Frequently asked questions (FAQs)

Omicron and Alpha variants: The impact of the 69-70del mutation in the Spike protein of SARS-CoV-2 on TaqPath COVID-19 testing assays

Background

The 69-70del mutation is a 6-nucleotide deletion (21765–21770) in the spike (S) gene of SARS-CoV-2 resulting in a deletion of two amino acids at sites 69 (histidine) and 70 (valine) in the spike protein. Until recently, the 69-70del mutation was predominantly observed in the B.1.1.7 lineage, designated by the World Health Organization (WHO) as the Alpha variant. The new SARS-CoV-2 Omicron variant (lineage B.1.1.529, also known as 21M), which was first reported on 24 November 2021 from a sample collected on 9 November 2021, has over 30 mutations across the S gene including the 69-70del mutation [1,2].

Since the discovery of the Omicron variant, isolates with different mutation profiles have been reported, leading to three sub-lineages: BA.1 (also known as 21K), BA.2 (also known as 21L), and BA.3 [3]. The BA.2 sub-lineage does not contain the 69-70del mutation (Table 1).

Table 1. Sub-lineages of Omicron B.1.1.529 (Pangolin lineage, also known as 21M) and their detection pattern.

Omicron sub-lineages	Also known as	69-70del mutation	S gene target failure (SGTF) detection pattern by TaqPath COVID-19 assay
BA.1	21K	Yes	Yes
BA.2	21L	No	No
BA.3	–	Yes	Yes

While there are several mutations besides 69-70del observed in other regions of the S gene as well as mutations in other regions of the SARS-CoV-2 genome, none of the other mutations have an impact on our Applied Biosystems™ TaqPath™ COVID-19 portfolio of assays.

Viruses constantly mutate leading to the emergence of new variants. As part of our post-market surveillance efforts, we routinely collect, review, and analyze data on the performance of our tests, including assessing whether any emerging mutations overlap with our assay design. Based on such analyses and communication with appropriate health authorities, we will communicate if and when any impact on test results is expected.

We assess the performance of the TaqPath COVID-19 tests and the TaqPath COVID-19 2.0 tests regularly against sequences in the Global Initiative on Sharing All Influenza Data (GISAID) public database to confirm *in silico* the detection of all known lineages, including Alpha and Omicron variants, without predicted loss in sensitivity.

Since some of our assays utilize the S gene (Table 2) as one of the targets for detection of SARS-CoV-2, this FAQ document will address how the S gene mutation might potentially affect our current TaqPath COVID-19 portfolio of assays.

Table 2. TaqPath COVID-19 portfolio and S gene dropout.

Kit	SARS-CoV-2 targets	Observable S gene dropout	Cat. No.
TaqPath COVID-19 CE-IVD RT-PCR Kit*	S, N, *orf1ab* (3 separate channels)	Yes	A48067 or A51738
TaqPath COVID-19 HT Kit for use with the Amplitude Platform*	S, N, *orf1ab* (3 separate channels)	Yes	A50883
TaqPath COVID-19, Flu A/B, RSV Combo Kit*	S, N (single channels)	No	A49867
TaqPath COVID-19 RNase P Combo Kit 2.0*	N, *orf1a*, *orf1b* (3 separate channels)	No	A51334
TaqPath COVID-19 Fast PCR Combo Kit 2.0*	N, *orf1a*, *orf1b* (3 separate channels)	No	A51605

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

Key facts on the 69-70del S gene mutation and how it may affect our products

- All viruses mutate over time naturally, and a high rate of mutations in response to selective pressure is especially common for RNA viruses. With that in mind, we design our products as multi-target assays with built-in redundancy.

- The Applied Biosystems™ TaqPath™ COVID-19 CE-IVD RT-PCR Kit (Cat. No. A48067 or A51738) and the TaqPath™ COVID-19 HT Kit (Cat. No. A50883) for use with the Thermo Fisher Scientific™ Amplitude™ Platform, detect SARS-CoV-2 infections by identifying the presence of three gene targets from the *orf1ab*, S, and N regions of the virus. The S gene target in the test is impacted by the presence of the 69-70del mutation (as present in Alpha variant and BA.1 and BA.3 Omicron variants), while the *orf1ab* and N gene targets have been determined to not be impacted by any of the mutations. 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 (*orf1ab* and N gene are detected for both Alpha and Omicron variants).

- The Applied Biosystems™ TaqPath™ COVID-19, Flu A/B, RSV Combo Kit (Cat. No. A49867) assay detects SARS-CoV-2 infections by identifying the presence of two gene targets from the S and N regions of the virus. The S gene target in the test is impacted by the presence of the 69-70del mutation, while the N gene target has been determined to not be impacted by any of the mutations. The Applied Biosystems™ Pathogen Interpretive Software algorithms utilize the results from two targets to generate a final call, and a positive result is called if at least one of the two SARS-CoV-2 targets is detected (N gene is detected for both Alpha and Omicron variants).

- The Applied Biosystems™ TaqPath™ COVID-19 2.0 tests (TaqPath™ COVID-19 RNase P Combo Kit 2.0 (Cat. No. A51334) and TaqPath™ COVID-19 Fast PCR Combo Kit 2.0 (Cat. No. A51605)) employ an advanced assay design targeting 8 sequences across 3 genomic regions (*orf1a*, *orf1b*, and N gene) to compensate for emerging SARS-CoV-2 mutations. The *orf1a*, *orf1b*, and N gene targets have been determined to not be impacted by any of the mutations. The 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 (*orf1a*, *orf1b*, and N gene for both Alpha and Omicron variants).

FAQs

Q. What is the difference between S gene dropout and S gene target failure (SGTF)?

A. There is no difference between the terms. Both refer to the fact that the S gene detection, as targeted in the TaqPath COVID-19 CE-IVD RT-PCR Kit and the TaqPath COVID-19 HT Kit for use with the Amplitude Platform, is impacted if the 69-70del mutation is present in the SARS-CoV-2 genome, as is the case for most of the Alpha and Omicron variants.

Q. What is the S Gene Advantage? Which kits can be used to support screening for the Omicron variant?

A. Using the TaqPath COVID-19 CE-IVD RT-PCR Kit and the TaqPath COVID-19 HT Kit for use with the Amplitude Platform, both the Alpha and Omicron variants (BA.1 and BA.3) will show the S gene dropout in the majority of the cases. The S Gene Advantage helped detect the Alpha variant, and both the WHO and the European Centre for Disease Prevention and Control (ECDC) report that the S gene dropout can be used in screening for the Omicron variant [3,4,5].

Q. Does S gene dropout necessarily indicate the presence of the Omicron variant?

A. The 69-70del is not exclusive to the Alpha and Omicron variants. In other words, the S gene dropout does not conclusively determine that a sample contains the Alpha or Omicron variant. In the case of the Omicron variant, the BA.2 lineage does not cause the S gene dropout pattern [3].

However, the Delta variant is not likely to display the S gene dropout. Furthermore, with the Alpha variant accounting only for <0.1% of the currently circulating strains worldwide [5], any observed S gene dropout is more likely to be indicative for Omicron.

Due to the novelty and potential risks associated with the new Omicron variant, the WHO and the ECDC recommend further characterizing specimens that show S gene dropout using sequencing [3,4].

Q. What is the difference between Omicron BA.1, BA.2, and BA.3?

A. Since the discovery of the Omicron variant (B.1.1.529), isolates with different mutation profiles have been reported, including a subset, referred to as the BA.2 sub-lineage, that lacks the 69-70del mutation. The lineages that include the 69-70del mutation are BA.1 and BA.3.

While the S gene dropout is observed among the BA.1 and BA.3 sub-lineages of Omicron, it is not observed in the BA.2 sub-lineage, which has therefore been referred to as a “stealth” version of Omicron. While BA.1 accounts for >99% of sequences submitted to GISAID as of 18 January 2021, BA.2 and BA.3 only account for a minority of the submitted sequences worldwide [3,5].

Q. What are the recommendations for those who are currently using the TaqPath COVID-19 CE-IVD RT-PCR Kit and the TaqPath COVID-19 HT Kit?

A. Since three SARS-CoV-2 targets are used in the TaqPath COVID-19 CE-IVD RT-PCR Kit and the TaqPath COVID-19 HT Kit, and a positive result is called when only two of the three targets are detected (per our Instructions for Use (IFU) and software algorithm), no adaptations to the test or changes in use are necessary at this time.

To date, the 69-70del S gene mutation has not been found to impact test results obtained using the TaqPath COVID-19 CE-IVD RT-PCR Kit and the TaqPath COVID-19 HT Kit.

Q. What is the potential impact of the 69-70del mutation on SARS-CoV-2 diagnostics?

A. Based on our *in silico* technical assessment, all TaqPath COVID-19 tests are expected to detect SARS-CoV-2 in samples containing the Omicron variant.

While the TaqPath COVID-19 CE-IVD RT-PCR Kit and the TaqPath COVID-19 HT Kit are not intended for use for the detection of the 69-70del mutation in SARS-CoV-2 variant strains, the ECDC has stated that the presence of the 69-70del mutation can be used as part of the screening process for the Omicron variant. Due to the novelty and potential risks associated with the new Omicron variant, the WHO and ECDC recommend further characterizing specimens that show S gene dropout using sequencing [3,4].

"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 ECDC reported [4].

Q. What happens if the S gene target drops out and only one other target is called positive?

A. Due to the built-in redundancy of the assay (TaqPath COVID-19 CE-IVD RT-PCR Kit and TaqPath COVID-19 HT Kit), it is unlikely that the S gene dropout occurs while only one of the other two targets (N gene and *orf1ab*) is detected. In an unlikely event that only one other target is positive, as per the IFU, the result will be called inconclusive. In case of an inconclusive result, the test needs to be repeated by re-extracting the original sample and repeating the RT-PCR. If the repeat result remains inconclusive, additional testing should be conducted if clinically indicated. Since at least one target is detected, the test will not report a false-negative result.

Q. What should I do if sample results are inconclusive?

A. Please refer to the IFU, which provides the following directions:

1. Repeat the test by re-extracting the original sample and repeating the RT-PCR.
2. After retesting one time, report results to the health care provider and appropriate public health authorities.

Important: Samples with an inconclusive result for SARS-CoV-2 shall be retested one time. If the repeat result remains inconclusive, the health care provider should conduct additional confirmation testing with a new specimen, if clinically indicated. If you would like to discuss your specific situation, please contact our technical support at **thermofisher.com/contactus**.

Q. What impact might the 69-70del mutation have for labs that use the TaqPath COVID-19 CE-IVD RT-PCR Kit and the TaqPath COVID-19 HT Kit?

A. The 69-70del in the spike protein results in S gene dropout in RT-PCR assays that use the S gene as one of the targets. The S gene dropout does not mean a result is negative; it only indicates that the S gene could not be detected by the assay. Due to the multi-target (N gene, S gene, and *orf1ab*) approach of the TaqPath COVID-19 CE-IVD RT-PCR Kit and the TaqPath COVID-19 HT Kit, the risk of a false negative is low. So far, there are no documented cases of false-negative COVID-19 results due to the 69-70del mutation.

While the TaqPath CE-IVD RT-PCR Kit and the TaqPath COVID-19 HT Kit are not intended for use for the detection of the 69-70del mutation in SARS-CoV-2 variant strains, the WHO and the ECDC recommend that the S gene dropout can be used as signal for the presence of a 69-70del mutant for further investigation [3,4]. Multi-target RT-PCR tests that utilize S gene regions impacted by the 69-70del mutation are quicker and cheaper than sequencing the entire virus and can help keep track of these mutant strains.

Q. How does the 69-70del mutation impact the TaqPath COVID-19, Flu A/B, RSV Combo Kit?

A. The TaqPath COVID-19, Flu A/B, RSV Combo Kit is a multi-target assay with built-in redundancy—two SARS-CoV-2 targets (N gene and S gene) in a single channel. Due to this assay design, the risk of a mutation in the 69-70del impacting test results is low. To date, there have been no valid reports of the S gene mutation impacting test results. However, due to the assay design wherein the N gene and S gene are detected in a single channel, the S gene dropout cannot be detected.

applied
biosystems

Q. How does the 69-70del mutation impact the TaqPath COVID-19 RNase P Combo Kit 2.0 and TaqPath COVID-19 Fast PCR Combo Kit 2.0?

A. The TaqPath COVID-19 2.0 tests, which include the TaqPath COVID-19 RNase P Combo Kit 2.0 and the TaqPath COVID-19 Fast PCR Combo Kit 2.0, use an advanced assay design targeting 8 sequences across 3 genomic regions (*orf1a*, *orf1b*, and N genes). The S gene is not targeted and the 69-70del mutation is not known to impact test results. Due to the assay design, the S gene dropout cannot be detected.

Q. Do other suppliers' assays target the S gene?

A. There are some other suppliers' assays that also target the S gene. However, we are not able to determine the impact of the 69-70del mutation on those assays. While most other assays only target either one or two genes, the TaqPath COVID-19 CE-IVD RT-PCR Kit and the TaqPath COVID-19 HT Kit for use with the Amplitude Platform, target three different genes (N gene, S gene, and *orf1ab*).

Q. Why were some TaqPath COVID-19 kits designed with the S gene as a target?

A. The S gene was chosen because it was considered a conserved sequence in the early phase of the pandemic and had the least sequence overlap with other coronaviruses.

Q. What is Thermo Fisher Scientific doing to monitor S gene mutations in their assays, and will there be product modifications in the future?

A. We regularly monitor post-market reports and public database updates to provide quality and accurate products for our customers. To date, there have been no reports of test results being impacted due to the S gene 69-70del mutation.

Q. Who should I reach out to if I have additional questions?

A. If you have additional questions or would like to discuss your specific situation, please contact our technical support team. You can identify your local technical support phone number or send an email to technical support at **thermofisher.com/contactus**.

References

1. Africa CDC. Africa Centres for Disease Control and Prevention's Statement regarding the new SARS-CoV-2 virus variant B.1.1.529. https://africacdc.org/news-item/africa-centres-for-disease-control-and-preventions-statement-regarding-the-new-sars-cov-2-virus-variant-b-1-1-529/
2. World Health Organization. Classification of Omicron (B.1.1.529): SARS-CoV-2 Variant of Concern. https://www.who.int/news/item/26-11-2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern
3. World Health Organization. Enhancing response to Omicron SARS-CoV-2 variant. https://www.who.int/publications/m/item/enhancing-readiness-for-omicron-(b.1.1.529)-technical-brief-and-priority-actions-for-member-states
4. European Centre for Disease Prevention and Control. Implications of the emergence and spread of the SARS-CoV-2 B.1.1.529 variant of concern (Omicron) for the EU/EEA. https://www.ecdc.europa.eu/sites/default/files/documents/Implications-emergence-spread-SARS-CoV-2%20B.1.1.529-variant-concern-Omicron-for-the-EU-EEA-Nov2021.pdf
5. World Health Organization. Weekly epidemiological update on COVID-19 - 18 January 2022. https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---18-january-2022

CE IVD

Find out more at **thermofisher.com/covid19**

ThermoFisher SCIENTIFIC