SARS-COV-2 VARIANTS IN THE REGION OF LISBON: COMPARISON AND VALIDATION STUDY BETWEEN RT-PCR AND NGS METHODOLOGIES

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Introduction

A novel coronavirus appeared in 2019 and it was termed severe acute respiratory syndrome coronavirus (SARS-CoV-2). The World Health Organization (WHO) named the infection, caused by this new coronavirus, Coronavirus Disease 2019 (COVID-19). On March 11th, this disease was considered a pandemic by the WHO [1].

Zoonotic coronaviruses were detected in the 1960s. They are viruses that usually exist in vertebrate animals but can also jump to humans and cause disease in both animals and humans [2, 3, 4]. They are single and positive stranded RNA viruses with a size between 80-200 nm in diameter with spikes of 12-24 nm that bring to mind a crown shape, hence the name “corona” [5]. The RNA of these viruses is wrapped by a nucleocapsid protein (N), which forms a coiled tubular structure. This structure is surrounded by the viral envelope (E) which has the matrix protein (M) and the spike structural protein (S) linked to it [1].

Through the pandemic, new variants kept emerging and with it the need to identify them and its possible consequences for the public health as fast as we could.

The aim of this project is to identify the main VOCs and VOIs circulating in the region of Lisbon, applying the methodology of real time RT-PCR in cadavers that tested positive for SARS-CoV-2. To meet this goal, we used three assays: Allplex™ SARS-CoV-2 Variants I, Allplex™ SARS-CoV-2 Variants II and Allplex™ SARS-CoV-2 Variants V. The first one detects defining mutations of the Alpha, Beta and Gamma variants (N501Y, E454K and HV69/70del), the second assay detects mutations present in the Delta, Beta, Gamma and California variants (L452R, K417T, K417N and W152C) and the third one detects defining mutations of the Delta and Lambda variants (L452R, P681R, L452Q and F690S) (Table 1). We also compared the results obtained through RT-PCR with two methods of NGS: NGS-ONT (Oxford Nanopore Technologies) e NGS-Sanger based.

Methodology

Sample collection, RNA extraction, RT-PCR and sequencing

The National Institute of Legal Medicine and Forensic Sciences, South Delegation (Lisbon) receives deceased bodies from Lisbon, Barreiro, Cascais and Torres Vedras. Before the autopsy, as routine due to the current pandemic, they need to be tested for the eventual presence of SARS-CoV-2 by the Laboratory of Virology and Infectious Diseases from the Institute. Nasopharyngeal swabs are collected from the corpses and placed in 1.5 mL of NaCl 0,9% in a microtube. The positive samples are stored in the refrigerator at -23ºC. Allplex™ SARS-CoV-2 and 3plex™ SARS-CoV-2 RT-PCR assays from SeeGene are used as a laboratory routine to detect the presence of SARS-CoV-2 RNA. To identity the genetic variants, Allplex™ SARS-CoV-2 Variants I, Allplex™ SARSCoV-2 Variants II and Allplex™ SARS-CoV-2 Variants V were used. The samples are inactivated by heat at 56ºC during 30 minutes before the RNA extraction. The extracted RNA was amplified using CFX96™ Real-time PCR Detection System (Bio-Rad). 72 samples were run on RT-PCR with SeeGene Allplex™ SARSCoV-2 Variants I, 33 samples with SeeGene Allplex™ SARS-CoV-2 Variants II and 14 samples with SeeGene Allplex™ SARS-CoV-2 Variants V assay.

To validate the RT-PCR results, each sample was sequenced by two methods of Next Generation Sequencing (NGS): NGS-Oxford Nanopore Technologies (NGS-ONT) and NGS-Sanger Based.

Data analysis

72 samples were tested with both methodologies (RT-PCR and NGS) but only 60 could be used for comparison purposes. In 12 samples it was not possible to obtain results with NGS. RT-PCR results were compared to at least one of the sequencing methods in 39 samples and compared to both methods in 21 samples.

Results and conclusions

With the three assays together we concluded that 18 samples belong to the Alpha variant and 42 to the Delta variant (Figure 2). In 12 samples it was not possible to compare the RT-PCR and NGS results.

The variants assigned by RT-PCR were coincident with the variants attributed by at least one of the sequencing methods in 100% of the samples (n=60) (Figure 3).

The only mutations that can be compared between RT-PCR and NGS results are N501Y, HV69/70del, L452R and P681R. With RT-PCR, N501Y was detected in 18 samples. These results matched at least one of the NGS methods in all samples (n=18) (Figure 1). With RT-PCR, HV69/70del was detected in 48 samples. However, these results only matched NGS results in 16 samples (Figure 1). This deletion alone does not affect the definition of the variant so I did not consider this discrepancy relevant. With RT-PCR, L452R was detected in 42 samples. These results matched at least one of the NGS methods in 41 samples (Figure 1). In the only sample where a match was not obtained, this mutation could not be detected by NGS-ONT and the reason might be due to the bad quality of the sample and low coverage of that part of the genome. With RT-PCR, P681R was detected in 14 samples. These results matched at least one of the NGS methods in 12 samples (Figure 1). In the two samples where a match was not obtained, this mutation could not be detected by NGS-ONT due to the same reason mentioned above.

Table 1. Spike mutations present in the four VOCs: Alpha, Beta, Gamma and Delta.

Alpha	Beta	Gamma	Delta
HV69/70del	D80A	L18F	T19R
Y145del	D215G	T20N	E156G
N501Y	241/243del	P26S	157/158del
A570D	**K417N**	D138Y	**L452R**
D614G	**E484K**	R190S	T478K
P681H	**N501Y**	**K417T**	D614G
T716I	D614G	**E484K**	**P681R**
S982A	A701V	**N501Y**	D950N
D1118H		D614G	
		H655Y	
		T0027I	
		V1176F	

Figure 1. Comparison of the detected mutations between RT-PCR and NGS.

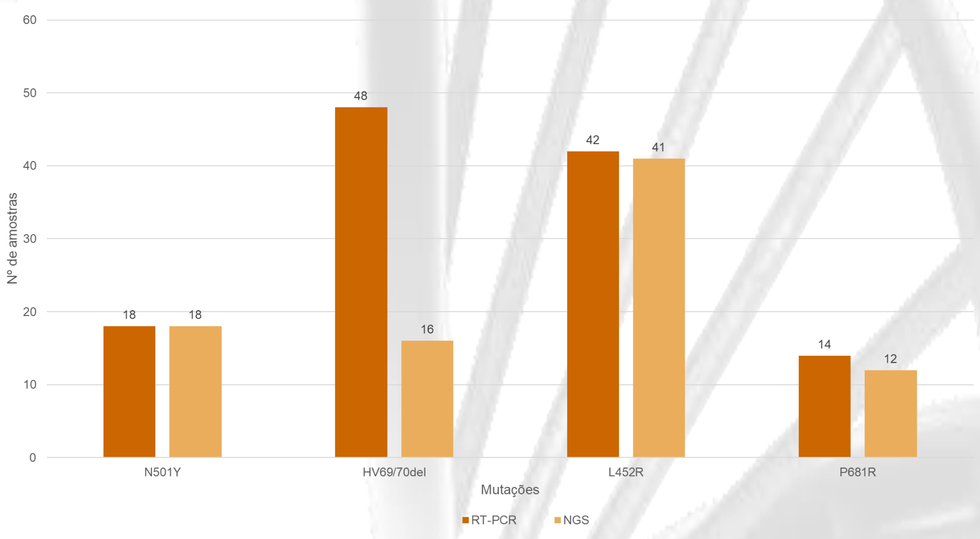

Figure 2. Percentage of variants assigned to the Alpha and Delta variants.

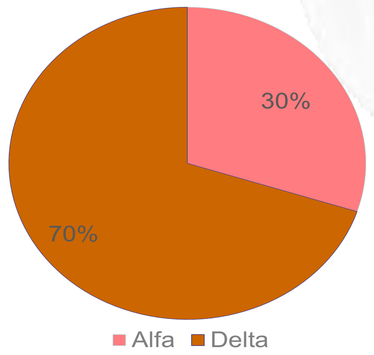

Figure 3. Number of samples assigned to Alpha and Delta variants by RT-PCR and NGS.

	Number of samples	
Variant	**RT-PCR**	**NGS**
Alfa	18	18
Delta	42	42

The percentage of the concordant results between the two methodologies suggests a huge efficacy of the three assays tested in this study in the correct identification of the different SARSCoV-2 variants.

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