Rapid screening assays of N501Y and E484K SARS-CoV-2 variants and identification of an emerging N501T variant in France

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Abstract

From September 2020, new variants of concern of SARS-CoV-2 were detected in Europe. In this context, our hospital center has led us to be particularly vigilant in the search for and detection of these variants. To this end, two SARS-CoV-2 Mutation Discrimination assays for the detection of 501 and 484 mutations on spike protein were developed. It allowed to quickly identify the United Kingdom variant 20I/501Y.V1 and the south-african variant 20H/501Y.V2 that were circulating in our region and surprisingly identify a mutation of interest (N501T), which reported for the first time the A.28 lineage in France.

Full Text

Since December 2019, the world has been in the grip of a pandemic of COVID-19, an infectious disease caused by SARS-CoV-2, a virus that emerged in China[1]. From September 2020, new variants of concern (VOC) of SARS-CoV-2 were detected in Europe [2]. The first was the United Kingdom variant 20I/501Y.V1 (lineage B.1.1.7) found in a retrospective study in the UK on September 20, 2020[3]. B.1.1.7 was characterized by an unusually large number of mutations including 14 non-synonymous mutations, 6 synonymous mutations and 4 deletions [4]. Three mutations in the S gene, N501Y located in the receptor-binding motif (RBM), 69–70 deletion lead to conformational changes of S protein and P681H adjacent to the furin cleavage site are particularly implicated in the dissemination of the variant. In parallel, the variant 20H/501Y.V2 (lineage B.1.351 called Beta) first detected in South Africa in August 2020, contained 10 mutations in the S protein and mutations N501Y and K417N enhanced viral transmission while E484K contributed to immune evasion [5, 6] . The variant 21A/478K (lineage B.1.617.2 called Delta) was first identified in the state of Maharashtra, India in October 2020, had multiple amino acid substitutions in the S protein including L452R and E484Q which cause enhancement of ACE2 binding, transmission and immune evasion [7, 8]. The variant 21K (lineage B.1.1.529 called Omicron) was identified in Botswana and South African in November 2021 and was designated as VOC by the WHO in the same month. It has 37 amino acid substitutions in the S protein, 15 of these being in the receptor-binding domain.

At the end of 2020, there is consistent epidemiological evidence that the United Kingdom variant 20I/501Y.V1 (lineage B.1.1.7) so-called “UK variant” is more efficiently transmitted [9] than the preexisting European strains, in particular in young patients. In this context, the geographical location of our hospital center has led us to be particularly vigilant in the search for and detection of this variant. We carried out enhanced surveillance in the fall of 2020 [10].

Here, we present an epidemiological analysis of SARS-CoV-2 variants circulating between December 21th, 2020 to May 2021 and the emergence during this period of a new variant, which included 69–70 deletion but characterized by the N501T mutation.

The 69-70 deletion, located in the spike protein, can be easily detected with the TaqPath™ COVID-19 RT-PCR kit (ThermoFisher scientific), which allows the determination of the three targets of SARS-CoV-2

(ORF1ab, protein S and protein N). Indeed, in the presence of the 69-70 deletion, the S target is not amplified. In order to confirm the presence of this variant, we sequenced the S gene.

The sequences highlighted the presence of two different variants including the 69-70 deletion circulated in our region. The first corresponds to VOC 20I/501Y.V1 (lineage B.1.1.7), and the second is characterized by another mutation at position 501 (N501T). After whole genome sequencing, this variant was identified as 19B/501T variant (lineage A.28/B.1.160) by the Pasteur Institut (Paris). We noticed that this variant was predominantly coming from one geographical zone. Furthermore, the South-African variant 20H/501Y.V2 characterized by the absence of the 69-70 deletion, the mutation N501Y and the presence of a mutation E/K in position 484 appeared in our region.

In order to facilitate the identification of these variants, we developed a test to quickly identify these mutations without sequencing. To this end, we performed two similar SARS-CoV-2 Mutation Discrimination assay (SARS-MD) for the detection of spike protein mutations on 501 and 484 positions.

This novel SARS-MD assay is based on TaqMan 5'nuclease chemistry for amplifying and detecting specific variants in purified genomic RNA samples. Each assay contained sequence-specific forward and reverse primers to amplify the target sequence region mutation and two TaqMan MGB probes, i.e. one VIC dye-labeled probe to detect the reference sequence and one FAM dye-labeled probe to detect the mutation sequence. The oligonucleotide primers and probes used in the study were designed from the spike gene of the Wuhan Strain (GenBank : NC_045512). Forward and reverse primers designed to cover both sides of the N501Y and E484K mutations had resulted in a product size of 94pb and 74pb respectively. The sequences for each assay provided by ThermoFisher Scientific are presented in Table 1. Briefly, 5µl of the total nucleic acid eluated on the MGISP-960 Platform (BGI) was added to 20µl total volume reaction mixture (1X SuperScript III Platinium One-Step qRT-PCR Reaction Mix, 4.0mM of $MgSO_4$, 500nM of Rox reference Dye, 0.9µM of each primer, 0.2µM of each probe and 0.5µl of Superscript III RT/platinum Taq mix). The RT-qPCR was carried out using the QuantStudio™ 5 Real Time PCR instrument (QS5) and the QuantStudio™ Design and Analysis Software V2.5 (Applied Biosystems) under the following conditions : a pre-read at 60°C for 30 sec, then 55°C for 15 min, followed by 2 min at 95°C, 40 cycles for 95°C for 15 sec and 60°C for 30 sec, terminated by a post-reading at 60°C for 30 sec. Fluorescence intensity is measured and plotted to determinate the reference and the mutant nucleotide allele. Samples that carry the reference sequence was cluster along the X-axis (VIC dye probe) and the mutant sequence along the Y-axis (FAM dye probe) respectively. For each SARS-MD assay, a negative control (water) was included. In the N501Y assay, two positive controls (501N and 501Y) were added on each run. In the E484K assay, two positive controls (481E and 484K) were also added on each run.

From December 21th, 2020 to May 12th, 2021 we selected 212 SARS-CoV-2 positive nasopharyngeal swabs previously analysed with the TaqPath™ COVID-19 RT-PCR Kit (ThermoFisher scientific) with a Cq value <28 (Table 2). Among these samples, 102 were positive with the three targets of the assay (ORF1ab, S gene and N gene) and 110 positive with only two targets (ORF1ab and N gene). All samples were

characterized by spike gene Sanger sequencing [11]. Among the 110 samples with a S gene target failure in PCR, all of them presented the 69-70 deletion.

The SARS-MD-501 results were completely consistent with the sequencing identification of the N501Y mutation (Fig 1.A and Table 2). Thus, the combinaison of RT-qPCR assay with SARS-MD N501Y assay successfuly predicted the presence of 57 20I/501Y.V1 (B1.1.7) variants with a quantification Cycle (Cq) mean value of 17.80 (range 11 from 28). For the 103 samples that do not carry the N501Y mutant sequence, there were distributed along the X axis and formed 2 distinct clusters. The first cluster concerned samples with sequence confirmed SARS-CoV-2 wild strain (N501). These 50 samples containing the N501 reference sequence strain were typed as 20A.EU2. The second group was concerned 53 samples with the N501T mutation. The N501T (A223064C) and N501Y (A23063T) are independant mutations that are located next to one another in the same codon of the spike protein. The probes designed of the assay to one mutation failed to bind to viral sequences that contain the other adjacent mutation. In the presence of N501T mutation, the sample exhibited weak amplification due to probe nonspecific activity and clustered near the negative control. By sequencing, these samples were identified as strain 19B/501T (A.28). As 20H/501Y strain, 19B/501T (A.28) has a deletion on the spike gene (H69/V70 deletion). The Cq mean value was 20.50 (range 10 from 27) for the 50 20A.EU2 strains and 19.60 (range 10 from 27) for the 53 19B/501T variants.

Results obtained by the SARS-MD-484 assay had 100% positive agreement when compared to the 52 samples presented the E484K mutation sequence and typed as 20H 501Y.V2 (B1.351) (FIG 1.B and Table 2). The Cq mean was 18.10 (range 14 from 25) for the 52 20H 501Y.V2 (B1.351) samples. The other samples distributed along the X-axis concerned 160 strains with sequence confirmed SARS-CoV-2 wild mutation (E484).

From December 2020, first N501Y and E484K SARS-CoV-2 variants appeared on French soil. We have identified the earliest case of 20I/501Y.V1 on December 21th, 2020, subsequently, these variants were found in every batch of samples tested. The first 20H/501Y.V2 in our population appeared on february 9th, 2021.

On January 12, 2021, a sample collected from a patient living in a nursing home in northern France was found to carry both 69-70 deletion and N501T mutations. The sequences of this sample concern the 19B/501T variant, which was the first 19B/501T (A.28/b.1.160) in France. We then observed a significant increase in these cases in our laboratory during the first quarter of 2021 (January to May). According to the data reported in GISAID's EpiCoV database, this A.28 VOC was found into 24 countries of the world with highest prevalence in the Persian Gulf countries. France was the European country where the most cases of A.28 were found.

In the present study, two molecular assays that detect SARS-CoV-2 mutations were developed. All assays were precise and robust, completely consistent with the Sanger sequencing. During the first quarter of 2021, the combination of the TaqPath™ COVID-19 RT-PCR kit with SARS-MD assays allowed our laboratory to quickly identify the United Kingdom variant 20I/501Y.V1 and the South-African variant

20H/501Y.V2 that were circulating in our region. Not only the assay is proved to be higly accurate in our clinical screening, it is also sensitive to new mutations within the probe binding site. This has been well exemplified by our discovery of the N501T mutation from the clinical specimens corresponding to the clade 19B/501T [12, 13]. Another important advantage for our SARS-MD assay is that primers and probe can be changed inexpensively and regularly as new SARS-CoV-2 mutations appear. However, a couple of limitations of this study need to be noted. This new screening test is slightly less sensitive than the widely used COVID RT-PCR diagnostic test. Samples with very low viral loads (Ct >28) may not show identifiable signals with this genotyping test [14–17]. However, it is also difficult to sequence these samples (Ct>28) [18].

For all samples sequenced, the correlation with this method was complete. These assays will allow laboratories and countries to screen the mutations of clinical interest in samples for SARS-CoV-2 in a rapid and cost-effective format.

In conclusion, this paper describes the development and validation of SARS-MD assay to detect some mutations of interest at the pandemic period. The impact of the assays was demonstrated by finding a mutation of interest (N501T) circulated in our region, which is reported for the first time and also allowed the identification of the first A.28 lineage in France.

Declarations

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Conflict of interest :

The authors have no competing interests to declare

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Tables

Table 1

Primers and probes used for Mutation discrimination

Use	Position	Name	(5'-3') Sequence	Gene
N501Y Discrimination	Sens primer	nCoV-N501Y_LEFT	GGTGTTGAAGGTTTTAATTGTTACTTTCCT	SARS-CoV-2
	Antisens primer	nCoV-N501Y_RIGHT	AGTACTACTACTCTGTATGGTTGGTAACC	S Gene
	Probe MGB-VIC	nCoV-N501_VIC	CAACCCACT**A**ATGGTG	
	Probe MGB - FAM	nCoV-501Y_FAM	CCAACCCACT**T**ATGGTG	
E484K Discrimination	Sens primer	nCoV-E484K_LEFT	GCCGGTAGCACACCTTGT	SARS-CoV-2
	Antisens primer	nCoV-E484K_RIGHT	GGGTTGGAAACCATATGATTGTAAAGG	S Gene
	Probe MGB-VIC	nCoV-E484K_VIC	AATGGTGTT**G**AAGGTTT	
	Probe MGB-FAM	nCoV-E484K_FAM	AATGGTGTT**A**AAGGTTT	

Table 2

Distribution of 484 and 501 mutations among the 212 positive samples

Mutation	501N	501Y	501T	Total
484E	50	57	53	160
484K	0	52	0	52
Total	50	109	53	212

Figures

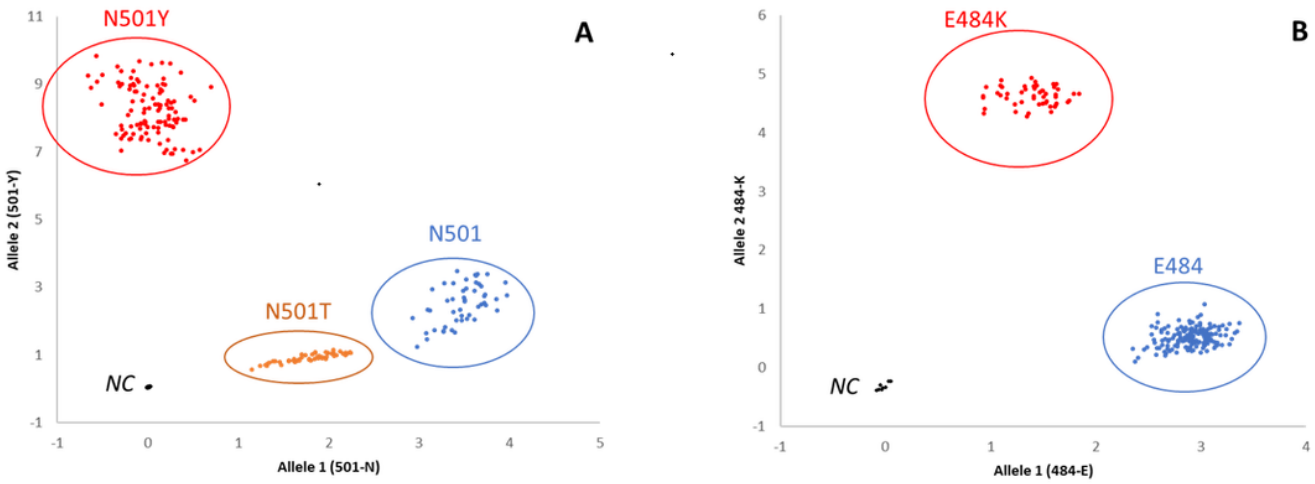

Figure 1

Mutation Discrimination assay. **A)** N501Y/T plot: samples with confirmed SARS-CoV-2 Wild strain N501 in blue, N501Y variant in red and N501T variant in yellow. **B)** E484K plot: samples with confirmed SARS-CoV-2 Wild strain E484 in blue, E484K variant in red.