Diagnosing Emerging Variants of SARS-CoV-2 for Effective Transmission Control

New variants have affected the accuracy of current testing methods, and there is urgent need to develop new tests to meet the constantly changing landscape of the COVID-19 pandemic

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Nearly two years after the emergence of COVID-19, the rapid spread of SARS-CoV-2 still presents a major global health threat that is devastating healthcare systems around the world. Increasing evidence suggests that transmission by infected individuals that do not display symptoms (asymptomatic) and individuals who are infectious before displaying symptoms (pre-symptomatic) is driving an increase in the infection rate (R value). It is therefore essential to routinely monitor the infection status of the general population, control transmission, and enable the lifting of restrictions on daily life while COVID-19 vaccinations and novel therapeutic agents are developed and implemented.

However, the continued persistence of SARS-CoV-2 in the population has facilitated the emergence of novel variants of concern (VOCs) with evolutionary advantages that potentially decrease the effectiveness of current diagnostic platforms and vaccines, while increasing transmissibility and virulence (1-2). VOCs are identified and continually monitored by international and national organisations, such as WHO, the European Centre for Disease Prevention and Control (ECDC), and Public Health England (Table 1) (3-4).

The SARS-CoV-2 Delta variant is among the newly emerged VOCs, with clear evidence that it is more transmissible than previous variants and spreading more quickly through the population, leading to a rapid increase in infection rates. Several studies also demonstrate that Delta variant infection results in higher hospitalisation rates in unvaccinated individuals (5-7). This highlights the critical need for highly specific and sensitive rapid diagnostic tests for large-scale population screening, that are routinely validated against new variants, to limit the transmission of SARS-CoV-2.

Types of Diagnostic Tools

Currently, SARS-CoV-2 can be detected using molecular tests that detect the viral genome, such as RT-PCR, isothermal amplification and CRISPR assays, or protein and immunological assays, such as ELISAs and lateral flow devices (LFDs).

LFDs are disposable rapid tests that detect an active infection by identifying the presence of viral antigens in oropharyngeal, nasal, or nasopharyngeal swab samples, using highly specific immunological reagents, such as antibodies and antibody alternatives (e.g., Affimer® technology) (8). Unlike other molecular or immunological assays, LFDs are simple to use, do not require trained staff or specialised equipment, and provide results rapidly. LFDs are, therefore, well positioned to support cost-effective, rapid population screening and point-of-care (POC) diagnostics. In addition, they are easy to mass-produce and transport worldwide.

Accuracy and reliability of these tests can be determined by assessing their sensitivity, which is defined as the proportion of infected individuals who test positive, and specificity; the proportion of non-infected individuals who test negative. A test

for mass screening needs to be sensitive enough to identify infectious individuals and specific enough to exclude people that are not currently infected.

Challenges in Monitoring SARS-CoV-2 Using LFDs

There are several factors affecting the accuracy of LFDs: low viral load at the time of testing, incorrect sample isolation, and user error resulting in false negative or false positive results. A false negative result, for example, may occur if a sample is taken from a recently infected individual with a currently undetectable viral load. Although low viral load is not usually associated with being infectious, early intervention can be advantageous as load tends to increase over time, and the individual may go on to subsequently infect other people, especially if they are not frequently testing due to an assumption that the initial negative result is correct. Tests vary in how sensitive they are at detecting viral levels from different stages of infection and, therefore, to negate this issue, the UK Government advice, at the time of writing, is to perform two self-administered LFDs per week (9).

Conversely, false positive results may arise from testing populations with LFDs that lack high specificity. RT-PCR, which offers more sensitive testing, but requires a longer time to see results, is recommended after a positive LFD to confirm

Figure 1: Diagnostic accuracy of AffiDX SARS-CoV-2 antigen lateral flow device in detecting SARS-CoV-2 Delta variant, in comparison with RT-PCR testing. In an independent study, AffiDX SARS-CoV-2 rapid antigen test was found to successfully detect SARS-CoV-2 in 100% of samples derived from a cohort of both symptomatic and asymptomatic patients infected with the Delta variant

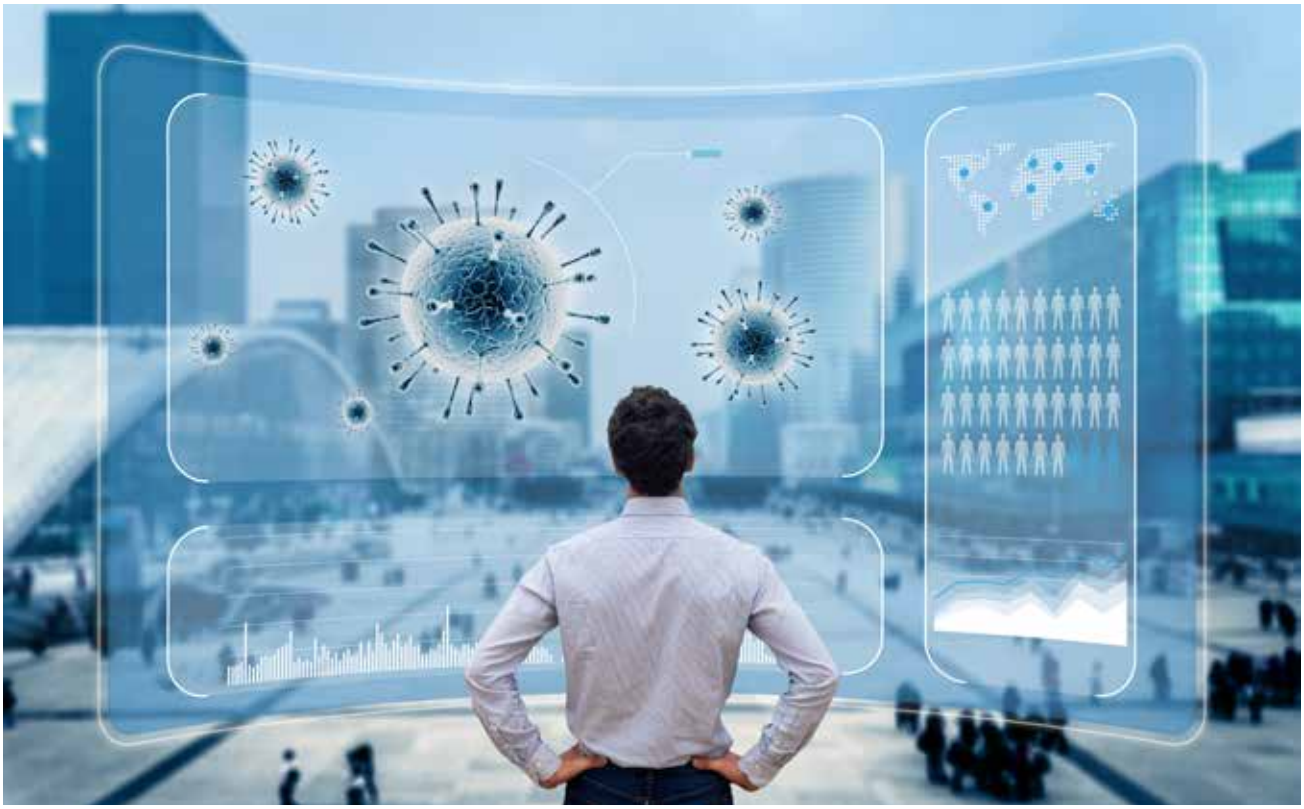
WHO label	Lineage	Spike mutations of interest	Earliest documented samples	Date of designation
Alpha	B.1.1.7	E484K, S494P, N501P	United Kingdom, Sep-2020	18-Dec-2020
Beta	B.1.351	K417N, E484K, N501Y, D614G, A701V,	South Africa, May-2020	18-Dec-2020
Gamma	P.1	K417T, E484K, N501Y, D614G, H655Y	Brazil, Nov-2020	11-Jan-2021
Delta	B.1.617.2	L452R, T478K, D614G, P681R,	India, Oct-2020	11-May-2021
Omicron	B.1.1.529	A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211-212, ins214EPE, etc.	South Africa, Nov-2021	26-Nov-2021

Figure 2: SARS-CoV-2 variants of concern (VOCs), according to the WHO. Table based on data on currently designated VOCs, published by WHO (4). Last updated November 29th, 2021

the infection status of an individual. This confirmation will either lead to the isolation of true positives or release of false positives. Sensitivity issues with LFDs occur when the viral loads are low. Viral loads in a sample are generally estimated by referring to the number of PCR amplification cycles needed to detect the virus. If the cycle threshold (Ct) is low (below 25) the levels of viable virus in the sample are high, suggesting that the individual is likely to be infectious (10).

Hundreds of SARS-CoV-2 LFDs are currently in development and several are already approved for either professional or self-administered use (11). LFDs detect SARS-CoV-2 by using monoclonal antibodies (or antibody alternatives) specific to viral proteins, such as the nucleocapsid (N) and spike (S) proteins. While the majority of approved LFDs detect the N protein, mutations in both the S and N proteins in circulating VOCs are a source of great concern, with researchers regularly monitoring vaccine efficiency against variants (1, 12).

Considering the increasing role of LFDs in routine population screening to identify and isolate potentially infectious individuals, evaluating rapid LFDs and their ability to accurately detect VOCs should be prioritised. Recent studies reported that a group of widely available LFDs targeting the N protein region of the virus fail to detect some variants of SARS-CoV-2 in certain samples (2, 12). Utilisation of antigen assays that are adversely



affected by SARS-CoV-2 mutations in the N protein may lead to positive selection for 'undetectable' and yet more transmissible variants (2). As a result, it is critical to develop tests that are able to detect alternative viral regions, such as the S protein.

Keeping up With Emerging SARS-CoV-2 Variants

Very few SARS-CoV-2 LFDs available on the market have been clinically evaluated against VOCs, and, more specifically, the Delta variant. Even those tested are usually evaluated based on an *in silico* laboratory approach, rather than using real-life patient samples.

The vast majority of LFDs rely on using monoclonal antibodies which, though invaluable in the field of diagnostics and therapeutics, are not without their limitations. Antibodies are large and complex molecules, weighing around 150 kDa, and are produced using animal models through time-consuming and costly workflows. Developing tests that can detect emerging SARS-CoV-2 mutations using monoclonal antibodies is therefore challenging since they are expensive to manufacture and take time to produce. Over the years there have been considerable efforts to develop non-antibody affinity ligands which demonstrate high specificity and sensitivity capabilities – such ligands include DARPins, affibodies, avimers, and Affimer reagents (13). Affimer reagents, for example, have been used to develop a novel LFD, specific to the SARS-CoV-2 S protein, with 98% sensitivity (Ct ≤ 31) and 99% specificity. In a recent independent validation, this Affimer-based technology was found to successfully detect SARS-CoV-2 in all samples, with 100% sensitivity, from a cohort of symptomatic and asymptomatic patients confirmed to be infected with the Delta variant (see **Figure 1,** page 41) (14). Manufacturers of LFDs, as well as governments utilising such tools for population screening, should, as a matter of urgency, ensure that LFDs currently on the market can detect emerging VOCs.

Curbing the COVID-19 pandemic requires the combined use of therapeutic reagents and diagnostic assays. At this stage, there is a global pressure to constantly combat new waves of SARS-CoV-2 infection led by the serial emergence of variants with higher transmissibility and virulence rates. Though RT-PCR assays were the gold standard for accurate diagnosis at the start of the pandemic, rapid POC testing has since proven itself to be an invaluable low-cost tool that provides nearly immediate results, without overwhelming diagnostic laboratories and healthcare services. With recent advancements in our understanding of SARS-CoV-2 pathology and epidemiology, regularly monitoring population infection rates will help limit exposure to both symptomatic and asymptomatic individuals. Routinely validating SARS-CoV-2 LFDs against emerging VOCs using clinical samples is of utmost importance to ensure continued diagnostic accuracy.

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