

TITLE: PREDICTIVE IDENTIFICATION OF SARS-COV-2 DELTA VARIANT USING SANGER SEQUENCING TECHNIQUE

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ABSTRACT:

The SARS-CoV-2 rapid worldwide spread has caused the emergence of new variants of great importance for the pandemic scenario. SARS-CoV-2 variants of concern (VOCs) are named as Alpha, Beta, Gamma and Delta. VOCs present main mutations in the spike protein, specifically in the receptor binding domain (RBD), responsible for viral binding to the host cell ACE2 receptors. The Delta lineage was first detected in India in October 2020 and now (August 2021) has been associated with an increased transmission worldwide in the last few months. New Generation Sequencing (NGS) plays an important role in the identification of SARS-CoV-2 lineages but it is expensive, time consuming and laborious. Nonetheless, the evaluation of the mutational profile, through Sanger sequencing of the RBD region could allow prediction and discrimination of VOCs. As the Sanger technique is faster and less expensive than NGS, it is preferred as a screening method to detect SARS-CoV-2 mutations. This work aimed to identify the Delta variant using Sanger sequencing technique. In August 2021, a patient was hospitalized with a suspicion of SARS-CoV-2 infection and had a nasopharyngeal swab sample collected. The sample was submitted to RT-qPCR technique and had a positive result. It was also submitted to the Sanger sequencing technique with a characterized Delta variant sample as a control, using primers that flank the RBD portion of the SARS-CoV-2 spike protein. These nucleotide sequences were analyzed against the reference sequence of the first SARS-CoV-2 complete genome (NC_045512.2). The following nucleotide exchanges were identified: T22917G, C22995A and A23403G, which lead to the spike protein mutations L452R, T478K and D614G. These alterations are consistent with the Delta variant. It is important to highlight that RBD region sequencing provides a presumptive result, since it is a virus genome partial sequencing and does not substitute NGS techniques for lineage characterization. The Sanger sequencing can be used as an important tool for VOCs screening and to perform SARS-CoV-2 genomic surveillance and monitoring, faster than NGS techniques. In addition, it allows other new variants to be detected in this same region, using the same protocols.

Keywords: variants of concern (VOC), Delta variant, Sanger sequencing, genomic surveillance.

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