Rapid Communication

SARS-CoV-2 Variant Testing

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Audience: Providers, Laypersons, and Generalists

Background for Variant Testing: Definitions and Epidemiology

Since all viruses mutate, it is common to find multiple strains (*aka* lineages or variants) of the same virus spreading simultaneously, and this is true of the COVID-19 pandemic.[1] Unique SARS-CoV-2 variants are grouped and defined as those strains containing a common set of genetic mutations. Emerging SARS-CoV-2 variants can be problematic if one or more of the independent mutations result in changes that make the virus more pathogenic, resistant to treatment, able to escape vaccines, or able to evade diagnostic tests.

Monitoring the spread of emerging SARS-CoV-2 variants in the United States relies on rapid molecular characterization of most, if not all, of the viral genome. This characterization is best accomplished using a sequencing method, commonly called next-generation or metagenomic sequencing or NGS.[2] NGS is a laboratory method that can identify SARS-COV-2 variants, which are reported to public health authorities for the purposes of strain surveillance and epidemiology.[3] In addition to NGS, detection methods, such as reverse transcriptase polymerase chain reaction (RT-PCR) can identify known mutations using rapid, accessible, and high-throughput methods. The Centers for Disease Control and Prevention's (CDC's) national SARS-CoV-2 Strain Surveillance program is comprehensive and population-based.[4] It monitors the SARS-CoV-2 genome and identifies emerging SARS-CoV-2 variants to determine implications for COVID-19 diagnostics, therapy, vaccines, and public health interventions.[5]

Identification of circulating viral variants can provide a link to epidemiological and biological events, and enable local and national tracking. These data can be used to inform national and state public health actions.[6] Variants are characterized as minor variants with no impact, variants of interest (VOI), variants of concern (VOC), or variants of high consequence (VOHC), the highest threat level.[5]

SARS-CoV-2 Variants with CDC Classification

B.1.1.7 lineage (VOC). Originating in the United Kingdom, the B.1.1.7 variant contains many genetic mutations. The variant was first identified in September 2020. Since then, the variant can be found in numerous countries worldwide. The strain was found in the United States at the end of December 2020. The strain has become the most common U.S. strain and is associated with increased transmissibility (i.e., more efficient with rapid transmission) and increased mortality.

B.1.351 lineage (VOC). In South Africa, the B.1.351 variant emerged independently of B.1.1.7, although the variant shares some genetic mutations with the strain. B.1.351 was first identified in South Africa in samples dating back to early October 2020. Cases have since been detected in various countries, including the U.S. in 2021.

P.1 lineage (VOC). The P.1 variant was first identified in Brazilian travelers. The variant has 17 unique genetic mutations, including three in the Receptor Binding Domain (RBD) region of the spike protein. Surveillance detected the variant in the US at the end of January 2021. The P.1 variant represents a branch from the B.1.1.28 lineage. Evidence suggests that some of the mutations in the P.1 variant may affect transmissibility and antigenic profile. The variant's emergence and association with a higher viral density raised concerns about potential increased transmission or a propensity for re-infection.

B.1.427 and B.1.429 (VOC). Both coronavirus strains from California are now officially characterized. The strains can be 20% more transmissible than common strains found in California, Nevada, Arizona, Wisconsin, and Virginia.

B.1.526 (VOI). First detected in New York in November 2020, the B.1.526 is a variant of interest. By February 2021 mutations appeared in around 25% of all the COVID genomes.

Multiple SARS-CoV-2 variants are circulating globally.[7] The CDC currently lists common strains, their defining mutations, and risk designation.[8] These are described briefly below. The World Health Organization (WHO) adopts a different nomenclature than the CDC.[9]

Why are sequencing and identification of variants important?

Identifying SARS-CoV-2 variants in clinical specimens serves two primary purposes that can be broadly classified into public health or clinical care domains. Within the public health domain, NGS is commonly used for whole-genome sequencing (WGS) of clinical specimens and provides a population-level unbiased analysis of the specific viral strains in circulation and monitors changes in the viral genome over time. These data can be used to track the spread of particular strains locally, regionally, and nationally, which can aid in outbreak investigation and studies of viral transmission within communities, healthcare facilities, schools, or other workplaces. Importantly, this type of surveillance can also identify the rapid emergence or introduction of a specific variant associated with increased transmissibility, reduced vaccine efficacy, or severe disease. State and national laboratories coordinate sequencing efforts for surveillance and public health purposes, and the sequencing results are not routinely reported to the patients or healthcare providers for direct patient care purposes.

Within the clinical domain, the goal of analyzing SARS-CoV-2 sequences is to improve the care of an individual patient. A growing list of specific mutations that either independently, or in conjunction with other mutations are associated with increased transmissibility, disease severity, reduced treatment efficacy, or potential for vaccine failure. Some of these mutations are more common in specific "variants" such as the "U.K. variant" (B.1.1.7) or the "New York variant" (B.1.526); however, a given mutation may not be present in all viruses within a specific variant type. [5] Sequence analysis may be accomplished by WGS, targeted sequencing of a portion of the genome (e.g., spike protein), or RT-PCR tests that target a specific mutation(s). These tests may be conducted by a hospital or other accredited reference laboratory, and results are reported to the patient and healthcare provider.[10] Notably, the specific contribution of these mutations to each variant's attributes is not entirely understood, and there is no definitive evidence that directly links a given mutation to poor outcomes, significantly reduced efficacy of SARS-CoV-2 therapies, or vaccine coverage. [11]

When should SARS-CoV-2 sequencing be used or requested, and for whom?

Currently, sequencing of SARS-CoV-2 is used primarily for public health applications. Identifying the circulating and prevailing variants of SARS-CoV-2 that may be associated with increased infectivity, transmission, or severity of infection in a given community or geographic region is essential for resource planning and implementation of effective mitigative measures to reduce the risk of infection.[12] Surveillance for specific mutations in the viral genomic sequences of circulating variants is also necessary to identify potential problems (e.g., false-negative test result) with diagnostic nucleic acid and antigen assays that are used to detect SARS-CoV-2.[13] Such testing is usually conducted by public health laboratories or large genomic sequencing centers who have the capability to perform high-throughput sequencing on positive clinical specimens among the infected population.

For direct medical care of a COVID-19 patient, sequencing of SARS-CoV-2 present in the positive clinical specimens may serve three possible functions: 1) to distinguish between persistent infection with the same viral strain and re-infection with a new viral strain, which aids in implementation of interventions to prevent re-infection in a patient with repeatedly positive SARS-CoV-2 results; 2) to detect and identify specific viral spike protein (S) gene mutations in certain variants that are potentially resistant or less susceptible to neutralizing antibodies or monoclonal antibodies in patients who are not responding to such therapy for COVID-19;[14, 15] and 3) to detect and identify viral S gene substitutions in specific variants that are potentially resistant or less susceptible to vaccine-induced S-protein neutralizing antibodies in individuals who develop COVID-19 after successful vaccination (i.e., detectable S-specific antibodies in serum or plasma after vaccination).[16] In each of the three above situations, identification of variants, and specific S codon substitutions could have direct impact on the affected patients' ongoing medical management.

What samples are used for NGS sequencing to identify variants?

Testing to identify which viral variant is present in a patient's specimen is not routine. Variant identification via genetic sequencing is a way to provide epidemiology results, and it is not usually intended for diagnosis of infection. Detecting a virus with a target-specific method is not the same as viral strain identification, which occurs by performing complete genetic sequence analysis.

Variant identification can only occur for positive specimens placed in viral transport media (e.g., universal transport media or viral transport media) and cannot be performed from dry swabs used in some point of care test methods. Likewise, certain tests methods use transport media that interferes with sequencing and would not be used for variant identification. Finally, pooled samples cannot be used for NGS analysis.

Clinical specimens used for strain identification should ideally be the same clinical specimens that were already found to be strongly positive for SARS-CoV-2 by target-based detection assays.[17] Since some routine molecular test methods can be more sensitive for viral detection than NGS; target specific mutation assays for VOCs are also being developed. Full sequencing would be required to identify novel mutations in strongly positive samples. Currently, no matter the method used, viral strain identification is most useful for epidemiology purposes.

There are situations when genetic variants might be suspected and testing positive samples might be warranted. For example, samples collected from:

Patients with reinfection(s): Reinfection with SARS-CoV-2 does occur and can occur with the same strain, a different strain, or with

multiple strains. Sequencing may be useful to assess the virus's complete genetic sequence for public health purposes.

Patients who develop breakthrough infections: Fully vaccinated patients can develop a new laboratory-confirmed COVID-19 infection with known strains or with new strain variants.

Hospitalized patients with re-infection or vaccine escape: Sequencing these samples may be a way to enrich the likelihood of identifying new viral variants via genetic sequencing.

Patients with treatment failure: As more treatment options become available, prospective sequencing may assist selection of the most effective therapy.

Disclaimer

Standard of practice is not defined by this article, and there may be alternatives. This article was developed to be of assistance to laboratory and other health care professionals by providing guidance and recommendations for a particular area of practice. It should not be considered inclusive of all proper approaches or methods, or exclusive of others. The guidance contained herein cannot guarantee any specific outcome, nor does it establish a standard of care. The article is not intended to dictate the treatment of a particular patient. Treatment decisions must be made on the basis of the independent judgment of health care providers and each patient's individual circumstances. The Association for Molecular Pathology (AMP), Infectious Diseases Society of America (IDSA), and Pan American Society for Clinical Virology (PASCV) each make no warranty, express or implied, regarding this article and specifically exclude any warranties of merchantability and fitness for a particular use or purpose. Neither AMP, IDSA, nor PASCV shall be liable for any direct, indirect, special, incidental, or consequential damages related to the use of the information contained herein.

Link for List of AMP member laboratories that offer variant testing.

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