

Guidance for surveillance of SARS-CoV-2 variants

Interim guidance

9 August 2021



Key points

- The public health risks of known and emerging variants of interest (VOIs) and variants of concern (VOCs) can be categorized into five main domains: increased transmissibility; a more severe clinical course; failure to be detected by diagnostic assays; escape to natural or vaccine-derived immunity and decreased susceptibility to therapeutics.
- Routine genetic sequencing is critical to follow the emergence and impact of VOIs and VOCs. Countries with limited capacity to perform sequencing are strongly encouraged to take steps to facilitate access to regional and international sequencing partnerships or increase their capacity through existing sequencing systems or laboratory networks.
- Sampling for genetic sequencing should consider all the following subsets, as feasible:
 - randomized samples, representative of geographic and demographic distribution of SARS-CoV-2 infections
 - targeted sampling focusing on specific subsets of cases associated with public health risks: diagnostic failures, vaccinated cases, reinfections, immunocompromised cases
 - outbreaks, alerts or other unusual events.
- Unexpected trends or signals from routine epidemiological surveillance (or other sources) such as increasing trends in epidemic course, with high impact on public health, can be an indication of a potential VOI or VOC.
- All reported sequences should be associated with a minimum set of linked information, called metadata, and include core details. If possible, descriptive metadata and metadata for characterization should be included.
- A combination of laboratory science, clinical manifestations and detailed epidemiologic investigations is required to accurately and rapidly characterize public health risks of SARS-CoV-2 variants.
- Prompt sharing of information around SARS-CoV-2 variant genomic sequences in public databases is integral to the global understanding and control of SARS-CoV-2.

Purpose of the document

This document aims to describe a minimum set of surveillance activities recommended at the national level to detect and monitor the relative prevalence of SARS-CoV-2 variants and outline a set of activities for the characterization and assessment of risk posed by these variants. A set of indicators is also provided to standardize monitoring and public reporting of variant circulation.

The document is primarily intended for national and sub-national public health authorities and partners who support implementation of surveillance for SARS-CoV-2 variants. Additional guidance has been published for laboratory stakeholders on [diagnostic testing for SARS-CoV-2](#) and [sequencing for public health goals](#), alongside an [implementation guide for SARS-CoV-2 sequencing](#).

Background

SARS-CoV-2 is an enveloped, positive-sense single-stranded RNA virus with a 30-kilobase genome, which, like all viruses, accumulates nucleotide mutations over time. These mutations result in the formation of distinct viral lineages. Since its characterization (1), genomic sequencing of SARS-CoV-2 has been conducted to identify mutations and any corresponding amino acid substitutions. Although the emergence of these new variants is expected and most have no impact on viral behaviour, some mutations may produce changes in phenotype.

The public health risks of known and emerging variants can be broadly categorized into five main domains:

- increased transmissibility, due to increased viral shedding, binding affinity for host cells, or stability of the virus
- atypical clinical course (e.g. increased severity, atypical signs and symptoms)
- diagnostic failure: decreased performance of some laboratory diagnostics, particularly molecular assays such as nucleic acid amplification testing (NAAT) (2) and approved antigen-detection rapid diagnostic tests
- decreased effectiveness of natural and vaccine-derived immunity: the ability of the variant to partially evade the host antibody response and, potentially, increase the likelihood of reinfection or vaccine breakthrough
- decreased susceptibility to therapeutics: the potential ability of a new variant to evade an antibody therapy is a cause for concern (3) and has led to changes in recommendations on the use of some therapeutics.

The World Health Organization (WHO) classifies “variants of interest” (VOIs) or “variants of concern” (VOCs) according to the global impact of these factors. As of 9 July 2021, WHO had designated seven VOIs and four VOCs (4).

Global genomic surveillance for SARS-CoV-2 is a critical public health function, with the primary objective to inform national and global decisions around public health and social measures (PHSMs), diagnostics, therapeutics, and vaccination. Surveillance of variants can be done through genomic surveillance as well as through detection of epidemiological signals and unexpected trends. These two strands of evidence should be brought together in a timely fashion to provide a broad understanding of viral evolution and its potential impact on disease control, in order to guide public health response.

Despite the concerning phenotypes of known VOIs and VOCs, WHO continues to recommend the implementation and adjustment of PHSMs to control transmission, as described in [existing WHO guidance](#). However, close monitoring of the impact of current variants on the efficacy of PHSMs is needed.

Guidance on diagnostic testing for SARS-CoV-2 can be found [here](#), and specific guidance on the use of antigen-detection rapid diagnostics tests can be found [here](#).

Evidence that vaccines may be less protective against a specific variant may be suggested by genomic and structural biology studies, animal studies and in-vitro neutralization testing. Lower effectiveness of a vaccine in protecting against infection and disease from a variant in humans, however, provides the strongest evidence. Epidemiological data on vaccine performance against new variants will primarily come from observational studies of vaccine effectiveness (VE); please see the [Addendum to Evaluation of COVID-19 vaccine effectiveness](#).

More variants will likely continue to arise as transmission continues, and they may be subject to selective pressures from natural immunity, vaccine use and therapeutics.

Methodology

This WHO interim guidance was written by WHO and the United States Centers for Disease Control and Prevention (US CDC) in consultation with the Africa Centers for Disease Control and Prevention (ACDC), the European Center for Disease Control and Prevention (ECDC), with additional feedback from expert advisory groups, such as WHO’s Epidemiology Technical Advisory Group. The guidance is based on a review of emerging evidence on variant epidemiology and characterization methods, covering all regions, using English language search engines. Search topics included: public health driven sampling strategy, genomic sequencing, phylogeny, genomic epidemiology and surveillance methods, genomic metadata and database management, genomic database analysis for public health purposes, as well as specific characterization evidence for specific mutations and variants of concern and interest. Evidence was synthesized following the thematic sections of the guidance. Additional references were provided by experts, and existing guidance documents from WHO and other partners were also referenced; see table below. This document is subject to updates as new evidence and methodologies on variant investigation emerge.

1. Surveillance for SARS-CoV-2 variants

1.1. Building capacity for genomic sequencing

Capacities for SARS-CoV-2 sequencing activities have expanded considerably as the pandemic has evolved. However, sequencing capacity varies significantly within and between countries. As a result, the amount of genetic sequence data (GSD), quality of metadata accompanying the GSD and the length of time from specimen collection to sequencing and reporting differ widely across countries. To help address this challenge, on 8 January 2021, WHO published two interim guidance documents on SARS-CoV-2 genomic sequencing to track the geographic spread of the virus over time and ensure that mutations with the potential to influence transmissibility, pathogenicity and medical countermeasures are identified and assessed quickly: [SARS-CoV-2 genomic sequencing for public health goals](#) and [Genomic sequencing of SARS-CoV-2: a guide to implementation for maximum impact on public health](#). The United States Centers for Disease Control and Prevention (US CDC) has also released a COVID-19 Genomic Epidemiology Toolkit (6).

Countries with limited capacity to perform sequencing are strongly encouraged to take steps to facilitate access to existing regional and international sequencing networks and partnerships. Countries may also choose to increase sequencing capacity through existing surveillance systems with sequencing capacity such as the **Global Influenza Surveillance and Response System (GISRS)** or existing regional networks. The [Shipping Fund Project](#) is designed to support the transport of samples for sequencing and data sharing. Collaborations with experienced laboratories and other potential partners may include public health departments, non-profits, academic centres or commercial entities. In addition to whole genomic sequencing, countries can also screen for known mutations utilizing targeted RT-PCR-based mutation detection assays; countries are encouraged to establish clear procedures for their use.

1.2. Variant definitions

WHO has released working case definitions for SARS-CoV-2 variants of interest and variants of concern. These may be updated regularly. Please refer to the [WHO variant webpage](#) for latest definitions, and list of latest VOIs and VOCs.

1.3. Variant alert triggers

Unexpected trends or signals from routine epidemiological surveillance (or other sources) indicating an increased impact of the course of the pandemic on public health can be an indication of a potential VOI or VOC.

1.3.1. Routine Epidemiological surveillance

WHO recommends the following minimum set of variables to be included in [weekly epidemiological surveillance](#).

- Number of confirmed cases
- Number of probable cases
- Number of confirmed deaths
- Number of probable deaths
- Number of individuals hospitalized (confirmed and probable)
- Number discharged (confirmed and probable)
- Number of health care workers infected (confirmed + probable) as a subset of total cases count
- Number of health care workers who died due to COVID-19 (confirmed + probable) as a subset of total death count
- Number of persons tested
- Number of persons tested by PCR
- Confirmed + probable cases by age group and sex (see below)
- Confirmed + probable deaths by age group and sex (see below)
- Transmission classification

The following age categories (in years) are recommended: 0-4, 5-9, 10-14, 15-19, 20-29, 30-39, 40-49, 50-59, 60-64, 65-69, 70-74, 75-79, and >80.

In addition to these variables, the monitoring of ICU occupancy and vaccination coverage of targeted population subgroups can enhance routine surveillance for alerts.

Weekly monitoring of epidemiological indicators at a high geographical granularity allows for timely detection of any departure from trends, or unexpected signals. This allows for early targeting of investigation and sampling for sequencing. Analysis should take into account public health and social measures, stringency index (7) and any other parameters that could impact transmission (e.g. mass gatherings).

Table 1 Examples of disease surveillance indicators, alert and trigger thresholds

Indicators	Alert
Cases	Increase / departure from trend
Age-disaggregated cases	Increase in specific age groups (under 18, under 65; to be determined locally)
Cases among health and care workers	Increase / departure from trend
Case fatality ratio	Increase / departure from trend
Age disaggregated deaths	Increase in specific age groups
Hospitalizations/ICU admissions or bed occupancy rate	Increase in specific age groups
Test positivity rate	Increase / departure from trend

These triggers and thresholds should be adapted to local situations, investigation capacity and desired sensitivity.

If routine surveillance systems are not in place to monitor hospital or ICU admissions or bed capacity, demand for oxygen and ventilators may indicate a surge in severe illness, which may or may not be driven by an emerging variant with increased virulence. Such indicators can be followed with joint monitoring from pharmaceutical and biomedical supplies providers.

Similarly, increases in transmission beyond which might be expected given population levels of immunity also warrant further investigation. For example, sustained community transmission in areas where vaccination coverage is high or there are high levels of past infection may indicate the presence of a variant able to evade the immune response. Please see [guidance on vaccine effectiveness in the context of new SARS-CoV-2 variants](#).

If robust case investigation and contact tracing protocols are in place, an increasing proportion of contacts that become cases (i.e. unexpectedly high secondary attack rate in comparison to studies in similar settings, such as households) might provide a similar signal.

Trends in mortality at the lowest available administrative level can reveal an increase in death rate in particular populations, and the case fatality ratio (CFR) may be estimated if case-based surveillance data covering the same time period and geographic region are also available (see [Case Fatality Ratio scientific brief](#)). Increases in CFR may warrant further investigation through genomic characterization, although trends in mortality are unlikely to reveal a variant with higher severity unless there is a drastic change in CFR. A decoupling between mortality and incidence trends (i.e., mortality higher than expected for a given incidence) could also be an indicator in increased disease severity.

1.3.2. Event-based surveillance

Reports of rapidly spreading outbreaks in healthcare facilities or communities might raise the concern that these events are due to a variant that spreads more easily from person to person. Similar reports from populations expected to have a high level of immunity (due to prior infections or high vaccination coverage) may indicate the presence of a variant able to evade the immune response.

Outbreaks causing unexpectedly high levels of morbidity and mortality (otherwise unexplained by the demographics and underlying conditions of the affected population, clinical case management or hospitalization capacity, medical supply shortage or other factors) may be due to a variant causing more severe disease.

Depending on capacity, such reports may trigger a field investigation. Specimens collected during such investigations may warrant prioritization for sequencing.

Reports of respiratory illness clusters meeting the suspect or probable COVID-19 case definition but testing negative for SARS-CoV-2 and without an alternative clinical diagnosis may also warrant investigation.

1.3.3. Environmental surveillance

If existing surveillance systems are in place to monitor wastewater for SARS-CoV-2 RNA, these might be leveraged for variant surveillance. Viral RNA can be sequenced directly from wastewater and may provide an early glimpse into whether a known VOC is being transmitted. There are several real-world examples of genomic sequencing of SARS-CoV-2 in wastewater revealing variants of concern (8,9),(10), but the temporal and quantitative association with community transmission requires further study (11).

1.4. Sampling strategies

Sampling strategies will vary depending on national variant surveillance objectives. Primary objectives may include:

- a) detecting variants circulating at low levels
- b) monitoring the relative prevalence of variants across time and geographic areas
- c) investigating particular cases of public health interest.

Broadly, objectives (a) and (b) can be met through routine surveillance of a randomized sample. Objective (c) requires a targeted sample.

For countries with high sequencing capacity, priority objectives should be a), detecting variants; and b), monitoring relative prevalence of variants. Countries with low sequencing capacity should focus on b), monitoring relative prevalence of variants.

1.4.1. Representative sampling for routine surveillance

Randomized representative sampling can be defined as a selection of a subset of a given target population, representative of the target population situation. Criteria accounting for the representative distribution of a sample should include at least age, sex, clinical spectrum, and geographical distribution.

Key considerations of surveillance systems outlined in other WHO documents (e.g. [GISRS guidance](#)) remain relevant, particularly: systematic sample collection, geographically relevant and sustainable sampling at regular frequency; sampling a representative population; and providing timely sample sequencing and analysis.

When conducting genomic surveillance, it is important to consider the time interval between infection and availability of sequence data. Contributors to a lack of timeliness include lags between sample collection and receipt of specimens by the sequencing laboratory; laboratory processing time; bioinformatics analysis; and time needed to provide data to public health authorities or to post sequence data on public databases. Efforts should be made to increase the timeliness of these actions at every stage. Routine collection of surveillance samples over a fixed, repeating time interval will ensure lagging data are regularly updated. Because the relative prevalence of variants can change rapidly, regular sample collection is recommended, preferably on a weekly basis. This also allows for time series to offer highly dynamic representativity.

The methods of selecting a representative sample may vary by country and draw on local surveillance systems, whether routine or sentinel, such as the GISRS sentinel sites network for influenza-like illness/ severe acute respiratory infection (ILI/SARI). Because incidence might fluctuate rapidly, sampling a fixed number of cases (as opposed to a fixed proportion of cases) may be more logistically feasible for submitting and sequencing laboratories to predict resource requirements and standardize protocols.

Table 2: Sensitivity and specificity of sequencing strategies

	Pros	Cons
1- Randomized representative sampling	High sensitivity	Large sample size: capacity challenge
2- Fixed sample from sentinel sites	Operationally practical; if stable, can allow to follow trend of circulating variants	Low sensitivity Low representativeness (geographical, population based)

1.4.1.1. Sampling methodologies

Sample size calculations assume that specimens are randomly sampled and thereby likely to be representative, assuming that positive samples are themselves a true representation of underlying infection rates. If diagnosed cases are a representative sample of all COVID-19 cases because diagnostic coverage is equally distributed across the country, an unadjusted sample of positive specimens identified through the clinical system may be sufficient to ensure reasonable representativeness.

However, in many countries, diagnostic coverage is uneven because of disparities in access to healthcare and diagnostics or extensive use of contact tracing to identify cases. If diagnostic coverage is not equally distributed, weighting the sample may partially adjust for this. This can be achieved by asking areas with lower diagnostic coverage to submit a higher proportion of specimens than areas with ready diagnostic access.

Options for representative specimen collection may include systematic sampling (sample selection at regular intervals) and random sampling (sample selection generated randomly). The choice of methods should be validated by comparing the distribution of representativeness criteria (e.g. age, sex, clinical spectrum, and geographical distribution) across the sample.

If a truly representative sample is difficult to obtain, sentinel surveillance from sites already enrolled in ILL, ARI or SARI surveillance might provide a valuable platform. Collecting a standard number of specimens from sentinel sites, rather than aiming to be ‘representative’ geographically, may provide greater stability and improve the quality of specimens and associated metadata, ensuring comparability over time to allow for monitoring of trends. However, depending on existing sentinel sites, this strategy could lead to biased estimations of the relative prevalence of variants and exclusion of some populations or settings.

The sampling methodology should be documented and taken into account during data analysis and interpretation.

1.4.1.2. Sampling size calculations

• Randomized representative sampling

Various sample size calculators (12,13) can help to refine the number of specimens from a representative sample that need to undergo genomic sequencing to detect variants circulating at low levels with a specified level of confidence. Given that sequencing capacity is highly variable across countries, and achievable sample sizes may be highly dependent on capacity, it is possible to use these same sample size calculators to ‘back-calculate’ the level of confidence and precision in available sequence data.

The ECDC has released [detailed guidance](#) on sample size calculations to detect and monitor the proportion of variants circulating at low levels, inclusive of tables showing the required sample size under various situations and parameters and the underlying equations to facilitate replication. Considerations when identifying a sample include:

- level of precision/sensitivity to detect
 - A variant circulating at a low level (e.g. 1%) will require a larger sample than that to detect a variant circulating at a higher level.
 - The ability to detect a change in the relative prevalence of a variant from 2.5% to 5% will require a larger sample than that required to detect a change from 2.5% to 10%.
- level of confidence required (e.g. 95% confidence)
- level of transmission within the country (a larger sample will be required when the incidence is high and there are many people with SARS-CoV-2 infection)
- unit of sampling time (regular, routine sampling weekly, every two weeks, or every month) is required, because the relative prevalence of lineages can change rapidly.

The required sensitivity to detect variants circulating at low levels, changes in the relative prevalence of variant lineages and the level of confidence of the surveillance findings, are country-level decisions. In general, for public health purposes, the sensitivity to detect variants circulating at low levels may be the primary driver of sample size decisions, because the public health significance of detecting a variant that was not detected before may be higher than detecting a modest change in the relative prevalence of a given lineage. Additionally, estimates of the sample size needed to monitor relative prevalence are complicated by the number of different lineages in local circulation.

Table 3: Sample sizes required to detect a significant change (at 95% confidence) of relative prevalence

Weekly number of SARS-CoV-2 detections	Sample size based on the difference in the proportion of a certain variant, from one week to another	
	From 2.5% to 5%	From 2.5% to 10%
>100,000	725	129
10,001–100,000	705–720	129
5,001–10,000	676	128
2,501–5,000	634	126
1,000–2,500	563	123
500–1,000	421	115
<500	296	103

As outlined above, identifying and sequencing a truly random sample is difficult. However, if biases are well understood, adjustment may be possible once sequence results are available, making it possible to provide less biased prevalence estimates. Additionally, given the inevitable delay between specimen collection and availability of sequence results, modelling approaches can project a current state of relative lineage prevalence based on the available sequence data and lineage growth rates, see [CDC MMWR](#) (15) and Galloway et al. on the emergence of Alpha (B1.1.7) (16).

- **Fixed sample sizes**

In countries with minimal laboratory capacity, sequencing a minimum of 15 specimens per week from sentinel sites provides a baseline on which to build ([WHO GISRS 2021](#)). Africa CDC and the Pathogen Genome Initiative (PGI) network aim to collect a random sample of at least 50 positive specimens from each country per week, with the goal of establishing a sustainable, routine sampling frame for African countries (14), whereas [the WHO Regional Office for the Americas/Pan American Health Organization \(PAHO\) recommends](#) that countries sequence at least 50 positive specimens a month. This is roughly equivalent to detection of at least one sample of a variant that has 5% prevalence for the determined sampling period. If the sample size is fixed, the confidence level of not detecting a specific variant can be ‘back-calculated’ (12).

1.4.2. Targeted sampling

Targeted sequencing of specimens with a higher pre-test probability of being a VOI or VOC might be beneficial in addition to the above strategies.

Potential triggers for targeted sequencing for surveillance include (see section 3.3):

- specimen-level characteristics [e.g. genomic sequencing based on results from screening assays such as PCR-based single nucleotide polymorphism (SNP) detection assays]
- individual-level characteristics (e.g. clinical characteristics; immunocompromised patients and selective sequencing of vaccine breakthrough)
- environmental characteristics (e.g. evidence of variant sequences from wastewater surveillance).

1.4.2.1. Specimen-level characteristics

A range of RT-PCR primers and probes specific for mutations common to VOCs are now available (17) (18). These assays rely on the detection of single or multiple single nucleotide polymorphisms (SNPs) that are characteristic of specific lineages or shared across multiple lineages and that are often believed to contribute to a phenotypic change. However, these mutations may also be present in non-VOCs, so verification by genomic sequencing is needed for definitive lineage assignment.

PCR-based approaches followed by whole genome sequencing (WGS) have several advantages. First, RT-PCR is more readily available and less resource intensive than sequencing and can therefore be done across a wider geographical area at higher volume. Second, RT-PCR results may provide information more rapidly than WGS, which often requires specimen transport to a reference laboratory. Third, if applied to a larger sample size than WGS, PCR pre-screening may allow detection of a lineage circulating at a low relative frequency.

However, restricting sequencing to samples that have been pre-screened using the SNP RT-PCR tests has limitations. First, PCR assays are biased towards mutations characteristic of known VOCs, and therefore are not likely to provide a representative picture of all circulating lineages. Similarly, if a known lineage acquires new mutations that are not targeted by the specific SNP PCR assay in use, these will not be detected. Second, if public repositories are being used to estimate lineage proportions, and PCR pre-screening is biasing the specimens which undergo WGS and subsequent upload to repositories, the publicly available data may be more skewed. Third, PCR pre-screening might delay time to obtain genome sequence data. Furthermore, if WGS is done on a subset of samples that have already been pre-screened using the SNP PCR assays, and also used to detect and monitor other variants, the computation of expected prevalence will need to be described and adjusted for bias.

1.4.2.2. Individual-level characteristics

Some variants have phenotypic characteristics that are potentially concerning due to their ability to spread more easily from person to person, cause more severe disease, or dampen the impact of available public health and social measures (PHSMs), diagnostics, therapeutics and vaccines.

Phenotypic characteristics identifiable by clinicians and public health agencies may be used to prioritize specimens for genomic sequencing. These include specimens from:

- cases of SARS-CoV-2 infection in people who have been fully vaccinated,
- cases of SARS-CoV-2 infection in people who have been previously infected;
- cases where there is unexpected discordance between diagnostic tests, such as in clusters of individuals testing positive by rapid antigen test but negative by RT-PCR (or vice versa); characteristic and recurrent drop-out in a single gene target in a multi-target PCR assay; or where sample compartment test results are discrepant (e.g. upper versus lower respiratory tract)
- patient groups with underlying conditions that increase the likelihood of prolonged viral replication and shedding, such as immunocompromised patients (19–21)
- case clusters with unusual clinical presentations (e.g. unusually severe disease, unusual symptoms)
- case clusters suggestive of zoonotic transmission (e.g. among people working with animals susceptible to SARS-CoV-2 infection)
- cases with unexpectedly poor response to therapeutics.

Alternatively, targeting based on epidemiologic characteristics, such as travel history, particularly recent travel to an area with a high incidence of a known VOC, might be used to prioritize specimens (22).

1.4.2.3. Environmental Characteristics

Detection of variant sequences in wastewater can flag the circulation of a variant and assist in targeting further investigations and sequencing in a given geographical area (e.g. informal settlement) or setting (e.g. prison, long-term care facility, passenger ship) where randomized sequencing might be a challenge.

1.5. Metadata for genomic surveillance

All sequences should be associated with a minimum set of linked information, called metadata, which are described in WHO's [Genomic sequencing of SARS-CoV-2: a guide to implementation for maximum impact on public health](#).

In addition to the metadata described in the above document, further variables are valuable for in-depth epidemiological analyses to characterize variants and their public health risk. Identifying these variables will likely require engagement with different stakeholders in disparate clinical and public health systems (e.g. medical records, diagnostic laboratory, vaccination services), and not all specimens are likely to have all associated metadata. However, the sharing of data publicly across systems will facilitate rapid and comprehensive evaluation of SARS-CoV-2 variants.

Table 4 below describes three tiers of metadata, with decreasing priority:

- **Highest priority:** Core metadata should always include at least the date and location of sample collection (country and state or province). Information on the place and time of specimen collection are necessary to track the spread of variants. The originating diagnostic laboratory, the laboratory conducting sequencing and the host species (human versus animal) are also minimum requirements.
- **Second priority:** The second metadata tier – which is key to triggering further investigations on characterization – is descriptive. It brings context to the genome sequence information and sequencing goals. Regardless of the sampling strategy used, the second tier of metadata includes patient characteristics (age, gender, race and ethnicity as relevant) and epidemiological characteristics (e.g. date of exposure and onset) associated with a VOC or VOI.
- **Third priority:** The third tier, which is metadata for characterization, is most helpful for analytic work to characterize the public health risk of a specific variant. Examples of variables here may include the diagnostic assay used to identify a laboratory-confirmed case, cycle threshold (Ct) value, markers of clinical severity, vaccination status, patient comorbidities, the number of secondary cases per case, travel history, association with a known outbreak or cluster or location of exposure, exposure to potentially or known infected animals, past history of SARS-CoV-2 infection and occupation as a health worker. These enhanced metadata might only be available in some settings but will greatly increase the ability to characterize the risk.

When uploading relevant metadata to public sequence data repositories, caution should be taken not to share metadata that will permit identification of individuals. It may be appropriate to share fewer data on public databases than on secure databases that are held and analysed by public health agencies.

Table 4: Recommended metadata standards to be collected for SARS-CoV-2 sequencing data

Metadata	Label	Details	Potential Analyses
Tier 1: Core metadata	Sample Identification number		
	Sample type	Examples: "sputum", "blood", "serum", "saliva", "stool", "nasopharyngeal swab", "wastewater"	
	Sample collection date		Introduction and evolutionary rates
	Country of collection		Introduction and transmission routes, using BEAST (Bayesian Evolutionary Analysis Sampling Tree)
	State/province of collection		
	Originating diagnostic lab	Where the clinical specimen or virus isolate was first obtained	
	Sequence submitting lab	Where sequence data have been generated	Sequencing capacity assessment
	Sampling method	Part of routine surveillance or focused sampling, representative or targeted sampling	
	Host	e.g. human, animal (specifics), environment, unknown	Transmission routes
Tier 2: Descriptive metadata	Age		Risk factors
	Sex	e.g. male, female, other, unknown	Risk factors
	Race and/or ethnicity*		Risk factors
	Health worker status	e.g. yes, no, unknown. See HW definition in surveillance protocol for Health Workers	Transmission routes, risk factors
	Travel History	Location(s) and timing	Introductions and transmission routes
Tier 3: Metadata for characterization	RT-PCR assay used (if any)		
	RT-PCR Ct value (if any)		
	Symptomatic	e.g. yes, no, unknown	Severity analysis
	Vaccination status (for humans)	Date of vaccination (dose 1 and/or dose 2, as needed), vaccine type, source of information (documented evidence such as vaccine register or vaccine card versus recall)	Vaccine failure
	Date of symptom onset		Delay between onset and sequence submission
	Hospitalization status	e.g. ever hospitalized, never hospitalized, unknown	Severity analysis
	Admission to intensive care unit (ICU)	e.g. yes, no, unknown	Severity analysis
	Mechanical ventilation	e.g. yes, no, unknown	Severity analysis
	Outcome	Deceased/recovered	Severity analysis
	Past history of SARS-CoV-2 infection and date		Reinfection risk
	Therapeutics received	COVID-19-specific	Therapeutic failure
	Location of exposure, link to known cluster/ outbreak		Cluster/outbreak analysis, transmission routes
	Contact with known animal reservoir	e.g. yes, no, unknown; and type(s) of animal(s)	Transmission routes
Comorbidities	List comorbidities known to increase COVID-19 severity	Risk factors	

*This item should be used in regard to local context and individual data collection laws

2. Characterization of SARS-CoV-2 variants

A combination of laboratory science and detailed epidemiologic investigations is required to accurately characterize SARS-CoV-2 variants. However, these studies are resource-intensive and often require a combination of financial support and technical expertise that may not always be available. Thus, in resource-limited settings, prioritization of these characterization efforts may be needed. Isolates of variants should be rapidly shared with reference laboratories to enable molecular and virologic characterization. Timely sharing of the results of characterization studies with WHO and the public are essential to the global understanding of emerging variants.

In resource-limited settings, the highest yield in characterization is provided through routine surveillance systems.

Special studies can also bring valuable evidence beyond inference based on surveillance data, including laboratory studies.

Table 5 below lists existing methods for specialized investigation and characterization of variants, such as analysis of neutralization activity or study in animal models, epidemiologic studies of transmissibility within households, and studies of the disease course. This table aims to help Member States prioritize characterization studies in relation to public health risk.

Table 5: Methods for investigating and characterizing emerging variants, with highest priority studies highlighted in blue.

Public health risk domain	Characteristics	Epidemiologic investigations		Laboratory investigations	
		Surveillance evidence	Epidemiological studies	In vitro	In vivo
Transmissibility	Risk of infection	Surveillance trends comparing variants (R_t), Contact tracing data (secondary infection rate)	Household transmission studies	Binding affinity (ACE-2)	Animal models
	Animal reservoir* and susceptibility to infection		First Few X (FFX) cases, animal surveillance and investigations		Animal models
	Disease course (incubation, onset, viral shedding, recovery, symptomatic vs asymptomatic)	Contact tracing (time from exposure to symptom onset/transmission)	First Few X (FFX) cases: clinical follow up, Cohort studies	Recurrent RT-PCR testing throughout disease course Viral culture	Animal models
Clinical course	Signs and symptoms (relation to case definition)		FFX cases: signs and symptoms, Sensitivity and specificity of symptom clusters	Compare detection in upper respiratory tract (URT) vs lower respiratory tract (LRT) samples	Animal models
	Severity	Age-disaggregated case fatality ratios Hospitalization ratios	First few cases follow up: hospitalization, CFR		Animal models
Laboratory diagnostic confirmation failure	Diagnostics detection			RT-PCR target failure or failure of other diagnostics	
Neutralization	(Pseudo)virus neutralization by monoclonal antibody treatments			Monoclonal antibody and antibody cocktail neutralization	
	(Pseudo)virus neutralization by polyclonal antibodies (sera)		Vaccine effectiveness studies (Test negative case control)	Convalescent sera Vaccinee sera	
	Length of immunity		FFX cases follow up Serological studies		

N.B. Some of these studies will require building (inter)national public health-academia partnerships. Not all public health agencies/laboratories should develop all these capacities.

*these would focus as the role of the animal reservoir towards human transmission

2.1. Laboratory studies

2.1.1. Mutations and evidence of associated phenotypical effects

Extensive genomic sequencing around the world has revealed several novel SARS-CoV-2 variants that share common mutations or constellations (i.e., combinations of mutations) that have arisen independently. Some of these mutations may confer a phenotypic advantage to the virus, and their independent emergence might represent convergent evolution. Several initiatives have been established to track and visualize SARS-CoV-2 variants and/or mutations and their effects, as referenced in Annex 2.

As new variants are discovered, some aspects of their phenotype, and associated public health risk, might be inferred based on their genome sequence, i.e., the presence or absence of specific mutations.

Final estimation of the public health risk from variants should involve a thorough review of all available laboratory and epidemiologic evidence, but additional characterization studies may be prioritized according to the expected impact of known mutations. Annex 3 summarizes the current evidence base surrounding some key mutations and their phenotypic impact.

2.1.2. Summary of laboratory investigations

Relevant laboratory investigations focus on understanding how particular variants behave, especially any changes in their replication or detection by the immune system. These include:

- 1) assessing the effectiveness of antibodies (either from individuals who were previously infected with SARS-CoV-2 or were vaccinated against the virus, or those being considered for therapeutic applications) in neutralizing the cell entry of the variant;
- 2) determining the binding affinity of a variant to the ACE-2 receptor, which is required for cell entry and replication; or
- 3) monitoring the amount of virus (viral load) in different specimen types or over time in the course of natural infection. As well, specialized laboratories can investigate viral replication, transmission and overall morbidity and mortality using animal models.

Finally, and easiest to monitor, is understanding how accurate current diagnostic tests are in detecting a particular variant. Public health laboratories are encouraged to monitor for RT-PCR target failure or other unexpected changes in test performance, which can indicate the circulation of a variant.

2.2. Epidemiologic evidence

The main sources for epidemiologic evidence are routine surveillance data; and, if surveillance data are insufficient, field epidemiologic studies.

2.2.1. Routine surveillance data

Routine surveillance data, if paired with genomic surveillance data, can provide valuable insights into the possible phenotype of variants. WHO recommends a baseline set of variables to be reported weekly in an [aggregated format](#) as a part of [public health surveillance for COVID-19](#) (see section 3.3).

Alongside this aggregated format, the collection of many valuable epidemiologic characteristics as part of a case based report can be captured in the [WHO COVID-19 surveillance case report form](#) or [the WHO Clinical case report form](#). The availability and completeness of relevant surveillance data, and the resulting ability to characterize emerging variants, will vary between countries. If surveillance systems routinely capture the recommended information, these data can be linked to sequence information. Subsequently, comparing patient, clinical and epidemiologic data across variant and non-variant cases may permit the estimation of phenotypic characteristics.

Even when lineage information is unavailable at the individual level, geographic and demographic associations between incidence and lineage proportion may be used to infer viral characteristics if relative prevalence of different lineages is known at the population level. If the relative proportion of different lineages is known, mathematical models may be used to estimate changes in transmissibility and severity using surveillance data (35).

Matching variant information with vaccination status (including the vaccine type) or a record of past infection(s) – or conducting focused sampling of vaccine breakthrough and reinfection cases to compare lineage information across cases and non-cases – may provide an indication of the ability of a variant to evade the immune system response. If case-based surveillance systems and vaccine registries are both in use, matching across data sources can permit these analyses.

2.2.2. Field Epidemiological studies

Although the timeliest epidemiological evidence would be obtained through existing surveillance data, field epidemiological investigations can provide valuable evidence. There is a paucity of high-quality field epidemiologic and clinical data on emerging variants, but these data are vital for assessing real-world impact. Epidemiologic studies of variants should be prioritized to settings (e.g. those variants or those populations) where there is a high degree of generalizability and findings are thus likely to have broad relevance.

Most epidemiologic characterization studies perform best when data from an emerging SARS-CoV-2 variant are directly compared to circulating extant lineages (i.e., before the emerging variant has become dominant), should the latter have a fitness advantage. If

characterization studies are conducted after the variant under investigation has become dominant, and it is consequently hard to enroll non-variant cases, historic data may serve as an appropriate comparator, although these may be confounded by temporal changes in public health strategies (e.g. altered case investigation and contact tracing frameworks or different PHSMs).

WHO, in collaboration with technical partners, has developed several standardized generic epidemiological investigation protocols branded as the [Unity studies](#). These studies aim to support national public health and social measures, promote the international comparability of research and address gaps in current knowledge regarding the COVID-19 pandemic. Several Unity studies may be useful in the investigation of emerging variants.

First Few X (FFX) cases

The primary objectives of an FFX investigation among cases and close contacts are to provide descriptions or estimates of:

- clinical presentation of SARS-CoV-2 infection and course of associated disease
- secondary infection rate (SIR) and secondary clinical attack rate of SARS-CoV-2 infection among close contacts
- serial interval of SARS-CoV-2 infection
- symptomatic proportion of COVID-19 cases (through contact tracing and laboratory testing)
- identification of possible routes of transmission.

The investigation can continue for as long as is determined feasible by the implementing country. The impact of emerging variants on ongoing studies will need to be assessed on a case-by-case basis. In the context of emerging variants, laboratory-confirmed cases could be enrolled retrospectively once WGS results are available, and a variant sequence is confirmed, or could be identified on the basis of a characteristic diagnostic test result (such as S gene target failures on TaqPath assay suggesting the Alpha (B.1.1.7) variant, or a variant-specific RT-PCR if available). Alternatively, cases could be enrolled without knowing the lineage of the virus and allocated to a variant cohort once WGS results are available. The protocol can be found [here](#).

Household transmission studies

A household transmission investigation is a case-ascertained prospective study of all identified household contacts of a laboratory-confirmed SARS-CoV-2 infection. It is intended to provide rapid and early information on the clinical, epidemiological and virologic characteristics of SARS-CoV-2. Given the time delay in receiving WGS results and assigning lineage information, household transmission studies of SARS-CoV-2 variants face many of the same challenges as FFX studies. Options include presumptive enrolment based on location, with variant cohort assigned once WGS results are available; enrolment based on a characteristic diagnostic test result; or retrospective enrolment of variant cases once WGS data are available. Additionally, due to the time taken to complete the study and associated genomic sequencing, the utility of these studies may be as much for documenting the mechanism of any phenotypic changes as for documenting the changes themselves.

The primary objectives of a household transmission study are to provide key epidemiological data to complement and reinforce the findings of FFX, including data on:

- proportion of asymptomatic cases and symptomatic cases
- incubation period of COVID-19 and the duration of infectiousness and of detectable shedding
- serial interval of SARS-CoV-2 infection
- reproduction numbers: R_0 and R of SARS-CoV-2
- clinical risk factors for COVID-19, and the clinical course and severity of disease
- high-risk population subgroups
- secondary infection rate and secondary clinical attack rate of SARS-CoV-2 infection among household contacts
- patterns of health care-seeking behavior

Duration of data collection between inclusion and the end of the publicly available study protocols is 28 days, but initial results can be produced in a few days to weeks. The initial stages of the survey design and implementation can take some time and are resource intensive. Countries are encouraged to establish surge capacity for these household surveys in advance of variant detection. The protocol can be found [here](#).

An example of a study protocol that has been used in the United States of America and adapted for Brazil has been published (36).

3. Reporting of SARS-CoV-2 variants surveillance data

Prompt sharing of information around SARS-CoV-2 variants is integral to global understanding and control of SARS-CoV-2. WHO has released guidance on reporting VOCs and VOIs (37).

The main actions by a Member State, if a potential VOI is identified, include:

- Inform WHO through established WHO Country or Regional Office reporting channels with supporting information about VOI-associated cases (person, place, time, clinical and other relevant characteristics).
- Submit complete consensus genome sequences and associated metadata to a publicly available database, such as [GISAID](#).
- Perform field investigations to improve understanding of the potential impacts of the VOI on COVID-19 epidemiology, severity, effectiveness of public health and social measures or other relevant characteristics.
- Perform laboratory assessments or contact WHO for support to conduct laboratory assessments on the impact of the VOI on diagnostic methods, immune responses, antibody neutralization or other relevant characteristics.

Main actions to be taken by a Member State if a VOC is identified:

- Reporting to WHO of first cases/clusters identified in a Member State associated with any VOC is required using International Health Regulations (IHR) mechanisms.
- Submit complete genome sequences and associated metadata to a publicly available database, such as GISAID.
- Where capacity exists and in coordination with the regional and international community, perform field investigations to improve understanding of the potential impacts of the VOC on COVID-19 epidemiology, severity, effectiveness of public health and social measures or other relevant characteristics.
- Perform laboratory assessments according to in-country capacity or contact WHO for support to conduct laboratory assessments on the impact of the VOC on diagnostic methods, immune responses, antibody neutralization or other relevant characteristics.

Overall, for all sequences, Member States are requested to:

- Share genomic sequences on public databases (e.g. GISAID).
- Regularly publish findings, including contextual information around cases.
- Report to WHO through the IHR mechanism of first cases/clusters identified associated with a VOC.
- Inform WHO of potential new VOIs/VOCs through established WHO Country Office and Regional Office channels/networks
- Include as much detail as possible to support assessments (e.g. person, place, time, clinical and evidence of phenotypic impacts).

The following indicators are recommended for national reporting and international sharing for each VOI or VOC and variants of national interest:

Table 6 Indicators recommended for VOC/VOI reporting

Label	Description	Method
Date reported to WHO	Date the variant strain was reported to WHO either through official channels like IHR notification, Emergency Warning and Response System, official announcements or unofficial event-based surveillance signal	See above for differences in reporting for VOI versus VOC
Date of first case in the country	Date the first case of the variant strain was reported in the country (date of onset if possible, or date of sequencing confirmation)	
Quantification method for variant	Subset sampling of whole genome sequencing, or screening with target PCR	
Number of variants in sequenced samples (numerator)	Proportion of variant strain identified from the total sample sequenced	This can also be done through target PCR: number of samples positive for target PCR
Number of sequenced samples (denominator)	Number of samples sequenced	If done through target PCR, this should be the number of cases screened

The **relative prevalence of each lineage** is calculated as the **number of sequences of that lineage (numerator)** divided by the **total number of sequences generated through routine surveillance (denominator)** in the same unit of time.

While the presence and relative prevalence of viral lineages with these characteristics has significant public health value, it is important to also consider how these fit in the broader epidemiologic context. For instance, if the *proportion* of incident cases due to a given VOC is rapidly increasing, yet overall incident case counts are decreasing, the actual population-level incidence of the VOC may not actually be increasing. Similarly, in the setting of increasing overall case incidence, the number of cases due to a VOC may actually be increasing even if its proportion is decreasing.

Annex 1. Existing SARS-CoV-2 sequencing and surveillance guidance from various agencies

Author	Title	Key Topics Addressed	Lab	Surv.	Policy
WHO	SARS-CoV-2 genomic sequencing for public health goals: Interim guidance, 8 January 2021	Guidance for national-level policymakers and stakeholders on how to maximize public health benefit of SARS-CoV-2 genomic sequencing	x	x	x
WHO	Genomic sequencing of SARS-CoV-2: a guide to implementation for maximum impact on public health	Comprehensive implementation guide on SARS-CoV-2 sequencing for those implementing sequencing programs	x		
WHO	Operational considerations to expedite genomic sequencing component of GISRS surveillance of SARS-CoV-2	Practical guidance to GISRS and other national laboratories to expand beyond virus detection to genomic sequencing of SARS-CoV-2 positive materials from sentinel surveillance programs.	x	x	
WHO	Weekly Epidemiological Update- Special Edition: Proposed working definitions of SARS-CoV-2 Variants of Interest and Variants of Concern	Specifies working definitions of VOIs and VOCs. Outlines WHO support for member states and recommended actions for member states regarding VOIs and VOCs.		x	x
US CDC	How CDC is responding to SARS-CoV-2 variants globally	Highlights global CDC SARS-CoV-2 variant response, continuous updates with new information.	x	x	
US CDC	Emerging SARS-CoV-2 Variants	Provides scientific updates on emerging SARS-CoV-2 variants.	x	x	
ECDC/ WHO EURO	Methods for the detection and identification of SARS-CoV-2 variants	Provides methods for detecting variants	x	x	
ECDC	Sequencing of SARS-CoV-2- first update	Technical guidance providing guidelines for making decisions on establishing sequencing capabilities. Addresses sequencing technologies with a standardization process to analyze/report findings.	x		
ECDC	Detection and characterization capability and capacity for SARS-CoV-2 variants within the EU/EEA	Reports detection and characterization sequencing capacities in EU countries, challenges, and recommendations.	x	x	
ECDC	Risk Assessment: Risk related to the spread of new SARS-CoV-2 variants of concern in the EU/EEA- first update	Information on risks of SARS-CoV-2 variants and options for country responses, including policy recommendations and PSHMs.		x	x
ECDC	Risk Assessment: SARS-CoV-2- increased circulation of variants of concern and vaccine rollout in the EU/EEA, 14th update	Update on the document above with additional guidance PSHMs and vaccine rollout.		x	x
ECDC	Guidance for representative and targeted genomic SARS-CoV-2 monitoring	Guidance on sample methods and sizes		x	
Genome Canada	CanCOGeN Interim Recommendations for Naming, Identifying, and Reporting SARS-CoV-2 Variants of Concern	Contains information on variant-naming, conventions for identifying, and conventions for reporting VOCs.	x		
WHO PAHO	PAHO Guidance for SARS-CoV-2 samples selection for genomic characterization and surveillance	Provides information for PAHO countries on the laboratory response for SARS-CoV-2 including sampling criteria for genomic characterization and surveillance.	x	x	
Africa CDC	Africa CDC New SARS-CoV-2 variants in Africa	Information for African countries on SARS-CoV-2 variants in Africa and outline of how VOCs affect specific diagnostic tests.	x		
APHL	Responding to the Coronavirus Disease (COVID-19) Pandemic	Provides continuously updated informatics and laboratory guidance for COVID-19.	x		
Johns Hopkins Center for Health Security	Staying Ahead of the Variants: Policy Recommendations to Identify and Manage Current and Future Variants of Concern	Assesses status of US SARS-CoV-2 surveillance, sequencing, variant characterization, and provides recommendations for increase capacity to respond to VOCs.		x	x

Annex 2. Variant/mutation trackers/tools

Several initiatives have been established to track and visualize SARS-CoV-2 variants and/or mutations and their effects, such as on diagnostic assays.

Name	Type of information	Nomenclature	URL
PANGO lineages	Global report of novel coronavirus haplotypes including maps of global spread, frequencies, first detection	Pango	https://cov-lineages.org/
CoVariants	Mutations and Variants of Interest/Concern and relevant literature	Nextstrain	https://covariants.org/
CovMT	Mutations and variants with focus on critical variants, top RBD mutation timeline (GISAID classification)	GISAID	https://www.cbrc.kaust.edu.sa/covmt/
CoV-GLUE	Database of amino acids replacements, insertions and deletions	Pango	http://cov-glue.cvr.gla.ac.uk/ - /home
BV-VRC	Tracking of variants of concern and provides genome browser	Pango	https://bv-brc.org/
COVID-19 Data portal ENA	Relevant datasets containing raw viral sequence reads, host sequences and more and is developing visualization tools	NA	https://www.covid19dataportal.org/
Outbreak.info Mutation Situation Reports	Lineage reports of VOC/VOI including visualization of mutations on genome and comparison between variants, global spread and daily prevalence	Pango	https://outbreak.info/situation-reports
Viroscience Primer Check tool	Primer Check tool	NA	https://viroscience-emc.shinyapps.io/primer-check/
Pha4ge	Sequencing protocols and analysis tools	NA	https://pha4ge.org/resources/

Annex 3. Examples of common key SARS-CoV-2 mutations in the spike protein and evidence of associated phenotypic effect

Key mutation	Public health risk domain	Phenotypic effects documented to date	Variant of Concern/ Variant of Interest
L452R	<i>Transmissibility</i>	Might increase infectivity by stabilizing the spike-angiotensin converting enzyme-2 (ACE-2) receptor interaction and has evolved independently in multiple lineages (23)	VOC Gamma
	<i>Antibody escape</i>	Reduced neutralization by convalescent plasma and monoclonal antibodies (mAbs); specifically, escapes neutralization by the antibody that forms the basis for bamlanivimab, a specific mAb therapeutic (24)	VOI Iota, Kappa, Lambda
E484K	<i>Transmissibility</i>	Increased binding affinity to (ACE-2) receptor, and binding may be stabilized by the presence of K417N. Has profound impact in shifting the main site of contact between viral receptor binding domain (RBD) and ACE-2 residues; in vitro evolution to select for greater ACE-2 receptor binding resulted in mutations S:E484K, S:N501Y, and S:S477N to be among the first selected (25,26)	VOC Alpha Beta Gamma
	<i>Antibody escape</i>	E484K was reported to be an escape mutation from a monoclonal antibody that neutralizes SARS-CoV-2; combination of E484K, K417N and N501Y (found in B.1.351 and P.1) induces conformational change greater than N501Y alone; selected when recombinant vesicular stomatitis virus (rVSV)/SARS-CoV-2 S was cultured in the presence of vaccine-elicited mAbs (27,28)	VOI Eta Iota Kappa
N501Y	<i>Transmissibility</i>	Increased binding affinity ACE-2 receptor. In vitro evolution to select for greater ACE-2 receptor binding resulted in mutations S:E484K, S:N501Y, and S:S477N to be among the first selected (25,26,29)	VOC Alpha Beta Gamma
	<i>Antibody escape</i>	The emergence and ongoing convergent evolution of the N501Y lineages coincides with a major global shift in the SARS-CoV-2 selective landscape. The precise cause of this selective shift is unknown but increases in seropositivity and/or the relaxation of transmission prevention measures are identified as obvious candidates. Selected when recombinant vesicular stomatitis virus (rVSV)/SARS-CoV-2 S was cultured in the presence of vaccine-elicited mAbs (29)	VOI
K417N	<i>Antibody escape</i>	In escape map study shown to be one of the mutations that could escape antibody recognition; selected when recombinant vesicular stomatitis virus (rVSV)/SARS-CoV-2 S was cultured in the presence of vaccine elicited mAbs; combination of E484K, K417N and N501Y (found in B.1.351 and P.1) induces conformational change greater than N501Y alone (25,27,30,31)	VOC Beta Delta Gamma
P681H/R	<i>Transmissibility</i>	Immediately adjacent to the furin cleavage site identified at S1/S2 linkage site. Enhances systemic infection and membrane fusion. In functional assay study, spikes containing P681H and P681R mutations were being more efficiently cleaved, and a role in enhancing transmissibility and pathogenicity is hypothesized (32,33)	VOC Alpha Delta VOI Kappa

References: [WHO Webpage on variants](#), [CDC webpage: Mutations with Impact on MoABs therapeutics](#), [Covariants](#) and [Outbreak Info](#)

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Acknowledgements

This document was developed in consultation with:

From the World Health Organization: Maya Allan, Brett Archer, Armanath Bapu, Lisa Carter, Jane Cunningham, Roger Evans, Daniel Feikin, Julia Fitzner, Masaya Kato, Biaukula Viema Lewagalu, Marco Marklewitz, Piers Mook, Minal Patel, Boris Pavlin, Richard Pebody, Emilie Peron, Mark Perkins, Olivier le Polain, Tika Ram, Lorenzo Subissi, Katelijn Vandemaele, Pushpa Ranjan Wijesinghe, Hattori Yuta, Judith Mandelbaum-Schmid

From the Pan American Health Organization (PAHO) / WHO Regional Office of the Americas (AMRO): Paula Couto, Lidia Redondo, Angel Rodriguez, Gaetano Marrone, Juliana Leite, Jairo Andrea Mendez Rico

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Management of conflicts of interest:

All external contributors cited below provided formal Declaration of Interest as required by WHO policy. No conflicts were declared.

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Bill and Melinda Gates Foundation: Jordan Tappero, Georgina Murphy

Funding: WHO Internal Funds

WHO continues to monitor the situation closely for any changes that may affect this interim guidance. Should any factors change, WHO will issue a further update. Otherwise, this interim guidance document will expire two years after the date of publication.

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WHO reference number: [WHO/2019-nCoV/surveillance/variants/2021.1](https://www.who.int/publications/m/item/WHO/2019-nCoV/surveillance/variants/2021.1)