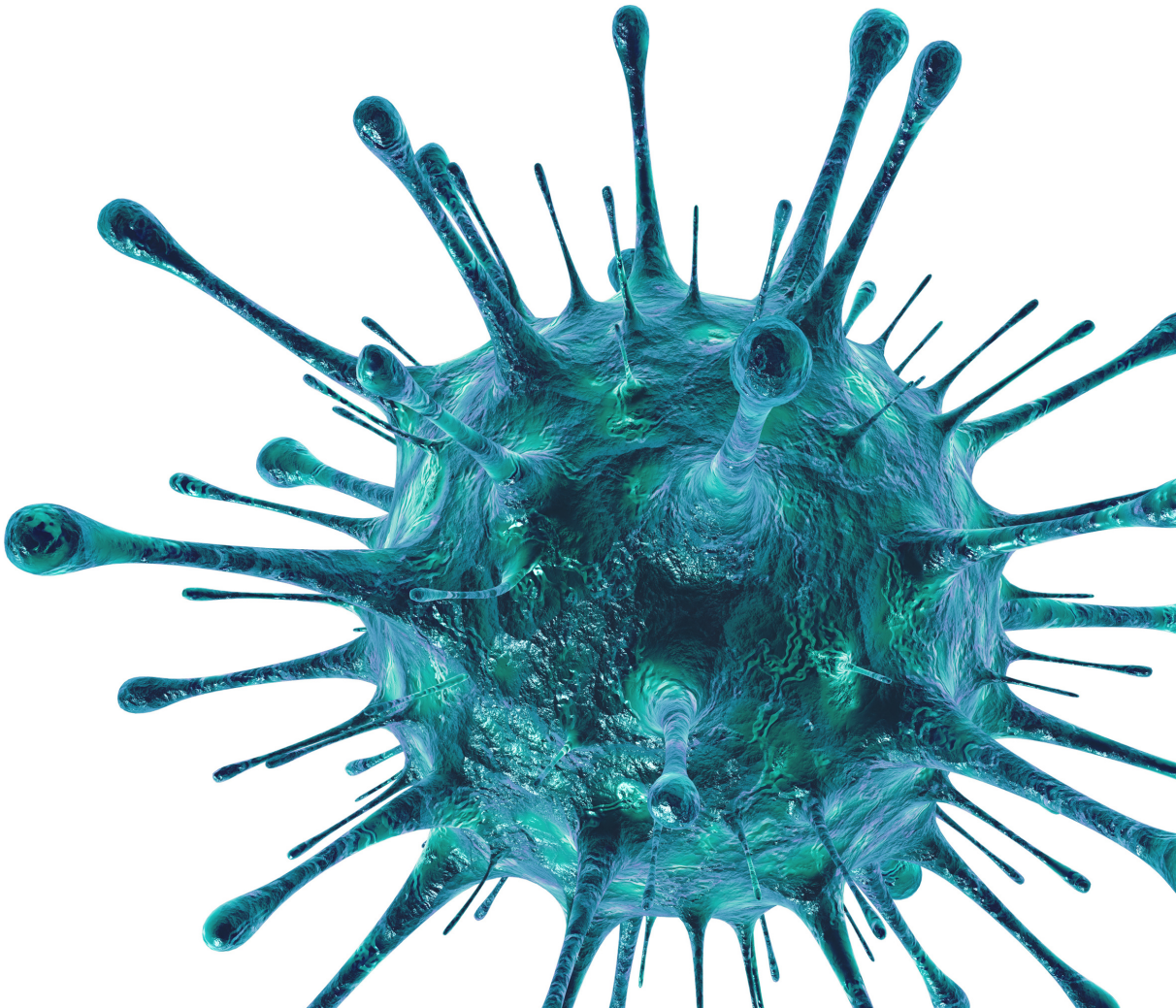
FIND

Because diagnosis matters

SARS-CoV-2 variants



ACKNOWLEDGEMENTS

This report was developed by PHG Foundation for FIND (the Foundation for Innovative New Diagnostics). The work was supported by Unitaid and UK aid from the British people.

We would like to thank all those who contributed to the development and review of this report.

Lead writers

Chantal Babb de Villiers (PHG Foundation)

Laura Blackburn (PHG Foundation)

Sarah Cook (PHG Foundation)

Joanna Janus (PHG Foundation)

Reviewers

Devy Emperador (FIND)

Jilian Sacks (FIND)

Marva Seifert (FIND/UCSD)

Anita Suresh (FIND)

Swapna Uplekar (FIND)

Publication date: 11 March 2021

URLs correct as of 4 March 2021

CONTENTS

1 INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in Wuhan, China in December 2019, and is responsible for the current pandemic of 'coronavirus disease 2019' (COVID-19) [1, 2]. As of 23 February 2021, there have been over 111,279,860 cases worldwide, with more than 2,466,639 confirmed deaths, affecting 223 countries [3]. SARS-CoV-2 is a positive-sense, single-stranded RNA virus, with a genome that is approximately 30kb in length and reported to contain 14 open reading frames encoding 27 proteins [4]. Genome sequencing of SARS-CoV-2 has taken place since the beginning of the pandemic, contributing to an understanding of viral evolution and enabling genomic epidemiology investigations into COVID-19 disease origins and spread. As of 23 February 2020, 595,339 SARS-CoV-2 genome sequences have been uploaded and shared via the GISAID database [5].

These sequencing efforts, and monitoring and surveillance of variation within the SARS-CoV-2 genome, enabled the rapid identification of the first of a number of variants of concern (VOCs) in late 2020, where genome changes were having observable impact on virus biology and disease transmission. Several initiatives that use available sequencing data, mostly from GISAID, to monitor variants and provide further analysis are summarized in Appendix 1.

Here we outline how genomic surveillance and sequencing have supported the recent identification of new SARS-CoV-2 variants and the impact that these variants have had on currently available diagnostic tests and public health measures. We also summarize the role of sequencing in the development of currently available vaccines.

2 SARS-COV-2 VARIANTS AND MUTATIONS

SARS-CoV-2, like all viruses, accumulates mutations – changes in its genetic code – over time as it replicates. This virus has inherent RNA repair mechanisms, and therefore accumulates mutations at a relatively slower rate than most other RNA viruses. On average, a genome from a virus collected in October 2020 has around 20 mutations compared to the first strain sequenced in January 2020 (Wuhan-Hu-1) [6]; the virus evolves at a rate of $\sim 1.1 \times 10^{-3}$ substitutions per site per year, corresponding to one substitution every ~11 days [7]. This compares to a rate of $\sim 4 \times 10^{-3}$ substitutions per site per year for the HIV virus [8]. Across all virus genomes sequenced to date, thousands of mutations have emerged since the start of the pandemic, which in turn have given rise to thousands of different variants. The majority have had no perceivable impact on the virus or disease biology and can act as a useful genetic 'barcode' for tracking viral spread and evolution. However, more recently, several variants have been identified that appear to increase transmissibility, and potentially have an impact on disease severity. As a result, they have been labelled VOCs.

Currently, four internationally confirmed VOCs have been identified − B.1.1.7, B.1.351, P.1 and Cluster 5 (table 1). These are the best characterized and studied so far, and there is supporting evidence of an impact on virus biology available from several countries. As the pandemic continues to unfold, it is likely that more VOCs will be identified, particularly in the presence of new selection pressures, such as vaccination.

2.1 Definitions of variants of concern

On 25 February 2021, WHO released a document outlining working definitions of VOCs and variants of interest (VOI), including recommended actions for member states if a VOI or VOC is identified [9]. A VOI is defined as an isolate of SARS-CoV-2 that has genotypic and/or phenotypic changes compared to the reference genome. The threshold for defining a VOI is quite low, in order to support surveillance efforts. A VOC is defined as a VOI which has a demonstrable increase in transmissibility, increase in virulence and/or is not being controlled effectively by current public health measures. The threshold of evidence for defining a VOC is quite high, to ensure focussed attention and resources on the variants with the greatest health implications. The full VOI and VOC working definitions as given by WHO are included in the glossary of this report.

Other contextual data, such as further characterization in the laboratory, will also support the identification of VOIs and VOCs, since this can help scientists understand whether there are phenotypic changes that improve viral fitness (replication capacity) or impact disease severity. Variants possessing one or more mutations suspected of improving viral fitness can be searched for, flagged, and monitored as VOIs. For example, mutations arising in the spike protein – a common vaccine target – or those which result in changes to an amino acid may be monitored and investigated further. However, due to the variety and number of mutations in the SARS-CoV-2 genome, it is not practical to monitor them all, and prioritizing which variant to follow up is important. In addition, collation of all the genomic, epidemiological and laboratory information about a new variant to determine if it is a VOC can take some time.

There is still some variability around the terms and naming systems used to describe the genetic diversity of SARS-CoV-2; for example, the words ‘variant’, ‘strain’ and ‘lineage’ have been used interchangeably. There is some debate around the use of the term ‘variant’, with some suggesting that a description of the ‘constellation’ of mutations is more important to define than just naming the variants themselves. A summary of these definitions is provided in the glossary.

Several naming systems for VOCs have also been proposed. Public health authorities have taken to naming VOCs by the date of their identification. For example, Public Health England dubbed the variant they identified that was enhancing transmission, VOC202012/01, after the date it was first identified. This is also known as VOC B.1.1.7.

Researchers in South Africa who identified a VOC named it after a defining mutation at the 501st amino acid site, 501Y.V2 (also known as VOC B.1.351) [10]. However, naming a variant after a mutation might cause confusion since it is not always clear, when it was first identified, and which mutations may be of concern, since it is possible that a combination of several mutations is more important in terms of defining the VOC.

A naming system proposed by researchers at NextStrain uses a year and letter system to define different SARS-CoV-2 clades [11]. In April 2020, Rambaut et al. proposed a lineage naming system for SARS-CoV-2 [12] which is currently the most commonly used when referring to current known VOCs, e.g., B.1.1.7, B.1.351 and P.1. This is the naming convention followed in this report. Discussions initiated by the WHO Virus Evolution Working Group are underway to produce a standardized nomenclature for SARS-CoV-2 [13].

2.2 Initial variants of concern identified

Genomic data is essential in supporting the identification of VOCs and having an effective genomic surveillance system in place can allow VOCs to be identified as rapidly as possible. Both the United Kingdom and South Africa, where VOCs have been identified, established genome sequencing initiatives early on in the pandemic: COG-UK [14] and NGS-SA, respectively [15]. Surveillance systems in Denmark, The Netherlands and Japan also contributed to the identification of VOCs.

In the UK, the **B.1.1.7** variant was first identified as a VOC by COG-UK in December 2020, as it was increasing in frequency during a nationwide lockdown, whilst other variants were decreasing in frequency. A retrospective examination of the data determined that the variant had been in circulation since September, but at that time there were insufficient data to suggest that it was a VOC. The B.1.1.7 variant is currently the most highly sequenced and well-characterized VOC, and has been shown to have increased levels of transmissibility at a rate of between 40 and 70% [16]. In addition, a paper from the New and Emerging Respiratory Virus Threats Advisory Group (NERVTAG) and presented to the UK Government's Scientific Advisory Group for Emergencies (SAGE) outlined the results from several preliminary analyses of B.1.1.7, suggesting that there could be an increase in mortality rates as a result of the variant [17, 18]. The COG-UK mutation tracker outlines the spike protein mutations seen circulating in the UK [19]. It also details the scientific evidence to date on the impact of different mutations on immune evasion.

In South Africa, the **B.1.351** variant was identified after frontline clinicians alerted NGS-SA to a rapid increase in cases, which prompted genomic investigation. The B.1.351 variant is a concern as it has been shown to have increased transmissibility and to reduce the efficacy of some vaccines [20]. For B.1.351, there may be some form of enhanced escape from immune pressure and onward transmission, generating a fitness advantage, but the evidence for this is still weak [21].

In the case of the **P.1** variant, Japan reported the variant via their surveillance system, after detection in four travellers who had returned from Brazil [22]. The variant was flagged to be of concern due to the presence of spike mutations also found in the B.1.351 variant: N501Y (which increases virus binding affinity to the ACE2 receptor on human cells), E484K (which renders the virus less susceptible to some monoclonal antibodies) and K417N/T (suggested to increase binding affinity to ACE2, in combination with N501Y). The set of mutations/deletions, especially N501Y, shared between the P.1, B.1.1.7 and the B.1.351 variants appear to have arisen independently.

P.1 and B.1.351 also appear to be associated with a rapid increase in cases in locations where COVID-19 disease rates were previously high. Therefore, it will be crucial to investigate whether there is an increased rate of recent re-infection, caused by these variants, in previously exposed healthy individuals.

The fourth variant, **Cluster 5**, was a VOC identified on mink farms in Denmark and the Netherlands. Swift action from local public health authorities in these countries stopped the spread of this VOC, and it is now believed to be extinct (see section 4.2).

Ongoing monitoring of variants B.1.1.7, B.1.351 and P.1 is being carried out globally.

Table 1: Variants of concern. Further information is available via online resources ([23, 24]) that are closely monitoring the main variants identified (B.1.1.7, B.1.351 and P.1). *Information extracted 19 January 2021 [24].

Variant (Bold indicates the most commonly used variant notation; other known notations for each variant recorded below**)**	**First identified (location)**	**Date identified**	**Notable mutations**	**Number of defining mutations (number in spike)**	**Clinical changes**			**Number of countries reporting variant**	**Sequence counts**
					Transmissibility	**Virulence**	**Antigenicity**		
B.1.1.7, VOC-202012/01, 20B/501Y.V1	United Kingdom	Nov 20	N501Y, 69–70del, P681H	23 (8)	Yes, evidence of increased transmissibility (PHE) >40%	Potential evidence emerging suggesting increased virulence	No evidence of change	83*	79,434*
B.1.351, 501Y.V2; 20C/501Y.V2	South Africa	Dec 20	N501Y, K417N, E484K	21 (9)	Yes, evidence of increased transmissibility (South African Department of Health)	No evidence of change	Undergoing investigation (E484K mutant)	41*	1436*
P.1, 501Y.V3, B.1.1.28.1	Brazil and Japan	Dec 20	N501Y, E484K, K417N	17 (10)	Undergoing investigation (N501Y mutant), evidence is suggesting increased transmissibility	unknown	K417T possibility escaping some monoclonal antibodies	20*	153*
Cluster 5, ΔFVI-spike	Denmark	Oct 20	Y453F, 69–70del	Not described (4)	No evidence of change	No evidence of change	Yes "Moderately decreased sensitivity to neutralizing antibodies"	Likely extinct	>300

2.3 Variants of interest

As the pandemic continues, new variants will emerge. Ongoing surveillance will therefore be required not only for known VOCs, but also for VOIs.

Variants of interest that are currently being closely monitored include the **P.2** variant in Brazil, of interest because it harbours a E484K mutation seen in other VOCs [25], and the **CAL.20C** variant in California, with an L452R mutation suggesting increased transmissibility [26]. Multiple variants with mutation Q677P, also suspected to increase transmissibility, have been identified in a number of states in the U.S. [27]. According to the Pan American Health Organization (PAHO) of WHO, three new variants have been detected in 14 countries in the Americas and flagged as possibly causing heightened spread and more severe disease [28]. In the UK, a second variant named **VOC202102/02** – that is B.1.1.7 with an additional E484K mutation – has been labelled a VOC by Public Health England, due to it retaining the characteristics of the original B.1.1.7 VOC, but with an additional mutation. It has been associated with a limited number of cases and is being closely monitored. Another variant with an E484K mutation is also being monitored [29]. These and other variants still need to be evaluated further to determine if they are VOCs and if they are having an impact globally.

3 IMPACT OF VARIANTS ON DIAGNOSTICS

Sequencing data can be used to understand whether current diagnostic tests remain fit for purpose, as well as inform the development of new diagnostics. As described below, virus mutations have the potential to reduce the accuracy of diagnostic tests. Regular analysis of sequence data can allow researchers to identify any mutations of particular concern, which can then be investigated to see if they impact on test function. Alternatively, if a test appears to not be working as expected, for example continually delivering false negative results, the samples could be sequenced to see if they contain a mutation responsible for test failure.

Routine testing for SARS-CoV-2 can be categorized according to the test target, including direct viral detection of either viral RNA or viral antigens indicative of current infection, or indirect detection of anti-SARS-CoV-2 specific antibodies, indicative of current or historic infection [30].

Nucleic acid amplification tests (NAATs), including those based on PCR, use primers (short DNA sequences) to bind and detect specific virus RNA target sequences. These tests remain one of the most accurate and widely used methods for SARS-CoV-2 diagnosis. However, if a mutation occurs in one of the primer target sequences used in these tests, it is possible that the primer may no longer be able to bind to the target, producing a false negative result. In general, most NAATs are designed to have multiple genetic targets [31]. This means that if a mutation does occur in one test target site, the overall test should still work and produce the correct results.

Antigen and antibody tests could also be affected by mutations, but for this to happen the mutation would have to cause an alteration to the protein or physical structure of the virus targeted by the test.

3.1 Impact of variants of concern on diagnostics

The most well-characterized VOCs, B.1.1.7, B.1.351 and P.1, have multiple mutations, including several in the spike gene (S-gene). If any of these mutations occur at primer binding sites or affect the structure of viral antigen targets that are detected by the antigen tests, they have the potential to impact the accuracy of diagnostic tests.

It is now well established that this is the case for the B.1.1.7 variant, due to the presence of a Δ69/70 mutation in the S-gene. This mutation prevents the primers in certain PCR tests with S-gene targets, notably the ThermoFisher TaqPath test, from binding to the S-gene target. This delivers a negative result for this target, widely known as S-gene target failure, or S-gene drop out [32]. However, the ThermoFisher TaqPath test contains three targets, with two targets still working as expected. Due to this built-in redundancy, there has been no impact on the accuracy of the overall test results. As most tests use multiple genetic targets, it is expected that most tests in widespread use should continue to be accurate in the face of viral evolution. There is no evidence to date that the S-gene mutations in the other VOCs alter the performance of PCR tests, although it will be important for users of tests that target the S-gene to monitor this.

The mutations in the other genes of the variants do not appear to have had an impact on diagnostic tests [33]. It is important to monitor the impact of mutations in all genomic regions, as most commercially available tests do not use the S-gene as a primary target. For example, the majority of commercially available antigen tests target the N-protein, and many PCR tests also include an N-gene target [34]. The B.1.1.7 variant has been shown to have no effect on five rapid antigen tests in an evaluation by the UK government, despite mutations in the N-gene which could potentially affect antigen structure [35]. The effect of the N-gene mutations observed in other variants on antigen test detection has not been evaluated, but currently no major changes to test performance are anticipated. Whilst there is potential for the mutations in the variants to impact antibody tests, this has not yet been evaluated for any of the variants [36].

3.2 How the impact on diagnostics has been managed

In terms of the impact of S-gene target failure in samples containing the B.1.1.7 variant, in many PCR diagnostic tests the S-gene is not used as a target, so many tests are unaffected by S-gene failure. In tests which do target the S-gene, it is typically only one of several targets, so overall test performance has not been affected. As two working test targets are perceived as sufficient, the ThermoFisher TaqPath test is still used. The overall impact on diagnostic test performance has therefore been minimal; however, the case of S-gene target failure does highlight the need for testing at least two independent targets, as recommended by WHO [37]. Thus, any diagnostic tests that currently have only one additional target may need to be redesigned. The S-gene should be avoided as a target in the future, due to the high potential for other mutations in this gene.

In the case of S-gene target failure, there has been an inadvertent positive impact on diagnostic testing. Comparison of sequencing results to S-gene target failure results showed that S-gene target failure can be used as a proxy for detection of the B.1.1.7 variant [16]. In areas where B.1.1.7 is common, S-gene target failure has allowed the variant to be monitored at scale via diagnostic testing programmes, allowing it to be better characterized and appropriate control measures to be introduced. For example, in England, analysis of S-gene target failure was used to show that the frequency of the B.1.1.7 variant increased relative to other variants during a lockdown period, which should have limited opportunities for transmission [16]. This suggested that this variant was more transmissible than other variants. In Portugal, S-gene target failure has also been used to track the frequency and geographical dispersion of the variant [38].

Use of S-gene target failure as a proxy for B.1.1.7 only works well if the prevalence of B.1.1.7 is high, as other variants also contain the Δ69/70 mutation, and so also cause S-gene target failure. In

countries where there are several circulating variants with the Δ69/70 mutation, such as the U.S., the use of S-gene target failure may not be a suitable proxy for B.1.1.7. However, the presence of S-gene target failure can still be useful to select potential cases of interest, which can then be prioritized for follow-up sequencing to characterize if VOCs are present [39, 40].

In summary, monitoring rates of target failure as part of routine testing can potentially be a useful signal that there may be changes in the variant distribution in a given population. A recent survey by the ECDC found that 16 European countries are now using S-gene target failure as a variant pre-screening strategy, to identify variants from positive PCR- test results, which may then be sequenced to confirm their identity [41]. This should be more widely encouraged as part of routine lab practice.

3.3 Use of sequencing to manage future impact of variants on diagnostics

The Δ69/70 mutation in the B.1.1.7 variant is not the only mutation to interfere with a diagnostic test, and other mutations have previously been detected in other genes which resulted in PCR target failure [42]. It is likely that as new variants and mutations continue to emerge, there will be additional impact on diagnostics. There are several ways in which sequencing can be used to help maintain the accuracy of diagnostic tests, as well as support the management of emerging variants.

Monitoring and evaluation of diagnostics: Sequencing has been an essential tool to confirm that the failure of specific PCR targets has been due to a mutation in the target site, rather than other test factors. It has been shown that there are now numerous mutations across virtually all virus genes, and therefore all current PCR test targets [43]. By keeping track of the number and type of mutations in the targets of the tests they are using, laboratories can understand if their diagnostics are likely to be affected, and if so, remain vigilant for any unexpected results, or if necessary, switch to a different test target. Several tools for monitoring relevant mutations are now available, as described in the WHO guidance for diagnostic testing of SARS-CoV-2 [37]. The U.S. FDA has also published recommendations for test manufacturers, particularly of molecular tests, to routinely monitor test performance by comparing primer/probe pairs to publicly available genomes [44].

Design of reliable tests: By using sequencing data to understand the rate of mutations in different regions of the genome, test developers can aim to target more stable regions of the genome. It is already recommended that tests do not primarily target the S-gene, due to high mutation rates in this region. Also, it is now understood that there is higher instability in other regions than at first assumed, including the often-targeted N-gene [43]. New tests may be required that avoid this region, or at least incorporate more targets to avoid the likelihood of overall test failure.

Design of variant-specific tests: The example of S-gene target failure has shown the utility of using routine diagnostic tests to track a new VOC in regions where the variant has a high prevalence. This approach is less suitable in areas with a more diverse range of variants. In addition, S-gene target failure is only applicable to variants containing the Δ69/70 mutation. Sequence data could be used to design diagnostics to specifically identify new VOCs rapidly and at scale through population testing programmes. Variant-specific assays covering several key mutations have already been developed and launched by several companies, including TibMolBiol, Eurofins and Novacyt [45-47].

Sequencing as a diagnostic test: Sequencing is most commonly being used as a surveillance tool during the pandemic, or occasionally to confirm uncertain diagnostic results. It is rarely used as a primary diagnostic tool. For most purposes, current PCR tests, and in some cases rapid antigen tests, are likely to remain the methods of choice, due to their relatively low cost and ease of use. By

monitoring and designing new tests as described above, it should be possible to ensure these tests continue to perform properly. However, sequencing-based diagnostics are now becoming available, including the Illumina COVID-Seq test authorized for use in the U.S., which covers the entire virus genome. The performance of WGS-based tests is less likely to be affected by variants. In addition, sequencing information can be obtained at the same time as the diagnostic result, without the need to send the sample separately for sequencing [48]. It is currently unclear whether it would be feasible to use sequencing-based diagnostics as a first-line diagnostic at a national level. However, they provide an alternative option to rapidly identify new variants, potentially for use within more targeted settings.

4 IMPACT OF VARIANT IDENTIFICATION ON PUBLIC HEALTH MEASURES

The new VOCs identified are more transmissible, leading to higher infection rates and higher levels of hospitalizations, increasing pressure on healthcare systems. Whilst current public health measures such as infection prevention, social distancing and mask wearing are still effective, there is a need for adjustment to counteract increased transmissibility.

There is also a concern that variants could arise that lead to immune escape, resulting in the possibility of reinfection within a shorter time frame compared to other variants, resulting in repeated infections. A UK-based study examining this issue, the SIREN project, is studying immunity and reinfection in healthcare workers. Any COVID-19 positive cases in this group are sequenced, which will allow for greater understanding of the risk of reinfection from different variants within communities [49].

One key response to the new variants in a number of countries has been stricter and enhanced public health and social measures [50], effectively reinforcing efforts to delay their spread. Responses can include school closures; workplace closures; cancellation of public events; restrictions on public gatherings; closures of public transport; stay-at-home requirements; public information campaigns; restrictions on internal movements; and international travel controls. With the situation changing rapidly on a daily basis, a summary of some of the measures being taken are described below; the *Our World in Data* website provides an overview of these measures globally [51]. Another response has been the implementation or enhancement of genomic surveillance efforts within countries, which is discussed at the end of this section.

4.1 Public health measures

Several public health measures were put in place, or existing measures strengthened, in response to the emergence of VOCs. However, a recent ECDC report noted that pandemic fatigue could adversely affect the continued acceptance of and compliance with public health and social measures by the population, which will need to be carefully considered and addressed as the pandemic progresses [33].

Stricter and extended lockdowns. European countries including Germany, the Netherlands and Denmark extended their lockdowns [52]. The UK introduced a tier system to enable implementation of regional restrictions where there were increases in cases, predominately where the B.1.1.7 variant was concentrated, namely in the south-east and east of England and emerging hotspots in south Wales and Cumbria [53]. Initial allowances for movement and household mixing over the Christmas period were reduced and made stricter with the emergence of the new variant. A national UK

lockdown was implemented in early 2021 once analysis demonstrated that these measures were not eliminating spread of the variant [16].

Travel restrictions and border closures. The suspension of travel and banning of flights from the UK, South Africa and Brazil occurred internationally to limit spread of VOCs [54]; South Africa closed all land borders and required testing before entry [55]. Restrictions on travel have started for countries where one of these variants is attributed to increasing cases, such as Portugal and Ireland. Many countries, such as Germany [56], now require a negative coronavirus test before entry.

Quarantine: While hotel quarantine has been a feature of many countries' pandemic responses, most notably Australia and New Zealand, more countries are starting to introduce this measure from locations they deem to be high-risk, including Germany [56], Ireland [57] and the UK. On 23 January 2021, a German hospital was put under quarantine after staff and patients tested positive for the B.1.1.7 variant [58].

Mask regulations. There is a growing body of scientific evidence indicating that mask use can help prevent the spread of the coronavirus [59]. In Germany, it has been made mandatory for people travelling on public transport, or in supermarkets, to wear medical style masks: either N95, KN95, FFP2, or a surgical mask [60]. Similar measures will also be introduced in Austria. The French health advisory council has discouraged the wearing of cloth and homemade masks, arguing they may not offer sufficient protection against the more highly transmissible variants.

School closures. The rate of infections in children has consistently been low, a phenomenon that is being investigated further. Early data on the B.1.1.7 variant had suggested that it was spreading more in children compared with other circulating variants. This resulted in some countries closing schools where previously they could have been left open. Research now suggests that B.1.1.7 is spreading more efficiently in all age groups, not just children. However more data is needed on how children transmit these new variants [61, 62].

Vaccination schedule. VOCs were reported around the same time as the first vaccination programmes were being established. In the UK, vaccinations started on 8 December 2020. On 30 December, the four UK Chief Medical Officers announced that delivering the first dose of the vaccine to as many people as possible would be a priority [63]. Whilst this decision was not attributed directly to the B.1.1.7 variant, the reasons given were to protect as many individuals as possible in as short a period of time [64]. The consequences of increasing the time between the first and second dose are unknown, with concerns that relatively weak immune responses induced by a single dose of vaccine could encourage the emergence of new variants of the virus – and that such variants could be more resistant to immune responses, particularly those generated by vaccines, increasing the risk that these variants could become a global threat [64]. Careful surveillance of variants will be required to monitor for the emergence of any that might weaken vaccine efficacy.

4.2 National and regional responses to VOCs

Variants linked to mink – zoonotic and anthroponotic transmission. Outbreaks of SARS-CoV-2 occurred on mink farms in the Netherlands and Denmark in late spring and early summer 2020. Genomic and epidemiologic investigation of an early outbreak in the Netherlands demonstrated human to mink, mink to mink, and mink to human transmission [6, 65]. In early November 2020, Danish authorities reported over 200 cases of COVID-19 associated with mink farms. Many SARS-CoV-2 sequences from the Netherlands and Danish outbreaks had a Y453F mutation, which was considered to possibly mediate increased binding affinity for mink ACE2. Eleven individuals from the

Danish outbreak had the variant termed Cluster 5, which had four additional spike mutations [6]. The apparent adaptation of SARS-CoV-2 to mink was alarming because continued evolution of the virus in an animal reservoir could potentially lead to recurrent spill-over events of novel SARS-CoV-2 from mink to humans and other mammals. For this reason, many countries have increased surveillance efforts and in some cases implemented large-scale culling of mink on mink farms [66].

As of January 2021, the virus has been detected at 400 mink farms in eight countries in the EU/EEA. The ECDC and the European Food Safety Authority (EFSA) released a report on 19th February 2021 that proposed monitoring strategies to help prevent and control spread of the disease [67]. This included the recommendation that all positive samples should be sequenced to monitor the evolution of the virus.

Response to two SARS-CoV-2 variants in Denmark. In addition to the SARS-CoV-2 outbreak associated with mink, Denmark first detected cases of the B.1.1.7 variant in mid-December. The proportion of cases caused by the variant climbed from 0.3% of all samples sequenced in November 2020 to 2.9% in early January 2021. The government reacted quickly by putting additional measures in place – in January 2021, Denmark's Staten Serum Institute (SSI) planned to sequence every new positive case with testing for the variant used for intensified contact tracing [68]. This concerted effort is in part driven by a desire to reduce the reproduction rate to below 0.7 to avoid exponential growth in February and March [56].

B.1.1.7 variant in a Dutch school. An outbreak of the B.1.1.7 variant was identified at a Dutch elementary school where 46 people with this variant were detected at the end of December 2020. Because of the unusually high number of cases at the school, Erasmus MC and GGD Rotterdam-Rijnmond launched a large-scale outbreak investigation that included testing the wider community [69, 70]. Within two weeks, 45,000 of the 63,000 residents of the town had been tested. At the time of this writing, 242 positive cases had been detected, of which 12% were the B.1.1.7 variant. In total, 70 individual cases were linked to the school. The original source of the B.1.1.7 variant at the school was not identified, and most transmissions appeared to be within households. With the extra measure of testing asymptomatic individuals, contact tracing and quarantining positive cases, the authorities are confident that this B.1.1.7 outbreak has been contained [71].

Response to variant B.1.351 in South Africa. Control measures were strengthened and in addition to closing the land borders to curb the spread of the second wave, South Africa has maintained restrictions such as a ban on sale of alcohol (partially lifted in early February) and a limit on the number of people allowed to attend public gatherings [55]. Strict guidelines on wearing masks in all public areas have been in place, with regulations published in February making it a criminal offence to not wear a mask [72].

Surge testing in the UK. In early February, Public Health England (PHE) announced the deployment of surge testing [73], which is increased testing (including door-to-door testing in some areas) and enhanced contact tracing in specific locations in England. This was in response to the B.1.351 variant being detected in various regions across the country, and more recently in response to other potential VOCs identified. In regions where these variants are present, asymptomatic testing is being offered to everyone, and all positive cases will be sequenced. This will enable a better understanding of this variant and identify if there are any more cases in affected areas.

4.3 Response of multi-national organizations

World Health Organization: WHO is working with Global Influenza Surveillance and Response System (GISRS) to expedite the sequencing component of sentinel surveillance for SARS-CoV-2 [74]. A 14 January meeting of the WHO emergency committee advised a number of actions that should be taken, including [75]:

- work collaboratively to develop standardized definitions and nomenclature of SARS-CoV-2 virus variants, and provide clear information on what constitutes a variant of concern – working definitions of VOIs and VOCs were released on 25 February [9];
- continue to increase worldwide capacities for molecular testing and genetic sequencing and encourage rapid sharing of sequence data and metadata;
- strengthen the risk monitoring framework for variants by supporting collaboration and research.

Africa CDC: The pandemic has highlighted the benefits of genomics for infectious disease management in Africa as well as the need to support and strengthen efforts to integrate genomics into public health surveillance systems [76]. In response to the new variants, the Africa CDC recommended a number of additional measures [77]. These include:

- public health laboratories and researchers strengthening collaboration and coordination with national and regional pathogen genomics laboratories to conduct genomic surveillance and sequencing of the virus in a timely manner. Africa CDC, through the Africa Pathogen Genomics Initiative (PGI), will support this coordination at the continental level;
- notification of any new SARS-CoV-2 variants to the Africa CDC;
- strengthening community-based surveillance, as well as surveillance and cross-border health measures at ports of entry.

European CDC: A recent survey by ECDC found that most of the EU/EEA countries are actively investigating the emergence of variants. However, the capacity currently available for sequencing is not sufficient to meet the recommendation of the European Commission to sequence 5-10% of positive samples. Many countries are increasing or planning to increase their sequencing capacity, but have indicated the need for support from ECDC [41].

A number of options in response to VOCs highlighted by ECDC include [78]:

- continued monitoring of local changes in transmission rates or infection severity to identify and assess the circulation and impact of variants, supported by increased surveillance and sequencing;
- preparing laboratories for increased testing turnover and healthcare systems for increased demand;
- increasing overall sequencing capacity by making use of all possible sequencing capacity in clinical, diagnostic, academic and commercial laboratories across different sectors;
- supporting ongoing public health measures, such as strengthened contact tracing, and restrictions on travel – based as appropriate on sequencing information about variant transmissibility;

- accelerating vaccination for high-risk groups, the most vulnerable, and key workers; monitoring vaccine effectiveness against new variants, using sequencing to monitor breakthrough infections.

4.4 Increase in global sequencing efforts

The description and monitoring of VOCs has demonstrated the usefulness of integrating genomic surveillance methods to document and help control SARS-CoV-2 spread in local and national settings. Genomics data can also be used in real time to inform and consolidate national outbreak investigation and response strategies [79]. There have been calls globally, including from WHO, to increase SARS-CoV-2 sequencing efforts to support detection of new variants and monitor their spread [80].

The **United Kingdom** is leading the way in terms of the number of SARS-CoV-2 genomes sequenced. The COG-UK consortium releases weekly reports that provide a summary on variants as well as mutations that have been observed in the UK. The consortium has been sequencing 5–10% of all positive cases, and aims to sequence 20,000 samples a week by March 2021 at a cost of £40 per sample at smaller sequencing sites, and £20 per sample at high volume sequencing sites [81]. They also support research and create data interpretation tools (such as Microreact), as well as protocols for sampling and sequencing. One project developed and funded by the consortium is the online platform CoV-GLUE [82]. It is a publicly accessible web application for the interpretation and analysis of SARS-CoV-2 genome sequences, which will allow easier identification of new variants.

Within **South Africa,** the Network for Genomics Surveillance in South Africa (NGS-SA) has been monitoring changes in the virus genome, sequencing 50-100 samples per week [83]. They have provided protocols, are regularly releasing data, and supporting other African countries to carry out sequencing. One of the NGS-SA members, KRISP, identified the B.1.135 variant in the country and is to receive ZAR 25 million (~USD 1.7 million) from the South African Department of Science and Innovation to sequence 10,000 samples in South Africa and the African continent [84].

As a response to the variants being detected in **Germany,** the government drafted regulations introduced in January 2021. These require laboratories and hospitals to sequence 5% of all samples with a positive PCR test and send the results to the Robert Koch Institute (RKI) [56, 85], the German public health institute. Once the number of new infections is below 140,000, plans are in place to sequence up to 10% of PCR-positive samples. The federal government in Berlin has earmarked a budget of €200 million for the programme [56].

In the **United States,** there have been calls to increase sequencing [86, 87]. In early February, CDC made a $200 million sequencing investment and the U.S. government has allocated $1.75 billion for this effort [88]. Approximately 1.4 million people in the U.S. test positive for the virus each week, but fewer than 3,000 of those weekly samples are sequenced by a variety of academic, state and commercial laboratories [89]. The U.S. CDC is monitoring the situation closely and sequence-based strain surveillance has been increasing [90]:

- National SARS-CoV-2 Strain Surveillance (NS3) – state health departments and other public health agencies have been sending at least 10 samples biweekly to CDC for

sequencing and further characterization. This system is now being scaled to process 750 samples nationally each week.
- As of 29 December 2020, CDC has agreements from national reference laboratories to sequence 1,750 samples per week and support surveillance.
- CDC has contracts with seven universities to conduct genomic surveillance in collaboration with public health agencies.
- Sequencing within state and local health departments – on 18 December 2020, CDC released $15 million in funding, together with COVID supplemental funds, through the Epidemiology and Laboratory Capacity Program to support these efforts [91].
- CDC leads a national consortium of laboratories sequencing SARS-CoV-2 (SPHERES), which also coordinate U.S. sequencing efforts outside the CDC. In 2020, $9 million from CDC supported this consortium [92].
- Commercial partnerships –Illumina and Helix, for example, announced a collaboration to track the B.1.1.7 variant in the U.S. and to augment the national surveillance infrastructure to track the emergence and prevalence of novel SARS-CoV-2 strains, with support from CDC. The companies are sequencing up to 1,000 genomes a week [93].

5 IMPACT ON VACCINES

Genomic sequencing has significantly advanced the process of modern vaccine design and development. Three types of vaccines hinge on accurate knowledge of the genetic sequence of the pathogen. These are:

- **Subunit vaccines**, where the genetic code of a pathogen is inserted into cells grown in the laboratory (typically yeast cells), while and the yeast cells synthesize the viral proteins *in vitro.*
- **Viral vector vaccines**, where the genetic material of the pathogen is inserted into a viral vector that can infect human cells without causing disease.
- **Nucleic acid vaccines** that deliver RNA or DNA to cells without the need for a viral vector.

5.1 Vaccines in development for SARS-CoV-2

The Regulatory Affairs Professional Society has an online COVID-19 vaccine tracker [94]. This web tracker comprehensively details the vaccines under development as well as those that are approved, including type of vaccine, developers, country of origin, clinical trial stage, and where it is approved for use. There are currently 57 vaccines in clinical or preclinical trials. The mechanism utilized by each vaccine varies and includes delivering the whole virus (either inactivated or attenuated), recombinant viral vectors, protein subunits, and nucleic acids. Vaccines using viral vectors (e.g., adenovirus or recombinant vesicular stomatitis virus) are most common, followed by nucleic acid vaccines and protein subunit vaccines [95].

Many of the vaccines under development target the spike glycoprotein. This is because it is found on the surface of the virion (virus particle) and mediates its access into human cells via the angiotensin-converting enzyme II (ACE2) receptor. The spike glycoprotein is an optimal target for vaccines; these glycoproteins are present on the virion surface and are the primary target of neutralizing antibodies, which protect cells from infection [96].

There are a few vaccines that specifically target other viral proteins, notably the recombinant vaccine VXA-CoV-2. This vaccine is not only unusual in its route of administration (oral), but it also delivers the viral nucleocapsid protein in addition to the spike protein [97]. Another vaccine taking this multi-antigen approach, which is yet to reach clinical trials, is CoVepiT. This has 11 targets, including structural and non-structural proteins [98]. It aims to produce long-lasting immunity by stimulating T-cell responses as opposed to just antibody production; these are a type of white blood cell responsible for immune memory, as well as supporting responses to acute infection.

5.2 Approved vaccines for SARS-CoV-2

There are eleven vaccines approved for use to protect against SARS-CoV-2 [94]. Five of these are inactivated virus vaccines, one is a subunit vaccine, three are viral vector vaccines and two are mRNA vaccines.

The mRNA vaccine Comirnaty (also known as BNT162b2) by Pfizer-BioNTech is approved in the largest number of countries – 30, plus the EU. This vaccine delivers the genetic material for human cells to make the spike protein of SARS-CoV-2. The other approved mRNA vaccine, Moderna's COVID-19 vaccine (also known as mRNA-1273) also uses this mechanism. The mRNA sequences for these vaccines were produced shortly after the public release of the first SARS-CoV-2 genome sequence in January 2020. Other COVID-19 vaccines with widespread use include those produced by AstraZeneca and Sputnik V, both of which use viral vectors containing the genetic information to produce the spike protein; and BBIBP-CorV, which is an inactivated virus vaccine.

5.3 Potential impact of new variants on vaccines

There is much focus on whether the recently identified variants and the mutations they possess are enabling the virus to escape from host immunity, in particular immunity conferred from vaccination. Determining whether variants have evolved to escape immunity is not possible by assessing sequence data alone, and further experimental investigation is required to determine the impact on viral phenotypes. Much of the currently available evidence is either published without peer review or not yet available for peer review and is therefore subject to change.

Studies are underway to evaluate whether VOCs reduce the efficacy and effectiveness of the approved vaccines. Researchers are using laboratory experiments and clinical data to assess whether individual mutations found in VOCs or VOCs themselves have an impact on vaccine efficacy. A popular laboratory method is to test antibody neutralization activity against genetically engineered pseudoviruses that possess mutations found in VOCs. Sera from individuals who have been vaccinated is used to determine whether antibodies produced from vaccination are able to effectively neutralize the antigens present on these pseudoviruses. Data from clinical trials is also becoming available, enabling analysis of the infection rates with VOCs in those who have been vaccinated.

There is mounting evidence to suggest that the B.1.351 variant possesses mutations that enable it to escape immunity from both previous infection and some vaccines [33]. The strongest and most conclusive evidence to date suggests that the COVID-19 Vaccine Oxford-AstraZeneca offers only minimal protection against B.1.351 infection [20]. A clinical trial involving 2,000 young adults in South Africa found that the vaccine did not protect participants against mild to moderate disease. Whilst it is undetermined if the vaccine still offers protection against severe disease and hospitalization, the

lack of efficacy found in this study has led South Africa to halt the roll out of the AstraZeneca vaccine [99].

Many other vaccines have been found to have a reduced effectiveness for the B.1.351 variant, however, early results from clinical trials suggest that they will still confer a good level of protection. Interim results from phase 3 clinical trials for the Novavax vaccine (NVX-CoV2373) [100] and the Johnson & Johnson vaccine (JNJ-78436735) [101] have reported 60% and 57% vaccine efficacy against B1.351, respectively. Importantly, the Johnson & Johnson vaccine has been found to be 85% effective against severe or critical disease with no decline due to the B.1.351 variant. This vaccine has recently gained approval for its use in Saint Vincent and the Grenadines, and recently received U.S. FDA emergency authorization [102].

Laboratory antibody neutralization experiments also suggest that the B.1.351 may reduce vaccine efficacy. For example, the Pfizer/BioNTech vaccine antibody neutralization was approximately two-thirds reduced [103] and Moderna observed a six-fold reduction in neutralizing titres with the B.1.351 variant relative to prior variants [104, 105]. Despite the apparent decrease in neutralization from antibodies produced in response to these vaccines, the manufacturers still expect them to be somewhat protective though clinical data is needed to draw stronger conclusions. Correlates of immunity and protection are required to allow extrapolation from neutralization reductions to the impact on immune protection and are currently being determined for COVID-19.

Plans are being made to develop booster versions of the AstraZeneca [106], Moderna [107] and Pfizer/BioNTech [108] vaccines that protect against emerging VOCs, including B.1.351. The regulatory environment is also adapting to facilitate rapid regulatory pipelines for updated vaccines. For example, the U.S. FDA have released guidance and implemented policy to streamline regulation for updated vaccines [109].

The evidence to date suggests that vaccines are effective against B.1.1.7. Whilst there appears to be some reduction in antibody neutralization by mRNA vaccines (Pfizer-BioNTech [110-112] and Moderna [104, 113]), the reductions are modest and not expected to translate into loss of efficacy. Clinical data from the AstraZeneca [114] and the Novavax vaccine [115] trials suggest these vaccines should also provide adequate protection against B.1.1.7. Generally, vaccination induces a multipronged immune response including the production of polyclonal antibodies, such that even if mutations do reduce the neutralization capability of the antibodies present in sera, there will still be some protection.

The E484K mutation has recently been detected in a small proportion of the B.1.1.7 variant samples [116]. This mutation has caused particular concern as it is a key mutation in the B1.351 VOC and thought to play a major role in evading host immunity [117]. The importance of its emergence in B.1.1.7 for vaccine efficacy is not yet determined. However, a preprint paper has found that the B.1.1.7 VOC carrying the E484K mutation has a ten-fold decrease in the titre of neutralizing serum antibodies by the Pfizer-BioNTech vaccine compared to B.1.1.7 without the mutation [110]. This suggests that the vaccine will be less effective against the B.1.1.7 variant if it possesses the E484K mutation.

There are no studies specifically looking at the efficacy of vaccines for VOC P.1, but the mutation profile of the spike protein in P.1 is similar to B.1.351, in particular that it also carries the E484K mutation. P.1 may therefore also have an impact on vaccine effectiveness.

5.4 How sequencing can help

Monitoring circulating variants for changes that could impact the effectiveness of vaccines is essential, especially as more of the population become vaccinated. As more people become vaccinated and develop immunity against the virus, there is a risk that the virus may develop immune escape mutations due to this selection pressure. It is therefore essential that the evolution and consequences of viral mutation are closely monitored.

Sequencing will play a vital role in determining if or when mutations accumulating in the viral population are leading to changes in an antigen that could render vaccines ineffective. Knowledge of the precise changes at the level of the viral genome will enable alterations to be made to vaccines, for example, changing the genetic code that is delivered in nucleic acid vaccines. As mentioned above, work is already underway to augment vaccine production in response to the B.1.351 variant.

Collaboration between those carrying out genomic surveillance, those conducting supporting experiments to assess the impact of variations, and vaccine manufacturers, will enable fast identification of detrimental changes and allow for adaptation of vaccine production to mitigate the impact of VOCs.

6 DISCUSSION AND CONCLUSIONS

Genomic surveillance has played an essential role in identifying and monitoring novel variants in SARS-CoV-2 that have had a direct impact on the control and spread of the virus. A range of public health measures have been implemented globally as a result of new information on variant transmissibility. In addition, the genetic data produced from surveillance has enabled further in-depth characterization of the variants to determine more precisely the roles of mutations, either individually or in combination, on virus biology. This information is, in turn, essential for informing future surveillance efforts, as well as identifying the impact mutations may have on vaccines, diagnostics, and therapeutics.

Multiple countries around the world have invested in genomic sequencing infrastructure and resources, in recognition of the fact that genomic surveillance forms the basis of a robust national and international response to infectious disease. The surveillance systems established so far represent an important step towards global genomic surveillance, but there remain areas of the world with a paucity of genomic data, despite high numbers of cases. There are likely to be many more variants of SARS-CoV-2 circulating globally, but countries with robust and routine genomic surveillance strategies are more likely to detect them. Moreover, the identification of a variant in a particular jurisdiction does not necessarily mean that the variant arose there.

It is therefore essential that infrastructure and resources are developed in countries where currently little or no sequencing is being carried out, and efforts must continue to be made to share data as swiftly and openly as possible. Initiatives such as the recently announced ‘New Variant Assessment Platform’ launched by the UK, could help support countries with limited resources to detect SARS-CoV-2 variants [118]. Genomic surveillance during the pandemic is an international effort and will be more effective if capabilities in this area are more evenly distributed globally. This will include ongoing support of data sharing through accessible international databases.

While there is a general need to increase the number and representativeness of the viral samples that are being sequenced, well-planned methods for sample selection and production of high-quality genomic sequences can provide valuable insights in countries where there are limited sequencing

capabilities. For example, in South Africa a VOC was identified through effective communication between local healthcare practitioners and careful consideration of their observations. Clinicians noticed an increase in the number of patients with COVID-19, and through sequencing a limited number of samples and coordination with other regions, they determined that a new variant was the likely driver behind this increase.

Much of the national and international SARS-CoV-2 sequencing efforts have been initiated via academic endeavours. These are most often set up for the primary purpose of surveillance, but some also contribute to clinical and epidemiological projects and investigations. Many of the examples outlined in this document demonstrate how a variety of skills and expertise from multiple backgrounds are needed to make genomic surveillance efforts a success. These include clinicians observing differences in the frequency and presentation of cases, geneticists sequencing and sharing information globally, epidemiologists identifying the specific context in which the virus is spreading, and policymakers acting upon the results. Good communication between these and other stakeholders is therefore essential. The COG-UK and NGS-SA initiatives are positive examples of how a broad network of engaged stakeholders, including national and global researchers, clinicians, public health authorities, health departments and health communities, can embed genomic surveillance into public health systems and ongoing public health action.

New SARS-CoV-2 variants will continue to arise as a result of the natural evolution of the virus, and potentially also in response to vaccination and other measures. Despite the increasing availability of vaccines, it will remain vital to ensure that the number of infections in populations are limited by ongoing public health measures, not least to reduce the likelihood of additional VOCs arising. The availability of viral genome sequencing data to understand ongoing changes in the viral genome will become increasingly important as SARS-CoV-2 vaccines and antivirals become more widely used.

Looking to the future, the use of sequencing to identify and monitor SARS-CoV-2 variants could also play a role in a number of areas:

- **Characterizing infection after historical infection or vaccination.** Determining if a particular variant(s) or mutation(s) is responsible for these cases can indicate that the virus has evolved to escape immunity.
- **Monitoring the new variants for their impact on therapeutics.** This will be required as early evidence suggests that some monoclonal antibody therapies are not as effective against the new variants [19].
- **Monitoring the impact of therapy on viral evolution**, to understand if therapies drive the selection of specific VOCs.
- **Monitoring viral evolution in those who experience prolonged infection**, including in those who are immunocompromised. This is because a prolonged infection where replication continues will lead to the virus accumulating more mutations, some of which could be a survival advantage.

While there are currently many uncertainties and knowledge gaps related to the impact of these VOCs, sequencing to support continued monitoring, evaluation, and investigation plays a key role in the understanding and management of these variants.

7 GLOSSARY AND ABBREVIATIONS

Glossary

Amino acid: An organic compound that acts as a building block of a protein, which is formed when multiple amino acids join together. A mutation can sometimes result in a different amino acid being produced, which in turn may affect the protein produced.

Antibody: Proteins produced by the body's immune system in response to detection of an antigen. Antibodies bind to and cause the elimination of antigens. Artificial antibodies may be manufactured, for use in antigen detection tests.

Antigen: A molecule or molecular structure, such as a protein, that is recognized as foreign and triggers an immune response in the body, which produces antibodies that recognise and bind to the antigen. Artificial antigens may be manufactured, for use in antibody detection tests.

Antigenicity: The ability of an antigen to bind to, or interact with, the products of the host immune response i.e., antibodies.

Assay: A method designed to assess specific characteristics of a substance or product. For example, a PCR based assay could be designed to detect SARS-CoV-2 mutations.

Base: see *nucleotide.*

Diagnostic: A tool or test used to help confirm or rule out a patient's condition. In the context of SARS-CoV-2, diagnostic tests are used to help confirm if a patient is infected or not.

DNA: Deoxyribonucleic acid, a double stranded molecule carrying genetic instructions required for organisms to grow, develop, and reproduce. It makes up the genomes of all living organisms and many viruses.

False negative: A test result that incorrectly reads negative (e.g., a test says a virus is not present in a sample) when the result should be positive (e.g., the virus is present).

First line test: The first choice of diagnostic test to be used, often decided due to a number of factors including sensitivity, specificity, cost, ease of use, and turnaround time.

Gene: a specific DNA or RNA sequence that encodes the synthesis of a gene product, either RNA or protein. Genes are the units in which a genome is inherited.

Genome: An organism's complete set of genetic instructions.

Genomic epidemiology: The study of how genomic variations influence health and disease. During outbreaks, the term is typically applied to pathogen genomics. Applications include tracking pathogen evolution and routes of transmission, as well as assessing functional impacts of pathogen variants.

Genomic surveillance: The systematic collection and analysis of pathogen genomes with the goal of understanding pathogen evolution and supporting genomic epidemiology efforts, for example to detect mutations that may have an impact on disease biology.

Immune escape: A strategy used by pathogens to evade a host's immune response to maximise their probability of being transmitted.

Immunogenicity: The ability of a foreign substance (such as a viral antigen) to provoke an immune response in an organism.

Lineage*: A group of sequences that share a distinct set of features and that can be associated with a particular epidemiological event.

Monoclonal antibody (mAb): An antibody that is produced from a unique parent white blood cell, and only recognizes one specific part of an antigen. They can also be synthesized in a lab for use in research and as targeted therapies.

Mutation: An alteration to or change in the RNA or DNA genome sequence, for example change of a nucleotide within the sequence, insertion or deletion of genetic material. A subset of mutations will cause changes in amino acid sequence and potentially in protein function, altering virus biology.

Next-generation sequencing (NGS): A “high-throughput, massively parallel” sequencing method used to determine the nucleotide sequence of a whole genome or part of a genome in a single biochemical reaction volume. NGS is performed by non-Sanger-based sequencing technologies that are capable of sequencing multiple DNA fragments in parallel, which are then pieced together and mapped to a reference genome using bioinformatics analyses.

Nucleotide: The molecules that make up RNA or DNA sequences, also known as bases. DNA sequences are made up of four nucleotides: adenine (A), cytosine (C), guanine (G), and thymine (T). RNA sequences are made up or A, C, G and U - uracil - instead of thymine.

Nucleic acid amplification test (NAAT): A test which uses primers to bind specific nucleic acid target sequences, which can then be amplified to allow their detection. PCR tests are the most commonly used NAATs.

Phenotype: The set of observable characteristics of an organism, resulting from the interaction of its genotype with the environment.

Phylogenetic analysis: Analysis of the evolutionary development and/or characteristics of a pathogen, which is then used to describe the relationships between different forms of that pathogen.

PCR: Polymerase chain reaction is a widely used technique in nucleic acid amplification tests (see glossary). It is used to detect specific regions of SARS-CoV-2 viral RNA, by first making a DNA copy of the virus RNA, then using primers (see glossary) to amplify specific targets through multiple cycles of heating and cooling.

Primer: Short DNA sequences that provides a starting point for synthesis; see also Nucleic acid amplification test (NAAT).

Protein: A molecule made up of multiple structural units called amino acids, which has a defined structure and performs one or more specific functions in the cell. The instructions to make proteins are encoded in DNA.

RNA: Ribonucleic acid, a molecule that transfers information from the genome to proteins in organisms with DNA genomes. Some viruses, such as SARS-CoV-2, have RNA rather than DNA genomes.

Real-time sequencing: Scientific technique that determines and reports DNA sequences simultaneously, as opposed to older methods that required separate processes to capture and report DNA sequence information.

S-gene target failure/S-gene dropout: Terms widely used to refer to the failure of some SARS-CoV-2 PCR tests to recognize their S-gene targets, due to a mutation (i.e., Δ69/70) in the S-

gene of SARS-CoV-2. Widely used as a tool to detect the B.1.1.7 variant, which contains this mutation.

Strain*: Genetic subtypes of a pathogen, which may or may not be biologically or functionally distinct from one another. Some virologists use the term strain to refer to biologically or functionally distinct subtypes only. The term strain has been used interchangeably with that of lineage.

Transmissibility: The extent at which a pathogen is transmitted from one organism to another.

Vaccine escape: When the genome of a pathogen changes such that the immune response resulting from vaccination to that pathogen is less effective or ineffective, and results in reduced vaccine efficacy or lack of prevention of infection and disease; see also viral escape.

Vaccine efficacy: Refers to how well a vaccine performs in a carefully controlled trial.

Vaccine effectiveness: Describes how well a vaccine performs in the real world.

Variant*: Refers to a genetically distinct form of the SARS-CoV-2 virus, characterized by specific mutation(s). This term has also been used to refer to the founding virus of a cluster/lineage, and/or the resulting variants that form collectively from a lineage.

Variant of concern (VOC)*: A variant of the SARS-CoV-2 virus that has a distinctive set of mutation(s) which confer a change in virus biology and epidemiology; for example, an increase in the number of disease cases, increased disease transmissibility, more serious disease and/or impact on vaccine efficacy. The WHO working definition of a VOC is: "*A VOI (as defined below) is a variant of concern (VOC) if, through a comparative assessment, it has been demonstrated to be associated with: increase in transmissibility or detrimental change in COVID-19 epidemiology; Increase in virulence or change in clinical disease presentation; or decrease in effectiveness of public health and social measures or available diagnostics, vaccines, therapeutics. OR assessed to be a VOC by WHO in consultation with the WHO SARS-CoV-2 Virus Evolution Working Group."* [9]

Variant of interest (VOI): The WHO working definition of a VOI: "*A SARS-CoV-2 isolate is a variant of interest (VOI) if it is phenotypically changed compared to a reference isolate or has a genome with mutations that lead to amino acid changes associated with established or suspected phenotypic implications; AND has been identified to cause community transmission/multiple COVID-19 cases/clusters, or has been detected in multiple countries; OR is otherwise assessed to be a VOI by WHO in consultation with the WHO SARS-CoV-2 Virus Evolution Working Group.*" [9]

Viral escape: When the genome of a pathogen changes such that existing immune responses are less effective against disease, or do not prevent disease; see also vaccine escape.

Virulence: The ability of a pathogen to overcome host defences and cause damage to the host.

*There are some differences within the wider research and clinical community in uses of the terms 'strain', 'variant' and 'lineage'. Differences in definitions are noted and working definitions by WHO of VOI and VOC have been provided. There is also not an agreed nomenclature for virus variants of concern – these issues continue to be under review by WHO and others.

Abbreviations

ACE2: angiotensin-converting enzyme II (a gene in SARS-CoV-2)
CDC: Centers for Disease Control and Prevention
COG-UK: The COVID-19 Genomics UK consortium
COVID-19: coronavirus disease 2019 caused by SARS-CoV-2
ECDC: European Centre for Disease Prevention and Control
EU/EEA: European Union/European Economic Area
GISRS: Global Influenza Surveillance and Response System
mAb: monoclonal antibody
mRNA: messenger RNA
N-gene: Nucleoprotein gene
NAAT: Nucleic acid amplification test
NGS-SA: Network for Genomics Surveillance in South Africa
PCR: Polymerase chain reaction
PHE: Public Health England
RNA: ribonucleic acid
S-gene: Spike gene (in SARS-CoV-2)
SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2
VOC: Variant of concern / **VOCs**: Variants of concern
VOI: Variant of interest / **VOIs**: Variants of interest
WGS: Whole genome sequencing
WHO: World Health Organization

8 APPENDIX 1: SARS-COV-2 VARIANTS – INFORMATION RESOURCES

This is a rapidly evolving field and advances are ongoing. To assist with the latest monitoring of sequencing and variants, a few resources that are currently actively monitoring VOCs are listed below. There are further resources using GISAID data listed on the GISAID homepage [5]. **Key: Data visualization** – Tools that display data collated and analysed by others. **Data analysis** – A tool that allows users to further analyse collated data and/or analyse their own data. **Report** – Regularly collated information, not linked live to data source

Type of resource	Resource	Description	Link
Data visualization	CDC: Global variants report	Global map showing which countries have reported variants based on data provided by the World Health Organization (WHO) Variant Tracker	https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/global-variant-map.html
Data visualization	U.S. COVID-19 Cases Caused by Variants	Reporting on the VOC in the United States. Content sourced from: National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases	https://www.cdc.gov/coronavirus/2019-ncov/transmission/variant-cases.html
Data visualization	COVID-19 virus mutation tracker system (CovMT)	Tool to track COVID-19 mutations globally, enabled by GISAID data	https://www.cbrc.kaust.edu.sa/covmt/
Data visualization	CoVariants	A global overview of variants, enabled by GISAID data, developed by researchers from Nextstrain	https://covariants.org/
Data visualization	Microreact	The Centre for Genomic Pathogen Surveillance maintain a Microreact website which permits continuous evaluation of variants circulating in the UK	https://beta.microreact.org/project/uuRSVf7sLRdNmRRogHkFuF-cog-uk-2021-02-16-uk-SARS-CoV-2/
Data visualization	ECDC PrimerScan	A free online resource of mutations in genomic regions targeted by Real-time PCR detection assays, using data from GISAID	https://primerscan.ecdc.europa.eu/?assay=Overview
Data visualization	CoVal	CoVal is a repository of amino acid replacement mutations identified. CoVal provides information on the demographic distribution of these mutations and reports co-occurring mutations. Uses GISAID data	https://coval.ccpem.ac.uk/
Data visualization	The Helix® COVID-19 Surveillance Dashboard	A surveillance dashboard from the Helix and Illumina partnership, sequencing 1000 samples per week in the US	https://www.helix.com/pages/helix-covid-19-surveillance-dashboard

Type of resource	Resource	Description	Link
Data visualization and analysis	Pango Lineages and Pangolin software	A tool tracking lineages of concern and interest. Pangolin is open-source software used to analyse sequence data	https://cov-lineages.org/global_report.html
Data visualization and analysis	CoVGLUE	CoV-GLUE contains a database of amino acid replacements, insertions and deletions which have been observed in GISAID	http://cov-glue.cvr.gla.ac.uk/#/home
Data visualization and analysis	Nextstrain Global SARS-CoV-2 analysis	Tool showing global genomic epidemiology of novel coronavirus, enabled by GISAID data	https://nextstrain.org/ncov/global
Data visualization and analysis	COVID-19 Viral Genome Analysis Pipeline	This website provides analyses and tools for exploring accruing mutations geographically and over time, with an emphasis on the Spike protein, using data from GISAID	https://cov.lanl.gov/content/index
Data visualization and analysis	Natural selection analysis of global SARS-CoV-2/COVID-19	Analyses of the natural selection of different mutations over time, using data provided by GISAID	https://observablehq.com/@spond/revised-SARS-CoV-2-analytics-page
Data visualization and analysis	Centre for Genomic Regulation (CRG) Covid Viral Beacon	CRG Covid Viral Beacon is a tool to find variability at genomic, amino acid and motif level. It offers the possibility to search in detail variants, filter queries and find unique cases, filter strain/country-specific variants, explore associated metadata and much more	https://covid19beacon.crg.eu/
Data visualization and analysis	Ensembl COVID-19	Webpage providing a variety of tools including variant analysis ranging from genetic locations to phenotypes and population context	https://covid-19.ensembl.org/info/website/index.html
Data visualization and analysis	Primer-Check	Compares sequence data from GISAID against common primers used to check for mismatches. Users can also input their own sequence data	https://viroscience-emc.shinyapps.io/primer-check/
Data analysis	Coronavirus typing tool (Genome Detective)	An online tool to identify the Coronavirus types, genotypes, and variants of a nucleotide sequence. Includes analysis of VOC, and lists mutations	https://www.genomedetective.com/app/typingtool/cov/

Type of resource	Resource	Description	Link
Data analysis	COG-UK mutation explorer (COG-UK-ME)	An interface to access data on SARS-CoV-2 mutations and variants of interest in the COG-UK genome sequence data set, from the MRC-CLIMB database. Largely focuses on spike gene mutations of potential or known importance based on epidemiological, clinical and/or experimental observations. It also collates scientific evidence pertaining to the impact of mutations on immunity or treatment with monoclonal antibodies	http://sars2.cvr.gla.ac.uk/cog-uk/
Report	New York Times New Variants Tracker	A compilation of information on variants and mutations, provided by the New York Times	https://www.nytimes.com/interactive/2021/health/coronavirus-variant-tracker.html
Report	WHO: Coronavirus disease (COVID-19) Weekly Epidemiological Update	Provides an overview of the global, regional and country-level COVID-19 cases and deaths, highlighting key data and trends, including updates on VOC	https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports
Report	ECDC: Situation updates on COVID-19	Includes risk assessments (some are variant specific when needed), situation reports and weekly surveillance reports (includes information on variants)	https://www.ecdc.europa.eu/en/covid-19/situation-updates
Report	PHE UK. Variants: distribution of cases data	PHE UK monitoring of VOCs or variants under investigation in the UK	https://www.gov.uk/government/publications/covid-19-variants-genomically-confirmed-case-numbers/variants-distribution-of-cases-data

9 REFERENCES

1. Weston, S., Frieman, M. B. COVID-19: Knowns, Unknowns, and Questions. mSphere. 2020. 5(2): pp. e00203-20.
2. Khan, S., Siddique, R., Shereen, M. A., et al. Emergence of a Novel Coronavirus, Severe Acute Respiratory Syndrome Coronavirus 2: Biology and Therapeutic Options. Journal of Clinical Microbiology. 2020. 58(5): pp. e00187-20.
3. Coronavirus disease (COVID-19) pandemic. World Health Organisation. 2020; Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019.
4. Wu, A., Peng, Y., Huang, B., et al. Genome Composition and Divergence of the Novel Coronavirus (2019-nCoV) Originating in China. Cell Host Microbe. 2020. 27(3): pp. 325-328.
5. Global initiative on sharing all influenza data. GISAID. 2021; Available from: https://www.gisaid.org/.
6. Rapid Risk Assessment: Detection of new SARS-CoV-2 variants related to mink. European Centre for Disease Prevention and Control. 2020; Available from: https://www.ecdc.europa.eu/en/publications-data/detection-new-sars-cov-2-variants-mink.
7. Martin, M. A., VanInsberghe, D., Koelle, K. . Insights from SARS-CoV-2 sequences. Science. 2021. 371(6528): pp. 466-7.
8. Andrews, S. M., Rowland-Jones, S. Recent advances in understanding HIV evolution. F1000Res. 2017. 6: p. 597.
9. Weekly epidemiological update - 25 February 2021. World Health Organization. 2021; Available from: https://www.who.int/publications/m/item/covid-19-weekly-epidemiological-update.
10. Tegally, H., Wilkinson, E., Giovanetti, M., et al. Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. medRxiv. 2020. p. 10.1101/2020.12.21.20248640.
11. Bedford, T., Hodcroft, E. B., Neher, R. A. Updated Nextstrain SARS-CoV-2 clade naming strategy. NextStrain. 2021; Available from: https://nextstrain.org/blog/2021-01-06-updated-SARS-CoV-2-clade-naming.
12. Rambaut, A., Holmes, E. C., O'Toole, A., et al. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. Nat Microbiol. 2020. 5(11): pp. 1403-1407.
13. Callaway, E. 'A bloody mess': Confusion reigns over naming of new COVID variants. Nature. 2021; Available from: https://www.nature.com/articles/d41586-021-00097-w.
14. The COVID-19 UK Genomics Consortium COG-UK. 2021; Available from: https://www.cogconsortium.uk/.
15. Network for Genomic Surveillance in South Africa. KRISP. 2021; Available from: https://www.krisp.org.za/ngs-sa/nga-sa_network_members_nhls_nicd_uct_ukzn_h3abionet_and_others/.
16. Volz, E., Mishra, S., Chand, M., et al. Transmission of SARS-CoV-2 Lineage B.1.1.7 in England: Insights from linking epidemiological and genetic data. medRxiv. 2021. p. 10.1101/2020.12.30.20249034.
17. NERVTAG paper on COVID-19 variant of concern B.1.1.7. Department of Health and Social Care and SAGE. 2021; Available from: https://www.gov.uk/government/publications/nervtag-paper-on-covid-19-variant-of-concern-b117.
18. Burki, T. Understanding variants of SARS-CoV-2. The Lancet. 2021. 397(10273): p. 462.
19. COG-UK mutation explorer. COG-UK Consortium 2021; Available from: http://sars2.cvr.gla.ac.uk/cog-uk/.
20. Madhi, S. A., Baillie, V., Cutland, C. L., et al. Safety and efficacy of the ChAdOx1 nCoV-19 (AZD1222) Covid-19 vaccine against the B.1.351 variant in South Africa. medRxiv. 2021. p. 10.1101/2021.02.10.21251247.
21. McNally, A. What makes new variants of SARS-CoV-2 concerning is not where they come from, but the mutations they contain. BMJ. 2021; Available from: https://blogs.bmj.com/bmj/2021/02/16/what-makes-new-variants-of-sars-cov-2-concerning-is-not-where-they-come-from-but-the-mutations-they-contain/.
22. Brief report: New Variant Strain of SARS-CoV-2 Identified in Travelers from Brazil. National Institute of Infectious Diseases, Japan. 2021; Available from: https://www.niid.go.jp/niid/en/2019-ncov-e/10108-covid19-33-en.html.

23. Miner, B. E., Thomas, M. K. Covid Variant Tracking Project: tracking the spread of alarming Covid variants. Tableau Public. 2021; Available from: https://public.tableau.com/profile/brooks.miner#!/vizhome/MapVariants/Dashboard.
24. PANGO lineages. SARS-CoV-2 lineages. 2021; Available from: https://cov-lineages.org/global_report.html.
25. Naveca, F. , Nascimento, V. , Souza, V., et al. Phylogenetic relationship of SARS-CoV-2 sequences from Amazonas with emerging Brazilian variants harboring mutations E484K and N501Y in the Spike protein. Virological. 2021; Available from: https://virological.org/t/phylogenetic-relationship-of-sars-cov-2-sequences-from-amazonas-with-emerging-brazilian-variants-harboring-mutations-e484k-and-n501y-in-the-spike-protein/585.
26. Zhang, W., Davis, B. D., Chen, S. S., et al. Emergence of a Novel SARS-CoV-2 Variant in Southern California. JAMA. 2021. p. 10.1001/jama.2021.1612.
27. Hodcroft, E. B., Domman, D. B., Snyder, D. J., et al. Emergence in late 2020 of multiple lineages of SARS-CoV-2 Spike protein variants affecting amino acid position 677. medRxiv. 2021. p. 10.1101/2021.02.12.21251658.
28. Epidemiological Update: Occurrence of variants of SARS-CoV-2 in the Americas - 26 January 2021. Pan American Health Organization. 2021; Available from: https://www.paho.org/en/documents/epidemiological-update-occurrence-variants-sars-cov-2-americas-26-january-2021.
29. PHE statement on Variant of Concern and new Variant Under Investigation. Public Health England. 2021; Available from: https://www.gov.uk/government/news/phe-statement-on-variant-of-concern-and-new-variant-under-investigation.
30. Kilic, T., Weissleder, R., Lee, H. Molecular and Immunological Diagnostic Tests of COVID-19: Current Status and Challenges. iScience. 2020. 23(8): p. 101406.
31. MacKay, M. J., Hooker, A. C., Afshinnekoo, E., et al. The COVID-19 XPRIZE and the need for scalable, fast, and widespread testing. Nat Biotechnol. 2020. 38(9): pp. 1021-1024.
32. Investigation of novel SARS-CoV-2 variant: Variant of Concern 202012/01 (technical briefing 1). Public Health England. 2020; Available from: https://www.gov.uk/government/publications/investigation-of-novel-sars-cov-2-variant-variant-of-concern-20201201.
33. Risk assessment: SARS-CoV-2 - increased circulation of variants of concern and vaccine rollout in the EU/EEA, 14th update. European Centre for Disease Prevention and Control. 2021; Available from: https://www.ecdc.europa.eu/en/publications-data/covid-19-risk-assessment-variants-vaccine-fourteenth-update-february-2021.
34. Nguyen, N. N. T., McCarthy, C., Lantigua, D., et al. Development of Diagnostic Tests for Detection of SARS-CoV-2. Diagnostics (Basel). 2020. 10(11).
35. SARS-CoV-2 lateral flow antigen tests: evaluation of VUI-202012/01. Public Health England. 2020; Available from: https://www.gov.uk/government/publications/sars-cov-2-lateral-flow-antigen-tests-evaluation-of-vui-20201201/sars-cov-2-lateral-flow-antigen-tests-evaluation-of-vui-20201201.
36. Novel variants of SARS-CoV-2 and the impact on diagnostic testing. FIND. 2021; Available from: https://www.finddx.org/covid-19/novel-variants/.
37. Diagnostic testing for SARS-CoV-2. World Health Organization. 2021; Available from: https://www.who.int/publications/i/item/diagnostic-testing-for-sars-cov-2.
38. Borges, V. , Sousa, C., Menezes, L., et al. Tracking SARS-CoV-2 VOC 202012/01 (lineage B.1.1.7) dissemination in Portugal: insights from nationwide RT-PCR Spike gene drop out data. Virological. 2021; Available from: https://virological.org/t/tracking-sars-cov-2-voc-202012-01-lineage-b-1-1-7-dissemination-in-portugal-insights-from-nationwide-rt-pcr-spike-gene-drop-out-data/600.
39. Moreno, G., Braun, K., Larsen, B. B., et al. Detection of non-B.1.1.7 Spike Δ69/70 sequences (B.1.375) in the United States. Virological. 2021; Available from: https://virological.org/t/detection-of-non-b-1-1-7-spike-69-70-sequences-b-1-375-in-the-united-states/587.
40. Bal, A., Destras, G., Gaymard, A., et al. Two-step strategy for the identification of SARS-CoV-2 variant of concern 202012/01 and other variants with spike deletion H69-V70, France, August to December 2020. medRxiv. 2021. p. 10.1101/2020.11.10.20228528.
41. Detection and characterisation capability and capacity for SARS-CoV-2 variants within the EU/EEA. European Centre for Disease Prevention and Control. 2021; Available from: https://www.ecdc.europa.eu/en/publications-data/detection-and-characterisation-capability-and-capacity-sars-cov-2-variants.

42. Artesi, M., Bontems, S., Gobbels, P., et al. A Recurrent Mutation at Position 26340 of SARS-CoV-2 Is Associated with Failure of the E Gene Quantitative Reverse Transcription-PCR Utilized in a Commercial Dual-Target Diagnostic Assay. J Clin Microbiol. 2020. 58(10).
43. Wang, R., Hozumi, Y., Yin, C., et al. Mutations on COVID-19 diagnostic targets. Genomics. 2020. 112(6): pp. 5204-5213.
44. Policy for Evaluating Impact of Viral Mutations on COVID-19 Tests. US Food and Drug Administration. 2021; Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/policy-evaluating-impact-viral-mutations-covid-19-tests.
45. Launch of PCR genotyping assay portfolio to detect COVID-19 variants. Novacyt Group. 2021; Available from: https://novacyt.com/2021/02/02/launch-of-pcr-genotyping-assay-portfolio-to-detect-covid-19-variants/.
46. GSD NovaType SARS-CoV-2 ID (96 reactions). eurofins. 2021; Available from: https://www.eurofins-technologies.com/gsd-novatype-sars-cov-2-id-96-reactions.html.
47. SARS Kits and VirSNiP Assays. TIB MolBiol. 2021; Available from: https://www.tib-molbiol.de/covid-19.
48. Illumina COVIDSeq test. Illumina. 2021; Available from: https://emea.illumina.com/products/by-type/ivd-products/covidseq.html.
49. SIREN: SARS-CoV-2 immunity and reinfection evalulation. Public Health England. 2021; Available from: https://snapsurvey.phe.org.uk/siren/.
50. Considerations for implementing and adjusting public health and social measures in the context of COVID-19. World Health Organization. 2020; Available from: https://www.who.int/publications/i/item/considerations-in-adjusting-public-health-and-social-measures-in-the-context-of-covid-19-interim-guidance.
51. Policy responses to the coronavirus pandemic. Our World in Data. 2021; Available from: https://ourworldindata.org/policy-responses-covid.
52. Henley, J., Oltermann, P., Jones, S. Germany extends Covid lockdown amid concern over variants in Europe. The Guardian. 2021; Available from: https://www.theguardian.com/world/2021/jan/19/germany-extends-covid-lockdown-amid-concern-variants-europe.
53. van Dorp, L. Coronavirus new variant – genomics researcher answers key questions. The Conversation. 2020; Available from: https://theconversation.com/coronavirus-new-variant-genomics-researcher-answers-key-questions-152381.
54. Mahase, E. Covid-19: What have we learnt about the new variant in the UK? BMJ. 2020. 371: p. m4944.
55. Makoni, M. South Africa responds to new SARS-CoV-2 variant. The Lancet. 2021. 397(10271): p. 267.
56. Bartsch, M., Bohr, F., von Bredow, R., et al. Can We Stop a Super Coronavirus? Spiegel International. 2021; Available from: https://www.spiegel.de/international/world/can-germany-stop-the-new-supervirus-a-e9ffc207-0015-4330-8361-b306f6053e15.
57. Covid-19: Ireland enforces hotel quarantine for travellers from South Africa. news24. 2021; Available from: https://www.news24.com/news24/world/news/covid-19-ireland-enforces-hotel-quarantine-for-travellers-from-south-africa-20210126.
58. German hospital under quarantine over more infections coronavirus variant. Reuters. 2021; Available from: https://www.reuters.com/article/us-health-coronavirus-germany-variant-idUSKBN29S0KQ.
59. Chu, D. K., Akl, E. A., Duda, S., et al. Physical distancing, face masks, and eye protection to prevent person-to-person transmission of SARS-CoV-2 and COVID-19: a systematic review and meta-analysis. The Lancet. 2020. 395(10242): pp. 1973-87.
60. Morris, L., Noack, R. Europe's growing mask ask: Ditch the cloth ones for medical-grade coverings. The Washington Post. 2021; Available from: https://www.washingtonpost.com/world/europe/europe-coronavirus-masks-regulations/2021/01/20/23463c08-5a74-11eb-a849-6f9423a75ffd_story.html.
61. Nogrady, B. How kids' immune systems can evade COVID. Nature. 2020; Available from: https://www.nature.com/articles/d41586-020-03496-7.
62. Lewis, D. What new COVID variants mean for schools is not yet clear. Nature. 2021; Available from: https://www.nature.com/articles/d41586-021-00139-3.
63. Vaccine deployment in the UK: letter to Chief Executives of all NHS trusts and foundation trusts, all PCNs and all GP practices. NHS England. 2020; Available from: https://www.england.nhs.uk/coronavirus/wp-content/uploads/sites/52/2020/12/C0994-System-letter-COVID-19-vaccination-deployment-planning-30-December-2020.pdf.

64. Editorial. Science advisers: publish evidence behind COVID vaccine dosing strategy. Nature. 2021; Available from: https://www.nature.com/articles/d41586-021-00045-8.
65. Oude Munnink, B. B., Sikkema, R. S., Nieuwenhuijse, D. F., et al. Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. Science. 2021. 371(6525): pp. 172-177.
66. Lauring, A. S., Hodcroft, E. B. Genetic Variants of SARS-CoV-2—What Do They Mean? JAMA. 2021. p. 10.1001/jama.2020.27124.
67. SARS-CoV-2 in mink: recommendations to improve monitoring. European Centre for Disease Prevention and Control. 2021; Available from: https://www.ecdc.europa.eu/en/news-events/sars-cov-2-mink-recommendations-improve-monitoring.
68. Denmark to test all positive Covid-19 swabs for infectious variants. The Local. 2021; Available from: https://www.thelocal.dk/20210113/denmark-to-test-all-positive-covid-19-swabs-for-infectious-variants.
69. The spread of the UK coronavirus variant in the Netherlands. National Institute for Public Health and the Environment, The Netherlands. 2020; Available from: https://www.rivm.nl/en/news/spread-of-uk-coronavirus-variant-in-netherlands.
70. Update on the spread of the UK coronavirus variant (VOC 202012/01) in the Netherlands. National Institute for Public Health and the Environment, The Netherlands. 2021; Available from: https://www.rivm.nl/en/news/update-on-spread-of-uk-coronavirus-variant-voc-20201201-in-netherlands.
71. All Lansingerland residents tested after finding British corona variant. Netherlands News Live. 2021; Available from: https://netherlandsnewslive.com/all-lansingerland-residents-tested-after-finding-british-corona-variant/59144/.
72. de Wet, P. Govt quietly made not wearing a mask a direct criminal act – 'reluctantly', says NDZ. Business Insider. 2021; Available from: https://www.businessinsider.co.za/not-wearing-a-mask-is-now-a-direct-criminal-offence-after-reluctant-rules-update-2021-2.
73. Surge testing to be deployed to monitor and suppress spread of COVID-19 variant. Department of Health and Social Care. 2021; Available from: https://www.gov.uk/government/news/surge-testing-to-be-deployed-to-monitor-and-suppress-spread-of-covid-19-variant.
74. COVID-19. Weekly epidemiological update - 16 February 2021. World Health Organization. 2021; Available from: https://www.who.int/publications/m/item/weekly-epidemiological-update---16-february-2021.
75. Statement on the sixth meeting of the International Health Regulations (2005) Emergency Committee regarding the coronavirus disease (COVID-19) pandemic. World Health Organization. 2021; Available from: https://www.who.int/news/item/15-01-2021-statement-on-the-sixth-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-coronavirus-disease-(covid-19)-pandemic.
76. Inzaule, S. C., Tessema, S. K., Kebede, Y., et al. Genomic-informed pathogen surveillance in Africa: opportunities and challenges. Lancet Infect Dis. 2021.
77. Statement on new SARS-CoV-2 variant with multiple spike protein mutations. Africa CDC. 2020; Available from: https://africacdc.org/news-item/statement-on-new-sars-cov-2-variant-with-multiple-spike-protein-mutations/.
78. Risk Assessment: Risk related to the spread of new SARS-CoV-2 variants of concern in the EU/EEA – first update. European Centre for Disease Prevention and Control. 2021; Available from: https://www.ecdc.europa.eu/en/publications-data/covid-19-risk-assessment-spread-new-variants-concern-eueea-first-update.
79. Tegally, H., Wilkinson, E., Lessells, R. J., et al. Sixteen novel lineages of SARS-CoV-2 in South Africa. Nat Med. 2021.
80. SARS-CoV-2 Variants - Disease Outbreak News. World Health Organization. 2020; Available from: https://www.who.int/csr/don/31-december-2020-sars-cov2-variants/en/.
81. Threat Assessment Brief: Rapid increase of a SARS-CoV-2 variant with multiple spike protein mutations observed in the United Kingdom. European Centre for Disease Prevention and Control. 2020; Available from: https://www.ecdc.europa.eu/en/publications-data/threat-assessment-brief-rapid-increase-sars-cov-2-variant-united-kingdom.
82. COV-GLUE. University of Glasgow. 2021; Available from: http://cov-glue.cvr.gla.ac.uk/#/home.
83. Cyranoski, D. Alarming COVID variants show vital role of genomic surveillance. Nature. 2021; Available from: https://www.nature.com/articles/d41586-021-00065-4.
84. DSI to pump R25m for KRISP as scientists grapple with COVID-19 variant. Devdiscourse. 2020; Available from: https://www.devdiscourse.com/article/health/1378309-dsi-to-pump-r25m-for-krisp-as-scientists-grapple-with-covid-19-variant.

85. Germany plans more tests, sequencing to deal with new coronavirus variants. Reuters. 2021; Available from: https://www.reuters.com/article/us-health-coronavirus-germany/germany-plans-more-tests-sequencing-to-deal-with-new-coronavirus-strains-idUSKBN29N0KN.
86. Lee, J. Fear of new virus variant pushes U.S. toward more genomic sequencing. MarketWatch. 2021; Available from: https://www.marketwatch.com/story/fear-of-new-virus-variant-pushes-u-s-toward-more-genomic-sequencing-11610738193.
87. US plays catch-up on genomic sequencing to track Covid variants. Financial Times. 2021; Available from: https://www.ft.com/content/1aa2f05c-1311-4480-a549-4ee22f9857c1.
88. C.D.C. Announces $200 Million 'Down Payment' to Track Virus Variants. New York Times. 2021; Available from: https://www.nytimes.com/2021/02/17/health/coronavirus-variant-sequencing.html.
89. U.S. is blind to contagious new virus variant, scientists warn. New York Times. 2021; Available from: https://www.nytimes.com/2021/01/06/health/coronavirus-variant-tracking.html.
90. Emerging SARS-CoV-2 Variants. Centers for Disease Control and Prevention. 2021; Available from: https://www.cdc.gov/coronavirus/2019-ncov/more/science-and-research/scientific-brief-emerging-variants.html.
91. Epidemiology and Laboratory Capacity for Prevention and Control of Emerging Infectious Diseases (ELC). Centers for Disease Control and Prevention. 2021; Available from: https://www.cdc.gov/ncezid/dpei/epidemiology-laboratory-capacity.html.
92. AMD (advanced molecular detection) Activities: 2020. Centers for Disease Control and Prevention. 2020; Available from: https://www.cdc.gov/amd/whats-new/amd-activities-2020.html.
93. Press release: Illumina and Helix Collaborate to Assess Prevalence of New SARS-CoV-2 UK Variant (B.1.1.7) in the US and Develop National Surveillance Infrastructure. Illumina. 2021; Available from: https://emea.illumina.com/company/news-center/press-releases/press-release-details.html?newsid=ba453369-4367-424f-8f5d-eacdff3ca5f0.
94. COVID-19 vaccine tracker. Regulatory Affairs Professionals Society. 2021; Available from: https://www.raps.org/news-and-articles/news-articles/2020/3/covid-19-vaccine-tracker.
95. There are four types of COVID-19 vaccines: here's how they work. Gavi, The Vaccine Alliance. 2021; Available from: https://www.gavi.org/vaccineswork/there-are-four-types-covid-19-vaccines-heres-how-they-work.
96. Walls, A. C., Park, Y. J., Tortorici, M. A., et al. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell. 2020. 183(6): p. 1735.
97. Clinical trial: Safety and Immunogenicity Trial of an Oral SARS-CoV-2 Vaccine (VXA-CoV2-1) for Prevention of COVID-19 in Healthy Adults. National Institutes of Health. 2020; Available from: https://clinicaltrials.gov/ct2/show/NCT04563702.
98. CoVepiT. OSE Immunotherapeutics. 2021; Available from: https://ose-immuno.com/en/ose-product/covepit/.
99. Covid: South Africa halts AstraZeneca vaccine rollout over new variant. BBC. 2021; Available from: https://www.bbc.co.uk/news/world-africa-55975052.
100. Mahase, E. Covid-19: Novavax vaccine efficacy is 86% against UK variant and 60% against South African variant. BMJ. 2021. 372: p. n296.
101. Johnson & Johnson Announces Single-Shot Janssen COVID-19 Vaccine Candidate Met Primary Endpoints in Interim Analysis of its Phase 3 ENSEMBLE Trial. Johnson & Johnson. 2021; Available from: https://www.jnj.com/johnson-johnson-announces-single-shot-janssen-covid-19-vaccine-candidate-met-primary-endpoints-in-interim-analysis-of-its-phase-3-ensemble-trial.
102. FDA Issues Emergency Use Authorization for Third COVID-19 Vaccine. US Food and Drug Administration. 2021; Available from: https://www.fda.gov/news-events/press-announcements/fda-issues-emergency-use-authorization-third-covid-19-vaccine.
103. Liu, Y., Liu, J., Xia, H., et al. Neutralizing Activity of BNT162b2-Elicited Serum - Preliminary Report. N Engl J Med. 2021. p. 10.1056/NEJMc2102017.
104. Wu, K., Werner, A. P., Moliva, J. I., et al. mRNA-1273 vaccine induces neutralizing antibodies against spike mutants from global SARS-CoV-2 variants. bioRxiv. 2021. p. 10.1101/2021.01.25.427948.
105. Moderna COVID-19 Vaccine Retains Neutralizing Activity Against Emerging Variants First Identified in the U.K. and the Republic of South Africa. ModernaTx. 2021; Available from: https://investors.modernatx.com/news-releases/news-release-details/moderna-covid-19-vaccine-retains-neutralizing-activity-against.
106. ChAdOx1 nCov-19 provides minimal protection against mild-moderate COVID-19 infection from B.1.351 coronavirus variant in young South African adults. University of Oxford. 2021; Available

from: https://www.ox.ac.uk/news/2021-02-07-chadox1-ncov-19-provides-minimal-protection-against-mild-moderate-covid-19-infection.
107. Hopkins Tanne, J. Covid-19: Moderna plans booster doses to counter variants. BMJ. 2021. 372: p. n232.
108. Parsons, L. Pfizer/BioNTech to develop a booster dose for their COVID-19 vaccine. PMLiVE. 2021; Available from: http://www.pmlive.com/pharma_news/pfizerbiontech_to_develop_a_booster_dose_for_their_covid-19_vaccine_1362048.
109. Coronavirus (COVID-19) Update: FDA Issues Policies to Guide Medical Product Developers Addressing Virus Variants. US Food and Drug Administration. 2021; Available from: https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-issues-policies-guide-medical-product-developers-addressing-virus.
110. Collier, D. A., De Marco, A., Ferreira, Iatm, et al. SARS-CoV-2 B.1.1.7 sensitivity to mRNA vaccine-elicited, convalescent and monoclonal antibodies. medRxiv. 2021. p. 10.1101/2021.01.19.21249840.
111. Muik, A., Wallisch, A. K., Sanger, B., et al. Neutralization of SARS-CoV-2 lineage B.1.1.7 pseudovirus by BNT162b2 vaccine-elicited human sera. Science. 2021.
112. Xie, X., Liu, Y., Liu, J., et al. Neutralization of SARS-CoV-2 spike 69/70 deletion, E484K and N501Y variants by BNT162b2 vaccine-elicited sera. Nat Med. 2021.
113. Wang, Z., Schmidt, F., Weisblum, Y., et al. mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. bioRxiv. 2021. p. 10.1101/2021.01.15.426911.
114. Emary, K. R. W., Glolubchik, T., Aley, P. K., et al. Efficacy of ChAdOx1 nCoV-19 (AZD1222) Vaccine Against SARS-CoV-2 VOC 202012/01 (B.1.1.7). SSRN. 2021. p. 10.2139/ssrn.3779160.
115. Novavax COVID-19 Vaccine Demonstrates 89.3% Efficacy in UK Phase 3 Trial. Novavax. 2021; Available from: https://ir.novavax.com/news-releases/news-release-details/novavax-covid-19-vaccine-demonstrates-893-efficacy-uk-phase-3.
116. Boseley, S. Kent Covid variant mutation must be taken seriously, warns UK scientist. The Guardian. 2021; Available from: https://www.theguardian.com/society/2021/feb/10/mutated-kent-covid-variant-must-be-taken-seriously-warns-uk-scientist.
117. Wise, J. Covid-19: The E484K mutation and the risks it poses. BMJ. 2021. 372: p. n359.
118. Press release. UK to support rest of the world to find COVID-19 virus variants. Department of Health and Social Care. 2021; Available from: https://www.gov.uk/government/news/uk-to-support-rest-of-the-world-to-find-covid-19-virus-variants.