World Health Organization
REGIONAL OFFICE FOR Africa

COVID-19 RESPONSE

WORLD HEALTH ORGANIZATION REGIONAL OFFICE FOR AFRICA SUPPORTS THE COVID-19 RESPONSE

Guidance on tools for detection and surveillance of SARS CoV-2 Variants

Interim Guidance

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1. Introduction

As the pandemic continues globally the SARS CoV-2 virus continues to mutate and the emergence of variants that differ from the original Wuhan strain will continue to arise, yet not all viral variants generated through mutation of the virus are important or have immediate public health relevance. However, variants with specific mutations that affect the way the virus behaves are now considered either Variants of Interest (VOI) or Variants of Concern (VOC) with respect to public health implications (1). Currently, there are 4 VOC circulating globally: Alpha, Beta, Gamma and Delta variants. There are additionally 4 VOI: Eta, Iota, Kappa and Lambda, that are being monitored and investigated to determine if they meet criteria for a VOC. The VOC differ from the original Wuhan virus as they have mutations across the genome. However, of specific interest are the mutations observed in the Spike gene (S) of the virus **(Table 1).** The viral S gene is important as it codes for the Spike protein which is the molecule that makes contact with, and allows entry of the virus into susceptible host cells, causing infection. Some mutations in the S gene may lead to changes in the spike protein which result in inhibition of contact and entry of the virus into human cells, however in the case of the VOC, they contain mutations in the S gene that enhance the process of contact and entry into human cells, increasing transmissibility of the virus.

Table 1 : Amino acid positions of mutations in S gene of 4 VOC (Alpha, Beta, Gamma and Delta variants) and their sub-lineages*

	Amino acid positions of mutation in S gene of VOC																								
VOC (Pango Lineage) *sub-lineage*	L18F	T19R	T20N	P26S	**del H69-V70**	D80A	D138Y	del 144-145	del 157-158	R190S	D215G	L242H	del 241-243	R246I	K417N	K417T	**L452R**	T478K	E484K	N501Y	A570D	P681H	P681R	A701V	S950N
Alpha (B.1.1.7)					✓			✓												✓	✓	✓			
B.1.1.7 +E484K					✓			✓											✓	✓	✓	✓			
B.1.1.7 +L452R					✓			✓									✓			✓	✓	✓			
Beta (B.1.351)						✓					✓	✓	✓	✓	✓				✓	✓				✓	
B.1.351.2	✓					✓					✓	✓	✓	✓	✓				✓	✓				✓	
B.1.351.3	✓					✓					✓	✓	✓	✓	✓				✓	✓				✓	
Gamma (P1)	✓		✓	✓			✓			✓						✓			✓	✓					
P.1.1	✓		✓	✓			✓			✓						✓			✓	✓					
P.1.2	✓		✓	✓			✓			✓						✓			✓	✓					
Delta (B.1.617.2)		✓							✓								✓	✓					✓		✓
AY.1		✓						✓	✓						✓		✓	✓					✓		✓
AY.2		✓						✓	✓						✓		✓	✓					✓		✓

*Mutations in other regions of the genome may also be informative for known VOI/VOC detection.

A number of tools are available for the detection of mutations that are characteristic of viruses defined as VOI/VOCs. The objective of this document is to outline the available methods and tools for screening and sequencing patient specimens for the detection of VOC or VOI, with a focus on those that offer a rapid turn-around time so as to support rapid implementation of public health actions for COVID 19 control.

2. WHO designated VOC and VOI

To date, WHO has designated 4 SARS CoV-2 genotypes that meet the working definition of a VOC: i) contain mutations divergent from the Wuhan strain and ii) that the mutations impact one or more of the aspects of viral infection that have public health significance. WHO has also designated 4 virus genotypes as VOI. However, the situation is extremely dynamic and information regarding the working definitions and designations of VOI and VOC, and the current list of VOI/VOC can be found here (1). Each variant will have characteristic mutations or a group of mutations that is/are unique to that particular variant. The mutation profiles VOCs are summarized below **(Table 1).**

3. Screening assays for detection of known VOC/VOI

Overall, these assays and approaches detect specific mutations or features such as insertions, deletions and point mutations in the SARS CoV-2 genome that are characteristic of a particular VOC/VOI using PCR. Currently, manufacturers are focusing on and targeting assays to mutations in the S gene. However, mutations that are characteristic of a specific VOC/VOI can occur anywhere in the SARS CoV-2 genome and can be targeted as a region for detection of VOC/VOI.

Note: these tests **CANNOT** be used for clinical diagnosis of SARS CoV-2 and are research use only (RUO).

Please refer to the section on **Choosing a PCR screening assay for VOC/VOI detection** for further information on how to select the test that suits your needs.

PCR target failure

Current diagnostic PCR assays target a variety of SARS CoV-2 genes and the vast majority target sequences in regions of the SARS CoV-2 genome that are highly conserved. Highly conserved regions are less likely to mutate, therefore there is a high likelihood that the diagnostic test will remain accurate in the presence of variants.

The S gene is one of the structural genes of the virus which encodes for a protein that sits on the surface of the SARS CoV-2 virus. This protein is a critical in the linking of the virus to a host cell it can infect. The S protein, due to its exposure on the outside of the virus is also one of the parts of the virus that is recognised as foreign by the immune system. As a result, this gene accumulates more mutations than other regions of the SARS CoV-2 genome. Since, more mutations will accumulate in the S gene as it is subject to selective pressure (Table 1), when compared to other areas of the genome, PCR diagnostic assays seldom target this gene. However, a few tests do have amplification targets in the S gene.

If the PCR assay includes primers or probes that bind in the area of a mutation(s) there may be a weaker signal produced or complete failure of the target to amplify, which is termed target failure of target gene drop out. If this occurs, it may be indicative that a variant is present in the sample being tested.

S gene target failure (SGTF)

One significant mutation in the S gene is the 6-nucleotide deletion at nucleotide positions 207-212 (Δ69-70) (highlighted in yellow in Table 1) of the S gene. This mutation is seen in the VOC Alpha (B.1.117) and its sub-lineages, but not in other VOC, therefore could be exploited for VOC detection purposes. However, it should be noted that this deletion is present in the VOI Eta and may be present in other variants not yet classified as a VOI/VOC. Currently, one commercial assay (approved through WHO EUL (2), ThermoFisher, TaqPath COVID-19 CE-IVD RT-PCR Kit) targets 3 SARS CoV-2 genes, including the S gene, specifically the region of the Δ69-70 mutation. Therefore, if the specimen being tested with this assay contains the Alpha VOC, the S gene target will be weakly amplified or not amplified at all, termed **S-gene drop out or S gene target failure (SGTF)**, thereby suggesting the presence of VOC Alpha in the specimen, or another variant that has this deletion.

It is worthwhile reviewing the details of the diagnostic NAAT assay you are using *in silico* to determine if the assay targets the S gene target(s) and if so determine whether the primers/probes are affected by specific S gene mutations present in the VOI/VOC. It is likely that this may be challenging due to primer/probe sequences being propriety information. GISAID enables checking of sequences against primers/probes from publicly-available PCR protocols (e.g. US CDC, Charité, Hong Kong, etc) or vice versa, if you know the primer/probe sequence of the assay you are using you can also verify whether sequences available or reference sequences have any mutations in the primer's binding site on the gene target[2].

Screening SNP assays

Screening for single specific nucleotide polymorphisms (SNP) can be performed to detect VOI/VOC and assays of this type are in the marketplace. Mutations targeted to specifically identify VOC may be in the S gene or other genes where signature mutations occur. Appropriate positive controls are necessary to ensure valid results. Careful choices should be made when considering mutations to target if using these screening assays as some circulating lineages of SARS CoV-2, including non-VOI/VOC lineages may contain some of the same mutations associated with VOI/VOC. For example, there are circulating lineages that contain the N501Y mutation but are not considered VOI/VOC.

[2] PrimerChecker is on the GISAID site (https://www.gisaid.org/) and requires a login to the GISAID site to access this tool.

Screening SNP assays using melting curve analysis

A number of real time PCR platforms have the capacity to use **melting curve analysis** to detect genotypes (3). Currently, assays have been developed to detect the following mutations using melting curve analysis: H66D, A67V, Δ69-70, D253G, K417N, K417T, N439K, L452R, Y453F, T478K E484K, E484Q, N501Y, A570D, D614G, P681H, P681R, F888L, Q949R and V1176F. These assays detect mutations that cover all 4 VOC: Alpha, Beta, Gamma and Delta and 2 VOI, Eta, and Iota (4). However, it should be noted that this list may expand or contract due to the dynamic nature of the situation.

Multiplex SNP assays, including S-gene drop out

Multiplex assays are commercially available for the detection of multiple mutations in VOC and VOI that may be able to differentiate between the probable presence of two or more VOI/VOC in a single reaction. These assays target mutations in the SARS CoV-2 genome that may be unique to the VOC or VOI. These mutations may or may not be in the S gene but also may be in other parts of the viral genome and are informative with respect to the detection of a VOC or VOI. Since the first characterization of the VOI/VOC many assays have entered the market and are available for screening of SARS CoV-2 positive specimens.

There are also lab-developed protocols which have been designed to screen for VOI/VOC. For example, a PCR assay was developed by a Brazilian laboratory that targets a deletion in nsp6 gene, that is common to Alpha, Beta, Gamma and Lambda VOI/VOC which can be used to screen for these VOI/VOC, but it is not able to differentiate between each of the VOI/VOC (5).

Please refer to the section on Choosing a PCR screening assay for VOC detection for further information on how to select the test that suits your needs.

Note: these tests **CANNOT** be used for clinical diagnosis of SARS CoV-2 and are research use only (RUO).

4. DNA Sequencing

Whole genome sequencing (WGS)

Whole genome sequencing has become more accessible to laboratories with advances in technology in particular next generation sequencing (NGS). WGS will, as the name implies result in the generation of a contiguous sequence that covers the entire viral genome. This methodology facilitates the detection of mutations across the entire SARS CoV-2 genome and can provide important information on mutations hat may impact the way the virus behaves. Whole-genome sequencing is the best methodology for confirming a VOC.

The table below taken from European Centres for Disease Control guidance document (**Table 2**) summarises example applications of

WGS and the available platforms that can be utilised. WGS is the most accurate method to detect known VOI/VOC however this approach is resource intensive, requires bioinformatic resources and results are not immediate - it may take a number of days to obtain results, depending on the protocol used. Comprehensive guidance on the use of sequencing can be found in the WHO Genomic sequencing of SARS CoV-2: a guide to implementation for maximum impact on public health.(6)

Partial/Targeted sequencing: Sanger or next generation amplicon-based sequencing

These methods are somewhat simpler and involve targeting specific regions of the genome for amplification and sequencing. Using these methods only regions that contain informative mutations for the detection of VOC are amplified, keeping in mind that the mutations may be in the Spike gene or in other regions of the SARS CoV-2 genome. To ensure detection of all known VOC, if focusing on the S gene, the amplicon generated for sequencing, if focusing on the S gene, to ensure detection of all known VOC should cover at a minimum the entire N-terminal domain and the receptor binding domain (amino acids 1-541, amplicon = 1623bp).

Additionally, amplicons covering the S1/S2 cleavage site (amino acid position 681) or the entire S gene (amino acids 1-800, 2,400bp) should be considered so as to monitor mutations at the S1/S2 cleavage site and other sites that may be of interest in the S gene.

Table 2. Use cases for NGS platforms in SARS CoV-2 surveillance and response SARS-CoV-2 genome sequencing applications and recommended technologies (7).

Application	Recommended sequencing platforms	Recommended library construction approaches	Recommended read length	Recommended minimum local coverage (approximate)
Transmission patterns, clade/lineage assignment, confirmation of reinfection, phenotypically relevant mutations, data reporting	MiSeq/NextSeq/iSeq/NovaSeq (Illumina), Ion Torrent (ThermoFisher), MinION (Oxford Nanopore), Sequel System (PacBio)	Amplicon-based (ARTIC, commercial, in-house)	>100 bp	>10x over >95% of genome
Confirmation of reinfection and/or direct transmission (in cases where minority variants are required)	MiSeq/NextSeq/iSeq/NovaSeq (Illumina), Ion S5 series/Genexus (Thermo Fisher)	Amplicon-based (ARTIC, commercial, in-house)	>100 bp	>500x over >95% of genome
In-depth genome analysis (large indels, recombination, rearrangements, quasi-species haplotypes)	MinION (Oxford Nanopore), Sequel System (PacBio)	Amplicon-based (>1000 bp fragments), capture-based, untargeted	>1000 bp	>500x over >95% of genome
Detection of unknown pathogens or highly divergent strains	MiSeq/NextSeq/iSeq/NovaSeq (Illumina), Ion S5 series/Genexus (Thermo Fisher), MinION (Oxford Nanopore)	Untargeted RNA sequencing, β-CoV-specific RT-PCR	>100bp	>5Gbp data per sample

5. Criteria for selecting a PCR screening assay for VOC Detection

The marketplace for assays for the detection of mutations characteristic of VOC or VOI is and will remain dynamic as the virus continues to change. To ensure you meet your testing/screening needs it is important to select assays to fit your purpose with respect to your equipment, laboratory expertise and objectives. Below are some features that you may need to consider when choosing an assay.

- Format used - single mutation detection/reaction or multiple mutation reaction/reaction (multiplex), consider how many reactions you need to do to detect a VOC, how is detection made
- Analysis method for mutation detection - does the assay require specialized or specific software to detect the mutations, is it a simple amplification/no amplification analysis, does it use melt curve analysis
- Fluorophores used in assay - it is important that the fluorophores in the assay can be detected by your real time PCR platform
- Validation on platforms - check to see if the assay has been validated for the PCR platform you are using or if there are publications which validate the assay on your platform

It should be noted that **none of the current SNP screening assays or sequencing protocols are intended to diagnose SARS-CoV-2** therefore it is critical as a first step to first identify SARS CoV-2 positive specimens using a quality assured (ideally WHO EUL approved) diagnostic test, e.g. PCR and/or Ag RDT **(see Algorithm).** Any PCR assay chosen to screen specimens for mutations must be used as a second step following detection of positives using an assay approved for clinical diagnosis e.g. WHO EUL PCR assay.

6. Implementation of PCR screening assays for VOC

The primary role of PCR screening assays is to expedite the process of identification of specimens containing mutations characteristic of VOI/VOC in countries to enable the rapid implementation of Public Health and Social Measures to abrogate transmission of the virus, to estimate the prevalence of a given mutation(s) characteristic of a VOI/VOC and to enhance the body of data on global circulation of VOC. **Using a PCR assay for detection of known VOC or VOI DOES NOT replace detection of cases by WHO EUL PCR assays or Ag-RDT.** In locations where DNA sequencing is not readily available, screening positive SARS CoV-2 specimens for VOC using PCR should be considered and accompanied by sequencing of a portion of specimens at Referral Laboratories for confirmation, if this aligns with national goals and objectives.

Practically, it is not necessary to screen all SARS-COV-2 positive samples for VOC. A selected proportion of samples can be screened that is representative of different geographical areas, clusters or clinical categories of patients. It is important to sequence a proportion

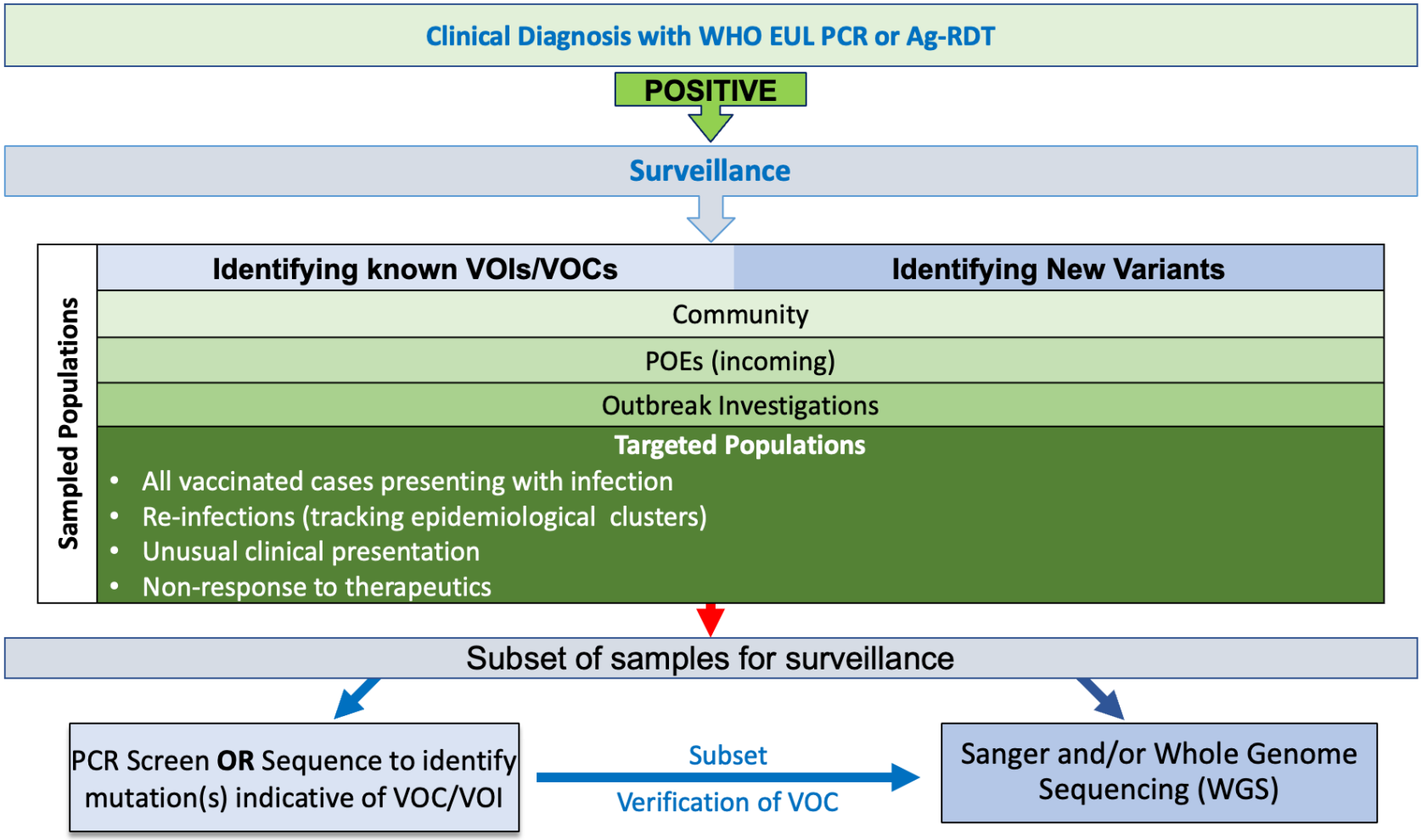
of samples with mutations characteristic of VOC for confirmation and identity of mutations in the VOI/VOC mutation profile, to detect any further mutations in the genome that may be informative and for quality control purposes.

The question of how many SARS CoV-2 positive specimens to PCR screen for VOI/VOC or to sequence for virus evolution tracking is complex. WHO AFRO has developed a guide that can be found here (8) to help you navigate this issue. However, when selecting a sampling strategy/size, particularly for PCR screening, consider the laboratory capacity to conduct additional assays and have in mind a plan of when to scale back or reduce VOI/VOCPCR screening, since once a more fit SARS CoV-2 variant enters a population, it will not take long to replace the existing variants in that population. If screening is scaled back it remains important to continue sequencing of proportions of positives to track viral evolution/detection of new mutations. As part of implementation please also consider establishing quality control systems such as inter laboratory comparisons, in addition to regular confirmation of a small subset of each VOC identified by sequencing.

7. References

1. *Tracking SARS-CoV-2 variants*. Geneva, Switzerland, World Health Organization, 2021 (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/, accessed 10 August, 2021)
2. *WHO Emergency Use Listing for in vitro diagnostics (IVDs) Detecting SARS-CoV-2,* Geneva, Switzerland, World Health Organization, 2021 (https://extranet.who.int/pqweb/sites/default/files/documents/210430_EUL_SARS-CoV-2_product_list.pdf, accessed 10 August, 2021).
3. Farrar, JS., Reed GH and Wittwer, CT, 2010 , High-Resolution Melting Curve Analysis for Molecular Diagnostics, in Patrinos, GP and Ansorge, WJ, (eds.), Molecular Diagnostics. 2nd ed., Elsevier Press; Cambridge, Massachusetts, United States.
4. SARS RT-PCR test kits**,** VirSNiP Assays, Berlin, Germany, TIB Molbiol, (https://www.tib-molbiol.de/covid-19#nav-644, accessed 16 June, 2021)
5. Naveca, F.G., Nascimento, V., de Souza, V.C. *et al.* COVID-19 in Amazonas, Brazil, was driven by the persistence of endemic lineages and P.1 emergence. *Nat Med* **27,** 1230–1238 (2021). https://doi.org/10.1038/s41591-021-01378-7
6. *WHO Genomic sequencing of SARS CoV-2: a guide to implementation for maximum impact on public health*. Geneva, Switzerland. World Health Organization, 2021. (https://www.who.int/publications/i/item/9789240018440, accessed August 1, 2021)
7. *Methods for the detection and identification of SARS-CoV-2 variants.* Copenhagen, Denmark. World Health Organization. Regional Office for Europe, March 2021. (https://apps.who.int/iris/handle/10665/340067, accessed July 11, 2021)
8. *Variant Surveillance Guidance: Executive Summary*, Brazzaville, Congo. World Health Organization Regional Office for Africa, 2021 (https://www.afro.who.int/sites/default/files/Covid-19/Techinical%20documents/Variant%20surveillance%20guidance%20-%20Executive%20summary.pdf, accessed July 30, 2021)

Strategy for use of DNA sequencing and PCR screening assays for identification of mutation(s) characteristic of VOC and tracking viral evolution



* Special groups may include patients with unusual presentation or illness, immunocompromised individuals etc. but should be determined by the country based on their epidemiological situation.