 [accessed 7 April 2021]. Available at: https://www.who.int/publications/m/item/covid-19-weekly-epidemiological-update.

10. European Centre for Disease Prevention and Control (ECDC). Case definition for coronavirus disease 2019 (COVID-19). ECDC [updated 3 December 2020]. Available at: https://www.ecdc.europa.eu/en/covid-19/surveillance/case-definition

11. European Centre for Disease Prevention and Control. Surveillance definitions for COVID-19. 15 March 2021. Stockholm: ECDC; 2021. Available at: https://www.ecdc.europa.eu/en/covid-19/surveillance/surveillancedefinitions

12. European Centre for Disease Prevention and Control (ECDC). Interim guidance on the benefits of full vaccination against COVID-19 for transmission and implications for non-pharmaceutical interventions. 21 April 2021. Stockholm: ECDC; 2021. Available at: https://www.ecdc.europa.eu/en/publications-data/interim-guidance-benefits-full-vaccination-against-covid-19-transmission

13. Davies NG, Abbott S, Barnard RC, Jarvis CI, Kucharski AJ, Munday JD, et al. Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. Science. 2021 Apr 9;372(6538) Available at: https://www.ncbi.nlm.nih.gov/pubmed/33658326

14. French Health Department: COVID-19: epidemiological update of April 15, 2021. Available from: https://www.santepubliquefrance.fr/maladies-et-traumatismes/maladies-et-infections-respiratoires/infection-a-coronavirus/documents/bulletin-national/covid-19-point-epidemiologique-du-15-avril-2021

15. European Centre for Disease Prevention and Control (ECDC). Risk related to the spread of new SARS-CoV-2 variants of concern in the EU/EEA – first update. 21 January 2021. Stockholm: ECDC; 2021. Available at: https://www.ecdc.europa.eu/en/publications-data/covid-19-risk-assessment-spread-new-variants-concern-eueea-first-update

16. European Centre for Disease Prevention and Control (ECDC). Risk of SARS-CoV-2 transmission from newly-infected individuals with documented previous infection or vaccination. 29 March 2021. Stockholm: ECDC; 2021. Available at: https://www.ecdc.europa.eu/en/publications-data/sars-cov-2-transmission-newly-infected-individuals-previous-infection

17. European Centre for Disease Prevention and Control (ECDC). Reinfection with SARS-CoV-2: considerations for public health response. Threat Assessment Brief. 21 September 2020. Stockholm: ECDC; 2020. Available from: https://www.ecdc.europa.eu/en/publications-data/threat-assessment-brief-reinfection-sars-cov-2.

18. European Centre for Disease Prevention and Control (ECDC). Coronavirus disease 2019 (COVID-19) data Reporting Protocol Version 4.4, 23 March 2021. Stockholm: ECDC; 2021. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/COVID-19-Reporting-Protocol-v4.2.pdf

19. European Centre for Disease Prevention and Control (ECDC). Sequencing of SARS-CoV-2: first update. ECDC; [updated 18 January 2021]. Available from: https://www.ecdc.europa.eu/en/publications-data/sequencing-sars-cov-2

20. European Centre for Disease Prevention and Control (ECDC). Methods for the detection and identification of SARS-CoV-2 variants. March 2021. ECDC, [accessed 7 April 2021]. Available from: https://www.ecdc.europa.eu/en/publications-data/methods-detection-and-identification-sars-cov-2-variants

21. World Health Organization. Diagnostic testing for SARS-CoV-2 2020. 11 September 2020. Available from: https://www.who.int/publications/i/item/diagnostic-testing-for-sars-cov-2

22. Wayne DW. Biostatistics: a foundation for analysis in the health sciences. 3rd ed. New York: John Wiley & Sons. 1999.

23. Wang H, Chow SC. 2007. Sample Size Calculation for Comparing Proportions. Wiley Encyclopedia of Clinical Trials.

Annex 1. Sample size calculation methodology

Tables 1, 2 and 3 show the sample size required for achieving Objectives 1 and 2. To compute the sample size we assumed that a representative sampling strategy was in place.

For the computation of the sample size required to detect a 95% confidence interval for the expected prevalence (Objective 1), the following formula was used [22]:

$$n = \frac{(Z\alpha_{/2})^2 * p(1-p)}{d^2}$$

where $Z\alpha_{/2}$ is the critical value of the Normal distribution at $\alpha_{/2}$ (with a confidence interval of 95%, α =0.05 and the critical value used was 1.96), p is the expected prevalence (1%, 2.5% and 5%), and d is the precision (d= 50% of the expected prevalence) for the confidence interval.

The finite population correction for the number of positive SARS-CoV-2 cases was calculated using the formula:

$$n_{corr} = \frac{n \times M}{n + M}$$

Where n is the computed sample size and M is the total number of positive SARS-CoV-2 cases per time unit for sampling.

To compute the sample size required n according to the expected increase in the prevalence (objective 2), we used the formula for detecting a difference between two proportions [23]:

$$n = \left(Z\alpha_{/2} + Z_\beta\right)^2 \times \frac{p_1(1-p_1) + p_2(1-p_2)}{(p_1 - p_2)^2}$$

where $Z\alpha_{/2}$ is the critical value of the Normal distribution at $\alpha_{/2}$ (to compute we assumed a confidence level of 95%, therefore α was 0.05 and the critical value used was 1.96), Z_β is the critical value of the Normal distribution at β (we considered a power of 80%, so β was 0.2 and the critical value was 0.87), and p_1 and p_2 are the proportions to compare (from 1% to 2%, to 3% and to 5%, and from 2.5% to 5% and 10%).

Annex 2. Estimated sample size required based on the mean, minimum and maximum weekly number of positive SARS-CoV-2 cases from week 48-2020 to week 7-2021 (30 November 2020 to 30 March 2021) by country

Table A1. Sample size needed to estimate the 95% confidence interval for the proportion of a certain circulating variant when its relative proportion reaches 1% (Objective 1) with a relative precision of 50% based on random selection of samples per week (example of time unit for sampling)

Country	Number of positive SARS-CoV-2 cases per week			Estimated sample size according based on expected prevalence of 1% of a variant per week		
	Mean	**Minimum**	**Maximum**	**Mean**	**Minimum**	**Maximum**
Austria	15 203	8 442	32 058	1 384	1 290	1 454
Belgium	15 256	12 585	17 684	1 384	1 358	1 402
Bulgaria	9 126	3 004	20 666	1 305	1 011	1 418
Croatia	10 716	2 252	25 533	1 333	909	1 437
Cyprus	1 692	747	3 376	802	502	1 050
Czechia	49 379	27 110	88 740	1 477	1 442	1 497
Denmark	10 182	2 661	24 676	1 325	969	1 434
Estonia	3 682	2 328	4 789	1 077	921	1 155
Finland	2 559	1 671	3 813	955	797	1 088
France	114 324	74 014	143 497	1 503	1 492	1 507
Germany	111 510	50 442	173 574	1 502	1 478	1 509
Greece	7 001	3 373	12 608	1 251	1 049	1 359
Hungary	17 959	7 715	39 170	1 404	1 272	1 466
Iceland	62	11	144	60	11	132
Ireland	10 737	1 780	45 726	1 334	821	1 473
Italy	107 719	83 706	176 310	1 501	1 495	1 509
Latvia	5 186	3 855	7 071	1 177	1 092	1 253
Liechtenstein	109	10	315	102	10	261
Lithuania	10 972	3 396	19 466	1 337	1 051	1 412
Luxembourg	1 895	852	3 711	844	547	1 080
Malta	925	548	1 299	576	403	701
Netherlands	43 724	24 069	80 594	1 471	1 432	1 494
Norway	2 786	1 729	4 758	985	810	1 154
Poland	59 625	35 266	123 744	1 485	1 460	1 504
Portugal	41 818	10 466	86 610	1 469	1 329	1 496
Romania	28 254	16 401	52 891	1 445	1 393	1 480
Slovakia	14 712	9 492	20 970	1 380	1 312	1 420
Slovenia	9 077	5 285	13 855	1 304	1 182	1 372
Spain	124 327	49 556	256 931	1 504	1 477	1 514
Sweden	31 354	19 618	46 178	1 452	1 413	1 474

Table A2. Sample size needed to estimate the 95% confidence interval for the proportion of a certain circulating variant when its relative proportion reaches 2.5% (Objective 1) with a relative precision of 50%, based on random selection of samples per week (example of time unit for sampling)

Country	Number of positive SARS-CoV-2 cases per week			Estimated sample size based on expected prevalence of 2.5% of a variant per week		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Austria	15 203	8 442	32 058	578	561	589
Belgium	15 256	12 585	17 684	578	573	581
Bulgaria	9 126	3 004	20 666	563	501	584
Croatia	10 716	2 252	25 533	569	474	587
Cyprus	1 692	747	3 376	443	333	510
Czechia	49 379	27 110	88 740	593	588	596
Denmark	10 182	2 661	24 676	567	490	586
Estonia	3 682	2 328	4 789	516	478	534
Finland	2 559	1 671	3 813	487	442	519
France	114 324	74 014	143 497	597	596	598
Germany	111 510	50 442	173 574	597	593	598
Greece	7 001	3 373	12 608	553	510	573
Hungary	17 959	7 715	39 170	581	557	591
Iceland	62	11	144	57	11	117
Ireland	10 737	1 780	45 726	569	449	593
Italy	107 719	83 706	176 310	597	596	598
Latvia	5 186	3 855	7 071	538	520	554
Liechtenstein	109	10	315	93	10	207
Lithuania	10 972	3 396	19 466	569	510	583
Luxembourg	1 895	852	3 711	456	353	517
Malta	925	548	1 299	364	287	411
Netherlands	43 724	24 069	80 594	592	586	596
Norway	2 786	1 729	4 758	494	446	533
Poland	59 625	35 266	123 744	595	590	598
Portugal	41 818	10 466	86 610	592	568	596
Romania	28 254	16 401	52 891	588	579	594
Slovakia	14 712	9 492	20 970	577	565	584
Slovenia	9 077	5 285	13 855	563	539	576
Spain	124 327	49 556	256 931	598	593	599
Sweden	31 354	19 618	46 178	589	583	593

Table A3. Sample size needed to detect a difference in the proportion of a certain variant, from 1% to 3% (Objective 2) within a week (example of time unit for sampling)

Country	Number of positive SARS-CoV-2 cases per week			Estimated sample size based on the expected increase in prevalence from 1% to 3% of a variant per week		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Austria	15 203	8 442	32 058	592	574	604
Belgium	15 256	12 585	17 684	592	587	595
Bulgaria	9 126	3 004	20 666	577	511	598
Croatia	10 716	2 252	25 533	582	484	601
Cyprus	1 692	747	3 376	452	338	521
Czechia	49 379	27 110	88 740	608	602	611
Denmark	10 182	2 661	24 676	580	500	601
Estonia	3 682	2 328	4 789	527	487	546
Finland	2 559	1 671	3 813	496	450	530
France	114 324	74 014	143 497	612	610	613
Germany	111 510	50 442	173 574	612	608	613
Greece	7 001	3 373	12 608	566	521	587
Hungary	17 959	7 715	39 170	595	570	606
Iceland	62	11	144	57	11	117
Ireland	10 737	1 780	45 726	582	458	607
Italy	107 719	83 706	176 310	612	611	613
Latvia	5 186	3 855	7 071	550	531	566
Liechtenstein	109	10	315	93	10	209
Lithuania	10 972	3 396	19 466	583	521	597
Luxembourg	1 895	852	3 711	465	358	528
Malta	925	548	1 299	370	290	418
Netherlands	43 724	24 069	80 594	607	600	611
Norway	2 786	1 729	4 758	504	454	545
Poland	59 625	35 266	123 744	609	605	612
Portugal	41 818	10 466	86 610	607	581	611
Romania	28 254	16 401	52 891	602	593	608
Slovakia	14 712	9 492	20 970	591	578	598
Slovenia	9 077	5 285	13 855	576	551	589
Spain	124 327	49 556	256 931	612	608	614
Sweden	31 354	19 618	46 178	604	597	607