SARS-CoV-2: the importance of mutation testing and variant surveillance

Abstract

Scientists and healthcare professionals have developed countermeasures against SARS-CoV-2 in record time since the global emergency began, so we are no longer defenseless against the virus. Tools like vaccines and antivirals have been developed that are highly effective at preventing infection with the virus or serious manifestations of illness. Even as we make progress to limit the spread of SARS-CoV-2 and its impact on personal and public health, the virus continually evolves and produces new variants. Mutations can alter viral transmissibility, pathogenicity, and virulence. They can also impact vaccine-induced or natural immunity, the effectiveness of antiviral treatment regimens, and the accuracy of diagnostic tests. Variant surveillance will thus remain a critical component of public health efforts. Antiviral treatments are most effective when administered in the early stage of illness, and it may become necessary to verify the presence or absence of mutations that confer resistance to them.

Introduction

Viruses mutate, and SARS-CoV-2 is no exception [1]. Several SARS-CoV-2 variants have emerged since early 2020, including the alpha, beta, gamma, delta, and omicron variants of concern (VOCs). VOCs are of global public health significance, as they harbor genetic changes that can increase transmissibility, worsen illness severity, and/or reduce the effectiveness of public health measures, diagnostics, treatments, and vaccines [2].

The alpha variant was first reported in November 2020 (Table 1). It was identified in a sample collected in the United Kingdom (UK) two months earlier [3], and spread globally in early 2021. The alpha variant was then replaced by the more contagious delta variant [4]. The delta variant caused more severe illness in unvaccinated individuals and breakthrough infections among people who had natural or vaccine-induced immunity. However, breakthrough infections were thought to be rare. Vaccines remained highly effective at preventing severe illness, hospitalization, and death. The omicron variant was first reported in South Africa in late November 2021, and it spread globally more quickly than any of the previous variants. The omicron variant is believed to be more transmissible than the delta variant and causes breakthrough infections more often. While the omicron variant is the most transmissible variant detected to date, it seems to cause less severe illness. It must be emphasized that the omicron variant is highly diverse with multiple sublineages, and natural immunity against one sublineage does not guarantee protection against another [5,6].

Table 1. Comparison of SARS-CoV-2 alpha, delta, and omicron VOCs.

WHO label	Alpha	Delta	Omicron
Pango lineage	B.1.1.7	B.1.617.2	BA.1, BA.2, BA.3, BA.4, BA.5
Nexstrain clade	20I	21A, 21I, 21J	21M, 21K, 21L, 22C, 22A, 22B
Earliest documented samples	UK, September 2020	India, October 2020	Multiple countries, November 2021
Designated VOC	December 18, 2020	May 11, 2021	November 26, 2021
Transmissibility	30% to 50% more transmissible than the original SARS-CoV-2 strain	80% to 90% more transmissible than the alpha variant	More transmissible than the delta variant
Illness severity	Believed to be more severe	More severe in unvaccinated patients	Less severe
Breakthrough of vaccine-induced immunity	NA	Rare	Frequent

In addition to altering transmissibility and illness severity, viral mutations can interfere with diagnostic test performance and confer resistance to antiviral therapeutics. Here we discuss the impact of mutations on nucleic acid amplification tests (NAATs), also referred to as molecular tests, rapid antigen detection tests (RADTs), and antiviral drug and antibody treatments. We also review various methods of identifying and tracking genetic changes in viral populations. Sequencing the whole SARS-CoV-2 genome with technology like next-generation sequencing (NGS) and characterizing new genetic mutations can support public health measures, and complementing NGS with PCR-based genotyping can help expand variant surveillance efforts [7]. Starting treatment early is generally the best strategy for patient management. PCR genotyping enables rapid verification of the presence or absence of resistance mutations, and it may become a crucial tool to support physicians who select treatments.

Impact of SARS-CoV-2 variants on diagnostic test performance

From a public health perspective, it is vital to identify circulating variants and to detect any new variants in a population. Though leaders agree that vaccination is a critical driver in the transition of SARS-CoV-2 to an endemic virus, it will be necessary to maintain diagnostic testing, screening, and surveillance during and after this transition. NAATs and RADTs will thus continue to be important tools [8]. A high-level comparison of NAATs and RADTs is shown in Figure 1.

For testing to be meaningful, virus detection must be reliable. However, mutations in the viral genome may reduce test sensitivity and impede detection of the virus in positive specimens, which can lead to false negative results. The majority of available RADTs for SARS-CoV-2 detection target the nucleocapsid (N) protein. Variants like omicron harbor genome-wide mutations that include mutations in the N gene, and such mutations can impact the antigen–antibody interactions that RADTs require for virus detection. There are several reports of reduced RADT sensitivity for the omicron variant [9,10], although it is important to note that mutations in this variant do not impact the performance of all RADTs. However, the risk of new mutations and variants impacting the performance of RADTs is real, and accurate identification of mutations and circulating variants is critical to ensure the reliability of test results.

Mutations can also interfere with the performance of NAATs, which utilize sequence-specific primers and probes to detect SARS-CoV-2 RNA. Even a single point mutation can potentially impact the performance of an NAAT. Like RADTs, the performance of commercially available molecular *in vitro* diagnostic (IVD) tests may be impacted by the omicron variant. The United States Food and Drug Administration (FDA) has published a list of molecular tests for which performance is impacted by known viral mutations. The list includes tests that are expected to be unable to detect the omicron variant [11].

As future SARS-CoV-2 mutations cannot be predicted, robust tests that are unaffected by viral mutations will be beneficial. All Applied Biosystems™ TaqPath™ COVID-19 assays* employ target redundancy to compensate for new mutations. For example, the Applied Biosystems™ TaqPath™ COVID-19 RNase P Combo Kit 2.0 (EUA)* contains unique fluorescent probes for detecting the SARS-CoV-2 N gene, *orf1a*, and *orf1b*. To ensure that new mutations in these regions do not interfere with detection, multiple targets within each region are monitored in the same fluorescence channel. Including multiple targets in different genomic regions helps compensate for known and future mutations.

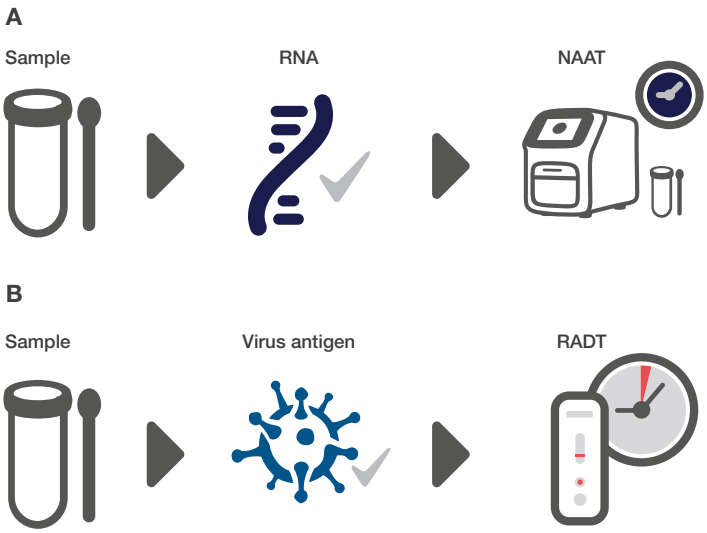

Figure 1. Comparison of nucleic acid amplification tests (NAATs) and rapid antigen detection tests (RADTs). (A) Viral RNA is extracted before detection with PCR-based NAATs. PCR requires sequence-specific oligonucleotides that are highly sensitive. Point-of-care NAATs can provide results in as little as 30 minutes, while a PCR run usually takes around 90 minutes once samples are purified. **(B)** Viral antigens are detected with antibodies in a RADT. Specimens can be used directly without prior purification. Many RADTs do not have the high level of analytical sensitivity that NAATs do, but they can be conveniently used at home and generally provide results within 15 minutes.

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Impact of variants on antiviral treatment

In this section we will focus on the mechanisms of antiviral drugs and monoclonal antibodies. We will not discuss procedures or treatment recommendations for SARS-CoV-2. Antiviral therapeutics for human infectious diseases can be directed against either viral or host targets. Targeting host factors has the advantage of minimizing the risk of drug resistance, and any virus that relies on the targeted cellular function may be inhibited. However, interfering with host function can have serious side effects [12]. In contrast, antivirals specifically designed for viral targets that lack cellular homologs may have few or no side effects. An antiviral drug that targets a protein with a key role in the life cycle of a virus may also be highly effective against a family of viruses that share the protein. An antiviral drug in this category may interfere with virus attachment, entry, uncoating, viral RNA translation, genome replication, viral assembly, or release from host cells. Key viral enzyme targets include polymerases, proteases, integrases, and reverse transcriptases [12]. The downside of these therapeutics is that viruses can mutate and potentially develop resistance to them.

Table 2 summarizes the SARS-CoV-2 antiviral drugs that have been recommended by the World Health Organization (WHO) and the FDA as of June 2022 [13,14]. Two of the drugs are nucleoside analogs that interfere with the function of SARS-CoV-2 RNA-dependent RNA polymerase. The other drug targets the SARS-CoV-2 protease M^{PRO}, which is a key enzyme in processing viral polyprotein into functional units. All three antiviral drugs are active against all known variants. However, a mutation that confers resistance to one of the nucleoside analogs has already been identified [15,16].

Some scientists are concerned that the virus will develop resistance to available antiviral therapeutics [17], but new antiviral drug candidates and combination therapies are currently under development [18]. Combination therapies generally reduce the potential for resistance, and synergistic combinations increase the likelihood of satisfactory treatment outcomes. However, if the virus develops resistance to one or more antiviral drugs, genotyping may be necessary before initiating treatment, to help ensure appropriate patient management.

Monoclonal antibodies (mAbs) are engineered antibodies that target specific and defined antigens. Antiviral mAbs can be administered for pre-exposure prophylaxis, known as passive immunization, or to treat an ongoing infection [19]. The SARS-CoV-2 spike protein is a common target, because it is key for receptor recognition and viral entry into host cells. Several mAb therapeutics designed to treat SARS-CoV-2 infection bind to the spike protein and neutralize the virus [20]. However, the omicron variant harbors mutations that alter the spike protein and interfere with certain mAb therapeutics (Table 3). The omicron variant is the most prevalent SARS-CoV-2 variant as of June 2022, and health authorities do not recommend treatment with antibodies that are known to be ineffective at neutralizing it [21,22].

Table 2. Overview of antiviral drugs for SARS-CoV-2 treatment.

Drug	Target	Route of administration
Remdesivir (nucleoside analog)	Interferes with RNA-dependent RNA polymerase	Intravenous injection
Molnupiravir (nucleoside analog)	Interferes with RNA-dependent RNA polymerase	Oral
Nirmatrelvir with ritonavir	Inhibits protease M^{PRO}. Ritonavir increases nirmatrelvir bioavailability by blocking cytochrome p450 3a4	Oral

Table 3. Overview of SARS-CoV-2 mAb therapeutics.

mAb therapeutic	Target	Anticipated clinical activity
Bebtelovimab	Spike protein	Active
Bamlanivimab with etesevimab	Receptor binding domain (RBD) of spike protein	Inactive or significantly reduced activity
Casirivimab with imdevimab	RBD of spike protein	Inactive or significantly reduced activity
Sotrovimab	RBD of spike protein	Inactive against BA.2 sublineage
Tixagevimab with cilgavimab	RBD of spike protein	Active against omicron BA.2 subvariant, but less active against BA.1 and subvariants

Methods for detecting SARS-CoV-2 mutations and variants

NGS is a powerful technology that has enabled scientists to detect SARS-CoV-2 and determine its genomic sequence in record time [23]. NGS makes it possible to routinely sequence >99% of the viral genome to accurately track circulating variants and identify newly emerging ones. Sanger sequencing is an alternative approach that is particularly useful for analyzing shorter, targeted segments of the SARS-CoV-2 genome like the spike gene. Another method for SARS-CoV-2 variant detection that has gained much attention is PCR-based genotyping. The three methods are compared in Table 4 [24].

The accuracy of PCR genotyping for identifying variants and subvariants was evaluated in a recent study initiated by the United States National Institutes of Health (NIH) [7]. The authors confirmed that PCR genotyping with a panel of specific markers could enable highly accurate variant identification. Eight- and twelve-marker panels were sufficient for identifying six and eight of the top ten WHO SARS-CoV-2 lineages, respectively. The study was executed before the omicron lineage emerged, and the authors reported that an increase in the number of undetermined calls, which occurred when variants could not be assigned to known positive samples, might signal the presence of a new variant.

PCR genotyping supports accurate and rapid detection of any point mutation in the viral genome, including resistance mutations. It can therefore act as a filter to select samples that should be sequenced by NGS to identify any potentially new mutations or variants. Once the genetic sequence of a new variant has been confirmed, new panel markers can be quickly added. In response to the emergence of the omicron variant, the team conducting the NIH study quickly developed a new genotyping panel to identify it. PCR genotyping with markers that are specific to mutations in omicron sublineages can enable detection of the BA.1, BA.2, BA.3, BA.4, and BA.5 subvariants.

Summary

Viral mutations can interfere with the effectiveness of antiviral treatment regimens and reduce the accuracy of viral diagnostic tests. Surveillance to detect mutations and variants is critical to supporting public health measures to combat SARS-CoV-2 and can directly impact treatment guidelines and recommendations. Testing laboratories have increased their sample throughput capabilities in response to SARS-CoV-2 by acquiring real-time PCR instruments, which has brought molecular testing to the forefront.

PCR genotyping is a powerful method that can enable scale-up of variant surveillance and the identification of resistance mutations. Given the wide availability of quantitative PCR technology, PCR genotyping can support early detection of new variants and quickly confirm known viral mutations. Identifying and tracking variants remains crucial in public health efforts to continually monitor vaccine and treatment efficacy and to assess the accuracy of diagnostic tests. Moving forward, identifying SARS-CoV-2 variants and key genomic mutations may support effective personalized treatment regimens. Consequently, rapid and accurate testing methods like PCR genotyping may become key for optimal patient management.

Table 4. Comparison of sequencing methods and PCR genotyping for SARS-CoV-2 surveillance.

	PCR genotyping	Sanger sequencing	NGS
Sample-to-result time*	Within one day	Within one day	Multiple days
Platform availability	High	Medium	Medium
Whole-genome sequencing	No	Usually targeted region(s)	Yes
Detection of known mutations	Yes	Yes	Yes
Tracking variants	Yes	Yes	Yes
Identifying new variants	Supportive	Yes	Yes

* Actual turnaround times vary.

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