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A novel single nucleotide polymorphism assay for the detection of N501Y SARS-CoV-2 variants

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ABSTRACT

The N501Y mutation in SARS-CoV-2 variants found in several strains from the UK, South Africa and Brazil has been linked to increased transmission. In order to discriminate N501Y variants quickly, a single nucleotide polymorphism (SNP) discrimination assay was designed and validated. It was then deployed prospectively in 757 nasopharyngeal swabs. Validation of the novel variant discrimination assay corroborated the results in all validation panel samples (n = 63) through sequencing. This novel variant discrimination assay was then deployed prospectively in 757 clinical nasopharyngeal swabs during the last week of January 2021. N501Y was found in 206 (27.4 %) of the samples: 94 (28.2 %) men and 112 (26.85 %) women (p = 0.73). The patients in whom it was identified had a mean age of 47.8 ± 25.9 (0–104) years, similar to that of patients without this variant: 51.7 ± 25.9 (0–104) years (p = 0.06). N501Y variant was confirmed in 34 samples by sequence method and 501 N wild type was confirmed in 67.

1. Introduction

During the evolution and adaptation of a virus it is very common for variants to be seen, particularly in RNA viruses like SARS-CoV-2 (van Dorp et al., 2020). The worrying thing about these variants is their potential capacity to elude the defense response (natural defense mechanisms as well as a vaccine).

The detection of SARS-CoV-2 variants has principally been through the use of the traditional Sanger sequencing. In order to achieve a faster and cheaper method a new one-step variant discrimination real time PCR method was designed and validated. It was then deployed prospectively in 757 nasopharyngeal swabs.

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PCR (VD RT-PCR) assay to detect the N501Y SARS-CoV-2 mutation was designed and developed, and then assayed in clinical samples.

2. Materials and methods

2.1. Nasopharyngeal samples

A total of 64 SARS-CoV-2 positive nasopharyngeal swabs collected in UTM media (Copan, Italy) between December 2020-January 2021 and 2021, under the same conditions as previous samples. Patients were 335 pharyngeal swabs collected prospectively between January 20 and 27 samples was below 30.

2.2. Processing of nasopharyngeal samples and SARS-CoV-2 detection

In the first step of the initial SARS-CoV-2 detection from the nasopharyngeal swabs, RNA was isolated using a MagNA Pure 96 System (Roche Diagnostics, Switzerland) following the manufacturer’s protocol. Amplification and detection were then carried out using in-house real-time (RT)-PCR developed to detect the Orf1ab and nucleoprotein genes of SARS-CoV-2.

Viral genomes were amplified using TaqMan® Fast Virus 1-Step Master Mix (Life Technologies, CA) and the primers and FAM/VIC-labelled MGB (minor groove binding) probes shown in Table 1. Amplifications and data analysis were performed using either a 7500 or a QS5 Real-Time PCR System (Applied Biosystems, CA) under the following conditions: retrotranscription at 50 °C for 15 min; denaturation at 95 °C for 5 min; 40 cycles at 95 °C for 5 s and 60 °C for 30 s. Pre- and post-readings were carried out at 50 °C and acquisition of other data was obtained during the annealing/extension stage of each cycle in both FAM and VIC filters. The total duration of the process was approximately 46 min.

As negative controls, four non-template controls (water) were included in each VD RT-PCR assay run. Three positive controls were also tested in each run: a confirmed N501 N variant sequence, a confirmed N501Y variant sequence and a mixture of the two.

2.4. Sequencing

For the identification of the SARS-CoV-2 N501 variants, a fragment of 450 bp from the spike gene (Nt 1412-1862; aa 472-620) was amplified with primers designed in our laboratory (Table 1). The PCR mix contained 25 pmol of each primer, 0.2 mM of each deoxynucleotide triphosphate, 1 μL reaction volume with the same primers using a BigDye Terminator (Applied Biosystems, Life Technologies Corporation, Foster City, CA, USA) and then removes unincorporated reaction components with the BigDye Terminator purification kit (Life Technologies Corporation, Bedford, USA). Genome sequences were obtained with an ABI PRISM 3130x Genetic Analyzer Avant Sequencer (Applied Biosystems, Life Technologies Corporation, Foster City, CA, USA). The chromatogram files were edited and assembled using Chromas.
amplify, but in the other 63, complete concordance was observed between the results of the RNA sequencing and the novel VD RT-PCR assay method was performed in 101 cases, 67 using Sanger sequencing and 34 using NGS.

2.5. Data and statistical analysis and ethical approval

All statistical tests were performed using GraphPad InStat version 3.00 for Windows 95 (GraphPad Software, San Diego, CA) following the manufacturer’s instructions.

This study was approved by the Local Ethical Committee.

3. Results

3.1. Validation panel

The validation panel was assembled using 29 SARS-CoV-2 501 N (wild) strains and 35 501Y variant strains. One wild variant did not amplify, but in the other 63, complete concordance was observed between the results of the RNA sequencing and the novel VD RT-PCR assay (Fig. 1).

NGS was performed on 11 of these 63 strain N501Y variants to ascertain the specific genotypes and linages. All of them where characterized like SARS-CoV-2 B.1.1.7 strain by Pangolin program (https://pangolin.cog-uk.io/).

3.2. Results for prospective determination of variant

Between January 20 and 27 2021, variant discrimination assays were performed on 757 nasopharyngeal swab samples. Conclusive results were achieved in 750 cases (99.07 %).

The N501Y mutation was present in 206 samples (27.4 %). These variants were found in 94 (28.2 %) men and 112 (26.85 %) women (p = 0.73).

Mean age of patients with N501Y was 47.8 ± 25.8 years (range 0–96) compared to 51.7 ± 25.9 years (range 0–104) for patients without this mutation (p = 0.06).

With the method described above, amplification of samples resulted in Cts of between 15 and 35. The N501Y mutation was present in 12 samples (5.8 %) with Ct of over 30 and wild type in 34 (6.25 %) such samples (p = 0.9).

In 101 sequenced, variants was present in 67 (36 by Sanger and 31 by NGS) and no present in 34 (31 by Sanger and 3 by NGS).

4. Discussion

Mutations in the SARS-CoV-2 spike protein could be linked to loss of natural/vaccine-induced immune response and increased virulence, which could have significant impacts on public health provisions. To this end, it is important internationally for health organizations to detect these variants quickly in order to take actions and adopt specific measures to minimize its community transmission (ECDC, 2021; WHO, 2021).

In 2020, the SARS-CoV-2 spike protein variant D614 G which replaced the original strains identified, was found to be associated with increased transmissibility and more serious pathology (Korber et al., 2020; Zhang et al., 2020; Volz et al., 2021). Variants with this mutation are now globally dominant (Conti et al., 2020).

Recently, the newly identified SARS-CoV-2 variants B.1.1.7, B1.351, and B.1.1.28.1 have come under scrutiny because they can increase transmissibility and reduce neutralization (Leung et al., 2021; WHO, 2021; Weisblum et al., 2020; Conti et al., 2020). All of these variants share the spike protein mutation N501Y, which is involved in virus binding, and is principally found in mutations associated with virus transmission (ECDC, 2020; Leung et al., 2021; WHO, 2021; Weisblum et al., 2020; Virological, 2021; Makowski et al., 2021).

The method designed was able to discriminate the variant in all but one instance: a wild type SARS-CoV-2 which was collected 20 days before the assay and had a Ct of 32. These characteristics could have influenced in the result.

In the other 63 samples (35 of them the N510Y variant), the correlation between the novel VD RT-PCR method and the sequencing method was complete.

Importantly, this VD RT-PCR method can be performed and provide results in less than one hour. What is more, this work indicates that it would be possible to design and develop a real time RT-PCR system that includes both diagnosis and variant detection. This would be a fast and simple system, the drawback being that more reagents would be needed.

The eleven samples analyzed by NGS confirmed that the N501Y variant found was most similar to strain B.1.1.7.

This method was implemented in clinical samples collected prospectively over 7 days at the end of January 2021. Results were possible in close to 100 % of cases. Only in 7 (0.9 %) samples with Ct over 30 was amplification not possible. N501Y variant was present in almost 30 % of these samples. Since the presence of these variants in our locality only

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**Fig. 1.** Variant discrimination assay. a) Variant discrimination assay plot: samples with sequence confirmed SARS-CoV-2 wild strain (N501) in blue, N501Y variants in either red, and mixed variants in green. b) Graph of variant amplification assay: amplification of wild type variant in green and N501Y variants in blue (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).
began in early January, these results suggest that the N501Y variant can now replace circulating wild-type strains very early. No differences in the presence of the N501Y variant were found in terms gender or mean age.

As before, in all samples sequenced, the correlation with this method was complete.

The method developed in this work to identify N501Y SARS-CoV-2 variants is fast and simple, and can be performed in any basic laboratory in less than one hour.

Author statement

Conceptualization; Santiago Melón García, Jose Antonio Boga, Marta Elena Alvarez Argüelles, Susana Rojo Alba, Santiago Melón García. Data curation; Marta Sandoval Torrientes, Jose Antonio Boga, Marta Elena Alvarez Argüelles, Susana Rojo Alba, Santiago Melón García. Formal analysis; Marta Sandoval Torrientes, Marta Elena Alvarez Argüelles, Susana Rojo Alba, Santiago Melón García.

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Declaration of Competing Interest

The authors report no declarations of interest.

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