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

2 August 2022

New in this update

- Information related to detection assays specific for Omicron variants BA.4 and BA.5 has been included.
- The chapter on rapid antigen detection tests (RADT) has been updated and includes available information on their performance for Omicron variants.
- The chapter on neutralisation assays has been updated with information on the isolation of SARS-CoV-2 BA.4/5 variants.

Summary

In the past year, several SARS-CoV-2 variants of concern (VOCs) have emerged and it is of key importance o monitor their circulation in all countries. Whole Genome Sequencing (WGS), or at least complete or partial spike (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 pre-screening to allow prevalence calculation of VOCs, variants of interest (VOI) and variants under monitoring (VUM). Many of these methods can accurately identify the different variants, while others will require confirmation by sequencing of at least the complete or partial S-gene genomic region in a subset of samples.

Genomic monitoring should be integrated into the overall respiratory virus surveillance strategies. Specific objectives for the detection and identification of variants include assessment of the circulation of different SARS-CoV-2 variants in the community by selecting representative samples for sequencing, and genetic characterisation to monitor 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 use these assay results as indicators of community circulation of virus variants, particularly 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. Variant typing results should be reported to The European Surveillance System (TESSy) and SARS-CoV-2 consensus sequences should be deposited in the Global Initiative on Sharing All Influenza Data (GISAID) database, or other public databases. If available, related sequencing raw data should be deposited in the European Nucleotide Archive (ENA) and raw data, if available, in the European Nucleotide Archive (ENA). This should be done in a timely manner (ideally on a weekly basis).

This document was developed by technical experts from ECDC and the World Health Organization (WHO) European Region and previous versions have been 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 years and it is of key importance that their circulation is monitored in all countries to prevent and control spread.
- Genomic monitoring should be integrated into the overall respiratory virus surveillance strategies.
- Countries are encouraged to remain vigilant for signals indicating new lineage/variant introduction or emergence. Countries should be prepared to increase testing and sequencing efforts if the introduction of a new VOC, VOI or VUM is imminent.
- Whole SARS-CoV-2 genome sequencing, or at least complete or partial S-gene sequencing, should be performed to confirm infection with a specific variant and to characterise the variant.
- For early detection and to calculate the prevalence of VOCs, or when sequencing capacity is limited, alternative methods can be used, such as diagnostic screening with NAAT-based assays.
- When employing NAAT-based methods, sequencing should be performed to characterise at least one subset of the variants.
- Sample and method selection are key and will depend on the objectives, such as assessing the circulation of different SARS-CoV-2 variants using representative samples from the community, early detection of novel variants, and genetic/antigenic characterisation to monitor 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 sequencing capacity is insufficient, priority should be given to sentinel samples from primary and secondary care sites, as well as sequencing and characterisation of suitable SARS-CoV-2 positive specimens from special settings, as these may provide important signals that novel variants with potentially changed characteristics are emerging. These can include clusters of cases, immunocompromised patients or patients with other underlying conditions associated with prolonged viral replication and shedding or cases with an unusual clinical presentation or poor response to therapeutics, including antiviral treatments. Further signals could be cases suggestive of zoonotic transmission or if any suspicion of changes in the performance of diagnostics.
- 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) and, if possible, through ENA to the COVID-19 data portal of the European Commission.
- SARS-CoV-2 clinical specimens and/or virus isolates should be shared with national and WHO COVID-19 reference laboratories for further antigenic characterisation and antiviral drug/treatment resistance monitoring.
- 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.
- Any virus characterisation laboratory data related to circulating VOCs in EU/EEA should be reported to the relevant EpiPulse events and shared with WHO European Region and ECDC as soon as it becomes available.

Introduction

During the COVID-19 pandemic, several SARS-CoV-2 VOCs emerged and it is of key importance to monitor their circulation in all countries to prevent and control spread [1]. The only way to identify and characterise new variants and unambiguously type existing variants is through genetic characterisation. 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 '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 published a 'Guidance for representative and targeted genomic SARS-CoV-2 monitoring', providing further information on the sampling and sequencing strategy [5]. Moreover, ECDC and WHO European Region have published a technical guidance on SARS-CoV-2 antigenic monitoring [6].

The time delay in obtaining WGS results can occasionally impede the public health response and real-time calculation of the prevalence of the different variants in the community. In some settings, Sanger sequencing of the S-gene can therefore be more feasible than WGS.

For early detection and prevalence calculation of VOCs, VOIs or VUMs [1], alternative methods are valuable, such as diagnostic NAAT-based screening assays that generate results in a few hours, with subsequent verification and/or 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.

Target audience

This document aims to provide interim guidance for microbiology and virology experts, other laboratory professionals, laboratory managers, infectious disease programme managers, public health professionals and other stakeholders that provide primary, confirmatory or advanced testing for SARS-CoV-2, including genomic sequencing, or are involved in making decisions on establishing or scaling-up capability and capacity to detect and characterise circulating SARS-CoV-2 variants.

Methodology

The original version of this document was developed by technical experts from ECDC and the WHO's Regional Office for Europe in consultation with experts from the WHO/EURO-ECDC joint SARS-CoV-2 Virus Characterisation Working Group (VCWG). Confidentiality and declarations of interest, collected from all VCWG members by both WHO and ECDC, were reviewed and no conflict of interest was identified.

In view of the dynamic situation with SARS-CoV-2 variants, it was not possible to perform systematic reviews. Targeted searches of PubMed, medRxiv and bioRxiv databases were conducted to identify both peer-reviewed and published and pre-print reports on studies of methods used to detect and characterise SARS-CoV-2 variants of concern. The searches included genomic sequencing methods, NAAT-based methods, rapid detection assays and neutralisation assays. Where appropriate, unpublished studies communicated as presentations during the VCWG meetings or through WHO European Region's COVID-19 protocol-sharing platform were also considered. The articles identified were assessed for quality of evidence based on the methods and results of the studies. Formal GRADE assessment was not performed due to time and resource constraints and because this was not a systematic literature review.

Other WHO and ECDC guidance documents were reviewed for recommendations on various laboratory methods and techniques as they apply for detection and characterisation of the emerging SARS-CoV-2 variants. Only the latest editions of ECDC and WHO guidance documents addressing surveillance and laboratory testing issues of the response to SARS-CoV-2, including its variants, were eligible for review.

Due to the highly dynamic nature of emerging SARS-CoV-2 variants, the amount of evidence available for review was limited. Furthermore, some ongoing studies on new variant detection and characterisation were not included as their results were not yet ready. It therefore remains imperative for ECDC and WHO to continue monitoring the latest developments in detection and characterisation of newly SARS-CoV-2 variants as they emerge.

Scope and objective

The objective of this document is to present the available methods for detection and characterisation of circulating SARS-CoV-2 variants. The document outlines quality assessment issues, as well as considerations relating to sample and method selection and results reporting based on the different testing objectives. It also provides advice to facilitate decision-making on the appropriate technologies to use and for which purpose.

This document has been updated to include more recent information and references on available assays for the detection and characterisation of emerging SARS-CoV-2 variants, with particular focus on Omicron variants BA.4 and BA.5.

Sequencing

Whole genome sequencing

WGS is essential to identifying, monitoring and assessing 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%) [7,7a].

WGS is a relatively resource-intensive method and it can take from hours to days to generate and analyse 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 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 be found in ECDC's guidance for representative and targeted genomic SARS-CoV-2 monitoring [5].

In general, specimens with a cycle-threshold (Ct) value of ≤25 are considered appropriate for obtaining good-quality whole genome sequences. Samples with Ct>30 may not give whole genome sequence results but may still be appropriate for determining SARS-CoV-2 lineage/variant [8].

Priority for sequencing should be given to sentinel samples from primary and secondary care sites, as well as sequencing and characterisation of suitable SARS-CoV-2 positive specimens from special settings, as these may provide important signals that novel variants are emerging, with potentially changed characteristics [9]. These can include outbreaks, immunocompromised patients or patients with other underlying conditions associated with prolonged viral replication and shedding and cases with an unusual clinical presentation or poor response to therapeutics including antiviral treatments. Other signals can be cases suggestive of zoonotic transmission or any suspicion of changes in the performance of diagnostics [8,8a].

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 [10].

Sanger or partial next-generation sequencing amplicon-based sequencing

Due to the many characteristic mutations of some of the VOCs, they can be identified from partial S-gene sequences. This should preferably include the receptor-binding-domain (RBD), but any region can be used, provided that it covers enough characteristic mutations to conclude that the virus is a specific variant. 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 [11,12]. Protocols have been developed for specific RT-PCRs relating to the marker regions of the S-gene region indicative of different VOCs, followed by sequencing [13]. 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 acids 1-541, 1 623 base pairs (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 (2 400 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 for these variants. The weaker signal or complete failure of the S-gene target is caused by a deletion at nucleotides (nt) 207-212 (deletion of amino acids 69-70, 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 [3,14-16]. 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 [17]. The spike proteins of BA.4 and BA.5 are identical, and comparable to BA.2 except for the addition of Δ69-70, L452R, F486V and the index virus amino acid at Q493. Therefore, SGTF may be used for pre-screening and as a proxy-marker to suggest the presence of BA.4 and BA.5 against the epidemiological background of a dominant variant that does not have these mutations (e.g. BA.2). There are, however, sequences of non-Omicron lineage viruses that also feature Δ69-70 and will therefore give an SGTF result [18]. This highlights the importance of sequencing for the characterisation of SARS-CoV-2 variants.

The US Food and Drugs Administration (FDA) has listed molecular tests that may be affected by mutations in the SARS-CoV-2 Omicron variant [19]. 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 which 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 instance 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 [18]. Confirmation of the deletion at nucleotides 207-212 (Δ69-70) by sequencing is highly recommended, especially in a low prevalence setting. However, irrespective of the prevalence, at least a subset of SGTF samples (minimum 10% or depending on available resources) should be characterised by sequencing [18]. 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 [20]. If the correlation is very high, SGTF can be used to approximate the frequency of the VOC with a deletion in the S-gene.

Multiplex RT-PCR

With a multiple channel real time RT-PCR device, the common E and/or N and/or ORF-1 target assays may be combined with the S-gene target, so the VOC screening can be integrated into the normal routine, in a single run [21].

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

Screening SNP assays

VOC-specific amino acid substitutions can be identified using specific RT-PCR assays targeting single nucleotide polymorphisms (SNP) that are present in some VOCs [21]. 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, at least one subset of samples should be verified 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 a specific SNP (e.g. N501Y) due to amino acid substitutions at sites affecting the primer/probe binding. For the Omicron variants in particular, some commercially available SNP assays for the

identification of T478K, N501Y and P681H are failing to reliably identify these mutations, despite the fact that these variants carry the mutations in the S-gene [19]. New assays have been developed for the identification of Omicron [22-25]. Laboratories need to remain vigilant to ensure that they detect any reduction in sensitivity or failure of specific assays to identify VOCs.

The Commission's Joint Research Centre (JRC) has compiled and published a COVID-19 In Vitro Diagnostic Devices and Test Methods Database, with information on COVID-19 test devices. JRC has also developed an Omicron-specific NAAT detection method and was recently able to confirm its validity [26].

Qualitative RT-PCR assays can identify the different Omicron sub-lineages, namely BA.1 through BA.5. The BA.1-specific assay is based on the deletion Δ69-70 and the insertion ins214EPE seen in the BA.1 S-gene. A specimen is identified as BA.1 only if both targets (Δ69-70 and ins214EPE) are positive [27]. Since BA.2 does not feature either Δ69-70 or ins214EPE in the S-gene, the BA.1-specific RT-PCR assay is negative for both targets Δ69-70 and ins214EPE when testing samples containing BA.2 [27]. Although lacking the ins214EPE, BA.3 through BA.5 feature the Δ69-70 in the S-gene (https://cov-lineages.org/lineage_list.html). Therefore, BA.3 through BA.5 are positive for Δ69-70 and negative for ins214EPE tested by the BA.1-specific RT-PCR assay [27]. In addition, BA.4 and BA.5 carry defining mutations L452R, F486V and R493Q (reversion) in the Spike protein. Against the current epidemiological background where BA.2 is the dominant variant, laboratories can use the presence of the spike changes L452R, or F486V, or SGTF (TaqPath™ COVID-19 qPCR assay [28]) as a pre-screening for the variant. However, at the very least the spike RBD should be sequenced for confirmation. The nucleotide substitution G12160A is a specific marker for the BA.4 and BA.5 variants, which can be used in cases where the whole RBD sequence cannot be recovered. To be able to differentiate between BA.4 and BA.5, assays targeting discordant parts of the viral genome or whole genome sequencing (WGS) are required.

Due to the Δ69-70 mutation in BA.4 and BA.5 variants, detection of that deletion can be used as a proxy for presence of these variants when other variants are circulating at low prevalence.

Table 1. Spike protein characteristic amino acid substitutions, deletions, or insertions for screening of different VOCs (list not exhaustive)

Spike amino acid variation	Alpha (B.1.1.7)	Beta (B.1.351)	Gamma (P.1)	Delta (B.1.617.2)	Omicron (B.1.1.529)				
					BA.1	BA.2	BA.3	BA.4	BA.5
Δ69-70	x				x		x	x	x
ins214EPE					x				
S371L/S373P					x				
S371F/S373P						x	x	x	x
K417T			x						
K417N		x			x	x	x	x	x
L452R				x				x	x
T478K				x	x	x	x	x	x
E484K		x	x						
E484Q	(x)								
E484A					x	x	x	x	x
F486V								x	x
N501Y	x	x	x		x	x	x	x	x
P681H	x				x	x	x	x	x
P681R				x					

IMPORTANT NOTE: Primer/probe mismatches at neighbouring sites in Omicron (or other) variants 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.

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. Δ69-70, 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	[22,23]
TIB MolBiol	ins214EPE	Melting curve	[22,23]
TIB MolBiol	E484A	Melting curve	[22,23]
TIB MolBiol	E484A, F486V	Melting curve	[29]
Thermo Fisher TaqPath	Δ69-70	SGTF	[30]
Seegene	E484A, N501Y, ΔH69/V70	RT-PCR	[31]
JRC	Multiple targets	RT-PCR - currently being validated	[32]
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	[17]
University Hospital Geneva	Two partial S-gene regions	RT-PCR and Sanger sequencing	[33]
Smorodintsev Influenza Research Institute (St. Petersburg, Russian Federation)	ORF1 deletion	RT-PCR	[34]
SSI, Denmark	Omicron specific 4-target PCR	RT-PCR	[35]

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 [36]. 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 or unavailable. However, their sensitivity is generally lower than for RT-PCR [37]. 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 or self-isolation to begin quickly. The EU Health Security Committee (HSC) has established a technical working group (TWG) on COVID-19 diagnostic tests which has agreed on a common, frequently-updated list of COVID-19 RADTs that meet defined performance criteria [38].

Since December 2021, the HSC TWG has been discussing the performance of RADT in the context of the emerging Omicron variant of concern. In particular, concerns have been raised about RADT that solely target the spike protein (and are therefore not combined with the nucleocapsid protein) as well as the viral load measured at different time points and at different sites (e.g. throat and nose) after Omicron infection. The HSC TWG will continue to monitor the situation, including emerging evidence on the potential impact of the Omicron variant of concern on the performance of COVID-19 RADT and, if necessary, amend the agreed criteria accordingly.

So far, no significant reduction in viral loads has been shown that could impact the RADT performance for individuals infected with Omicron (as compared to individuals infected with Delta) [39,40].

Data on the sensitivity of antigen tests for the Omicron variant are not conclusive: while there are some studies indicating a tendency towards lower sensitivity compared to earlier variants [41,42], there are other studies using virus isolates [43-45] or clinical sample material [46-51], in which no substantial difference in sensitivity was found. It is, however, important to note that all available data are based on Omicron variants BA.1 and BA.2 and corresponding studies on BA.4 and BA.5 are yet to be carried out. At present, properly validated RADT are even considered to perform well for the Omicron variant [52].

It should be noted that RADTs mainly aim to detect the viral nucleocapsid (N)-protein and, in the Omicron variants, this demonstrates less variation than the Spike (S)-protein. For the time being, RADTs that target the S-protein or for which the target protein is unknown have been marked in the EU common list [53]. 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

ECDC and WHO European Region have jointly published technical guidance on antigenic SARS-CoV-2 monitoring [6].

The VOCs and VOIs need to be assessed 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). For laboratories to assess how well the antibodies have been developed through humoral immunity in response to natural infection and through vaccine-induced immunity, which may protect against the circulating variants, 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.

As for other SARS-CoV-2 viruses, virus isolation is essential for further antigenic characterisation. Isolation of BA.4/5 virus variants should take place in biosafety level (BSL) 3 conditions. African green monkey kidney cells overexpressing human ACE2 and TMPRSS2 (Vero E6-ACE2-TMPRSS2; Glasgow University) cells remain efficient for virus culture of Omicron [54]. This includes the BA.4 and BA.5 virus variants (Bruno Lina, University Hospital Lyon, personal communication). However, replication defects in comparison to Delta variant have been reported [55]. Plate centrifugation (37°C for 30 minutes at 900G) enhances cell infection and optimal growth is observed between 72 and 96 hours (Bruno Lina, University Hospital Lyon, personal communication). 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) [56].

Multiple laboratory methods have been developed to determine virus neutralisation capacity. Some examples are plaque reduction neutralisation (PRNT), microneutralisation and pseudovirus neutralisation assay [57-59]. 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 BSL-3 laboratories and are often labour-intensive. Alternatively, assays using replication-defective pseudotyped viral particles can be performed under BSL-2 conditions, but they depend on creating the variant-specific pseudotype virus 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 [60].

To assess the neutralisation capacity of sera for different patient situations, the serum panels should 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 [18].

To compare the neutralisation assay results with other laboratories, WHO International Antibody Standard (WHO IS) or, if the WHO IS unavailable, the so-called NIBSC working reagent (21/234) or high titer-reference serum (20/150) should be used for neutralisation assays [57,61-63]. 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 (index virus, D614G, Alpha, Beta, Gamma, Delta and Omicron, with preference for D614G, Alpha, Beta, Delta and Omicron as a minimum) in the neutralisation assays. The assays should also ideally be performed in duplicate or triplicate reactions and repeated in at least two to three independent experiments.

Several studies have already looked at VOC and VOI antigenic properties through various neutralisation assays - e.g. SARS-CoV-2 501Y.V2 [64], Alpha [65], Beta [66], Delta [67], Eta [66], Gamma [68], Lambda[65] and Omicron variants [66,69,70]. Additional B-cell and T-cell assays will give insight into the immune responses against different VOCs and immune correlates beyond neutralising antibodies.

EU/EEA countries can request support in setting up antigenic characterisation assays or instructions on how to forward clinical samples or virus isolates for antigenic characterisation by contacting 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 and the list of reference laboratories can be found here. Antigenic characterisation results for new VOCs should immediately be shared with ECDC and WHO European Region. EU/EEA data can be shared and discussed by country nominated users on the confidential and secure network platform EpiPulse (https://epipulse.ecdc.europa.eu).

Considerations for sample and method selection

Genomic monitoring should be integrated into overall respiratory virus surveillance strategies to ensure reliability and interpretability of findings. Sample and method selection are key and will depend on the surveillance objectives to be met.

- Countries are encouraged to remain vigilant for signals of new lineage/variant introduction or emergence and to increase testing and sequencing efforts if the introduction of a new VOC, VOI or VUM is imminent.
- A selection of positive SARS-CoV-2 samples can be screened for VOC identification. Screening of travellers is ineffective in preventing 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, travel-related cases should ideally be screened to allow quick isolation and contact tracing of all cases, and sequenced to assess the prevalence of a VOC in this group [71]. 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 [18].
- 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 [18]. Sequencing should be the preferred method for testing representative samples, however screening methods can also be useful, as fast result turnaround time is important for informing public health interventions [72].
- To use the screening method as an indicator of the overall situation, sequencing can be employed to assess the fraction of viruses with S-gene target failure, or VOC detections positive with other screening methods [18].
- WGS should be done in parallel for virus genetic characterisation to monitor the virus evolution. For this purpose, representative samples from new clusters should be targeted, as well as samples from immunocompromised patients or patients with other underlying conditions associated with prolonged viral replication and shedding, and cases with an unusual clinical presentation or poor response to therapeutics including antiviral treatments. Similarly, cases suggestive of zoonotic transmission should also be targeted, or if there is suspicion of changes in the performance of diagnostics (antibody, antigen, molecular assay) [73].
- 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, potentially indicating changed virus characteristics (e.g. increased transmissibility or immune escape) for initial identification of novel VOCs [73].
- 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 reference or international reference laboratories [6]. Priority should be given to specimens from outbreaks, immunocompromised patients or patients with other underlying conditions associated with prolonged viral replication and shedding, and cases with an unusual clinical presentation or poor response to therapeutics including antiviral treatments. Priority should also be given to cases suggestive of zoonotic transmission, or if there is suspicion of changes in the performance of diagnostics (antibody, antigen, molecular assay).
- Additional B-cell and T-cell assays will give insight into other aspects of the immune response against different VOCs and potential establishment of immune correlates of protection beyond neutralising antibodies.

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 [74]. 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 with other laboratories for a subset of samples [74]. ECDC and WHO European Region are planning a molecular External Quality Assessment 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 some 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 European Region 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] [75].

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 [76]. Detection of a suspicious signal related to a SARS-CoV-2 lineage considered of relevance by the reporting county should be reported immediately by nominated users of EU/EEA countries through EpiPulse, and by nominated users of all countries of the region through the Early Warning and Response System (EWRS) and IHR. Detections of variants should be reported to TESSy on a weekly basis.

SARS-CoV-2 consensus sequences should be submitted to GISAID and if feasible also in the COVID-19 data portal 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).

With regard to BA.4 and BA.5 variants, ECDC has invited nominated users of EU/EEA countries to use the EpiPulse event for BA.4 and BA.5 to informally discuss and share information on these two variants, including partial and preliminary data. Information that should be shared includes country-specific assessment of epidemiological and microbiological situations; information on variant emergence, if not yet reported to TESSy; information on access to virus isolates and ongoing characterisation work including any new evidence on disease severity, virus transmissibility and immune evasion, effects on diagnostics and therapeutics. Case reporting should continue through TESSy, preferably using the case-based NCOV record type, if possible.

Variables for reporting of variants in TESSy have been implemented within the aggregated and case-based 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 facilitate 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.

[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/

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 European Region 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 has set-up the BioHub for the purpose of material sharing.

Protocol and information sharing

WHO European Region and ECDC have jointly set up a protocol/information sharing platform EZCollab for 'COVID-19 protocol sharing' and registration can be carried out here: https://ezcollab.who.int/euroflu/flulab/covid19_protocols.

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