Molecular Diagnostics

How COVID-19 testing and surveillance tools target a changing virus

Highlighting the critical role of PCR technology in identifying SARS-CoV-2 infections and tracking mutations

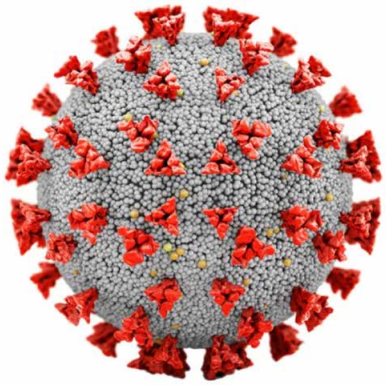
Introduction

Since it was first discovered in late 2019, SARS-CoV-2 has undergone several genetic changes. These mutations have affected how contagious the virus is, the severity and symptoms associated with COVID-19, and the efficacy of vaccines and treatments.

The introduction of variants also raises concerns around the reliability of testing and surveillance solutions that are critical to understanding and limiting the spread of disease. How can we help ensure existing detection platforms remain accurate in identifying COVID-19–positive patients as the virus mutates? And what is the most efficient way to track and learn about variants with these new strains circulating in communities? Since the start of the pandemic, Thermo Fisher Scientific has been working to address these central questions by developing tools that support mitigation efforts with mutations in mind.

In the following articles, we address crucial uncertainties pertaining to the robustness of existing solutions as the virus evolves. This e-book,* which consists of blog posts written by our scientists, outlines how Thermo Fisher's leadership in COVID-19 testing and surveillance technologies has proven reliable over time in the face of a dynamic pathogen. It also includes an explanation of the S gene advantage that is helping to identify variant trends.

* Disclaimer: This e-book was created using a series of blogs that were each accurate and current at the original time of publication.



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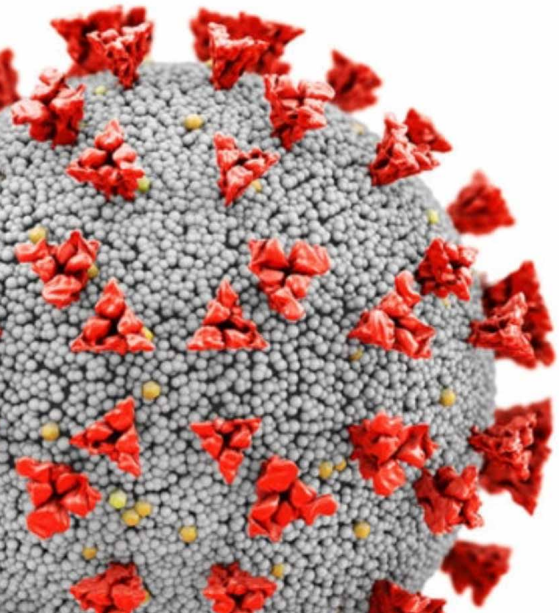


Chapter 1

As the SARS-CoV-2 virus evolves, PCR diagnostic and surveillance solutions help stay ahead of viral variants

This blog was originally published in March 2022

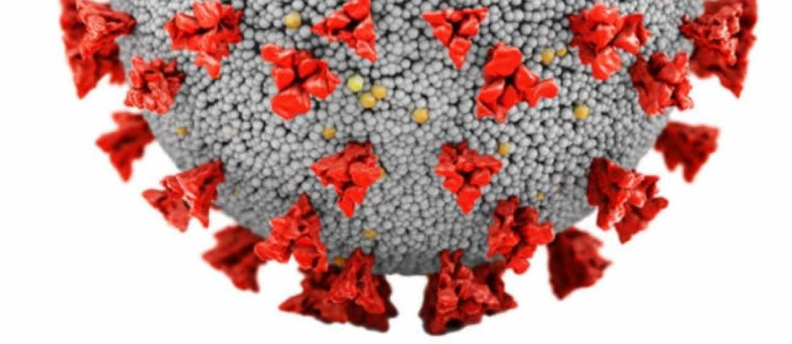
When SARS-CoV-2 first started rapidly spreading just a little over two years ago, the ability to identify and track positive cases within communities became critical to helping gain control of the pandemic. The molecular diagnostics community met this global challenge by developing accurate, reliable PCR solutions for COVID-19 monitoring and detection at lightning speed. As the virus has circulated around the world infecting over 400 million individuals and changed along the way [1], these tools and technologies have gotten faster, smarter, cheaper, and more accessible to keep up with unexpected variants and unprecedented demands.

During **a recent Labroots webinar**, Thermo Fisher Scientific's Vanessa Lacey, PhD,* and Jelena Feenstra, PhD,** recapped highlights from the COVID-19 detection and surveillance journey thus far, offering insight into how existing tools were designed to remain resilient as the fate of this unpredictable virus remains uncertain.

Molecular tests, such as PCR, remain the most reliable method for COVID-19 detection

PCR and other nucleic acid amplification tests work by targeting and amplifying sections of RNA specific to SARS-CoV-2. These tests are highly sensitive, with the PCR method remaining the gold standard for COVID-19 detection. Antigen tests, which work by binding to specific proteins on the surface of the virus, are also widely available in the community but require more of the virus to be present in a sample to trigger a positive result [2]. In fact, the World Health Organization (WHO) recognizes the reduced sensitivity of antigen tests and enhanced reliability of PCR options by differentiating between the two when confirming an infection [3]. A positive PCR test alone constitutes a confirmed case, while an antigen test is only counted if other criteria are met.

As the SARS-CoV-2 virus evolves, Thermo Fisher's lab-based PCR solutions for detecting COVID-19 remain robust in the face of variants. These tests were designed with mutations in mind and target multiple SARS-CoV-2 genes—if a genetic change causes one target to fail, the other targets can still be successfully detected to deliver an accurate positive result.



With S gene mutations, diagnostic COVID-19 tests can offer insight into key SARS-CoV-2 changes

The 69-70del mutation in the S gene, which codes for the SARS-CoV-2 characteristic spike protein, was found in the Alpha variant of concern and is also present in the Omicron strain. The spike protein plays a key role in COVID-19 infection and is also the cornerstone of vaccine-induced immunity, making changes within this gene of particular interest.

The **Applied Biosystems™ TaqPath™ COVID-19 multiplex diagnostic solution**† offers a unique advantage in tracking specific variants carrying the 69-70del in the S gene. The test is designed to detect three different regions of the SARS-CoV-2 genome (S, ORF1ab, and N genes). Although the 69-70del mutation prevents a signal from being generated for the S gene, the test remains reliable in detecting COVID-19 overall with robust amplification of the ORF1ab and N gene targets.

When laboratories start to see trends in S gene failure, or "dropout," with COVID-19 positive tests, it can be indicative of variant patterns. After S gene dropout was noted at the start of the Alpha wave, the subsequent return to results identifying all three gene targets was indicative of the pandemic's shift to the Delta variant. The S gene dropout pattern returned with Omicron, and continued observations of S gene target failures have been a sign of this variant's dominance. If labs start to notice more positive S gene results, it could indicate a new variant is taking hold. This capability may prove especially useful as cases of the Omicron subvariant BA.2, which lacks the 69-70del mutation, begin to rise around the world [4]. While S gene target failure may be used as an indicator of the presence of specific variants, the actual variant in the sample must be confirmed with additional testing using genotyping or sequencing approaches.

Genotyping assays provide a quick, accessible option for surveillance of known variants

As COVID-19 cases fluctuate, a strong surveillance system will remain crucial to gaining and maintaining control of the pandemic. PCR solutions designed specifically for surveillance of known variants, such as Thermo Fisher's **Applied Biosystems™ TaqMan™ SARS-CoV-2 Mutation Panel**,‡ can help track the spread of new, known virus strains over time. These assays provide a fast option for mutation analysis using the same PCR equipment as diagnostic tests. Labs have flexibility to build the panel they want for variant detection and can adapt over time as needed. Encouragingly, a recent study showed 100% agreement between genotyping analyses and whole-genome sequencing in the ability to identify the Omicron variant within a sample, backing this solution as a reliable option for variant-specific surveillance activities [5].

To learn more about how Thermo Fisher is working to provide a continuous supply of reliable solutions for the testing and monitoring of COVID-19, watch the Labroots webinar **on demand**.

* Vanessa Lacey, PhD, Staff Scientist, Molecular Biology, Clinical Applications, Thermo Fisher Scientific.

** Jelena Feenstra, PhD, Sr. Manager of Global Scientific Communications for Genetic Sciences at Thermo Fisher Scientfiic.

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‡ For Research Use Only. Not for use in diagnostic procedures.

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Chapter 2

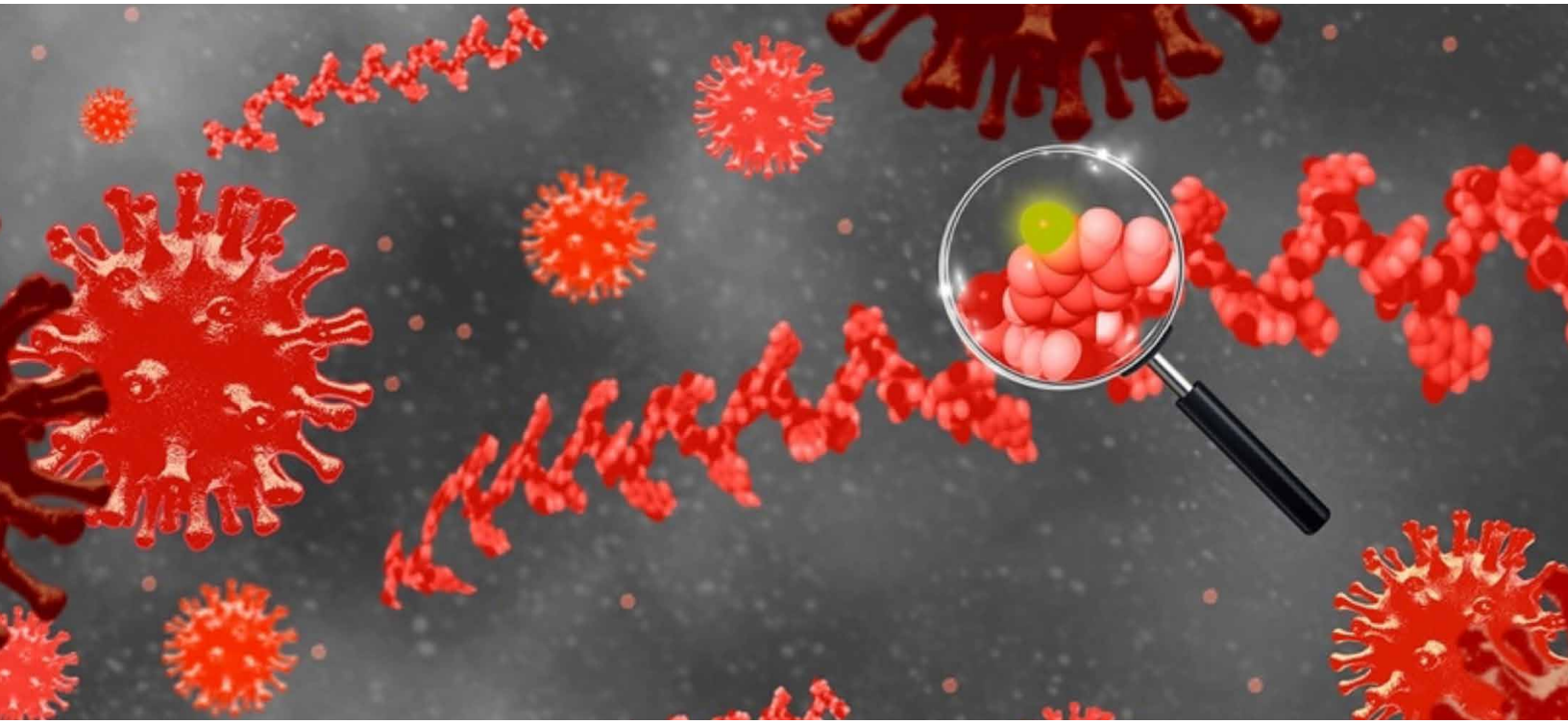
Omicron: Where do we go from here?

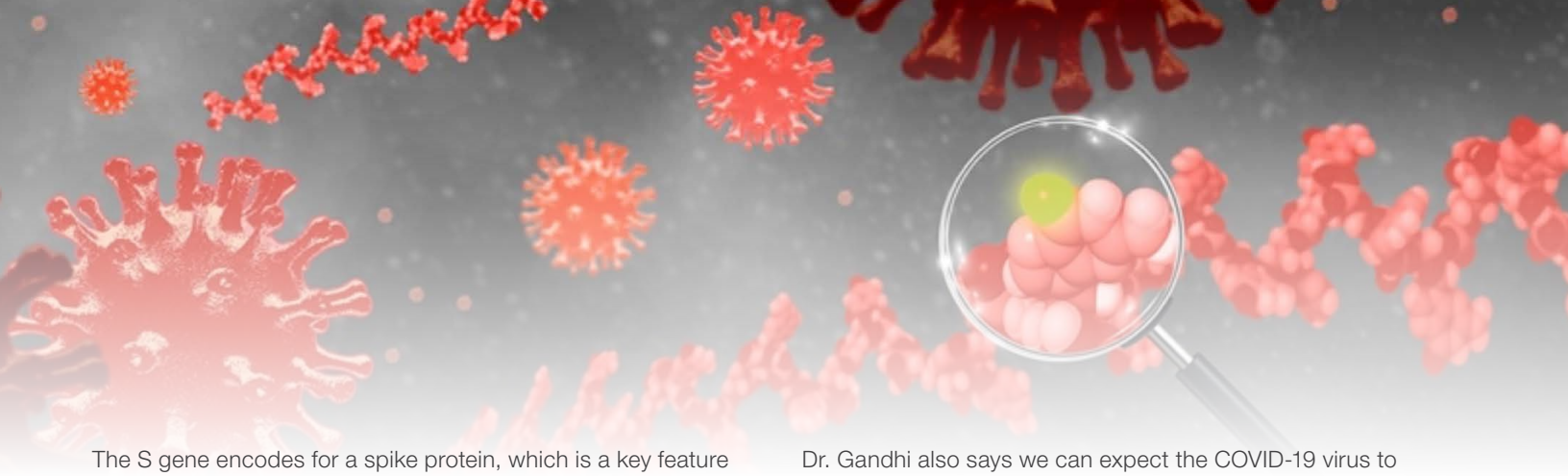
This blog was originally published in January 2022

Since the initial COVID-19 outbreak in 2020, the SARS-CoV-2 virus has mutated, resulting in a range of variants such as Alpha, Beta, Delta, and most recently, Omicron. While much about Omicron remains uncertain, public health organizations and medical professionals are watching the potential impacts of this new variant closely, warranting the question—what makes it different? And, how concerned should we be about both Omicron and future mutations or variants?

To help increase understanding of the Omicron variant and how we can prevent its spread, Manoj Gandhi, MD, PhD, Thermo Fisher's senior director of medical affairs, Genetic Sciences, recently spoke with the BBC on the essential role of testing in response to emerging variants. Dr. Gandhi highlighted the impact of surveilling the viral genome, the informative "S gene dropout" finding, and why public health organizations around the world are calling on labs to take advantage of a distinctive feature of Thermo Fisher Scientific's **Applied Biosystems™ TaqPath™ COVID-19 diagnostic testing solution**.*

As Dr. Gandhi explained, we can examine test patterns originally noted with the Alpha variant to gain insight into the Omicron variant. Certain COVID-19 tests are designed to analyze multiple areas within the viral genome to deliver a positive or negative result. According to Dr. Gandhi, this redundancy is built into multiplex tests, so even if the virus mutates the test can still detect its presence. The TaqPath COVID-19 diagnostic testing solution, for example, analyzes three parts of the genome: the orf1a/1b region, the S gene, and the N gene. While each of these regions holds significance to the virus's ability to be infectious, the S gene has proven to be an essential tool for tracing COVID-19 infections and identifying changes to SARS-CoV-2.





The S gene encodes for a spike protein, which is a key feature of the virus that allows for entry into healthy human cells and subsequent infection [1]. In December of 2020, experts started seeing an unusual pattern in COVID-19 tests, where the result would be positive for SARS-CoV-2, but the S gene part of the test would be negative. This "S gene dropout" became a key feature in identifying the Alpha variant. Labs and public health officials soon realized the TaqPath test offers a unique advantage when examining COVID-19 test results, as it can easily detect the S gene dropout and be used to proactively flag samples for confirmatory sequencing.

When scientists began seeing a similar S gene pattern in November of 2021, the initial thought was that the Alpha variant was reemerging. However, after closer examination, experts found a variety of mutations across the genome that are not present in previously identified variants, ultimately leading to the discovery of Omicron [2].

While Dr. Gandhi says it is still too early to draw specific conclusions about the Omicron variant's impact on the pandemic, the many mutations present raise concerns that the virus will continue to mutate and potentially evade vaccine-mediated protection. Early data indicates that current vaccines and boosters are not as effective against Omicron as they are against previous variants, but the increased number of antibodies in the body from vaccine doses helps protect against severe Omicron infections and hospitalization [3].

Dr. Gandhi also says we can expect the COVID-19 virus to continue to mutate and evolve, finding new ways to infect people and evade vaccines. At the same time, our collective immunity will likely continue to adjust to the changing virus. Similar to the flu, as COVID-19 becomes endemic, experts predict that in the future, we will likely see some years with severe infections, and some years that have a lower incidence of infections and milder disease [4]. Regardless, the only way to reliably track these mutations and predict future COVID-19 outbreaks is by testing and sequencing SARS-CoV-2.

For more information on Omicron, variants, and the importance of testing, please listen to the full **BBC podcast** featuring Dr. Gandhi.

* For Emergency Use Authorization (EUA) Only. For Prescription Use Only. For *In Vitro* Diagnostic Use. Regulatory requirements vary by country; products may not be available in your geographic area.

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Chapter 3

How COVID-19 tests are staying ahead of SARS-CoV-2 variants

This blog was originally published in December 2021

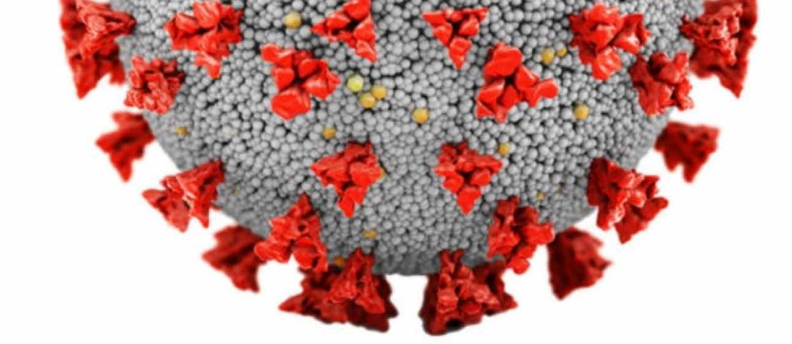
Thermo Fisher Scientific has been working with clinical labs and public health officials since tracking COVID-19 cases became a top priority at the start of the pandemic. From the time SARS-CoV-2 was first identified as a potential global health threat in early 2020, one of the biggest concerns has been how easily the virus seemed to spread within communities and across geographies.

As the pandemic has progressed and case counts fluctuate, **multiple variants** of concern have emerged that are even more transmissible than earlier strains. Delta, the most widespread variant thus far, is reportedly five times more infectious than the original strain, for example [1]. Most recently, the World Health Organization designated Omicron as a variant of concern, putting public health organizations around the globe on alert [2]. While the impact of this new version of the virus remains to be seen, it is a reminder that SARS-CoV-2 will continue to mutate with ongoing spread, leading experts to caution that future variants could be even more virulent.

Health experts warn that unvaccinated populations, in particular, are at high risk of falling sick, thereby creating a potential breeding ground for new variants and, in turn, a dangerous public health situation [3]. Due to a combination of access issues and consumer hesitancy, vaccination rates are only in the single digits in some parts of the world and, although the US Food and Drug Administration (FDA) recently authorized the vaccination of children ages five and older, parents are still waiting for an option to immunize younger children.

Another concerning issue is that while vaccines have largely been shown to be effective even against new strains, there have been "breakthrough" infections in vaccinated individuals. In fact, the Centers for Disease Control and Prevention (CDC) has confirmed that fully immunized people can still get infected and spread COVID-19 [4].

It is important to understand the difference between the terms "infection" and "disease." Vaccines don't always prevent someone from getting infected with SARS-CoV-2, but they are highly effective at preventing someone who is infected from falling sick due to COVID-19 and having their disease progress to severe.



While overall risks are reduced, no intervention is perfect, and there have been cases of people being hospitalized despite being fully vaccinated. Additionally, waning immunity post-vaccination until a booster is administered introduces the opportunity for previously vaccinated individuals to become more susceptible to infection. It is therefore extremely important to continue to seek testing in the event of illness or exposure, even for those who are vaccinated.

As COVID-19 continues to evolve, testing was designed with it to stay ahead of the changing virus. Thermo Fisher designed the **multiplex COVID-19 Applied Biosystems™ TaqPath™ tests** to be resilient from the start by targeting three different areas of the SARS-CoV-2 genome, ensuring the tests can detect the virus even as it mutates. This built-in redundancy was put to the test last fall when labs started noticing early evidence of the Alpha, or B.1.1.7, variant—a new COVID-19 strain that would soon start spreading around the globe. Since the test looks at multiple targets, the mutation in this strain did not affect its accuracy. In fact, labs began using what they termed the "S gene dropout," or "S gene target failure," as a signal to detect the presence of the Alpha variant and to track the spread of this variant in support of global surveillance efforts.

The TaqPath test and S gene dropout are proving invaluable yet again with the emergence of Omicron. As with the Alpha variant, a mutation in the spike region of the Omicron strain causes an S gene target failure when a sample is analyzed with the TaqPath multiplex test. Labs are now using this test as an early warning signal that Omicron may be present in samples. In fact, public health officials have asked labs to use Thermo Fisher's TaqPath test to search for the Omicron variant and prioritize samples with the S gene target failure for genomic sequencing [5].

Omicron will likely not be the last variant to emerge from COVID-19. Scientists can't predict exactly how SARS-CoV-2 will mutate in the months ahead, but we do know existing mutations have primarily emerged in the "S" gene, or the area of the genome that codes for the virus's notable spike protein. To account for this new understanding and ensure future availability of effective tests, Thermo Fisher developed the next generation of TaqPath COVID-19 tests to look at multiple targets across three genomic regions outside of the S gene. The **Applied Biosystems™ TaqPath™ COVID-19 2.0 tests** now have eight targets across three genes; the orf1a, orf1b, and the N gene. The new tests' increased redundancy safeguards their effectiveness against future SARS-CoV-2 mutations.

The health and scientific communities have made great strides in the fight against COVID-19. As more of the population gets vaccinated, there will be evolutionary pressure on the virus to mutate and form new variants. These new variants could threaten to upend that progress, but health systems, test developers, and drug and vaccine researchers are constantly adjusting course to stay ahead of the virus and put an end to this global pandemic.

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Chapter 4

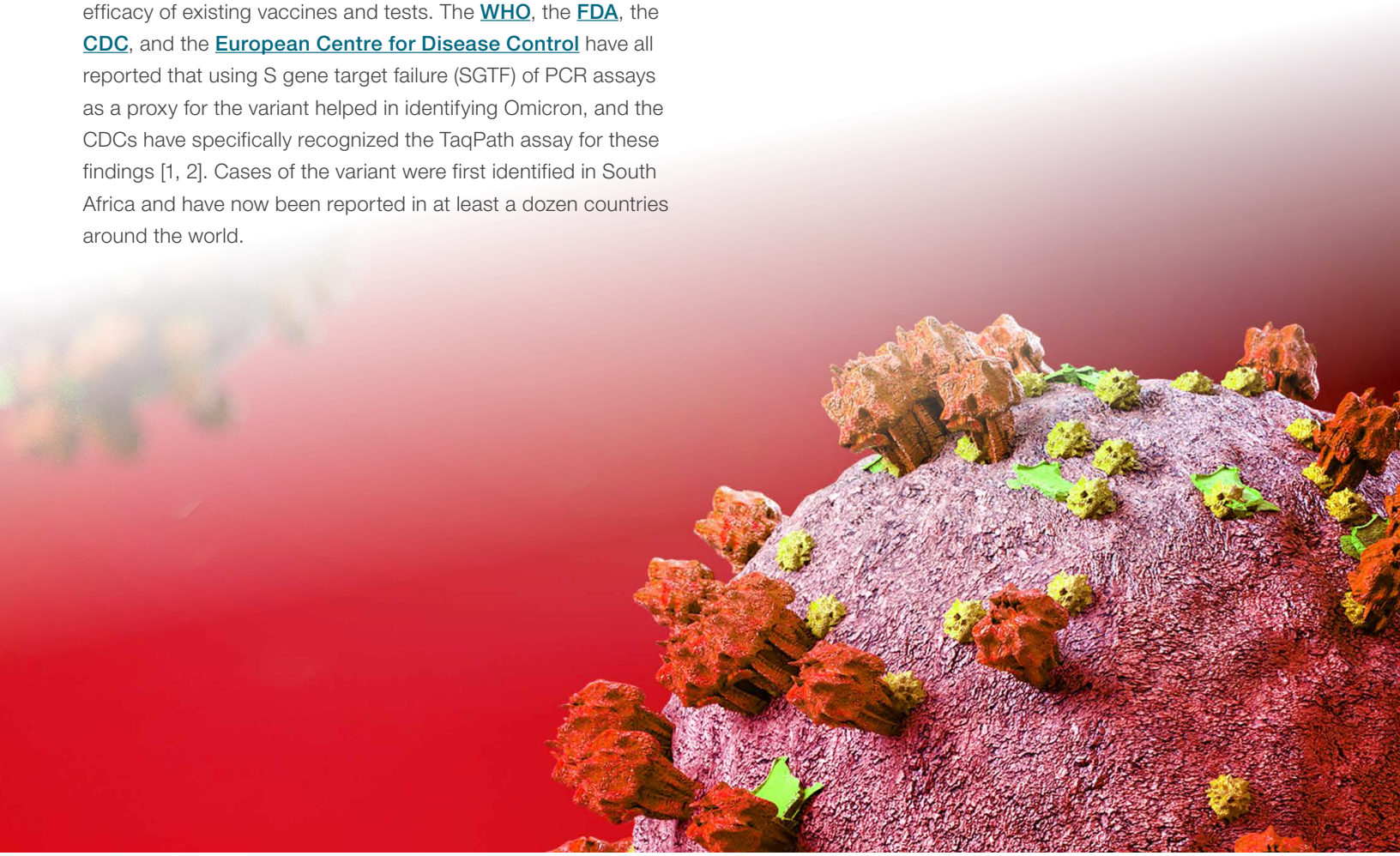
TaqPath COVID-19 tests support detection of SARS-CoV-2 in samples containing the Omicron variant

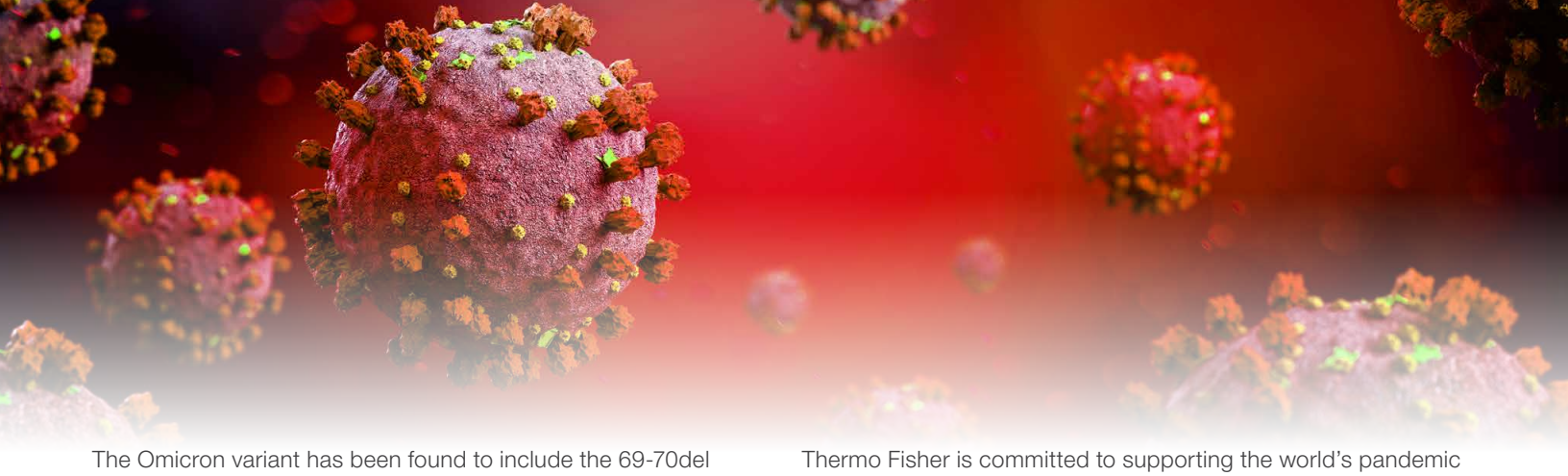
This blog was originally published in November 2021

The Applied Biosystems™ TaqPath™ COVID-19 Combo Kit,* Applied Biosystems™ TaqPath™ COVID-19 CE-IVD RT-PCR Kit,** and the Thermo Fisher Scientific™ Accula™ SARS-CoV-2 Test,* which test for the presence of SARS-CoV-2, are not impacted by the emerging B.1.1.529, or Omicron variant, enabling accurate test results.

The Omicron variant, which was designated a "variant of concern" by the World Health Organization (WHO), has more than 30 mutations in the spike protein alone. The WHO has reported that preliminary evidence suggests an increased risk of transmission compared to other variants of concern. This designation and information are leading to renewed travel restrictions and research to examine the variant's impact on efficacy of existing vaccines and tests. The **WHO**, the **FDA**, the **CDC**, and the **European Centre for Disease Control** have all reported that using S gene target failure (SGTF) of PCR assays as a proxy for the variant helped in identifying Omicron, and the CDCs have specifically recognized the TaqPath assay for these findings [1, 2]. Cases of the variant were first identified in South Africa and have now been reported in at least a dozen countries around the world.

The **TaqPath COVID-19 assays** detect SARS-CoV-2 infections by identifying the presence of three gene targets from the orf1a/b, S, and N regions of the virus. By surveying across multiple genes, the test can report out accurate results even in the case where one of the targets is impacted by a mutation. While the S gene target in the test is impacted, the orf1ab and N gene targets in the TaqPath COVID-19 tests have been determined to not be impacted by any of the mutations in the Omicron variant, based on assessment of sequences in the GISAID public database. As a result, the overall accuracy of the TaqPath COVID-19 assays is not impacted.





The Omicron variant has been found to include the 69-70del mutation of the S gene, first identified as a mutation in the Alpha variant. This mutation causes a dropout of the S gene target in results from the TaqPath test, which could indicate to clinicians and researchers a possible Omicron variant infection. Confirmation must then be performed by sequencing the sample. Thermo Fisher provides both **Sanger sequencing** and **next-generation sequencing** solutions for SARS-CoV-2 sequencing.

In addition to the lab-based TaqPath COVID-19 PCR tests, Thermo Fisher Scientific offers the rapid RT-PCR, point-of-care **Accula SARS-CoV-2 Test**. The Accula test targets a separate part of the genome, the Nucleocapsid (N) gene. Based on sequences published in GISAID database, the Omicron variant contains an ancestral 28881-28883 (GGG to AAC) mutation in the region of the N gene targeted by the Accula test. This mutation has been circulating in SARS-CoV-2 sequences since January 2020 and has been demonstrated to have no impact on Accula SARS-CoV-2 test performance [3].

Specific genotyping assays to detect the Omicron variant are being developed for Thermo Fisher's **Applied Biosystems™ TaqMan™ SARS-CoV-2 Mutation Panel**.† The panel, which is currently used for research purposes, already has a menu of over 50 assays to assess confirmed COVID-19 cases for the presence of known variants and mutations.

Thermo Fisher is committed to supporting the world's pandemic response through the monitoring of new SARS-CoV-2 variants and developing and producing innovative, adaptive, and resilient testing solutions using gold-standard PCR technology. This work helps track and subsequently limit the spread of COVID-19, helping communities stay healthy.

* TaqPath COVID-19 Combo Kit and Accula SARS-CoV-2 Test: For Use Under Emergency Use Authorization (EUA) Only. For Prescription Use Only. For *In Vitro* Diagnostic Use.

** For *In Vitro* Diagnostic Use.

† For Research Use Only.

The Accula SARS-CoV-2 Test has not been FDA cleared or approved. It has been authorized for emergency use by the FDA for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high, moderate, or waived complexity tests. The test is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation. The Accula SARS-CoV-2 Test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens. The emergency use of this test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

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Chapter 5

The S gene advantage: TaqPath COVID-19 tests may help with early identification of Omicron variant

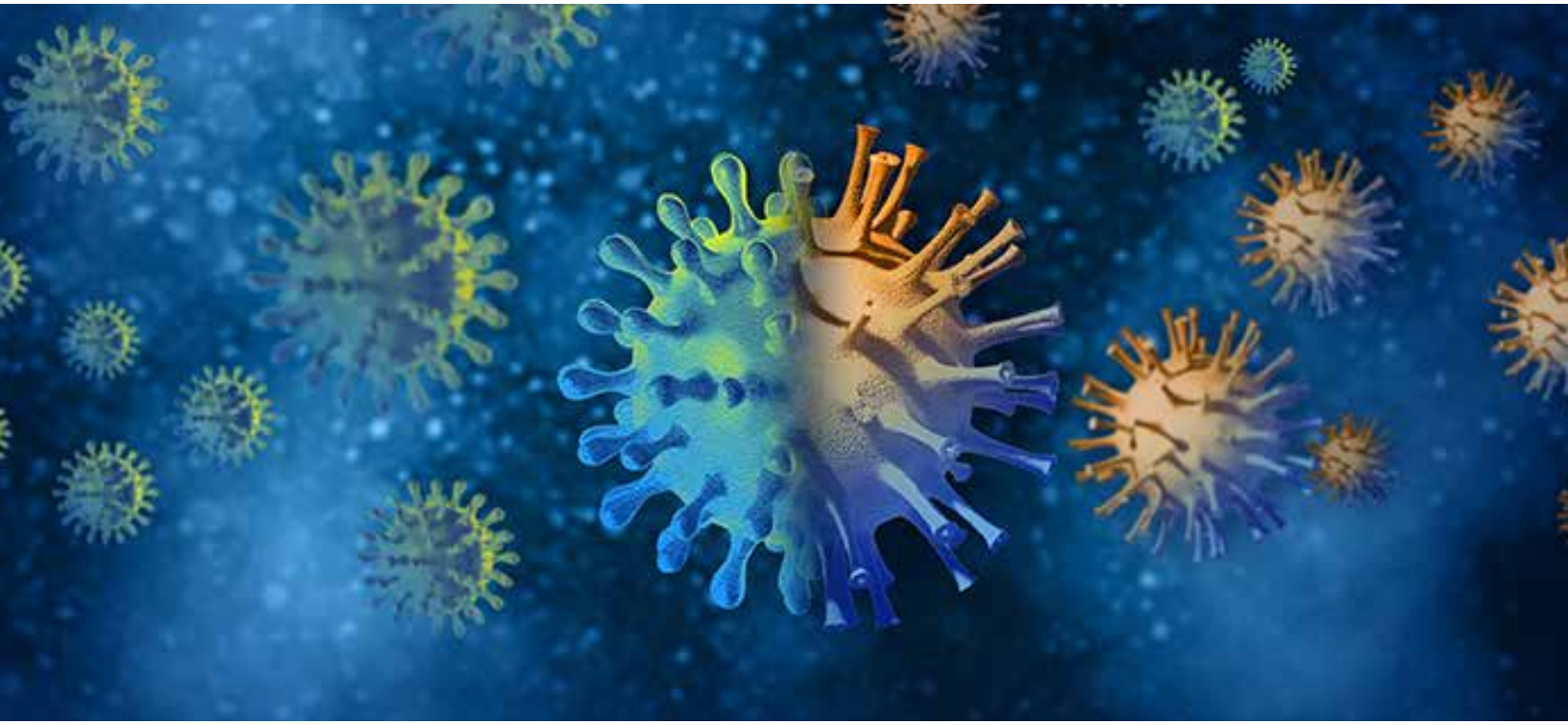
This blog was originally published in November 2021

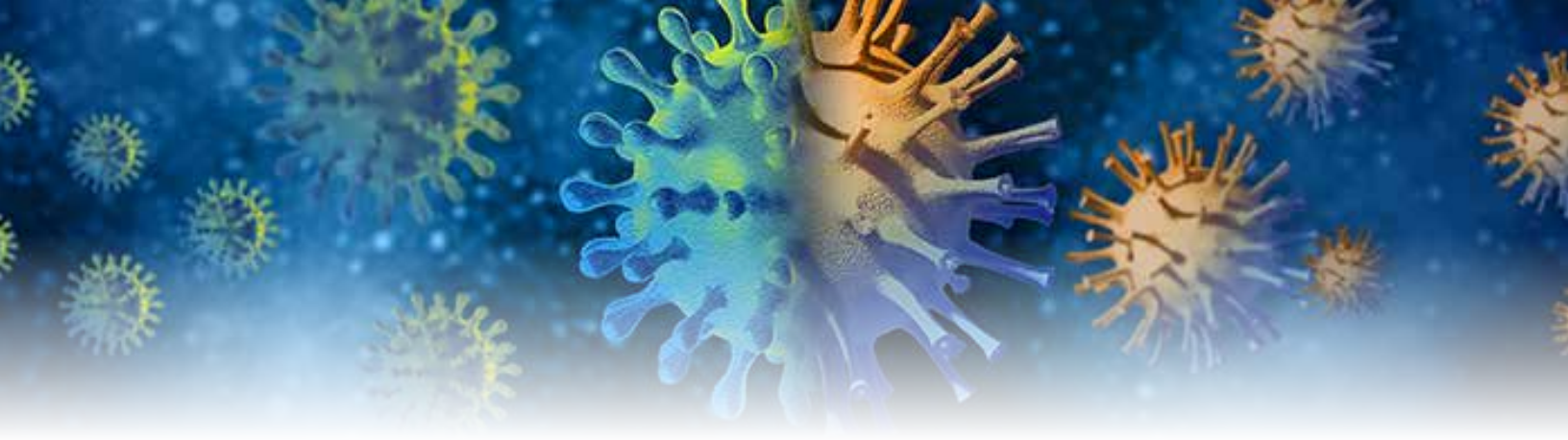
A new, potentially highly transmissible variant of SARS-CoV-2 discovered in South Africa, B.1.1.529 (or Omicron) has public health organizations and governments on alert. B.1.1.529 is the most divergent variant identified in significant numbers, with over 30 mutations across the S gene, which raises concerns for disease control and prevention. Due to concerns around a detrimental change in COVID-19 epidemiology, the **WHO designated** B.1.1.529 as a variant of concern on November 26, 2021. Health officials indicate that more information is needed to understand if Omicron is more transmissible or severe than other variants, including Delta. The WHO is currently asking countries to:

- Enhance surveillance
- Submit sequence information to publicly available databases, such as GISAID
- Report initial cases/clusters
- Perform field investigations and laboratory assessments to improve understanding of the potential impacts

The S gene advantage

The **Applied Biosystems™ TaqPath™ COVID-19 diagnostic tests** use a **multi-target design** to compensate for emerging SARS-CoV-2 variants and mutations. Furthermore, the TaqPath tests are unique among the most commonly used molecular tests in that the multi-target design includes an S gene target. The Omicron variant has been found to include the 69-70del mutation of the S gene, first identified as a mutation in the Alpha variant. This mutation causes a dropout of the S gene target in results from widely used TaqPath COVID-19 detection kits. An S gene failure does not mean a result is negative, only that the S gene was not detected. Multiple public health organizations have noted that this pattern of detection (i.e., S gene dropout) can be used as marker for this variant, pending sequencing confirmation.





WHO, European CDC highlight S gene dropout for early identification of B.1.1.529

As previously indicated with the Alpha variant, the WHO, European CDC, and African CDC all report that the S gene dropout from selected TaqPath COVID-19 kits can be used as a screening method for Omicron. Per the European CDC, "The presence of the deletion Δ69-70 means that S gene target failure (SGTF) for the Thermo Fisher TaqPath assay can be used as a screening method for Omicron. In a setting with the Delta variant dominating, this can be used as a proxy for Omicron after confirmation of a subset of samples by sequencing." The WHO also reports, "Several labs have indicated that for one widely used PCR test, one of the three target genes is not detected (called S gene dropout or S gene target failure) and this test can therefore be used as marker for this variant, pending sequencing confirmation. Using this approach, this variant has been detected at faster rates than previous surges in infection, suggesting that this variant may have a growth advantage." Both the WHO and ECDC recommend further characterizing specimens that show S gene dropout using sequencing.

Reliable detection

By surveying across multiple genes, the TaqPath COVID-19 detection kits can report SARS-CoV-2 results in the case where one of the targets is impacted by a mutation (as long as the other targets are not impacted and the positive control passed). While the S gene target in the test is impacted, the orf1ab and N gene targets in the TaqPath COVID-19 tests have been determined to not be impacted by any of the mutations in the Omicron variant, based on assessment of sequences in the GISAID public database.

Identify potential variants early to help curb the spread

Being able to distinguish between different, potentially more transmissible SARS-CoV-2 variants is of value for gathering preliminary data on the appearance and spread of SARS-CoV-2 variants and lineages. Because the detection patterns of the TaqPath COVID-19 tests may differ between different variants, test results from these kits may provide initial insight into developments in SARS-CoV-2's continued evolution. Further genetic surveillance studies using targeted genotyping assays or whole viral genome sequencing techniques should be employed to more thoroughly track this deadly pathogen. As recommended by the WHO, reporting of initial cases/clusters can help us understand current and future COVID-19 epidemiology and inform public health and social measures.

Chapter 6

TaqPath COVID-19 diagnostic tests detect the Omicron variant and all its lineages

This blog was originally published in January 2022

Based on a **multi-target design** that compensates for emerging SARS-CoV-2 variants and mutations, the Applied Biosystems™ TaqPath™ COVID-19 diagnostic tests can detect the Omicron variant of concern (VOC) and all its currently known lineages. In fact, the TaqPath COVID-19 diagnostic tests can detect all currently known SARS-CoV-2 among patients' samples.

Independent of the currently known variants, the tests can determine whether a patient's sample is positive or negative for the virus. The TaqPath COVID-19 diagnostic tests listed in Table 1 will show a specific detection pattern for the majority of Omicron variant isolates. Using this detection pattern as a proxy can help identify the Omicron variant.

The first case of Omicron and its subsequent lineages

The first case of the Omicron VOC was reported in November of 2021 and designated as B.1.1.529 (pangolin lineage, also known as 21M). This new variant has more than 30 mutations in the spike protein alone. Since its first discovery, multiple variations of the Omicron virus have been identified that share the majority of mutations but also harbor their own unique sequence changes. The three sub-lineages identified so far include BA.1 (also known as 21K), BA.2 (also known as 21L), and BA.3 (Table 2) [1].

Table 1: TaqPath COVID-19 Diagnostic Tests

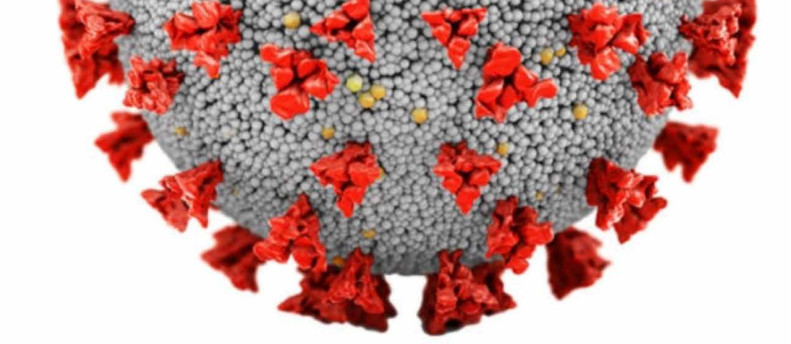
TaqPath COVID-19 Assay		Cat #	Detects SARS-CoV-2 (Including Omicron B.1.1.529, BA. 1, BA.2 and BA. 3)	Detects S Gene Target Failure (SGTF) (Detection Pattern Suggestive of Omicron BA. 1 and BA. 3)
Products with US FDA's Emergency Use Authorization (EUA)*				
TaqPath COVID-19 Combo Kit*	EUA	A47814	**YES**	**YES**
TaqPath COVID-19 Combo Kit Advanced*	EUA	A47813		
TaqPath COVID-19 High-Throughput Combo Kit for use with Amplitude Solution*	EUA	A49869		
TaqPath COVID-19 Pooling Kit*	EUA	A49918		
Products Complied with EU's CE-IVD Requirements**				
TaqPath COVID-19 CE-IVD RT-PCR Kit**	CE-IVD	A48067	**YES**	**YES**
TaqPath COVID-19 HT Kit for use with Amplitude Platform**	CE-IVD	A50883		

* For In Vitro Diagnostic Use. For Emergency Use Authorization Only.

**CE-IVD. For In Vitro Diagnostic Use.

Table 2: Sub-Lineages of Omicron B.1.1.529 (Pangolin Lineage, Also Known as 21M) and Their Detection Pattern

Name of Omicron Sub-Lineages	Also Known As	Shows SGTF Detection Pattern by TaqPath COVID-19 Assay
BA.1	21K	**YES**
BA.2	21L	**NO**
BA.3	–	**YES**



The 69-70 deletion in the spike protein, a mutation that presents in the majority of the currently circulating Omicron cases worldwide, helps identify the potential presence of the VOC when using any of the TaqPath COVID-19 diagnostic tests listed in Table 1 on patients' samples. This mutation is present in BA.1 (21K) and BA.3, but not in BA.2 (21L) lineage [1].

The worldwide and country-specific prevalence of the Omicron variant and its lineages can be tracked using:

- World Health Organization (WHO)'s "Tracking SARS-CoV-2 variants"
- WHO's "Technical brief and priority actions for member states"
- Outbreak.info hosted by Scripps Research
- The ROSALIND tracker hosted by ROSALIND, Inc.

The BA.1 (21K) lineage of Omicron, which has the 69-70 deletion in the spike protein and presents the S gene target failure (SGTF) detection pattern by TaqPath COVID-19 assay, is currently the most prevalent worldwide. It accounts for >98% of sequences submitted to Global Initiative on Sharing Avian Influenza Data (GISAID) as of 7 January 2022. BA.2 and BA.3 only account for a minority of the submitted sequences worldwide [1].

The WHO, the US Food and Drug Administration (FDA), the US Centers for Disease Control and Prevention (CDC), and the European Centre for Disease Prevention and Control (ECDC) have all reported that using SGTF of PCR assays as a proxy for the variant helps in identifying Omicron [1, 2, 3, 4].

TaqPath assay detects all currently known strains of SARS-CoV-2, including different lineages of the Omicron variant

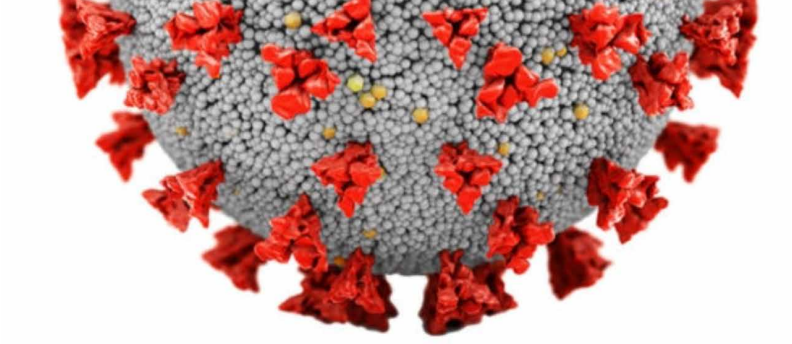
The TaqPath COVID-19 diagnostic tests are capable of detecting samples infected with different Omicron variants. The tests detect SARS-CoV-2 infections by identifying the presence of three gene targets from the orf1a/b, S, and N regions of the virus. The Applied Biosystems™ COVID-19 Interpretive Software algorithms utilize the results from all three targets to generate a final call, and a positive result is called if at least two of the three SARS-CoV-2 targets are detected.

Figure 1: Multi-target design of the TaqPath COVID-19 diagnostic tests listed in Table 1.

For the BA.1 and BA.3 lineages of the Omicron variant B.1.1.529, the TaqPath COVID-19 diagnostic tests detect both the orf1ab and N genes while showing a negative signal for the S gene target. This **SGTF** is also known as **S gene advantage** as it can be used as a marker suggestive of the Omicron variants. The Delta variant, currently the predominant variant worldwide, does not have the S gene 69-70del mutation in the majority of the cases and the S gene is detected by the TaqPath COVID-19 kits. It is important to note that the 69-70del mutation may also exist in other mutants, and a dropout of the S gene signal could also be caused by other reasons.

For the Omicron variant BA.2 and other SARS-CoV-2 variants without the spike protein mutations, the TaqPath COVID-19 diagnostic tests detect all three gene targets from the orf1a/b, S, and N regions of the virus.

In summary, the TaqPath COVID-19 diagnostic tests can detect all currently known SARS-CoV-2 variants. The S gene advantage has already helped detect the Alpha variant, and the WHO, US CDC, European CDC, and African CDC have all reported that using SGTF of PCR assays as a proxy for the variant helps in identifying Omicron. The CDCs have specifically recognized the TaqPath assay for these findings. Both the ECDC and US FDA recommend further characterizing specimens that show S gene dropout using sequencing.



What to do if SGTF is observed?

Samples that are associated with the S gene dropout reflect either a resurgence of the Alpha variant, a new or emerging mutant that interferes with the S gene detection, or the presence of the new Omicron variant (B.1.1.529, BA.1, and BA.3). Given that the majority of currently circulating SARS-CoV-2 is the Delta variant, which does not produce the SGTF profile, any sample with S gene dropout should be seen as signal to characterize the sample for the presence of Omicron. Due to the novelty and potential risks associated with the new Omicron variant, the WHO, CDC, and ECDC recommend further characterizing specimens with S gene dropout using sequencing.

For surveillance purposes, confirming various variants by genotyping may be performed using the following:

For genotyping with existing qPCR equipment:

- TaqMan SARS-CoV-2 Mutation Panel†

Thermo Fisher's Applied Biosystems™ TaqMan™ Mutation Panel, which is currently used for research purposes, is compatible with real-time PCR instruments already commonly used in laboratories around the world. Laboratories can choose from a menu of over 50 known mutations of SARS-CoV-2 to assess confirmed COVID-19 positive samples for the presence of known variants and mutations. The panel includes a set of assays specifically designed to identify the Omicron variant (mutations: S.G339D. GGT_GAG, S.Q493R.CAA_CGA, S.T547K.ACA.AAA, orf1ab. A2710T, and orf1ab.T13195C).

Confirming various variants via sequencing

Thermo Fisher Scientific supports variant and lineage confirmation by sequencing:

- Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay†
- Sanger sequencing solutions for SARS-CoV-2 research†

Learn how we can help your emerging SARS-CoV-2 variant and mutation surveillance efforts with our Sanger sequencing and NGS technologies.

† For Research Use Only. Not for use in diagnostic procedures.

To keep track of SARS-CoV-2 variants and their distributions:

- World Health Organization's "Tracking SARS-CoV-2 variants"
- World Health Organization's "Technical brief and priority actions for member states"
- Omicron variant report at Outbreak.info by Scripps Research
- Real-time tracker to support rapid genotyping "Project ROSA" program for SARS-CoV-2 in the United States

References:

1. who.int/publications/m/item/enhancing-readiness-for-omicron-(b.1.1.529)-technical-brief-and-priority-actions-for-member-states
2. fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/sars-cov-2-viral-mutations-impact-covid-19-tests
3. cdc.gov/csels/dls/locs/2021/12-03-2021-lab-alert-CDC_Update SARS-CoV-2_Omicron_Variant.html
4. ecdc.europa.eu/sites/default/files/documents/SARS-CoV-2-variant-multiple-spike-protein-mutations-United-Kingdom.pdf

Chapter 7

Viruses mutate, and SARS-CoV-2 is no exception

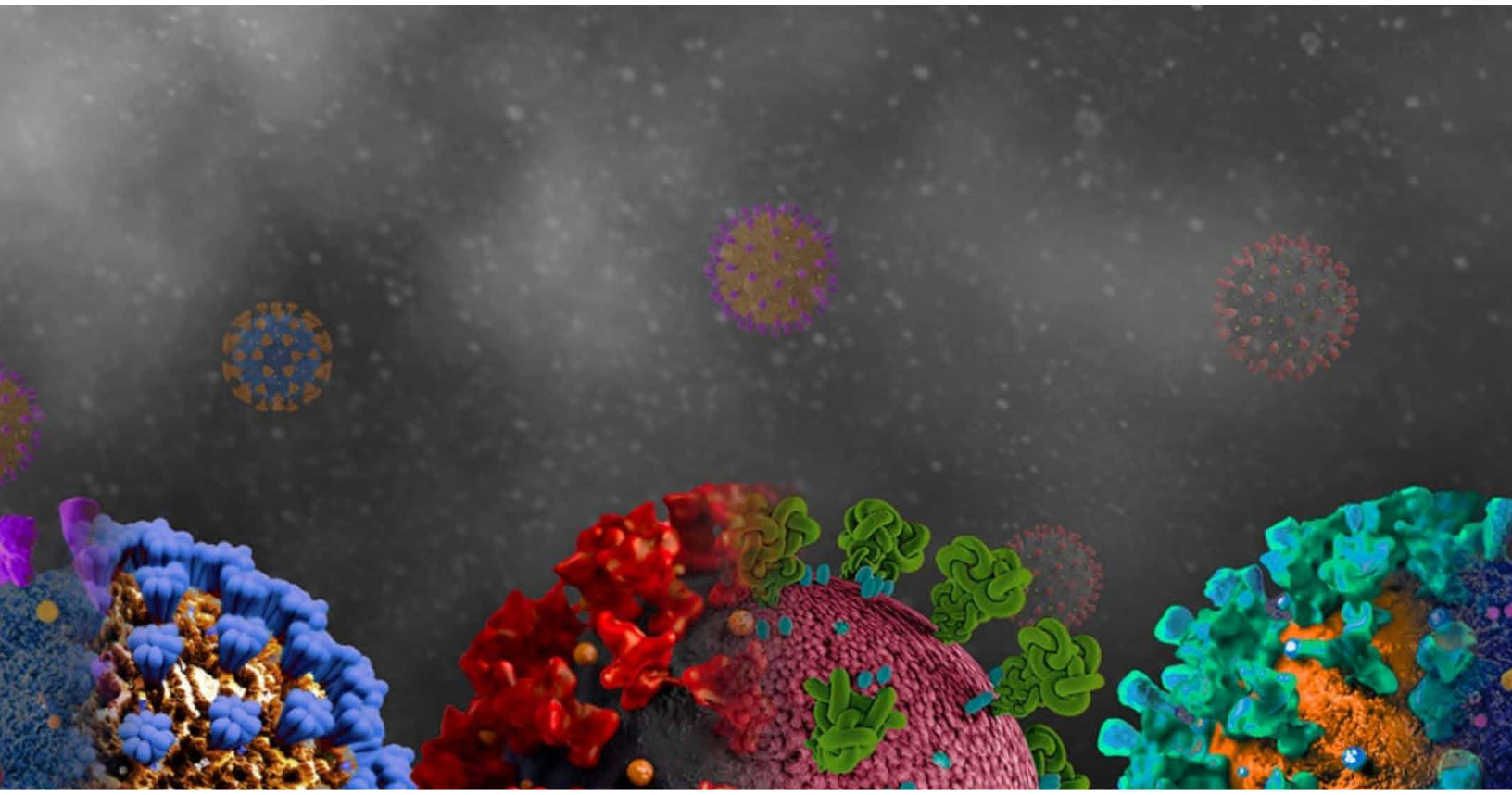
This blog was originally published in October 2021

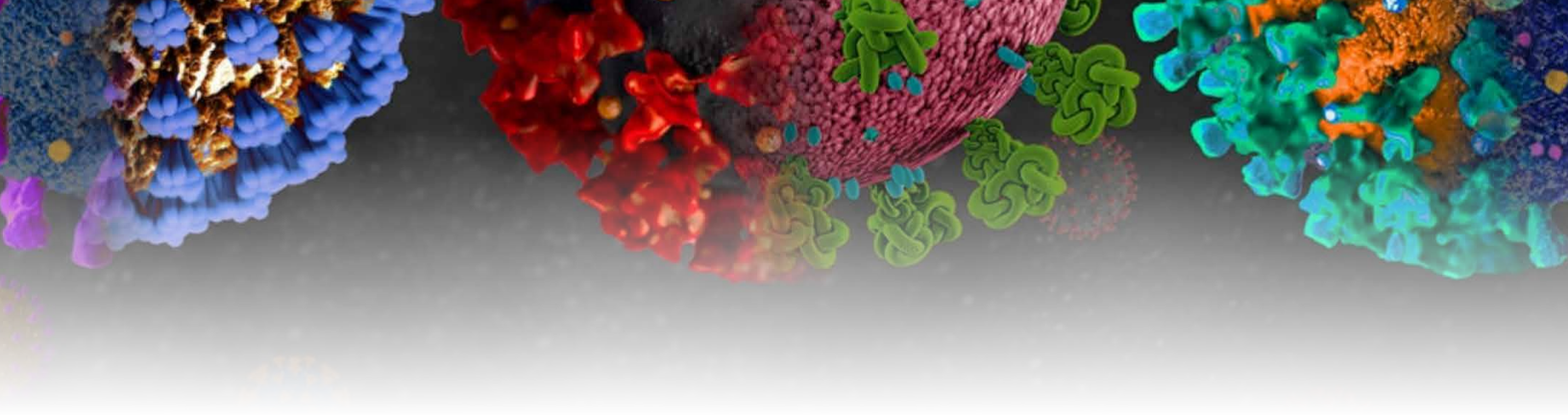
With COVID-19 cases resurging around the globe, experts warn that vaccination does not offer complete protection against infection. A special envoy to the World Health Organization recently cautioned in an interview with Bloomberg TV, “Variants that can beat the protection offered by vaccines are bound to emerge all over the world in the coming months and years.” [1]

The European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) is one of the most comprehensive and influential congresses in the field of infection. This year, infectious disease researchers, test developers, and heads of global laboratories met at a symposium at ECCMID to discuss emerging mutation surveillance and the best approaches available to rapidly identify SARS-CoV-2 variants. Despite increasing concerns about highly transmissible new strains, the presenters highlighted how testing is evolving to stay ahead of the virus.

Staying ahead of SARS-CoV-2 mutations

“Over the course of the pandemic, there has been a growing interest in test features that could improve sample analysis,” Jelena Feenstra, senior manager for global scientific communications, shared during her talk at the conference. In the face of emerging variants, Feenstra highlighted the need for tests that analyze multiple targets and have increased redundancy to minimize the potential impact of mutations on assay performance. Thermo Fisher designed the Applied Biosystems™ TaqPath™ SARS-CoV-2 2.0 assay menu to compensate for current and future SARS-CoV-2 mutations.





Both assays in the menu look at three genomic regions and eight total targets not including the S gene, which has been shown to have a high risk of mutation. The menu includes the **Applied Biosystems™ TaqPath™ COVID-19 Fast PCR Combo Kit 2.0**,* a fast, direct-to-PCR raw saliva solution for widespread, high-frequency testing. Not only is saliva sample collection easy and non-invasive, it can be performed without the need for a trained healthcare professional, limiting risk of infection. The menu also includes the **Applied Biosystems™ TaqPath™ COVID-19 RNase P Assay 2.0**,* featuring an updated assay design using RNase P as an internal control to both confirm a human specimen and ensure nucleic acids in the sample are preserved during transport.

Identifying new and emerging variants

For many laboratories, the goal is not just to stay ahead of SARS-CoV-2 mutations, but also to aid global efforts to detect new and emerging variants of concern. At ECCMID, Simone Vanoni, PhD, a molecular biologist at PharmGenetix GmbH based in Austria, shared results from a recent study evaluating a dual workflow for identifying variants using whole-genome sequencing (WGS) and Thermo Fisher's **Applied Biosystems™ TaqMan™ SARS-CoV-2 Mutation Panel**.**

With WGS, Dr. Vanoni's lab was able to determine if a sample contained a variant of concern, but the results were not available for three days or more—a turnaround time that is less than optimal when rapid insights are needed to curb new outbreaks before they spread. In addition, as Dr. Vanoni shared during his talk, WGS requires significant resources and is not easily scalable.

Dr. Vanoni's lab tested a workflow where all samples positive for SARS-CoV-2 as determined by the Applied Biosystems™ TaqPath™ COVID-19 CE-IVD RT-PCR Kit† were sequenced with the standard WGS protocol and also analyzed with a custom mutation panel covering seven mutations of interest. In an analysis of 99 samples, his team found 100% agreement between the mutation panel screening and parallel WGS. While WGS remains an important tool for continuous monitoring for novel and undetermined strains, Dr. Vanoni's data show that the TaqMan SARS-CoV-2 Mutation Panel can enable reliable detection of lineages that impose the highest threat to the public health in less than 24 hours, making the panel an ideal solution to aid surveillance efforts in areas where variants of concern or interest are circulating.

To watch the on-demand talks, as well as additional ECCMID presentations, please visit **thermofisher.com/eccmid2021**.

* For Emergency Use Authorization (EUA) Only. For Prescription Use Only. For *In Vitro* Diagnostic Use.

** For Research Use Only. Not for use in diagnostic procedures.

† For *In Vitro* Diagnostic Use.

Reference:

1. https://www.bloomberg.com/news/articles/2021-09-13/who-envoy-warns-of-increasing-risk-of-variants-evading-vaccines

Conclusion

As case counts fluctuate and COVID-19 possibly moves toward becoming endemic, diagnostic testing and monitoring of SARS-CoV-2 genetic changes will continue to play critical roles in disease control strategies. Thermo Fisher Scientific's extensive COVID-19 and SARS-CoV-2 solutions are designed with mutations in mind. Our PCR products leverage the gold standard for COVID-19 testing, with each diagnostic option relying on multi-gene targets to remain reliable in detecting the virus as it changes.

The **Applied Biosystems™ TaqPath™ multiplex diagnostic solution*** test offers the added advantage of identifying the characteristic S gene dropout pattern observed with some noteworthy variants, including Alpha and Omicron. By quickly screening samples for known mutations, the **Applied Biosystems™ TaqMan™ SARS-CoV-2 Mutation Panel**** provides local and global organizations with an accessible way to support critical surveillance efforts using the same equipment needed for PCR testing platforms. These robust technologies, along with lessons learned along the pandemic journey, will help to address future COVID-19 outbreaks more efficiently, even as the virus evolves over time.

Explore our **Clinical Conversations** blog space for additional resources highlighting the significance of variants and their impact on COVID-19 testing and surveillance solutions.

If you would like more information, please **contact us**.

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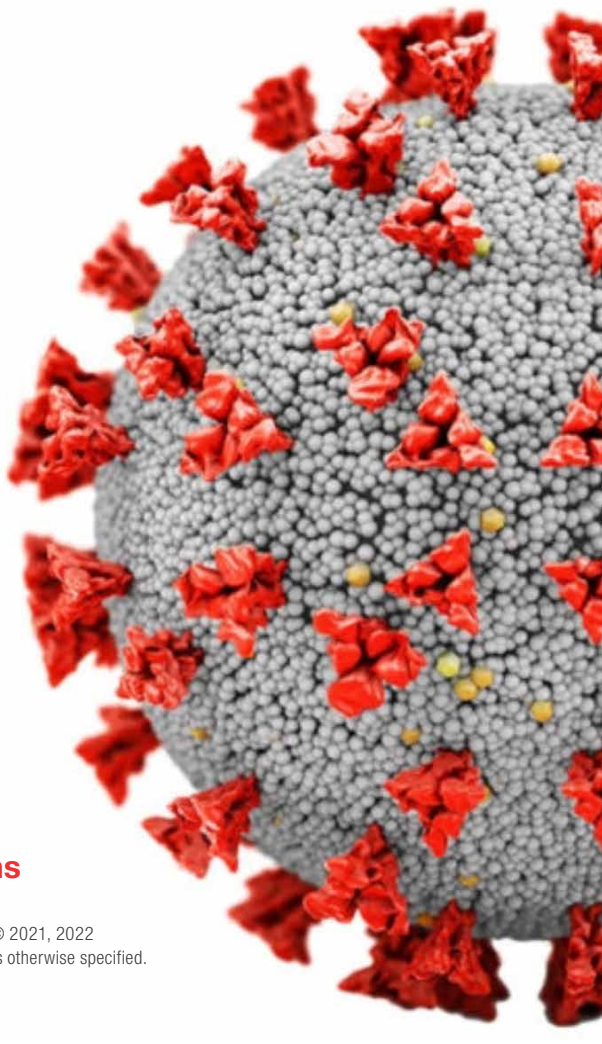
Resources

To learn more about any of the following topics, please visit the resources provided below:

- **Thermo Fisher Scientific's COVID-19 testing solutions**
- **COVID-19 testing technologies**
- **Emerging SARS-CoV-2 variants and mutations**
- **SARS-CoV-2 epidemiological surveillance**
- **SARS-CoV-2 research solutions**



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