TITLE PAGE

Full-length title:

Implementation of an in-house real-time reverse transcription-PCR assay to detect the emerging SARS-CoV-2 N501Y variants

Short title (for the running head): qPCR for SARS-CoV-2 N501Y variants

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ABSTRACT

The SARS-CoV-2 pandemic has been associated with the emergence of several variants with a mutated spike glycoprotein that are of substantial concern regarding their transmissibility and ability to evade immune responses. This warrants implementing strategies for their detection and surveillance. We have set up an in-house one-step real-time reverse transcription-PCR (qPCR) assay that specifically detects SARS-CoV-2 spike N501Y variants. Our assay was positive for all 6 patients found spike N501Y-positive by genome sequencing. Ten cDNA samples for each of the 10 Marseille variants identified by genome sequencing and three nasopharyngeal samples of a spike N501Y-negative variant (Marseille-4) that predominates locally tested negative. All negative controls among which 5 SARS-CoV-2-negative nasopharyngeal samples tested negative. First use in the setting of diagnosis on 51 nasopharyngeal samples from SARS-CoV-2-positive but Marseille-4-negative patients showed positivity in 5 cases further confirmed by sequencing as from spike N501Y variant-infected patients. Thus, our in-house qPCR system was found reliable for the detection of the N501Y substitution and allowed estimating preliminarily that spike N501Y variant prevalence was 4% among SARS-CoV-2 diagnoses since January 2020.

TEXT

The SARS-CoV-2 pandemic has been associated with the occurrence of several viral variants with a mutated spike glycoprotein (S). Those currently of greatest concern carry N501Y substitution within the spike (S) receptor binding domain (RBD). Indeed, they have become predominant in England (20I/501Y.V1) [1] and were detected in South Africa (20H/501Y.V2) [2] and Brazil [3]. The 20I/501Y.V1 variant has started to spread worldwide including in France [4]. It has been reported as 50-74% more transmissible than preexisting strains, suspected to evade anti-spike antibodies [1], and it caused a reinfection [5]. Its real-time detection is critical to manage patients appropriately, monitor and assess its epidemiological and clinical features, and survey cases of immune escape post-infection or vaccination. An alternative comprehensive detection strategy is warranted considering the very large number of SARS-CoV-2 cases.

We have implemented an in-house one-step real-time reverse transcription-PCR (qPCR) assay that specifically detects SARS-CoV-2 N501Y variants by targeting nucleotide position 23,063 within S gene where A>U leads to N501Y. SARS-CoV-2 genomes from the GISAID database with or without N501Y were used to design primers and a hydrolysis probe. The sequences of these latter and the qPCR conditions are shown in Table 1. Our N501Y-specific assay was positive for all 6 patients identified as 20I/501Y.V1-positive by genome sequencing and for all 3 additional contact patients for whom sequences were not obtained due to low viral load (Ct of qPCR diagnosis test= 32.0-34.0) leading to no amplification by two conventional PCR. Ten cDNA samples, one for each of the 10 Marseille variants [6] identified by genome sequencing, and three additional nasopharyngeal (NP) samples of the Marseille-4 variant that predominates locally (≈two-thirds of diagnoses) tested negative. All negative controls (5 SARS-CoV-2-negative NP samples and 20 RNA extract-free qPCR

mixes) tested negative. The first use for diagnosis purpose conducted on 51 NP samples from SARS-CoV-2 positive (Ct= 10.2-30.8) but Marseille-4-negative patients showed positivity for 5 samples, which were confirmed by sequencing as from N501Y variant-infected patients.

Our in-house qPCR system was found reliable to detect specifically the N501Y substitution and preliminarily allowed estimating 20I/501Y.V1 variant prevalence to 4% among our current SARS-CoV-2 diagnoses since January. This assay showed better sensitivity than conventional PCR and should be able to detect different N501Y variants. A commercialized RT-PCR diagnosis assay (TaqPath RT-QPCR test) allows the indirect identification of the 20I/501Y.V1 variant by detecting its ORF1a and N genes but not its S gene due to a deletion at positions 21,766-21,772 [4]. However this deletion is also present in strains devoid of the N501Y substitution and was reported in 0.6% of recent SARS-CoV-2 diagnoses in France [4]. Moreover, it is absent from the South African and Brazilian N501Y variants, which prevents their identification. Finally, our in-house qPCR test can be widely and easily deployed in laboratories as it runs on any open qPCR microplate platform, does not require technical workers' training, and is as cheap as other in-house qPCR assays. Such approach should allow adapting continuously diagnosis strategies for new SARS-CoV-2 variants.

Author contributions

Conceived and designed the study: PC, DR. Contributed materials/analysis tools: MB, PEF, LH, PC. Analyzed the data: all authors. Wrote the paper: PC, DR.

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

Ethics

This study has been approved by the ethics committee of the University Hospital Institute Méditerranée Infection, Marseille, France, with the registration number 2020-029.

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TABLE

Table 1. Primers, probe and qPCR conditions

Name	Sequence (5'-3')	Positions *
Primers:		
Pri_IHU_N501Y_F1	ATCAGGCCGGTAGCACAC	22,980-22,997
Pri_IHU_N501Y_R1	AAACAGTTGCTGGTGCATGT	23,135-23,116
Probe (6FAM-labelled):		
Pro_IHU_C_GB_1_MBP	CCACTTATGGTGTTGGTTACCAA	23,058-23,080

* in reference to SARS-CoV-2 genome GenBank Accession no. NC_045512.2 (Wuhan-Hu-1 isolate). The nucleotide carrying the mutation is covered by the probe and underlined.

The qPCR was performed by adding 5 µL of extracted viral RNA to 15 µL of reaction mixture containing 5 µL of 4X TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher Scientific, Grand Island, NY, USA), 0.5 µL of forward primer (10 pmol/µL), 0.5 µL of reverse primer (10 pmol/µL), 0.4 µL of probe (10 pmol/µL), and 8.6 µL of water. PCR conditions are as follows: reverse transcription at 50°C for 10 min, then a hold at 95°C for 20 sec followed by 40 cycles comprising a step at 95°C for 15 sec and a step at 60°C for 60 sec. This qPCR was run on a LC480 thermocycler (Roche Diagnostics, Mannheim, Germany).