Screening of the H69 and V70 deletions in the SARS-CoV-2 spike protein with a RT-PCR diagnosis assay reveals low prevalence in Lyon, France

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Abstract

The H69 and V70 deletions in SARS-CoV-2 spike protein have been detected in mink and human infections. We found that these deletions resulted in a false negative result for the spike target of a commercial RT-PCR assay. From August 3rd to November 1st, 39/6964 (0.56%) of positive tests performed in Lyon, France, displayed a S negative profile suggesting a limited circulation of the deleted variant. This RT-PCR assay could be useful to address the risk of the mink variant’s spreading.
A SARS-CoV-2 variant detected both in minks and humans in Denmark has raised concerns about mutations associated with potential reduced susceptibility to neutralizing antibodies [1, 2]. This variant harbors a set of mutations identified in the spike (S) glycoprotein including Y453F, I692V, M1229I as well as two amino acid deletions H69del/V70del. To address the risk of spreading, an easy-to-use method enabling a fast large-scale screening is urgently needed.

**H69del and V70del identification during routine genomic surveillance**

As part of routine SARS-CoV-2 genomic surveillance performed at national reference centre for respiratory viruses (Lyon, France), a 6-nucleotide deletion (21765-21770) within the S gene was identified in two nasopharyngeal samples. The SARS-CoV-2 whole genomes corresponding to these samples were sequenced using the ARTIC amplicon based protocol combined with Illumina sequencing (Illumina, San Diego, USA) on NextSeq500 platform (GISAID accession numbers : EPI_ISL_582112, EPI_ISL_582120). Using CoV-GLUE resource [3], we found that the S deletion 21765-21770 led to the removal of 2 amino acid (H69del and V70del) in the N-terminal domain of the S protein (Figure 1A). Importantly Y453F, I692V and M1229I mutations were not detected.

These samples were collected from two SARS-CoV-2 infected health-care workers (HCW) working in the same geriatric unit on September 1st and 7th, respectively. They were 58 and 59 years and developed mild COVID-19. No serological testing nor RT-PCR testing were performed before this episode. For the HCW sampled on September 1st, serological testing performed on November 4th was positive using the Architect SARS-CoV-2 IgG assay (Abbott, North Chicago, USA). The SARS-CoV-2 detection had been performed with the Applied Biosystems TaqPath COVID-19 kit (Thermo Fisher Scientific, Waltham, USA) which includes the ORF1ab, S and N gene targets. For these two samples, the S target was negative while ORF1ab and N targets were positive with Ct values < 25 (Figure 1B).
H69del and V70del screening with RT-PCR kit

In order to assess the use of TaqPath RT-PCR kit for the screening of the H69del and V70del, 15 additional nasopharyngeal SARS-CoV-2 positive samples were sequenced. Among them, 4 were negative for the S target while 11 presented an amplification of the S target. The sequencing results were fully concordant with the RT-PCR profiles (100% of the S negative profile had the H69 and V70 deletions while 100% of the S positive profile did not have these deletions). We then performed a retrospective analysis of RT-PCR results performed in our lab with the TaqPath kit from August 3rd to November 1st among HCW and general population. To avoid misinterpretation, we selected only positive samples with a Ct value < 25 for the N target, the most sensitive target of the test. By doing so, we found that 39/6964 (0.56%) of positive tests performed with the TaqPath kit had no amplification of the S gene. No significant increase of the S negative profile was noticed over time with a proportion ranging from 0% (week # 32, 33, 34, and 42) to 2.91% (week # 35) (Figure 1C).

Discussion and conclusion

In the present study, we explore the ability of a commercial RT-PCR assay for screening the H69 and V70 deletions in S, previously associated with mink and human infections [2]. The prevalence of the S negative profile was relatively low and steady in Lyon, France, from August to November 2020. However, this result should be interpreted with caution. As a limited number of samples with the S negative profile in RT-PCR were sequenced, we could not exclude the presence of other S mutations associated with this profile. Therefore the prevalence of these S variant could be lower than 0.56 %. Moreover we could not determine whether the deletion affected the primer or the probe binding region as their coordinates were not available. Of note, the present diagnostics kit did not lead to a false negative conclusion as the two other targets remain positive. Few manufacturers have included a S target which is characterized by
a high genetic diversity [4, 5]. Importantly, the receptor binding domain (RBD) in the S protein is the main target of SARS-CoV-2 neutralizing antibodies [6].

According to CoV-GLUE resource [3] (last update from GISAID: November 2\textsuperscript{nd}), the S deletion 21765-21770 has been identified in 855 other sequences worldwide (>95\% in Europe) corresponding to a detection rate of about 0.5\% (187, 770 SARS-CoV-2 sequences have been deposited on GISAID as of November 2\textsuperscript{nd}). Interestingly, only 10 sequences including 7 European sequences were sampled before September 1\textsuperscript{st}. Among these European sequences, 5/7 were sampled in March including a French sequence sampled in Marseille on March 23\textsuperscript{th} France [7].

It should be underlined that, except the mutation Y453F within the RBD, the other mutations detected in mink and humans in Denmark have not been associated with significant changes both in SARS-CoV-2 pathogenesis and host-immune response [8, 9]. Nonetheless, H69del and V70del are in N-terminal domain which might play a role in COVID-19 pathogenesis, by interacting with new identified lung receptors [10] or by being a target of neutralizing antibodies [11]. The S variants need to be early identified and characterised as recently done for the circulating S variant N439K which shows evidence of immune evasion [12, 13].

In conclusion our study emphasizes (i) the crucial role of routine genomic surveillance for identifying S variants (ii) that RT-PCR assay could be a gateway for S deletions screening and a high throughput tool to monitor their spread in the global population. For the SARS-CoV-2 positive samples but negative for the S target, whole genome sequencing should be performed in order to characterize all the mutations of the S protein. Further studies are needed to assess the impact of H69del and V70del on the host-immune response and vaccine efficiency.
Figure 1

A. Visualisation of the spike deletion 21765-21770 (in green) compared with the reference sequence Wuhan-Hu-1 (in blue) using genome visualization tool from the CoV-GLUE online resource. The 21765-21770 deletion results in H69 and V70 deletions, with ATC (21764-21771-21772) coding for an isoleucine amino acid (I) B. Amplification curves obtained with TaqPath COVID-19 RT-PCR Kit for samples with the S deletion 21765-21770. The three targets included in the RT-PCR kit are represented by a different colour. The amplification curve of the internal control is also represented (MS2, red curve) C. Prevalence of the S negative profile with TaqPath COVID-19 RT-PCR Kit from August 3rd (week 32) to November 1st (week 44).
Data availability

SARS-CoV-2 genomes sequenced in this study were deposited in the GISAID database (EPI_ISL_582110, EPI_ISL_582111, EPI_ISL_582112, EPI_ISL_582113, EPI_ISL_582114, EPI_ISL_582115, EPI_ISL_582116, EPI_ISL_582117, EPI_ISL_582118, EPI_ISL_582119, EPI_ISL_582120, EPI_ISL_582508, EPI_ISL_623098, EPI_ISL_623099, EPI_ISL_623100, EPI_ISL_623101, EPI_ISL_623102)

Ethics statement

Samples used in this study were collected as part of an approved ongoing surveillance conducted by the national reference centre for respiratory viruses in Lyon, France (WHO reference laboratory providing confirmatory testing for COVID-19). The investigations were carried out in accordance with the General Data Protection Regulation (Regulation (EU) 2016/679 and Directive 95/46/EC) and the French data protection law (Law 78–17 on 06/01/1978 and Décret 2019–536 on 29/05/2019). Samples were collected for regular clinical management during hospital stay, with no additional samples for the purpose of this study. Patients were informed of the research and their non-objection approval was confirmed. This study was presented by the ethics committee of the Hospices Civils de Lyon (HCL), Lyon, France and registered on the HCL database of RIPHN studies (AGORA N°41).

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