Reverse Transcription Polymerase Chain Reaction Pattern of SARS-CoV-2 Beta Variant

Padrão da Variante Beta do SARS-CoV-2 por Biologia Molecular (RT-PCR)

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Palavras-chave: COVID-19/epidemiologia; COVID-19/virologia; Mutação; Portugal; Reação em Cadeia da Polimerase Via Transcriptase Reversa; SARS-CoV-2

To the Editor:

In March 2021, the presence of the SARS-CoV-2 Beta (B.1.351) variant was suspected and detected in our hospital by real-time reverse transcription polymerase chain reaction (RT-PCR), later confirmed by the National Institute of Health Dr. Ricardo Jorge (INSA) by genomic sequencing. With the increase in the prevalence of SARS-CoV-2 variants in Portugal[1] and the fact that not all laboratories have specific kits to detect these mutations, we believe that it is relevant to share the findings that led us to suspect the presence of a variant in these patients. This variant is considerably more contagious and can quickly worsen the underlying disease of inpatients that share the same ward if they become infected.[2]

The RT-PCR test can be performed using various kits, which can detect various viral genes. These genes code for different structures such as the spike glycoprotein (S), the envelope protein (E), the nucleoprotein (N) and the ORF1ab sequence that contains RNA-dependent RNA polymerase (RdRP).[3]

Among the SARS-CoV-2 variants currently in circulation in Portugal, there are some raising attention. The Alpha (B.1.1.7) variant, originally identified in the United Kingdom in September 2020, the Gamma (P.1) variant circulating in Brazil since mid-2020 and the Beta (B.1.351) variant described in South Africa at the end of 2020.[4] The three variants share the *N501Y* mutation and the Beta and Gamma variants share the *E484K* mutation.[5]

Samples from our patients, obtained by nasopharyngeal swabs, were tested using the Allplex™ (Seegene®; Werfen) RT-PCR kit that detects the presence of the E, RdRP/S and N genes. The samples had low cycle thresholds (Ct) values (< 25 - 30) and we noticed a RT-PCR amplification pattern in which the N gene amplified two to six cycles later in relation to the other genes. This raised the suspicion that these samples could contain a SARS-CoV-2 variant. The results are described in Table 1.

In order to clarify these findings, we tested the samples with the Novaplex™ I (Seegene®, Werfen) RT-PCR kit that can detect the presence of the specific mutations in each variant. All samples were positive for the *E484K* and *N501Y* mutations and negative for the 69/70 deletion. In order to differentiate the Gamma and Beta variants, samples were sent to *INSA* for genome sequencing, which revealed that all samples had the SARS-CoV-2 Beta variant (B.1.351) Therefore, we identified 12 new cases of the Beta mutation. Until March 2021, there were only five reported cases of this variant in Portugal. In the most recent report, there are 103 reported cases.[1]

The aim of this letter is to raise awareness regarding this variant, which can be suspected when there are lower cycle threshold values and the N gene has two to six amplification cycles later than the other genes tested, which is relevant since genome sequencing is a time consuming process. Kits that detect SARS-CoV-2 variants should be implemented as reflex testing when this pattern is present.

AUTHORS CONTRIBUTION

DFS: Draft of the paper, critical review, and approval of the final version of the paper.

PDS: Draft of the paper and critical review.

IB: Critical review and approval of the final version of the paper.

Table 1 – Real-Time RT-PCR results

Kit (Analyzer)	**Samples**	**Gene**		
		E (ct)	**RdRP/S** (ct)	**N (ct)**
AllplexTM (Seegene®, Werfen)	A	15.17	17.36	22.53
	B	20.16	22.08	25.89
	C	18.29	18.20	21.29
	D	16.06	15.77	20.87
	E	19.06	18.03	22.87
	F	19.57	18.98	22.23
	G	20.85	20.93	27.14
	H	22.17	22.59	26.43
	I	19.80	20.05	25.90
	J	20.35	20.70	25.00
	K	26.21	25.74	31.49
	L	23.03	21.75	25.20

ct: cycle threshold

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PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association updated in 2013.

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patients' data publication.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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