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

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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 the N501Y substitution within the spike receptor binding domain. Indeed, they have become predominant in England (20I/501Y.V1) [1] and were detected in South Africa (20H/501Y.V2) [2] and Brazil (20 J/501Y.V3) [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. Sequencing is a detection strategy that is difficult to implement exhaustively considering the very large number of SARS-CoV-2 cases.

We 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 (Table 1). Ten cDNA samples, one for each of the 10 Marseille variants [6,7], including Nextclades 20A.EU2 and 20E, identified in our institute by genome next-generation sequencing, and three nasopharyngeal samples of the Marseille-4 variant/Nextclade 20A.EU2 that predominated locally [8] tested negative. Then we tested SARS-CoV-2 positive nasopharyngeal samples (cycle threshold value of qPCR diagnosis test= 10.2–32.9) collected in January 2021 from 161 patients and for which the viral genotype was determined by next-generation sequencing, as previously described [8]. All 112 and 26 samples from patients infected with a SARS-CoV-2 20I/501Y.V1 and a 20H/501Y.V2 variant, respectively, were positive with the N501Y-specific qPCR, while all 23 samples from patients infected with a N501Y-negative SARS-CoV-2 (Marseille-2/Nextclade 20E (n = 20); Marseille-8 (n = 2); Marseille-4/Nextclade 20A.EU2 (n = 1)) tested negative with our N501Y-specific qPCR.

Our in-house qPCR system was found reliable to detect the N501Y substitution. It allowed estimating the 20I/501Y.V1 variant prevalence among SARS-CoV-2 diagnoses to be 18.0% overall between January 1st and February 28th, 2021, and to increase from 3.4% (57/1683) during the two first weeks of January 2021 to 47.3% (745/1572) during the two last weeks of February 2021. 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 20H/501Y.V2 and 20 J/501Y.V3 N501Y variants, which prevents their identification. Finally, our in-house qPCR test can be widely and easily deployed in laboratories as it can potentially run 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.

CRediT authorship contribution statement

Marielle Bedotto: Methodology, Investigation, Formal analysis, Writing - review & editing. **Pierre-Edouard Fournier:** Methodology, Investigation, Formal analysis. **Linda Houhamdi:** Methodology, Investigation. **Philippe Colson:** Conceptualization, Supervision, Methodology, Investigation, Formal analysis, Writing - review & editing. **Didier Raoult:** Conceptualization, Supervision, Formal analysis, Writing - review & editing.

Declaration of Competing 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.

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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

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 s followed by 40 cycles comprising a step at 95 °C for 15 s and a step at 60 °C for 60 s. This qPCR was run on a LC480 thermocycler (Roche Diagnostics, Mannheim, Germany).
*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.

Ethics

This study has been approved by our institution's ethics committee (N°2020-029).

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