Methods for the detection and characterisation of SARS-CoV-2 variants – first update

20 December 2021

What is new in this update:

- The reference list for available assays has been updated.
- The Delta and Omicron variant assays have been included.
- The rapid antigen detection test chapter has been updated to include information related to the Health Security Committee's list of mutually recognised tests in the EU/EEA countries.

Summary

In the past year, several SARS-CoV-2 variants of concern (VOCs) have emerged and monitoring their circulation in all countries is of key importance. Whole Genome Sequencing (WGS), or at least complete or partial S-gene sequencing, is the best method for characterising a specific variant. Alternative methods, such as diagnostic screening nucleic acid amplification technique (NAAT)-based assays, have been developed for early detection and prevalence calculation of variants of concern (VOCs), variants of interest (VOI) or variants under monitoring (VUM). Many of these methods can also accurately identify the variants, while others will require confirmation of at least a subset by sequencing.

Testing strategies should be flexible and rapidly adaptable to changes in the epidemiological situation across the WHO European Region, local epidemiology, population dynamics and available resources. Adequate sample and method selection is a key factor in the successful implementation of the testing strategy and is highly dependent on the specific public health objectives of the testing strategy. Specific objectives include assessment of the circulation of the different SARS-CoV-2 variants in the community, selecting representative samples for sequencing, and genetic characterisation to monitor the virus evolution and inform vaccine composition decisions or outbreak analyses. When NAAT-based assays are used, confirmatory sequencing of at least a subset of samples should be performed to be able to use these assay results as indicators of community circulation of the VOCs. Before introducing a new testing method or a new assay, a validation and verification exercise should be carried out to ensure that the laboratory testing system is reliably detecting the circulating viruses. Results should be reported to The European Surveillance System (TESSy) and the sequences to the Global Initiative on Sharing All Influenza Data (GISAID), or other public database, and raw data, if available, to the European Nucleotide Archive (ENA) in a timely manner (ideally on a weekly basis).

This document was developed by technical experts from ECDC and the WHO Regional Office for Europe and reviewed by experts at WHO's referral laboratories and in the SARS-CoV-2 Characterisation Working Group.

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Key messages

- Several SARS-CoV-2 VOCs have emerged in recent months and monitoring their circulation in all countries is of key importance to prevent and control the spread of VOCs.
- Whole SARS-CoV-2 genome sequencing, or at least complete or partial S-gene sequencing, should be used to confirm infection with a specific variant and characterise the variant.
- For the early detection and prevalence calculation of VOCs (or when sequencing capacity is limited) alternative methods should be used, such as diagnostic screening NAAT-based assays. To contain or delay introduction of a VOC, positive samples should ideally be screened using a NAAT-based assay that can offer the advantage of rapid results.
- When employing NAAT-based methods, sequencing should be used to characterise at least a subset of the variants.
- In the event of low prevalence of a VOC in the population and when the objective is to delay the introduction and spread of the VOC, ideally all NAAT-based results indicative of the VOC should be confirmed by sequencing.
- Sample and method selection are key and will depend on the objectives (e.g. to assess the circulation of the different SARS-CoV-2 variants using representative samples from the community, or genomic characterisation to monitor the virus evolution and inform vaccine composition decisions or outbreak analyses).
- Assay validation should be carried out to ensure that the laboratory testing system is performing adequately for the circulating viruses.
- Laboratories should remain vigilant to ensure that they detect reduction in sensitivity or failure to detect/identify circulating variants by the different PCR or antigen-based assays.
- If the diagnostic capacity is insufficient, priority should be given to severe cases, fatal cases, and cases with suspected high contagiousness of the pathogen that caused the outbreak, especially among those vaccinated or individuals with a history of COVID-19.
- SARS-CoV-2 consensus sequences should be submitted to GISAID or other public databases. Raw sequences should also be submitted to the European Nucleotide Archive (ENA).
- Detection of novel VOCs or outbreaks of currently circulating VOCs should be reported immediately through the Early Warning and Response System (EWRS), while variant detections should be reported to TESSy on a weekly basis.

Introduction

In the past year, several SARS-CoV-2 VOCs have emerged and monitoring their circulation in all countries is of key importance [1]. The only way to identify and characterise new variants and unambiguously type existing variants is through genomics. To be able to confirm infection with a specific variant, it is necessary to sequence the whole SARS-CoV-2 genome, or at least the complete or partial S-gene for the current variants. Guidance on sequencing of SARS-CoV-2 can be found in ECDC's technical guidance on sequencing of SARS-CoV-2, WHO's 'Genomic sequencing of SARS-CoV-2: a guide to implementation for maximum impact on public health' and WHO's 'SARS-CoV-2 genomic sequencing for public health goals: Interim guidance' [2-4]. ECDC has also published 'Guidance on representative and targeted genomic SARS-CoV-2 monitoring', providing further information on the sampling and sequencing strategy [5].

Occasionally, the time delay in obtaining WGS results can impede the public health response (e.g. contact tracing) and real-time calculation of the prevalence of the different variants in the community. In some settings Sanger sequencing of the S-gene can be more feasible and timely than WGS.

For early detection and prevalence calculation of VOCs, VOIs or VUMs [1], alternative methods are valuable – e.g. diagnostic NAAT-based screening assays that generate results in a few hours, with subsequent verification/confirmation by sequencing. Laboratories should remain vigilant to ensure that they detect reductions in sensitivity or failure to detect the circulating or emerging variants due to mismatches in primer/probe sequences.

Scope and objective

This technical report provides guidance to laboratories, microbiology experts and relevant stakeholders in making decisions on establishing or scaling up capability and capacity to detect and identify circulating SARS-CoV-2 variants. It will also facilitate decision-making on the appropriate technologies to use and for which objective.

The objective of this document is to present the available methods (screening and sequencing) for detection and characterisation of circulating SARS-CoV-2 variants. The document also outlines quality assessment issues, as well as considerations relating to sample and method selection and results reporting based on the different testing objectives.

This document has been updated to include more recent references and information on available assays for the detection and characterisation of emerging VOCs (e.g. Delta and Omicron SARS-CoV-2 variants).

Sequencing

Whole genome sequencing

WGS is essential to identify, monitor and assess virus variants that may be more transmissible and associated with increased disease severity, or may have adverse effects on public health and social control measures. Using either a tiled amplicon approach or shotgun sequencing, the entire genome of the virus will be sequenced and can be compared with other circulating strains [2]. WGS can be used to efficiently detect VOCs as it represents an unbiased approach, without the need for prior knowledge on the presence of certain mutations in the viral genome.

Wastewater SARS-CoV-2 surveillance can be performed using WGS. While it can be a useful tool for SARS-CoV-2 prevalence estimation, extracting information at the variant level is complicated and requires specialised bioinformatics pipelines. Results should be interpreted with care, especially for variants detected at low proportions (below 5%).

WGS is a relatively resource-intensive method that can take from hours to several days to generate results, depending on the protocol. Data storage issues and bioinformatics support also need to be considered. Guidance on the implementation of WGS can be found in ECDC's technical guidance on sequencing of SARS-CoV-2, and WHO's documents 'Genomic sequencing of SARS-CoV-2: a guide to implementation for maximum impact on public health' and 'SARS-CoV-2 genomic sequencing for public health goals: Interim guidance' [2-4]. Further guidance on sampling and sequencing strategy to ensure representativeness and reliability of findings can be also found in ECDC's 'Guidance for representative and targeted genomic SARS-CoV-2 monitoring' [5].

In the above-mentioned guidance, ECDC recommends that to accurately estimate and monitor the prevalence of VOCs and to assess the level of circulation of known VOCs in the community, a representative number of positive samples, reaching a precision of 1−2.5%, needs to be sequenced on a weekly basis. With the emergence of a VOC that needs to be contained or its introduction to Europe delayed, ECDC recommends that countries enhance their sequencing efforts and sequence a number of samples that allows detection at 1% prevalence level. In this case (VOC early detection, containment and delaying spread), it is also important to prioritise targeted sampling of travel-related cases, or at least all cases with a travel history (14 days prior to the positive test result) to areas with suspected wide-spread transmission of the VOC, or where the epidemiological situation is unclear. However, this should be in addition to the representative sampling described above. Severe cases, reinfections, outbreaks and unusual events should also be sequenced.

At times, some amplicons of specific assays may fail to detect certain circulating VOCs, due to primer-template mismatches. This can lead to missed calls at the spike protein residues. For example, in silico analysis has shown that amplicon 76 of the ARTIC v4 protocol could fail for the Omicron VOC due to primer-template mismatches, leading to missed calls at spike protein residues 417, 440 and 446. If this region is not covered, other characteristic mutations can be used to identify the variant. There is an updated v4.1 version of ARTIC that aims to mitigate primer-template mismatch for Omicron (ref ARTIC v4.1).

Sanger or partial next-generation sequencing amplicon-based sequencing

Due to the many characteristic mutations of some of the VOCs, they can be identified based on partial S-gene sequences. This should preferably include the receptor-binding-domain (RBD), but any region covering enough characteristic mutations to conclude that the virus is a specific variant can be used. Particular attention should be paid to the presence of single nucleotide polymorphisms (SNP) of known mutations leading to a change in the biological characteristics of the virus. Sanger or next generation sequencing (NGS) amplicon-based sequencing of selected parts of the viral genome are alternative methods for the identification of VOCs. With these techniques,

targeted whole or partial S-gene sequencing can be performed. The NGS method comes with the same challenges as WGS regarding equipment and bioinformatics analysis. Protocols have been developed for specific RT-PCRs for marker regions of the S-gene region indicative of different VOCs, followed by sequencing [6]. Such protocols (also for the Omicron variant) can be found on WHO's EZCollab platform for download by national public health laboratories. If Sanger sequencing is the preferred method, the region to be sequenced should cover at least the entire N-terminal and RBD (amino acid 1-541, 1623 bp) to reliably differentiate between the circulating variants. Variant-specific signature mutations should be present in the sequenced region. Ideally, S-gene amino acids 1-800 (2400 bp) or the entire S-gene should be sequenced to also monitor the S1/S2 cleavage site and other regions of interest. The characteristic amino acid substitutions of variants can be found in ECDC's updated list of VOCs, VOIs and VUMs here.

ECDC and WHO can support countries with WGS and bioinformatics analyses. Please contact covid.microbiology@ecdc.europa.eu or euinfluenza@who.int for more information.

Diagnostic screening assays of known VOCs

RT-PCR assays and S-gene target failure

Nucleic acid amplification tests (NAAT) based on reverse transcriptase PCR (RT-PCR) are generally used as the gold standard detection method for SARS-CoV-2. Such RT-PCR tests can use one or multiple target genes for amplification.

Some of the SARS-CoV-2 VOCs (e.g. Alpha [B.1.1.7] and Omicron [B.1.1.529]) generate a negative or significantly weaker positive S-gene result in multiplex RT-PCR assays, with positive results for the other targets. This has been used as an indicator or screening method to identify these particular variants. The weaker signal or complete failure of the S-gene target is caused by a deletion at nt207-212 (Δ69-70) in the respective gene and is called the S-gene target failure (SGTF). By coincidence this occurs in some assays that include an S-gene target (e.g. ThermoFisher TaqPath assay), but not all [3,7-9]. In particular, Alpha and the majority of Omicron variants give a positive signal in ORF1 and N-gene targeted RT-PCRs, but not in S-gene targeted RT-PCRs [10].

The US Food and Drugs Administration (FDA) has listed molecular tests that may be affected by mutations in the SARS-CoV-2 Omicron variant [11]. Similarly, the European Commission Joint Research Centre (JRC) is monitoring the performance of RT-PCR assays and displays the information on the JRC Dashboard. Laboratories are urged to verify the efficiency of protocols used on the dashboard that is based on in silico analysis. It should be noted that SGTF is not exclusive to Alpha or Omicron variants. Therefore, the presence of the SGTF may identify other non-VOC variants, while it may also fail to detect some other VOCs (including the Omicron sub-lineage BA.2). SGTF itself cannot identify specific variants. In addition, samples with high Ct values may show a pattern of SGTF by chance, with a weak signal in the other targets as well. Samples with lower Ct (i.e. below 30) can be assessed more reliably. The cut-off can be chosen based on whether SGTF is used for screening to detect single cases at an early stage (and all positives are subsequently sequenced), or as a proxy when the SGTF-positive VOC (e.g. Omicron) is prevalent. In the first instance, sensitivity should be maximised, while in the second specificity is more important and therefore a more conservative cut-off can be chosen. It is preferable for SGTF to be used as an indicator when the specific VOC is already circulating at high prevalence in a specific setting, and rapid laboratory results are needed, or sequencing capacity is limited. Confirmation of the deletion at nucleotides 207−212 (ΔH69/V70) by sequencing is highly recommended, especially in a low prevalence setting. However, irrespective of the prevalence, at least a subset of SGTF samples (i.e. minimum 10% or depending on available resources) should be characterised by sequencing. This is necessary to increase the confidence and reliability of the results and should be closely monitored. In settings where other non-VOC variant(s) with the same deletion are circulating, sequencing of all SGTFs is necessary.

Increasing the numbers of sequenced samples screened by SGTF can be considered to assess the regional correlation between SGTF and the specific VOC, as this varies with the regionally circulating variants [12]. If the correlation is very high, SGTF can be used to approximate the frequency of the VOC with a deletion in the S-gene.

It should be noted that Omicron sub-lineages have been described that share common mutations with the Omicron sub-lineage BA.1 [13]; however the BA.2 viruses do not carry the Δ69-70 mutation and therefore will not be identified by SGTF assays. There are also a very low number of sequences of non-Omicron lineage viruses that carry Δ69-70 and will therefore give an SGTF result. The above highlights the importance of sequencing for the characterisation of SARS-CoV-2.

Multiplex RT-PCR, including S-gene target failure

With a multiple channel real time RT-PCR device, the normal E and/or N and/or ORF-1 target assays may be combined with the S-gene target, so the VOC screening could be integrated into the normal routine, in a single run [14].

It is important to emphasise that results should not be over-interpreted and must be checked and continuously validated through the use of genomics.

Screening SNP assays

VOC-specific amino acid substitutions can be screened using specific RT-PCR assays targeting single nucleotide polymorphisms (SNP) that are present in some VOCs [14]. Appropriate positive controls will be needed. This method allows for a quick estimation of the prevalence of the specific mutation-positive variants in the community.

It should be noted that there are amino acid substitutions (e.g. N501Y) present in more than one SARS-CoV-2 lineage that do not belong to currently circulating VOCs. Several currently circulating VOCs share common mutations. Therefore, verification of at least a subset of samples should be done using sequencing.

It is important to note that existing SNP assays (e.g. N501Y SNP assays) may fail to detect/identify newly emerging variants that do carry the specific SNP (e.g. N501Y) due to amino acid substitutions in sites affecting the primer/probe binding. Specifically, for the Omicron variant, it has been observed that some commercially available SNP assays for the identification of T478K, N501Y and P681H are failing to reliably identify these mutations, despite the fact that this variant carries the mutations in the S-gene [11]. New assays have been developed for the identification of Omicron [15,16]. Laboratories need to remain vigilant to ensure that they detect any reduction in sensitivity or failure of specific assays to identify VOCs.

The European Commission's JRC [17] has developed an Omicron-specific NAAT detection method that is currently being validated [18]. Specific assays for omicron detection have also been developed and published in pre-prints [10,19].

Table 1. Amino acid substitutions/deletions/insertions used to screen different SARS-CoV-2 VOCs (list not exhaustive)

Spike amino acid Variation	Alpha B.1.1.7	Beta B.1.351	Gamma B.1.1.28	Delta B.1.617	Omicron B.1.1529*
ΔH69-V70	x				x
ins214EPE					x
S371L/S373P					x
L452R				x	
N501Y	x	x	x		x
K417T			x		
K417N		x			(x)
E484K		x	x		
E484Q	(x)				
E484A					x
P681H	x				x
P681R				x	
T478K				x	

Important note: Primer/probe mismatches at neighbouring sites in Omicron (or other) variant may cause failure to detect the amino acid substitution even if the variant carries this substitution. Validation is therefore recommended for detection/characterisation of new variants.

(x): Some of the VOC sequences carry this amino acid substitution.

** Some of the amino acid substitutions are different for the Omicron sub-lineages BA.1 and BA.2 (e.g. S371L/S373P will identify only BA.1 viruses).*

Screening single nucleotide polymorphism using specific real time RT-PCR melting curve analysis

Many real time RT-PCR platforms allow for melting curve analysis. Commercial assays have been developed to use this genotyping method to identify specific amino acid substitutions (e.g. ΔH69/V70, S371L/S373P, K417N, N439K, Y453F, E484K, N501Y, A570D, D614G, P681H or V1176F). Such assays can be used for the identification of VOCs.

Table 2. List of available assays/protocols for identification of SARS-CoV-2 Omicron variant (not exhaustive)

Commercial or in-house assay	Spike gene amino acid substitution	Methodology	References
TIB MolBiol	S371L/S373P	Melting curve	[15,16]
TIB MolBiol	ins214EPE	Melting curve	[15,16]
TIB MolBiol	E484A	Melting curve	[15,16]
Thermo Fisher TaqPath	ΔH69/V70	SGTF	[20]
Seegene	E484A, N501Y, ΔH69/V70	RT-PCR	[21]
JRC	Multiple targets	RT-PCR - currently being validated	[18]
Israel Ministry of Health Central Virology Laboratory (CVL) and Israel Institute for Biological Research (IIBR)	nsp6 (Orf1a)	RT-PCR assay – as of 16 December 2021	[10]
University Hospital Geneva	Two partial S gene regions	RT-PCR and Sanger sequencing	[22]
Smorodintsev Research Institute of Influenza (St. Petersburg, Russia)	ORF1 deletion	RT-PCR	[23]
SSI, Denmark	Omicron specific 4-target PCR	RT-PCR	[19]

Reverse transcription loop-mediated and transcription-mediated amplification isothermal amplification

Reverse transcription loop-mediated isothermal amplification (RT-LAMP) and transcription-mediated amplification (TMA) on Panther Hologic techniques have emerged as an alternative molecular detection method for SARS-CoV-2. RT-LAMP technique has some advantages, such as faster test results and the need for fewer resources, while maintaining high sensitivity and specificity, although currently available protocols will not differentiate between specific VOCs [24]. However, some protocols (e.g. LamPORE) provide a possible pathway to sequencing.

Proper clinical validation studies are needed to evaluate the new techniques and assess the potential role they could play in the different settings.

Rapid antigen detection tests

Rapid antigen detection tests (RADTs) can contribute to overall SARS-CoV-2 testing capacity, offering the advantage of shorter turnaround times and reduced costs, especially in situations where NAAT testing capacity is limited. However, their sensitivity is generally lower than for RT-PCR [25]. RADTs may detect the presence of SARS- CoV-2 (including variant viruses) but cannot identify/differentiate the VOC. They can, however, help reduce further transmission through early detection of highly infectious cases, enabling contact tracing to begin quickly. The EU Health Security Committee (HSC) has established a technical working group on COVID-19 diagnostic tests which has agreed on a common, frequently-updated list of COVID-19 RADTs that meet defined performance criteria [26].

Despite the emergence of virus variants, no reduction in RADT test sensitivity has been reported so far [25]. Preliminary results of the rapid assessment conducted by the Foundation for Innovative New Diagnostics (FIND) suggest that the accuracy of RADTs has not been impacted by the emergence of the Omicron variant [27]. Furthermore, initial laboratory validation of lateral flow devices in use by UK's NHS (National Health Service) Test and Trace has determined similar sensitivity in detecting Omicron to that for Delta [28]. It should be noted that RADTs are mainly targeted at detecting the viral N protein which in the Omicron variant appears less divergent than spike. Wider performance studies of RADTs for the Omicron variant in settings with high transmission have not yet been conducted. Further studies are ongoing, and laboratories should remain vigilant to ensure that they detect reductions in sensitivity of the RADTs used for different VOCs.

Neutralisation assays and antigenic characterisation

The VOIs and VOCs need to be assessed more broadly through a risk assessment process looking into various risk elements (e.g. increased transmissibility, morbidity/mortality among those previously vaccinated against COVID-19 or individuals with a history of COVID-19 or vaccine escape). In order for laboratories to assess how well the antibodies developed through humoral immunity in response to natural infection and from vaccine-induced immunity may protect against the circulating viruses, it is important to perform virus neutralisation assays with convalescent plasma/sera from infected and vaccinated individuals. These assays should include international standards (see below) to assess the antigenic characteristics of the circulating variants.

Multiple laboratory methods have been developed to determine virus neutralisation capacity. Some examples are plaque reduction neutralisation (PRNT), microneutralisation and pseudovirus neutralisation assay [29-31]. Assays with replication competent SARS-CoV-2 isolates are normally either plaque reduction/focus forming assays or TCID50 (Median Tissue Culture Infectious Dose)-based assays. However, they have the disadvantage that they require biosafety level (BSL)-3 laboratories and are often labour intense. On the other hand, assays using replication-defective pseudotyped viral particles can be performed under BSL-2 conditions, but they depend on having the variant-specific pseudotype construct. As all neutralisation assays require living cells, they are more difficult to standardise than molecular assays. Therefore, testing the robustness of these assays is a crucial step [32]. A comparison of four different types of neutralisation assays has shown that these SARS-CoV-2 neutralisation assays were robust. Results were comparable and produced highly reproducible neutralisation titres [32,33]. Excellent concordance between the surrogate virus neutralisation test and the gold standard PRNT has also been demonstrated [29].

To assess the neutralisation capacity of sera for different patient situations, the serum panels could include serum from asymptomatic, symptomatic and convalescent individuals after severe disease with different sampling intervals (e.g. 14 days post symptom start or sampling for asymptomatic, and after three to six months, or later), if available to the testing laboratory. For vaccinee sera, different time and vaccination regimens could include 14 days, three to six months post second dose and 14 days, or three to six months post booster vaccination. Heterologous prime-boost or infection plus any vaccination sera would also be beneficial for comparison purposes.

The laboratories working on neutralisation assays and therefore cultivating SARS-CoV-2 viruses should consider that serial propagation of SARS-CoV-2 variants in Vero E6 or other cell types may lead to furin cleavage site mutations that affect how the virus grows and behaves in vitro or in vivo. Propagation of unwanted mutations can be mitigated by growth in cells such as Vero/hSLAM and by frequent sequence confirmation (deep sequence methods preferred) [34].

For comparing the neutralisation assay results with other laboratories internationally, WHO International Antibody Standard (WHO IS) or, if WHO IS is unavailable, the so-called NIBSC working reagent (21/234) or high titer reference serum (20/150) should be used for neutralisation assays [29,35-37]. It should be noted that the WHO IS performs differently for each variant and therefore, any data presented comparing the WHO IS should always identify the variant being tested. It is important to include representatives of different variant strains (Wuhan-like, D614G, Alpha, Beta, Gamma and Delta with preference as a minimum for D614G, Alpha, Beta and Delta) in the neutralisation assays. The assays should also ideally be performed in duplicate or triplicate.

Several studies have already looked at VOI and VOC antigenic properties through various neutralisation assays, - e.g. on SARS-CoV-2 501Y.V2 [38], Alpha [39], Beta [40], Delta [41], Eta [40], Gamma [42] and Lambda variant [39]. Assay details have been shared by scientists without peer review for the Omicron variant as well [43,44]. Additional B-cell and T-cell assays will give insight into the immune responses against different VOCs.

EU/EEA countries that need support in setting up antigenic characterisation assays or would like to send samples for antigenic characterisation can contact covid.microbiology@ecdc.europa.eu and countries outside the EU/EEA can contact euinfluenza@who.int. The WHO COVID-19 reference laboratories for the WHO European Region can support countries with antigenic characterisation; the list of reference laboratories can be found here. Antigenic characterisation results for new VOCs should immediately be shared with WHO Regional Office for Europe and ECDC.

Considerations for sample and method selection

Testing strategies should be flexible and rapidly adaptable to change, depending on the local epidemiology, population dynamics and resources. Sample and method selection are key and will depend on the objectives below.

- Timely testing of those with symptoms remains important to enable rapid initiation of infection prevention and control measures. This can be fostered by improving access to testing and encouraging people to seek testing as soon as possible after symptom onset. Targeted (travel-related, outbreak, hospitalisation, reinfection) sampling for VOC identification is important for early detection and response activities.
- To contain or delay the introduction and spread of a new VOC in the community, circulating VOCs can be sequenced and screened for early detection using one of the aforementioned methods. This will rely on the capacity to generate rapid results.
- To contain a VOC or delay the introduction of new VOCs in the community, it is recommended that NAAT-based assays are used to screen positive samples for the presence of the VOC. NAAT-based assays offer the advantage of rapid results for immediate public health action.
- All or a selection of positive SARS-CoV-2 samples can be screened for VOC identification. Screening of travellers is ineffective in containing the introduction of a new variant, however when there is a need to contain or delay the introduction and spread of a new VOC in Europe from other parts of the world, ideally all travel-related cases should be screened or sequenced. If an NAAT-based method is used, all (when the VOC is detected sporadically or in low prevalence) or at least a subset of samples screened (when the VOC has spread in the community) should be selected for further confirmatory sequencing.
- According to ECDC's sequencing guidance [5], a representative sample should be collected on a weekly basis, allowing for detection of variants at a prevalence of 1–2.5%. This will help to assess the level of circulation of known variants in the community.
- To delay the spread of new VOCs, ECDC recommends that sequencing volumes are adjusted to allow for detection of a 1% prevalence, in accordance with the ECDC sequencing guidance [5]. In addition, sequencing of travel-related cases should be enhanced, especially from areas where there is community transmission of the new VOCs.
- There is a risk of bias in the sequencing results if the sample selection is not representative - e.g. when the selection of samples for sequencing is based on samples for confirmation of SGTF screening. Sequencing should be the preferred method for testing representative samples, however screening methods can also be useful, as fast result turnaround time is important to inform public health interventions.
- To be able to use the screening method as an indicator of the overall situation, sequencing can be used to assess the fraction of viruses with S-gene target failure, or VOC detections positive with other screening methods.
- In parallel, WGS should be done for genetic characterisation to monitor the virus evolution. For this purpose, samples should target prolonged/chronic infections and severe infections across the disease spectrum and for different demographics, reinfections, zoonotic infections and outbreaks [2].
- Depending on the resources available, WGS sequencing can be performed for additional objectives, such as outbreak analyses, phylodynamic analyses and other research studies.
- It may be necessary to sequence viruses from areas with observed overall higher incidence for the initial identification of novel VOCs.
- To monitor the antigenic properties of the circulating viruses and the vaccine match, a representative sample of viruses should be evaluated in neutralisation assays at national or international reference laboratories. Additional B-cell and T-cell assays will give insight into other aspects of the immune response against different VOCs.

Guidance on sample selection and how to calculate the minimum number of viruses to be sequenced for surveillance purposes can be found in ECDC's 'Guidance for representative and targeted genomic SARS-CoV-2 monitoring' [5].

Quality assessment

Before introducing a new testing method or a new assay, a validation and verification exercise should be carried out to ensure that the laboratory testing system is performing adequately for the circulating viruses [45]. In general, laboratories should have a quality assurance system in place and are encouraged to participate in external quality assessment (EQA) schemes or perform result comparison between laboratories for a subset of samples [45]. ECDC and WHO's Regional Office for Europe are planning a molecular External Quality Assessment (EQA) for national COVID-19 reference laboratories in 2022. Please contact covid.microbiology@ecdc.europa.eu for more information.

Genomics is the best tool for identifying new variants. Diagnostic laboratories need to remain vigilant to detect any mismatches of NAAT-based assay (e.g. RT-PCR or SNP assays) primers and probes against circulating virus genomes and detection capability of other assays such as RADTs, and to adapt Sanger sequencing protocols. The vast majority of primer/probe binding sites of commercial assays are not publicly known. It is important to note that it is coincidental that detection assays targeting the S-gene can be used as a proxy to screen for some variants. For all assays, it is vital to keep track of possible incidents of sub-optimal performance and to inform the manufacturer of a commercial assay and international SARS-CoV-2 public health networks, ECDC and WHO Regional Office for Europe of any concerns regarding a specific assay.

JRC is monitoring the performance of RT-PCR assays and displays the information on the JRC Dashboard[1]. Laboratories are urged to verify the efficiency of protocols used on the JRC dashboard. FIND is also performing independent evaluation studies to verify the limit of detection (LOD) and results are displayed on their website[2] [46].

Reporting results

Detections of SARS-CoV-2 should be reported on a weekly basis to The European Surveillance System (TESSy) by the designated data managers of national public health authorities in the reporting countries using the most recent version of the COVID-19 reporting protocol [47]. Detection of a suspicious signal related to a SARS-CoV-2 lineage or outbreaks of currently circulating VOCs or variants considered of relevance by the reporting county should be reported immediately through the Early Warning and Response System (EWRS) and IHR, while detections of variants should be reported to TESSy on a weekly basis.

SARS-CoV-2 sequences should be submitted to GISAID or other public databases in a timely manner (i.e. ideally within one week of sample collection.) If available, raw data should be deposited in the COVID-19 data portal through the European Nucleotide Archive (ENA).

Variables for reporting of variants (i.e. VirusVariant) have been implemented within the aggregated (NCOVARIANT) and case-based (NCOV) TESSy record types. SGTF results should be reported using the respective coded value. In the case-based record type NCOV, sequence ID numbers (GISAID identifiers) should be reported as well. Raw sequencing data ENA/Sequence Read Archive (SRA) accession numbers, if available, should also be submitted to TESSy by filling in the respective variable. Any epidemiological data available, including the setting from where the sample was obtained, whether the sample originated from representative or targeted surveillance and whether the case was imported or locally acquired, and/or the probable country of infection should also be reported (some variables can only be reported using the case-based record type, see reporting protocol for detailed instructions). This will enable more accurate data analysis and interpretation by identifying those representative cases that reflect the prevalence of variants in the community.

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

Laboratory support

ECDC and WHO's Regional Office for Europe are coordinating their support for 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 covid.microbiology@ecdc.europa.eu for more information. Countries wishing to receive support from WHO Regional Office for Europe may contact euinfluenza@who.int.

Reference viruses for neutralisation assays, constructs for pseudovirus assays and control material for NAAT assays can be found through the European Virus Archive Global (EVAg) and National Institute for Biological Standards and Control (NIBSC). WHO is currently also setting up a BioHub for the purpose of material sharing.

Protocol and information sharing

WHO's Regional Office for Europe and ECDC have jointly set up a protocol/information sharing platform EZCollab for 'COVID-19 protocol sharing'. Registration can be done here: https://ezcollab.who.int/euroflu/flulab/covid19_protocols.

[1] JRC dashboard available at: https://covid-19-diagnostics.jrc.ec.europa.eu/devices?device_id=&manufacturer=&text_name=&marking=&method=1&rapid_diag=&target_type=5&field-1=HSC+common+list+%28RAT%29&value-1=1&search_method=AND#form_content

[2] FIND website available at: https://www.finddx.org/covid-19/sarscov2-eval-molecular/molecular-eval-results/

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