SARS-CoV-2 variant risk evaluation

30 August 2023
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List of contributors and affiliations

Lorenzo Subissi1, James Richard Otieno1, Homa Attar Cohen1, Nathalie Worp2, Bas B. Oude Munnink2, Laith J. Abu-Raddad3, Amal Barakat4, Wendy S. Barclay5, Jinal N. Bhiman6,7, Leon Caly8, Meera Chand9, Mark Chen10, Ann Cullinane11, Jane Cunningham1, Tulio De Oliveira12, Christian Drosten13, Julian Druce8, Paul Effler14, Ihab El Masry15, Adama Faye16, Elodie Ghedin17, Rebecca Grant1, Bart Haagmans2, Belinda L. Herring8, Manish Kakkat19, Zyleen Kassamali1, Rebecca J. Kondor20, Yee Sin Leo10, Marco Marklewitz21, Jairo Mendez-Rico22, Nada Melhem23, Karen Nahapetyan21, Djin-Ye Oh24, Boris I. Pavlin1, Thomas P. Peacock5,9, Malik Peiris25, Zhibit Peng26, Leo Poon25, Andrew Rambaut27, Senjuti Saha28, Yinzhong Shen29, Marilda M. Siqueira30, Sofonias K. Tessema31, Erik Volz5, Volker Thiel32,33, Henda Triki34, Sylvie van der Werf35, Sylvie Briand1, Anne von Gottberg6,7, Marion P.G. Koopmans2, Anurag Agrawal36, Maria D. Van Kerkhove1

1. World Health Organization, Geneva, Switzerland
2. Erasmus Medical Centre, Rotterdam, Netherlands (Kingdom of the), and Pandemic and Disaster Preparedness Research Centre, Rotterdam/Delft, Netherlands (Kingdom of the)
3. Weill Cornell Medicine – Qatar of Cornell University, Doha, Qatar
4. World Health Organization Regional Office for the Eastern Mediterranean, Cairo, Egypt
5. Imperial College London, London, United Kingdom of Great Britain and Northern Ireland
6. National Institute for Communicable Diseases, Johannesburg, South Africa,
7. School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa,
8. Victorian Infectious Diseases Reference Laboratory (VIDRL), Melbourne, Australia,
9. UK Health Security Agency, London, United Kingdom
10. National Centre for Infectious Diseases, Singapore, Singapore
12. Centre for Epidemic Response and Innovation (CERI), Stellenbosch University, Stellenbosch, South Africa
13. Charité Medical Center, Berlin, Germany
14. University of Western Australia, Perth, Australia
15. Food and Agriculture Organization, Rome, Italy
16. Institute for Health and Development, Dakar, Senegal
17. National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, United States of America
18. World Health Organization Regional Office for Africa, Brazzaville, Congo
19. World Health Organization Regional Office for South-East Asia, New Delhi, India
20. United States Centers for Disease Control and Prevention, Atlanta, United States of America
21. World Health Organization Regional Office for Europe, Copenhagen, Denmark
22. World Health Organization Regional Office for the Americas, Washington, United States of America
23. American University of Beirut, Beirut, Lebanon
24. Robert Koch Institute, Berlin, Germany,
25. School of Public Health, LKS Faculty of Medicine, The University of Hong Kong, China, Hong Kong SAR
26. Chinese Center for Disease Control and Prevention, Beijing, China
27. University of Edinburgh, Edinburgh, United Kingdom
28. Child Health Research Foundation, Dakka, Bangladesh
29. Fudan University, Shanghai, China
30. Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, Brazil
31. Africa Centers for Disease Control and Prevention, Addis Ababa, Ethiopia
32. Multidisciplinary Center for Infectious Diseases, University of Bern, Bern, Switzerland
33. Institute of Virology and Immunology, Mittelhäusern and Bern, Switzerland
34. Institut Pasteur Tunis, Tunis, Tunisia
35. Institut Pasteur, Université Paris Cité, Paris, France
36. Ashoka University, New Delhi, India
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACE-2</td>
<td>angiotensin-converting enzyme 2</td>
</tr>
<tr>
<td>COVID-19</td>
<td>coronavirus disease 2019</td>
</tr>
<tr>
<td>CoV</td>
<td>coronavirus</td>
</tr>
<tr>
<td>ICU</td>
<td>intensive care unit</td>
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<tr>
<td>MERS</td>
<td>middle east respiratory syndrome</td>
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<tr>
<td>PISA</td>
<td>Pandemic influenza severity assessment</td>
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<tr>
<td>RBD</td>
<td>receptor binding domain</td>
</tr>
<tr>
<td>SARS-CoV-2</td>
<td>Severe acute respiratory syndrome coronavirus 2</td>
</tr>
<tr>
<td>SARI</td>
<td>severe acute respiratory infection</td>
</tr>
<tr>
<td>TAG-VE</td>
<td>Technical Advisory Group on SARS-CoV-2 Virus Evolution</td>
</tr>
<tr>
<td>TMPRSS-2</td>
<td>Transmembrane protease, serine 2</td>
</tr>
<tr>
<td>VOC</td>
<td>Variant of concern</td>
</tr>
<tr>
<td>VOI</td>
<td>Variant of interest</td>
</tr>
<tr>
<td>VUM</td>
<td>Variant under monitoring</td>
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<td>WHO</td>
<td>World Health Organization</td>
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Executive summary

There is always a degree of uncertainty pertaining to the significance of any emerging SARS-CoV-2 variant. It is critical to provide a regular evaluation of the public health risk posed by the variant as evidence emerges so that appropriate public health action can be taken. Assessing the strength of the evidence and associated confidence is a complex process that requires multidisciplinary expertise. This document, which provides the method for SARS-CoV-2 variant risk evaluation that WHO will use in collaboration with experts in countries, helps with the interpretation of the evidence and provides a framework to evaluate risk considering three main indicators: clinical severity, growth advantage and immune escape. The framework also includes impact on diagnostics and therapeutics. While this approach is specific to SARS-CoV-2, many elements can be used for evaluation of emerging coronaviruses or even other emerging respiratory pathogens.

Purpose of this document

This SARS-CoV-2 risk evaluation tool – which WHO will use in collaboration with experts from Member States – is designed to assist in evaluating the risk posed by emerging SARS-CoV-2 variants based on the available evidence and the level of confidence in the evidence. While the tool focuses specifically on the risk posed by SARS-CoV-2 variants in the human population, it also provides relevant information for the evaluation of any newly emerging coronavirus variants or other respiratory pathogens that demonstrate human-to-human transmission. This approach aims to address the challenge of summarizing and evaluating existing evidence to inform timely public health responses and decision making. Guidance is provided by proposing a set of relevant risk indicators and a set of studies for each indicator to support timely and balanced weighting of information for an overall risk evaluation.

Intended audience

The World Health Organization (WHO) will be the key user of this tool and will share outputs with Member States, partners and the public through publications on the WHO SARS-CoV-2 variants’ tracking website. The evaluation at the global level will be conducted in consultation with international experts in the fields of public health, virology, epidemiology, genetics, immunology, bioinformatics, diagnostics, clinical management and others, as necessary. The tool can also be used for risk evaluation at the national or regional level, in line with the following three recommendations:

- Experts from diverse sectors and disciplines should consider and weigh different risk elements for a balanced, multidisciplinary evaluation of risk.
- The risk assessment should be based on the latest and most comprehensive knowledge about the virus and should go beyond local, national and regional considerations.

The tool provides a structured, comprehensive, and transparent framework and guidance for evaluation, and should be used without adaptations, except for country- or region-specific elements, such as population immunity, resources, and available countermeasures.
**Introduction**

During the last two decades, three coronaviruses (CoVs) – Severe acute respiratory syndrome (SARS)-CoV-1 in 2003, Middle East respiratory syndrome (MERS)-CoV in 2012, and SARS-CoV-2 in 2019 – have caused substantial morbidity and mortality in humans. SARS-CoV-2 emerged in late 2019 and caused a pandemic of acute respiratory disease (COVID-19) with significant consequences on human health and the global economy. SARS-CoV-2 continues to circulate and evolve due to its propensity for mutation and recombination, along with natural selection/immune selection for variants with increased fitness. This genetic diversification can lead to the emergence of variants with different characteristics (e.g. replication rate, cell tropism, immune escape), resulting in increased transmissibility and possibly distinct disease patterns and severity.

WHO's Technical Advisory Group on Virus Evolution (TAG-VE) monitors the evolution of SARS-CoV-2 and assesses emerging variants based on evidence from global genomic surveillance and research. Currently, the highest alert level is the designation of a new variant of concern (VOC), which may lead to actions such as issuing recommendations to update the composition of vaccines, change treatment guidelines, or heighten public health and social measures. Greek letters have been assigned to variants of interest (VOI) or variants of concern (VOC) which are deemed likely to cause epidemic waves globally and require public health action and health care preparedness actions. Prior to the changes of the WHO variant tracking system in March 2023, five VOC’s and eight VOI’s were designated with a Greek letter.

The emergence and dominance of Omicron VOC

VOCs and VOIs have been designated based on their potential to replace previously circulating variants and cause new waves of increased transmission globally, requiring adjustments to public health actions. Within the Strategic Preparedness, Readiness and Response Plan published early 2022, WHO outlined some planned scenarios based on the potential characteristics of newly emerging variants. The emergence of Omicron was a remarkable evolutionary step for SARS-CoV-2, because this variant displayed substantial genetic differences from previous variants. Soon after its emergence, Omicron became the globally predominant variant. As of April 2023, it represented over 99% of all publicly available sequences. In line with the original VOC definition, all Omicron sublineages were considered part of the Omicron VOC. Given the worldwide predominance of Omicron, the working definitions of VOCs and VOIs were subsequently adjusted so that Omicron sublineages could be independently classified as variants under monitoring (VUM, VOI or VOC). This “reset” of the variant tracking system was done to identify sub-lineages that could potentially pose a greater threat to public health than the Omicron viruses already in circulation.

Experts from the TAG-VE reached a consensus that Omicron represented a new SARS-CoV-2 ‘type’, compared to previous VOCs, based on its distinct genetic profile, comparison of antigenic cross-reactivity using animal sera, replication studies in experimental models of the human respiratory tract and evidence from clinical and epidemiological studies in humans. Omicron viruses continuously evolve genetically and antigenically with an expanding range of sublineages which, so far, have all been characterized by properties of evasion from existing population immunity and a preference to infect the upper respiratory tract (versus the lower respiratory tract).

We propose referring to pre-Omicron variants as SARS-CoV-2 type 1 variants (which therefore include Alpha, Beta, Gamma, Delta, Epsilon, Zeta, Eta, Theta, Iota, Kappa, Lambda and Mu), and Omicron variants as SARS-CoV-2 type 2 variants, given their different genotypic and phenotypic properties. Despite waning of immunity, infections due to type 1 variants or type 1 vaccines provide higher protection against infection due to type 1 variants than against infections due to type 2 variants. All type
2 variants are VOCs that can cause massive surges of infections, especially in regions lacking immunity against type 2 viruses, and irrespective of vaccination coverage. This can be explained by the rapid antigenic evolution of Omicron and its descendant lineages, leading to short-lasting protection from infection or by vaccination with type 1 antigens. Somewhat more durable immunity against infections with type 2 variants may arise from SARS-CoV-2 type-2 infection or new vaccines incorporating SARS-CoV-2 type 2 antigens. Importantly, vaccination with any antigen (type 1 or type 2) continues to provide protection against severe disease.

In light of the dominance of type 2 viruses since the beginning of 2022, the working definitions for SARS-CoV-2 variants were updated in March 2023. The main objective of the update was to refine the definition of VOC to only encompass new “types” of SARS-CoV-2 that are substantially different from existing SARS-CoV-2 “types” both genotypically and phenotypically. Prior protective immunity achieved against existing SARS-CoV-2 types means that the emergence of type 1 or type 2 subvariants is not likely to cause an increased public health risk.

Scope and objectives

This tool is designed to offer a comprehensive framework for evaluating and communicating the risks and potential impact of emerging SARS-CoV-2 variants. By providing a structured and transparent method, it facilitates the harmonization of national and international preparedness and response efforts. The tool also highlights the key roles and responsibilities of WHO in providing global leadership and support for evidence-based decision-making, and of Member States in conducting SARS-CoV-2 surveillance, risk assessment, and mitigation measures.

The specific objectives of the tool are:

- provide a transparent evidence-based risk evaluation tool for emerging SARS-CoV-2 variants to inform public health decision making
- harmonize data collection and sharing and rapid assimilation of evidence from countries and regions for a global risk evaluation
- describe knowledge gaps in our understanding of emerging SARS-CoV-2 variants.
- allow for evidence-based communication on risks associated with emerging variants.

A stepwise approach to SARS-CoV-2 variant Risk Evaluation

Step 1: Automated screening of variants based on publicly available genetic sequence data

This approach assumes that the publicly available genetic data are representative of SARS-CoV-2 circulation in a defined population. While this may hold true for some countries, it may not be the case at the global level. For this variant risk evaluation to represent a robust national representative and balanced global analysis, it is essential that countries maintain surveillance activities and work towards timely sharing of genetic sequence data and associated metadata that are representative of SARS-CoV-2 circulation in target populations, with representativeness prioritized over quantity (see Table 1). Countries’ capacity to perform these activities is strongly influenced by available resources. Selecting a specific sample size of sequences and transmission scenario to apply to every Member State is highly challenging. The WHO COVID-19 surveillance guidance has provided guidance for Member States; and the Global Influenza Surveillance and Response
System (GISRS) has provided such guidance in the context of influenza sentinel surveillance. Novel approaches, such as integrating wastewater-based epidemiology into SARS-CoV-2 surveillance strategies could support such an objective.

**Genetic evidence**

The following genetic criteria should be used to shortlist variants for which an in-depth growth advantage analysis will be undertaken:

1. genetic distance compared to previously circulating variants (phylogenetic diversity)
2. spike RBD mutations
3. specific mutations in B and/or T cell epitopes
4. mutations that are predicted or known to confer substantial resistance to tools in use (vaccines, therapeutics, diagnostics).

**Evidence of growth advantage of variants**

Growth advantage may pose a public health threat and can be caused by a combination of changes in the intrinsic properties of the virus (i.e., cell tropism, replication rate, generation time) and immune escape. Automated growth advantage analyses using methods such as multinomial regression and/or phylodynamics using publicly available sequence data can be used to identify VOI, which then undergo a risk evaluation.

<table>
<thead>
<tr>
<th>Weekly number of SARS-CoV-2 detections</th>
<th>Sample size based on the difference in the proportion of a certain variant, from one week to another</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>From 2.5% to 5%</td>
</tr>
<tr>
<td>&gt;100,000</td>
<td>725</td>
</tr>
<tr>
<td>10,001-100,000</td>
<td>705-720</td>
</tr>
<tr>
<td>5,001-10,000</td>
<td>676</td>
</tr>
<tr>
<td>2,501-5,000</td>
<td>634</td>
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<tr>
<td>1,001-2,500</td>
<td>563</td>
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<tr>
<td>500-1,000</td>
<td>421</td>
</tr>
<tr>
<td>&lt;500</td>
<td>296</td>
</tr>
</tbody>
</table>

Table 1: Sample sizes required to detect a significant change (at 95% confidence) of relative prevalence. From WHO Public health surveillance for COVID-19: interim guidance.
Step 2: Evaluation of risk elements and confidence assessment

2A Growth advantage

Overall transmissibility is a function of various parameters such as intrinsic transmissibility (i.e., the transmission potential in an immune-naive population), immune escape (i.e., the transmission potential in an immune population) and other parameters that may differ by variant (i.e., generation time). Laboratory studies can best achieve the evaluation of virus traits while epidemiological evidence can best address virus-host interactions.

Epidemiological evidence

Comparative evidence from prospective cohort studies (e.g. secondary attack rates) can provide solid evidence of an overall higher transmissibility of a variant, as compared to a previous or co-circulating variant (see Fig. 1). However, these studies are time consuming, costly and require knowledge of exposure history – which is becoming increasingly difficult to obtain – and such evidence, consequently, is increasingly rare.

A practical way of looking at growth advantage is to generate growth rate estimates based on publicly available sequences, provided (or assuming) that the country or region under study submits viral sequences that are representative of the virus circulating in target populations of the country or region. Different analyses of growth rates provide different insights into the overall transmissibility of a variant. A consensus across the different methods to estimate growth rates is required to provide more robust evidence for growth advantage. Growth rates are not predictable from the genetic profile, and they are among primary early evidence available to infer/predict how successful a variant could become.

In addition to sequence-based growth advantage, reproduction rates (overall Rt), viral load data, or increase in case numbers in the general population are assessed to estimate transmissibility due to the emergence of a new variant. One aspect that can define transmissibility is the viral load in the upper respiratory tract (URT). Comparative viral load based on PCR has also been used to compare transmissibility of variants, but this can be less reliable as there are biases and confounders that may occur (e.g. differences due to selection bias in choosing study population or sampling after symptom onset). Confounders may be addressed through statistical models that control for them. To assess whether the virus found in the URT is still infectious, virus culture can be used. However, this can only be done in a highly standardized routine setting with proper biosafety measures in place, where Ct values are consistently monitored and the patient population is more or less stable. Of note, continued testing and case reporting – for instance of sentinel patient groups – is required to assess potential changes in case numbers.

Approaches to inferring community spread from other sources such as wastewater surveillance, may fill an important gap and should be explored. Wastewater analysis to survey changes in virus quantitative indicators (e.g. copies/mL) may be used in some countries (provided the wastewater system is a closed and robust source of information) to indicate an early signal of increase in viruses in the community. It can also be used to estimate the magnitude of underreporting of cases due to reduced testing. However, inferring increased transmissibility needs comparative evidence.

Laboratory evidence

As noted earlier, overall transmissibility (growth advantage) is an outcome that may depend on both immunity and on the intrinsic viral characteristics. Specific viral traits can be measured in laboratory studies (in vitro and in vivo), and transmissibility can be modelled in animal experiments. Competition assays in relevant animal models are the most exhaustive methods to infer intrinsic transmissibility, and infection in pre-immunized animals can provide information on overall transmissibility (growth advantage) in the context of population immunity in humans. Although SARS-CoV-2 spike proteins from index viruses bound to ACE2 from several animal species (for example, hamsters, ferrets and nonhuman primates), they did not bind mouse ACE2, which explained why laboratory strains of mice could not be infected by SARS-
CoV-2. Mice could become susceptible through expression of hACE2 via a transgene, a viral vector or under regulation of the mouse ACE2 promoter. Later in the pandemic, some variants such as VOCs Alpha, Beta and Gamma acquired a mouse-adapting spike substitution (N501Y), which allowed engagement of mouse ACE2 and productive infection of mice without hACE2 expression. Omicron, which also carries N501Y, was subsequently shown to cause attenuated disease both in mice and hamsters.

Following the emergence of Omicron, the immune escape potential of a variant has been somewhat predictive of its success, and therefore it is believed that the intrinsic transmissibility has played a less relevant role, as compared to its critical role in the emergence of the Alpha and Delta variants.

2B Immune escape

There are various types of immune responses that provide different levels of protection against SARS-CoV-2 infection and COVID-19. Innate immunity is the first line of defence and it is not pathogen-specific but is critical to activate adaptive immunity, which is a pathogen-specific line of defence. In this context, immune escape refers to escape from adaptive immunity, which includes both humoral and cell-mediated immunity. While humoral responses (e.g. neutralizing antibodies) are associated with protection against infection as well as mild and severe illness, cellular responses (i.e. CD4+ and CD8+ T cells) may also play an important role in protection against severe disease. Neutralizing antibodies mainly target the receptor binding domain of the spike protein, while cellular responses target a broader, overall more conserved spectrum of virus epitopes, and are therefore less likely to be subject to immune escape. As a consequence, antibody escape might have a lower impact on COVID-19 morbidity and mortality than escape to CD4+ and/or CD8+ T cells, though the latter is less likely. As for transmissibility, immune escape studies refer to both virus traits and virus-host interactions and should provide outcomes thereof. A suite of standardized assays and clinical and epidemiological observations are needed to test the full spectrum of the immune escape profile of a variant, with virus traits being best studied in the laboratory and virus host interactions in human studies. The preliminary risk evaluation specifically reports on antibody escape, as that is the type of data that can be generated quickly.
Clinical and epidemiological evidence

Many indicators may support estimations of immune escape at the population level. First, it has been helpful to know vaccine and infection-derived background immunity levels at the population level using cross-sectional seroprevalence studies. Mathematical models could provide an alternative method when sero-epidemiological data are not available, untimely, or insufficient.

With the emergence of Omicron, population immunity has built up to very high levels, and an increasing number of people have hybrid immunity (they experienced both infection and vaccination). In this scenario, seroprevalence based on current serological tests that measure binding antibodies would need to be based on much more specific tests (e.g. tests able to detect Omicron-specific antibodies) to be relevant. Currently, such tests are only available in specialized laboratories and are not yet suited for large scale testing. Estimates of reinfection rates through cohort studies, case test-negative designs (similar to vaccine effectiveness) or rolling indicators of reinfection rates from surveillance systems or other signals from outbreak investigations have been useful to better understand the extent of immune escape in the population (see Fig. 2).

Severity associated with reinfection has also been measured in comparative studies.

In silico and laboratory evidence

Immune escape predictions from structural models can provide important preliminary insights into the immune (especially antibody) escape potential of newly emerging variants, especially for those with mutations arising in the receptor binding domain and the N-terminal domain, which are the main (primary and secondary) targets of the neutralizing antibody response. Those predictions always need to be confirmed by laboratory evidence. There is consensus that the simplest assays to measure antibody escape are virus neutralization assays. This is because neutralizing antibodies correlate well with vaccine protection from infection. However, it is also important to assess cross-recognition of T cell responses against newly emerging variants.

Neutralizing antibodies can be measured in vitro using pseudo viruses or live viruses, and they can therefore be used to characterize the antibody escape profile of variants. This requires the use of both well-characterized and representative virus isolates that can be shared and well-characterized sera, because antigenic characterization is relevant only when it
compares a newly emerging variant to reference and precursor viruses using a set of standardized assays and sera. For this purpose, as a minimum, sets of sera samples representative for the current exposure history (both vaccination and infection) in a given population should be used. It’s important to stress that population immunity profiles can differ significantly in different regions of the world, depending on the extent and impact of experienced waves and vaccination efforts.

Antigenic differences may be difficult to discern with new antigenically diverse variants using sera against earlier variants such as the index virus (or index vaccine), but monospecific human sera against recent variants (i.e., sera from unvaccinated individuals who have experienced a primary infection with a recent variant) will become increasingly difficult to obtain as population (often hybrid) immunity rapidly builds up. For this reason, the use of animal models to infect with reference viruses to generate high levels of antibodies directed against a specific variant in the absence of any other SARS-CoV-2 antibody response is critical for SARS-CoV-2 variant antigenic characterization. Thus far, mice and hamsters have been used for this purpose; the potential of larger animals to support this work could be explored, as mice and hamsters provide relatively small volumes of serum. So far, the advantage of mice and hamsters has been that they develop very high homologous titres following infection. High homologous titres are needed to study new variants as they make it possible to measure up to large drops (fold reduction) in the neutralizing antibody titres. Monospecific sera are essential to process neutralization data into antigenic cartography, which is currently the best way to visualise antigenic differences in SARS-CoV-2 variants.

While assessing antibody escape in the preliminary risk evaluation can be based on convenient sets of human sera and related to vaccine effectiveness and protection against severe disease, a comprehensive risk evaluation should also include additional human or animal sera against the novel emerging variants. This is crucial for better characterization of the cross-neutralization potential of divergent antigenic variants and remains critical data to inform vaccine composition.

A preliminary risk evaluation thus transitions to a comprehensive evaluation with time and data.

**2C Increased clinical severity**

Just like the other risk elements, severity can be assessed as a virus trait and as a result of virus-host interaction. Inferring clinical severity based on virus characterization in the laboratory alone is challenging as there is no single specific assay/ marker that can be used reliably. Except for pre-immunized animal models, these markers can exclusively provide insights into intrinsic severity, while they aim at inferring clinical effective severity. Laboratory studies can, however, provide evidence of a change in viral phenotype, which adds confidence to epidemiological findings. Moreover, laboratory studies may provide critical insights into specific virus traits, such as a change in tropism, which cannot be easily determined through epidemiological evidence.

**Clinical and epidemiological evidence**

Random community sampling and longitudinal follow up provide the most robust inference for disease severity, but this is increasingly difficult, because community sampling is now absent in most settings.

Ecological models can also be used to infer disease severity, as they are fast, simple, widely applicable, and do not need genomic and clinical data linkage or require community sampling. They do not require individual-level data and can compare populations or groups using a multiple-group design, periods of time using a time-trend design, or groups and time using a mixed design. However, they often cannot adjust for relevant confounders and may generate
many false signals. Therefore, they can only generate hypotheses that then need to be further tested. However, they remain important because combining such evidence with that generated using one or more approaches may increase our confidence in the available information. Importantly, a simple shift in age distribution in hospitalizations should also be considered as a serious indicator to further investigate, as a variant causing more severe disease might produce more hospitalizations in younger age groups. This does not require community sampling, but it can give false signals because new variants typically show younger age profile regardless of severity, and the variation in immunity between age groups could produce an association independent of intrinsic severity.

Preliminary risk evaluation of new circulating variants in a country with low prevalence initially poses a challenge to statistical analysis due to small sample sizes. Rigorously matched studies of diagnosed cases could be used to infer differences in disease severity. Surveillance systems or studies that rely on routine data – which can come from sentinel-based or more comprehensive approaches depending on available resources - can also provide critical information on clinical severity if they are systematic, well-designed and collect data on confounding factors.

Using wastewater as a proxy for community transmission does not replace hospital surveillance, but it may fill a gap in community sampling and testing. Approaches to infer community spread such as wastewater surveillance could be used to measure a denominator and create a rolling ratio that represents a proxy of the infection hospitalization ratio. Of note, time lags between epidemic waves in different age groups and between infection and hospitalization can complicate such analysis. Leveraging existing systems such as sentinel surveillance systems for influenza viruses or the Pandemic Influenza Severity Assessment (PISA) framework should play an important role in establishing severity indicators for newly emerging variants of SARS-CoV-2 or any other respiratory pathogen.

Going forward, rolling indicators for severity are a more realistic approach to severity assessment. While hospitalization can be a timely severity indicator, reasons for it may differ between hospitals and can depend on clinical judgement. Moreover, with widespread testing (e.g. entry screening), hospitalizations with a concurrent SARS-CoV-2 positive test can be confused with hospitalizations due to COVID-19. Indicators like oxygen or ventilation requirement, ICU admission or any other parameter in the WHO clinical case definition for severe COVID-19 are more objective indicators but require large catchment populations and may be less timely than hospitalization. Some require detailed patient data which are often unavailable. For variant severity assessment, targeted sequencing of severe cases is of critical importance to be able to link the variant causing the infection with the clinical outcome.

Deaths can also be used to survey severity by using COVID-19-associated deaths or overall excess mortality. Attributing the cause of death can be subjective, and differences in COVID-19-associated deaths between countries may have been at least partially driven by differences in the ways they are assigned. Excess deaths have been used to assess the impact of the COVID-19 pandemic. While excess deaths do not depend on testing availability, they reflect many more factors than just the emergence of a new variant (e.g. circulation of other pathogens, environmental factors).

**Laboratory evidence**

Laboratory evidence serves to support and mechanistically explain observations from epidemiological evidence. The scientific community is still working to find the most useful markers of disease severity. Although variability across laboratories for severity outcomes has not been systematically measured, there is likely a need for harmonizing approaches. One obstacle is the access to agreed reference viruses, which remains challenging and time consuming due to internationally different interpretations of existing legislation, and shipping rules and costs. Reference cell lines, procedures for handling viruses, and other reference functions would be easier to standardize across laboratories. Of note, laboratory evidence that supports inference of disease severity will also generate data on the...
intrinsic transmissibility of a variant, as those characteristics are intertwined. The following virus properties are relevant to infer disease severity and can be studied in the laboratory: intrinsic fitness, virulence/pathogenicity (usually assessed in the absence of immunity), and virus life cycle traits affecting virulence. Of note, immune escape is a component of effective fitness and disease severity, but it is covered in the previous section 2B.

**Intrinsic fitness**

This can be measured in competitive fitness experiments (comparing growth curves) and is usually based on confluent cell cultures. There are issues with standardization and choice of models. Organoids, tissue culture or organ explants can be used, but those present significant challenges (reproducibility, infectious dose, and interpretation of subtle differences in fitness). In addition, surrogates of cell entry process such as protein affinity and diffusion (e.g. ACE-2 binding may be used). 78,79 Pseudo type entry is not a real measure of fitness, but it has been used to generate laboratory evidence about infectivity. 80,81 Finally, competitive fitness experiments in relevant animal models are used to assess the intrinsic viral fitness of emerging variants. 82

**Virulence and pathogenicity**

While virulence is a context-independent feature of the pathogen (intrinsic virulence), it is mostly used to compare variants and is only useful in a comparative setting. It cannot be systematically linked to a certain feature of the life cycle or virus. On the other hand, pathogenicity refers to how sick an animal or human gets, which organs are affected and at what level of severity. It is context-dependent and can be ascribed to an epidemic rather than a pathogen itself but does need a virulent pathogen involved. It can be measured through survival time, symptoms and metabolism (weight gain/loss) in relevant animal models, or tissue tropism or tissue damage in relevant animal models, organoids, ex vivo culture of organ explants (see Fig. 3) or tissue models. 83–85 Virus excretion and viral load can also be measured in relevant animal models, explants and tissue models. Type, timing and level of immunity, including immunopathology, can only be studied in relevant animal models. 85

**Virus life cycle traits affecting virulence**

Aspects of the virus life cycle like cell entry (and damage due to it) may affect virulence. Entry pathways (e.g. use of TMPRSS2-dependent pathway), dynamics and syncytium induction are virus life cycle features that can be measured in the laboratory, but they only provide indirect evidence about intrinsic severity, and therefore should not be overinterpreted. 86 Another important aspect is viral action against cellular defences, such as cytokine induction evasion and antagonism, interferon response evasion and antagonism, and other defences like apoptosis, autophagy and stress response. 87 Descriptive or functional studies of changes of host-cell milieu are also of use, such as omics studies 1 and morphological and ultrastructural studies (e.g. membrane utilization). 88

**Other virus properties**

Physical stability, preference in temperature, biophysical determinants of infectivity (e.g. linked to optimal timing of glycoprotein processing in light of biophysical environment such as pH or ion strength), virus attachment as a function of cell surface charge, glycoprotein properties and diffusion through mucus can be measured. 83,89,90

**2D Failure of diagnostics and therapeutics**

**Clinical and epidemiological evidence**

The triggers to investigation of diagnostics and therapeutics failure can come from trials or observational studies with detailed patient characterization and good linkage between genomic and clinical data but can also simply come from clinical suspicion of diagnostics or therapeutics failure from case reports or case series. For other disease like influenza, these come from routine surveillance systems. 91 Once potential failure or resistance is flagged, this can be easily studied by laboratory testing. It is also

---

1 genomics, epigenomics, transcriptomics, proteomics, and metabolomics studies
the responsibility of the end-users of diagnostic and therapeutic tools to report unusual patterns that would trigger further laboratory investigations into their failure to diagnose or treat a newly emerging variant. Evidence from analysis of mutational patterns in large publicly available databases can be useful to generate a hypothesis, which then needs confirmation using patient-level data.92

In silico and laboratory evidence

Evidence for escape from diagnostics and therapeutics can be generated in two steps. The first is an in silico prediction, which takes into account the nucleotide mutations in the new variant and investigates whether those mutations may impact target genes for diagnostics or therapeutics. While this is relatively straightforward and should be highly predictive for molecular diagnostics, it is less so for antigen-based diagnostics and for antivirals, depending on what is known about their mode of action. For example, a mutation outside the protease catalytic active site can still affect efficacy of a protease inhibitor drug.93 For monoclonal antibodies, in silico predictions have also been quite accurate (e.g. to predict resistance of BA.1 to casirivimab/imdevimab and of BA.2 to sotrovimab), but laboratory evidence is required, just as for the other antivirals.94 For antivirals, laboratory evidence of resistance can be generated in vitro and in vivo. Monitoring diagnostics and therapeutics failure is a responsibility of the corresponding manufacturers, especially considering that primers and antigen targets are often not disclosed. When applicable, WHO can facilitate monitoring processes when required through prequalification processes and laboratory networks.

Levels of evidence

Levels of evidence from clinical and epidemiological studies

Many different study designs can be used to gather evidence for the risk evaluation of emerging SARS-CoV-2 variants; and some studies provide stronger evidence than others for a change in the characteristics of the virus under investigation.
Table 2: Risk elements and relative scores for clinical and epidemiological studies in humans

<table>
<thead>
<tr>
<th>Type of evidence</th>
<th>Score*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth advantage</strong></td>
<td></td>
</tr>
<tr>
<td>Comparative evidence from retrospective cohort studies with sequencing data on one or more variants. Outcomes include comparison of hospitalization and fatality rates within a cohort[^{95}]</td>
<td>5</td>
</tr>
<tr>
<td>Whole genome sequencing studies on variants, with analysis focused on COVID-19 outcomes; reinfection, hospitalization and death[^{96,97}]</td>
<td>4</td>
</tr>
<tr>
<td>Comparative viral load data for multiple variants in same population based on PCR or culture[^{98}]</td>
<td>4</td>
</tr>
<tr>
<td>Transmissibility, viral load and minimal infective dose studies, including modelling studies to estimate viral emissions based on viral load data. Indicators measured are viral load in upper respiratory tract among two or more variants[^{99,100}]</td>
<td>3</td>
</tr>
<tr>
<td>Outbreak reports showing a significant rise in number of sequences for a particular variant in 2 or more countries[^{101}]</td>
<td>3</td>
</tr>
<tr>
<td>Outbreak report with no genetic data</td>
<td>1</td>
</tr>
<tr>
<td><strong>Immune escape</strong></td>
<td></td>
</tr>
<tr>
<td>Comparative evidence of reinfection or breakthrough infection risk from large case controls studies, cohort studies, meta-analysis or similar studies&lt; indicators include rates of hospitalization, vaccination protection (as measured by vaccination status in infected participants)[^{102,103}]</td>
<td>5</td>
</tr>
<tr>
<td>Comparative evidence of decreased vaccine effectiveness or protective effectiveness of prior infection against hospitalization or severe disease. Indicators include rates of hospitalizations, severe disease (i.e., serious symptoms that require hospitalization, ICU admission, and death)[^{103,104}]</td>
<td>5</td>
</tr>
<tr>
<td>Comparative evidence of decreased vaccine effectiveness or protective effectiveness of prior infection against infection or symptoms[^{104}]</td>
<td>4</td>
</tr>
<tr>
<td>Case control studies estimating vaccine effectiveness with one or more circulating variants; indicators include hospitalization and ICU admission[^{105}]</td>
<td>4</td>
</tr>
<tr>
<td>Evidence of reinfection or breakthrough infections case reports, case series, outbreak reports, post-marketing surveillance or similar studies</td>
<td>2</td>
</tr>
<tr>
<td><strong>Clinical severity</strong></td>
<td></td>
</tr>
<tr>
<td>Comparative evidence from prospective cohort studies (data on multiple variants)[^{106}]</td>
<td>5</td>
</tr>
<tr>
<td>Evidence from retrospective studies comparing different time periods[^{107,108}]</td>
<td>4</td>
</tr>
<tr>
<td>Matched studies using national databases of diagnosed cases (e.g. lab-confirmed, confirmed by antigen rapid test)[^{109}]</td>
<td>4</td>
</tr>
<tr>
<td>Evidence from comprehensive surveillance data[^{110-116}]</td>
<td>4</td>
</tr>
<tr>
<td>Clinical evidence of shift in symptomatology (e.g. increase in reports of pneumonia, vasculitis)</td>
<td>4</td>
</tr>
<tr>
<td>Prospective cohort studies or case control studies (single variant)[^{117}]</td>
<td>4</td>
</tr>
<tr>
<td>Ecological studies</td>
<td>2</td>
</tr>
<tr>
<td><strong>Diagnostics</strong></td>
<td></td>
</tr>
<tr>
<td>Cohort of clinical suspicion of diagnostic failure; e.g. false negatives using antigen RDTs; absence/ presence of S gene target failure (many patients)[^{118}]</td>
<td>3</td>
</tr>
<tr>
<td>Case series of clinical suspicion of diagnostic failure (few patients)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Therapeutics</strong></td>
<td></td>
</tr>
<tr>
<td>Clinical trials with detailed patient characterization</td>
<td>5</td>
</tr>
<tr>
<td>Comparative evidence from prospective cohort studies (multiple variants)</td>
<td>4</td>
</tr>
<tr>
<td>Prospective cohort studies (single variant)</td>
<td>3</td>
</tr>
<tr>
<td>Clinical suspicion of therapeutics failure. Prospective observational of patients receiving therapies (e.g., monoclonal antibodies). Indicators include hospitalization, oxygen requirement, ICU admission, extracorporeal membrane oxygenation (ECMO) requirement and death[^{119}]</td>
<td>3</td>
</tr>
<tr>
<td>Case control studies using a test-negative design. Main outcome measures include vaccine or antiviral effectiveness against a particular variant, disease severity[^{120}]</td>
<td>2</td>
</tr>
<tr>
<td>Case reports and literature reviews. Indicators include elevated inflammatory markers, altered pathology as evidenced by scans[^{121}]</td>
<td>1</td>
</tr>
</tbody>
</table>

*Scores are from 1 to 5, with 5 representing strongest evidence and 1 weakest evidence. Of note, 5 does not represent ‘perfect’ evidence, but rather the strongest evidence that can be generated based on current knowledge.
Levels of evidence from in silico and laboratory studies

Each laboratory study is helpful in gathering evidence for the risk evaluation of emerging SARS-CoV-2 variants, however some studies provide stronger evidence than others for a change in the characteristics of the virus under investigation.

Table 3: Risk elements and relative levels of evidence for most relevant laboratory studies

<table>
<thead>
<tr>
<th>Type of evidence</th>
<th>Score*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transmissibility (intrinsic only)</strong></td>
<td></td>
</tr>
<tr>
<td>Competition assay or transmission study in relevant animal model(^1)(^2)(^2)</td>
<td>4</td>
</tr>
<tr>
<td>Transmission study in relevant animal model</td>
<td>4</td>
</tr>
<tr>
<td>Evidence for host range expansion in relevant animal model</td>
<td>4</td>
</tr>
<tr>
<td>Competition assays or comparative infection in LRT and URT cell lines (shift to URT)</td>
<td>3</td>
</tr>
<tr>
<td>Competition assays or comparative infection in tissue cultures/organoids/organ explants(^8),(^9),(^2)(^2)(^3)</td>
<td>3</td>
</tr>
<tr>
<td>Human ACE-2 binding affinity(^7),(^8),(^2)(^4)</td>
<td>3</td>
</tr>
<tr>
<td><strong>Immune escape</strong></td>
<td></td>
</tr>
<tr>
<td>Reduced B cell responses: neutralization assays with authentic virus from patient isolates(^1)(^2)(^5),(^6),(^2)(^6)</td>
<td>4</td>
</tr>
<tr>
<td>Immune escape or vaccine escape studies in relevant animal model</td>
<td>4</td>
</tr>
<tr>
<td>Reduced T cell responses: Activation Inducer Marker assay, intracellular cytokine staining flow cytometry assay, or ELISPOT assay(^1)(^2)(^7)</td>
<td>4</td>
</tr>
<tr>
<td>Pseudoneutralization assays using pseudotyped viruses(^1)(^2)(^8)</td>
<td>3</td>
</tr>
<tr>
<td>Evidence from antigenic mapping using animal sera(^1)(^2)(^9),(^2)(^0)</td>
<td>3</td>
</tr>
<tr>
<td>Reduced B cell responses: binding assays (e.g. chemiluminescence immunoassay, enzyme-linked immunosorbent assay)</td>
<td>2</td>
</tr>
<tr>
<td><strong>Disease severity (intrinsic only)</strong></td>
<td></td>
</tr>
<tr>
<td>Disease severity studies in relevant animal model (e.g. viral load, viral excretion, weight gain/loss etc)(^1)(^4),(^1)(^1)(^1)</td>
<td>4</td>
</tr>
<tr>
<td>Evidence for expanding organ tropism (e.g. in tissue culture, organoids, organ explants)</td>
<td>3</td>
</tr>
<tr>
<td>Competition assays or comparative infection in tissue cultures/organoids/organ explants</td>
<td>3</td>
</tr>
<tr>
<td>Competition assays or comparative infection in LRT and URT cell lines (shift to LRT)</td>
<td>3</td>
</tr>
<tr>
<td>Fusogenicity studies(^1)(^3)</td>
<td>1</td>
</tr>
<tr>
<td>Descriptive or functional studies of changes of host-cell milieu (e.g. -omics studies)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Diagnostics</strong></td>
<td></td>
</tr>
<tr>
<td>Molecular or antigenic testing failure</td>
<td>5</td>
</tr>
<tr>
<td><strong>Therapeutics</strong></td>
<td></td>
</tr>
<tr>
<td>Virus inhibition studies in relevant animal models(^1)(^3)(^3)</td>
<td>4</td>
</tr>
<tr>
<td>Virus inhibition studies in organoids or cell lines(^1)(^3)(^4)</td>
<td>3</td>
</tr>
<tr>
<td>In vitro target enzyme assays (e.g. protease, polymerase, etc.)</td>
<td>2</td>
</tr>
<tr>
<td>Evidence from analysis of mutational patterns in large publicly available databases</td>
<td>1</td>
</tr>
</tbody>
</table>

* Scores are from 1 to 5, with 5 representing strongest evidence and 1 weakest evidence. Of note, 5 does not represent ‘perfect’ evidence, but rather the strongest evidence that can be generated based on current knowledge.
Overall Confidence Assessment

The expert team will need to set criteria and (possibly) thresholds for public health action for each of the risk elements described in step 2 (see also Supplementary Tables 1 and 3). Unfortunately, there is currently no consensus for any of such thresholds and this therefore remains a decision-making process that predominantly relies on expert opinion, which is informed by evidence from a very dynamic space.

Tables 1 and 2 attempt to provide relative strength for data obtained from the above-described studies based on current knowledge. These scores reflect the median value of the responses to the questionnaire shared with experts across the various fields of research, which were also given the option to rate studies from 1 to 5. As the questionnaire was designed in 2021, minor adaptations were done to the list of studies to capture evidence generated in the last two years and useful indicators moving out of the pandemic phase. For such minor adaptations, the scores were inferred based on similarities/differences with studies reported in the original questionnaire. It is critical to acknowledge that evidence weighting at this stage remains very dynamic and this list may not be exhaustive. It is an approach that compares datasets to show how scoring can support the evaluation of available data. Importantly, levels from laboratory studies should not be related to levels from clinico-epidemiological studies in humans as they both provide critical evidence for variant risk assessment and should not compete with each other.

While studies in humans provide more direct evidence for specific risk elements, they can be prone to biases and confounders. In silico and laboratory studies are therefore essential to tackle questions that cannot be answered in human studies and that require targeted answers. As SARS-CoV-2 surveillance transitions from the pandemic phase to an endemic phase, the data generated from human studies have become and will continue to become less available, shifting focus on at-risk groups and severe disease. Therefore, laboratory studies will become even more important to answer specific questions. Data from one single group will have much less weight than converging evidence generated by multiple laboratories or clinical-epidemiological studies.

Step 3: Impact on society

The risk elements described in step 2 may lead to significant impact on public health and may require public health actions. However, this might not be enough to declare a new VOC, which is the highest level of warning. The working definition for VOC updated on 15 March 2023 can be found on the WHO variant tracking website. Of note, the assessment of severity of an emerging variant is a key aspect of the new VOC definition and variants that could still trigger changes in vaccine composition Recommendations to update treatment guidelines, advice to update diagnostic assays or any other change in public health and social measures should be considered in the presence of VOIs. Such variants are unlikely to be declared VOCs unless they are assessed as putting significant pressure on health systems.

Step 4: Risk Communication

Risk communication and community engagement (RCCE) play a critical role in providing timely and accurate information about the risk of a disease or threat, including what is known and unknown. The emergence of new variants of SARS-CoV-2 calls for targeted and nuanced RCCE strategies to help manage public risk perception, manage pandemic fatigue, support the uptake and maintenance of protective behaviours and maintain trust in interventions and authorities. The impact of new variants of SARS-CoV-2 on at-risk communities must be understood within the context of attitudes, behaviours, practices, knowledge, trust, rumours, and misinformation linked to COVID-19. Socio-behavioural data should be collected for COVID-19 and for the new variant in an ongoing and iterative basis, and this data should be used to inform RCCE strategies on a local and national level, alongside data on the known and unknown characteristics of a new variant. Ongoing on- and offline social listening should also be conducted.
The emergence of a new variant may lead to increased fear and mistrust or land in an environment of pandemic fatigue where the motivation to change behaviour to limit the impact of COVID-19 is minimal. The dissemination of accurate and timely information about the new variant to the public through various trusted communication channels is essential (including but not limited to social media, traditional media, web pages, and community engagement materials). Key messages should include what is new about this variant, such as changes in symptoms, transmission, vaccine efficacy, and protective behaviours. Key messages should be adapted for audiences at a local level. It is important to acknowledge what is unknown, and what is being done to find out more, as well as using empathy to acknowledge the potential impact a new variant may have on the lives of those affected. Community engagement should be conducted with community leaders, NGOs, CSOs, workplaces, etc. so that communities are included in the planning and implementation of interventions, and feedback loops used to hold two-way dialogues with those affected. Guidance should be provided directly to affected countries and communities on how to respond to the emerging variant. Strong RCCE in the early days of an emerging variant can have a significant impact on the potential public health risk of a disease and should be prioritised.\textsuperscript{136}


References

10. World Health Organization. Statement on the update of WHO’s working definitions and tracking system for SARS-CoV-2 variants of concern and variants of interest.


64. Rössler, A. et al. BA.2 and BA.5 omicron differ immunologically from both BA.1 omicron and pre-omicron variants. *Nat Commun* 13, 7701 (2022).


Annex: Evaluation methods

A novel variant may be tagged for preliminary risk evaluation by review of WHO growth advantage models, epidemiological, laboratory-based and vaccine status surveillance data, and discussions with the TAG-VE. Once a novel variant is selected for evaluation, data and evidence are collected for three main indicators: growth advantage, immune evasion and clinical severity. In addition, treatability (i.e., susceptibility to therapeutics) and detectability (i.e. ability of available diagnostics to detect the variant) are also assessed. The evaluation of each indicator is based on evidence gathered from various studies and across different countries and regions. This includes published data and data reported to WHO by Member States, through Regional Offices or through TAG-VE membership as well as other WHO expert networks. The confidence level of the evaluation starts low and increases as more evidence is gathered. Two factors contribute to the level of confidence: first, the robustness and reproducibility of results from different study designs and second, independent observations in different countries or regions.

Supplementary Table 1, below, is organized in the order we anticipate evidence will become available and outlines a structured framework to gather, evaluate and communicate the public health risk associated with a new emerging SARS-CoV-2 variant. Assessments are to be conducted within two-time frames: first, a preliminary risk evaluation attempted within the first four weeks following the designation of a new variant as a variant of interest; and, a second, more comprehensive evaluation conducted after four to 12 weeks to allow for more detailed studies, such as vaccine effectiveness studies in human populations (as opposed to in vitro studies). Some studies will take longer to be performed and will contribute to our understanding of the virus characteristics but are unlikely to be timely enough for public health action.

This tool’s outputs can be used to prioritize and target research, studies, surveillance efforts, and mitigation measures.
## Supplementary Table 1: Preliminary and comprehensive risk evaluations based on available evidence

<table>
<thead>
<tr>
<th>Overall risk evaluation (yellow, orange or red colour)</th>
<th>Including overall view of threat in the wider context, confidence level in the assessment, and identification of urgent priority studies.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indicator (in order of earliest evidence)</td>
<td>Indicator and type of study for preliminary risk evaluation (0-4 weeks) and comprehensive risk evaluation (4-12 weeks)</td>
</tr>
<tr>
<td></td>
<td>Risk evaluation indicator</td>
</tr>
<tr>
<td></td>
<td>LOW</td>
</tr>
<tr>
<td><strong>Growth advantage</strong></td>
<td>PRELIMINARY: Evidence of a growth advantage likely to lead to dominance</td>
</tr>
<tr>
<td></td>
<td>• A major increase in variant-specific reproduction rate (Rt) over a short time interval</td>
</tr>
<tr>
<td></td>
<td>• WHO growth advantage models (compared to currently circulating variant)</td>
</tr>
<tr>
<td></td>
<td>COMPREHENSIVE: Evidence of growth advantage and spread leading to dominance</td>
</tr>
<tr>
<td></td>
<td>• Variant increasing in prevalence and geographic spread in multiple countries</td>
</tr>
<tr>
<td><strong>Antibody escape</strong></td>
<td>PRELIMINARY:</td>
</tr>
<tr>
<td></td>
<td>A. Signals from outbreak investigations (e.g. shift in affected age group, differences in reinfection pattern)</td>
</tr>
<tr>
<td></td>
<td>B. Rolling reinfection rates through surveillance system</td>
</tr>
<tr>
<td></td>
<td>C. Genomic (predictive) and structural biology assessment</td>
</tr>
<tr>
<td></td>
<td>D. Pseudovirus neutralization using vaccinee sera or pre-banked population serosurveys</td>
</tr>
<tr>
<td></td>
<td>COMPREHENSIVE:</td>
</tr>
<tr>
<td></td>
<td>1. Population or surveillance-based vaccine effectiveness; reinfection rates from observational studies (protective effectiveness of prior infection or hybrid immunity)</td>
</tr>
<tr>
<td></td>
<td>2. Live virus neutralization using human sera</td>
</tr>
<tr>
<td></td>
<td>3. Antigenic cartography using monospecific animal sera (processes neutralization data from multiple laboratories)</td>
</tr>
</tbody>
</table>
### Severity and Other Clinical/Diagnostic Considerations

#### Preliminary & Comprehensive:
- Change in a rolling surveillance metric for severity synchronised with increase in variant e.g.
  - Infection hospitalization ratio
  - Severity indicators from a sentinel hospital network (influenza surveillance such as SARI, PISA)
  - ICU admission per hospitalized case
  - Death per hospitalized case
  - Comparison of hospital and/or ICU admission trends with previous variants
- Change in the demographic profile of hospital admissions due to SARS-CoV-2
- Change in all-cause mortality
- Change in clinical phenotype or unexpected disease manifestations
- Clinical evidence from shift in tissue tropism
- Change in severity from observational studies (e.g. case control, cohorts) or clinical trials linked to genomic data

#### Other Clinical/Diagnostic Considerations:
- Major therapeutics issues such as reduced susceptibility
- Major diagnostics issues such as false negative results

<table>
<thead>
<tr>
<th>Severity and Other Clinical/Diagnostic Considerations</th>
<th>No or Limited Evidence of Increased Severity or Mortality</th>
<th>Some Evidence of Increased Severity or Mortality, but the Magnitude of the Effect is Small or Uncertain, OR Limited Data on Severity but Suggestive of Increased Severity</th>
<th>High Increase in ICU Admission or Mortality OR More Severe Clinical Syndrome or New Severe Symptoms That Are Not Present in Other Circulating Variants</th>
</tr>
</thead>
</table>
The matrix shown below outlines the structure and the weighting of evidence used by WHO and external experts as a framework to assess the overall public health risk across the three selected indicators (in order of importance): clinical severity, growth advantage, and antibody escape. Based on available evidence, each indicator is assigned a level of risk: low, moderate, or high. Supplementary Table 2 then combines these three indicators to present all possible combinations of public health risk levels associated with a variant. Importantly, some combinations may be more likely than others.

Supplementary Table 2: Risk-ranking matrix

<table>
<thead>
<tr>
<th>Evaluation of public health risk, per indicator</th>
<th>Public health risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical severity</td>
<td>Growth advantage</td>
</tr>
<tr>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Low</td>
<td>Moderate</td>
</tr>
<tr>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Low</td>
<td>Moderate</td>
</tr>
<tr>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Low</td>
<td>Moderate</td>
</tr>
<tr>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Moderate</td>
<td>Low</td>
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<td>Moderate</td>
<td>Low</td>
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<tr>
<td>Moderate</td>
<td>Moderate</td>
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<tr>
<td>Moderate</td>
<td>Low</td>
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<tr>
<td>Moderate</td>
<td>Moderate</td>
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<td>Moderate</td>
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<td>Moderate</td>
<td>High</td>
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<td>Moderate</td>
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<td>High</td>
<td>Low</td>
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<td>High</td>
<td>Low</td>
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<tr>
<td>High</td>
<td>Moderate</td>
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<tr>
<td>High</td>
<td>Moderate</td>
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<td>High</td>
<td>High</td>
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<td>High</td>
<td>Moderate</td>
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<tr>
<td>High</td>
<td>High</td>
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<tr>
<td>High</td>
<td>High</td>
</tr>
</tbody>
</table>
Overall public health risk

In the overall risk evaluation, clinical severity is given more weight than growth advantage and antibody escape because of its greater potential impact on health systems and population health. Treatability (impact on therapeutics) and detectability (impact on diagnostics) are also assessed, and any information is included in the indicator “severity and other clinical considerations” of the risk evaluation. A variant with high clinical severity could lead to increased hospitalizations, severe illness, and deaths. By contrast, a variant with high growth advantage or antibody escape may be more transmissible or able to evade the antibody response but may not result in severe illness or require hospitalization. However, a substantial rise in total cases within a short period of time could lead to a significant strain on the health system, and therefore the public health risk associated to it could be high, even if severity is not significantly affected (e.g. in the case of the emergence of Omicron).

In the risk-ranking matrix, if clinical severity is assessed as high, the overall public health risk will be considered at least as high, since the severity of the illness caused by the variant is likely to have a significant impact on public health. Conversely, if clinical severity is assessed as low, the grading of the overall public health risk will be low or moderate since the potential impact on public health is expected to be limited.

It is important to note that these risk levels are not fixed for a VOI and may change as more evidence becomes available and following consultations with technical experts.

The evaluation will rigorously and comprehensively examine quantitative and qualitative information generated through the studies outlined in Supplementary Table 1, which will be triangulated to lend greater strength to the conclusions. In some situations, this context assessment – which should take into account qualitative knowledge of local capacities and vulnerabilities – may result in an upward or downward adjustment of the calculated situational level. For the morbidity and mortality assessment, particular attention will be paid to sub-populations at highest risk of severe disease, rather than the general population.

Actions to be taken for each public health risk level

Once the preliminary (or comprehensive) risk evaluation is completed, specific actions need to be taken based on the overall public health risk level associated with a variant.

- **Low:** Variants assessed as low risk need ongoing monitoring, but they do not require further action beyond routine surveillance.
- **Moderate:** Variants assessed as moderate risk require immediate reporting, increased monitoring, and consultation with the WHO TAG-VE experts. Further research and investigation are needed to determine the potential impact of the variant on public health. Measures such as enhanced surveillance and targeted testing may also be necessary. If additional information suggests a higher risk, the variant should be re-evaluated.
- **High:** Variants assessed as high risk require immediate reporting and action. The variant should be closely monitored and regularly reassessed. Close consultation with TAG-VE experts is essential to determine the appropriate response and consider the designation of a new VOC. Enhanced public health measures, such as increased testing, contact tracing, and targeted public health messaging may be necessary.
- **Very high:** Variants assessed as very high risk require urgent reporting and action. This may include increased sequencing and investigation, targeted public health measures, and potential change in pharmaceutical interventions. Consultation with TAG-VE experts is critical. Designation of the variant as a new VOC is likely, and this can also be achieved by a preliminary risk evaluation (e.g. Omicron was declared 4 days after the date of submission of the earliest publicly available Omicron sequence).

Of note, a change in vaccine antigen composition may be recommended for variants that are assessed as having low or moderate public health risk. The main objective of such change is to enhance vaccine-induced immune responses to circulating SARS-CoV-2 variants, and does not focussing exclusively on improving protection against severe disease. 137
**Supplementary Table 3: Assessment of confidence in available evidence**

<table>
<thead>
<tr>
<th>Indicator (in order of earliest evidence)</th>
<th>Indicator and type of study for preliminary risk evaluation (0-4 weeks) and comprehensive risk evaluation (4-12 weeks)</th>
<th>Confidence in the evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth advantage</strong></td>
<td>PRELIMINARY: Evidence of a growth advantage likely to lead to dominance</td>
<td>LOW</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multiple models using data derived from one country</td>
</tr>
<tr>
<td></td>
<td>COMPREHENSIVE: Evidence of growth advantage and spread leading to dominance</td>
<td>Not applicable*</td>
</tr>
<tr>
<td><strong>Antibody escape</strong></td>
<td>PRELIMINARY:</td>
<td>One indicator (reinfection, neutralization or structural model)</td>
</tr>
<tr>
<td></td>
<td>• Signals from outbreak investigations</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Rolling reinfection rates through surveillance system</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Genomic (predictive) and structural biology assessment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Pseudovirus neutralization using vaccinee sera or pre-banked population serosurveys</td>
<td></td>
</tr>
<tr>
<td></td>
<td>COMPREHENSIVE:</td>
<td>Not applicable*</td>
</tr>
</tbody>
</table>
**Severity and clinical considerations**

<table>
<thead>
<tr>
<th>PRELIMINARY &amp; COMPREHENSIVE:</th>
<th>One metric, one country</th>
<th>Multiple metrics, one country OR same method in multiple countries</th>
<th>Multiple metrics, multiple countries in multiple regions</th>
</tr>
</thead>
</table>
| • Change in a rolling surveillance metric for severity synchronised with increase in variant e.g.  
  ▪ Infection/ hospitalization ratio  
  ▪ Severity indicators from a sentinel hospital network (influenza surveillance such as SARI, PISA)  
  ▪ ICU admission per hospitalized case  
  ▪ Death per hospitalized case  
  ▪ Comparison of hospital and/or ICU admission trends with previous variants  
  ▪ Change in the demographic profile of hospital admissions due to SARS-CoV-2  
  ▪ Change in all-cause mortality  
  ▪ Change in clinical phenotype or unexpected disease manifestations  
  ▪ Clinical evidence from shift in tissue tropism  
  ▪ Change in severity from observational studies (e.g. case control, cohorts) or clinical trials linked to genomic data  
  ▪ Other clinical/diagnostic considerations:  
    ▪ Major therapeutics issues such as reduced susceptibility  
    ▪ Major diagnostics issues such as false negative results | |

*There is no such level of confidence in the assessment.*

N.B. It is essential to integrate these indicators in a systematic and sustained manner to allow early detection of the emergence and spread of a new variant. This requires the integration of processes into routine surveillance at the national level.

The preliminary risk evaluation becomes more comprehensive with time and as evidence from more countries with different immunity landscapes accumulates. The confidence levels of the comprehensive risk evaluation per indicator are defined based on the available evidence and/or the degree of widespread distribution in different WHO Regions.