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TECHNICAL ADVANCE

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Genome Sequencing from Post-Mortem Formalin-Fixed, Paraffin-Embedded Lung Tissues



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Implementation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) testing in the daily practice of pathology laboratories requires procedure adaptation to formalin-fixed, paraffin-embedded (FFPE) samples. So far, one study reported the feasibility of SARS-CoV-2 genome sequencing on FFPE tissues with only one contributory case of two. This study optimized SARS-CoV-2 genome sequencing using the Ion AmpliSeq SARS-CoV-2 Panel on 22 FFPE lung tissues from 16 deceased coronavirus disease 2019 (COVID-19) patients. SARS-CoV-2 was detected in all FFPE blocks using a real-time RT-qPCR targeting the *E* gene with crossing point (Cp) values ranging from 16.02 to 34.16. Sequencing was considered as contributory (i.e. with a uniformity >55%) for 17 FFPE blocks. Adapting the number of target amplification PCR cycles according to the RT-qPCR Cp values allowed optimization of the sequencing quality for the contributory blocks (i.e. 20 PCR cycles for blocks with a Cp value <28 and 25 PCR cycles for blocks with a Cp value between 28 and 30). Most blocks with a Cp value >30 were non-contributory. Comparison of matched frozen and FFPE tissues revealed discordance for only three FFPE blocks, all with a Cp value >28. Variant identification and clade classification was possible for 13 patients. This study validates SARS-CoV-2 genome sequencing on FFPE blocks and opens the possibility to explore correlation between virus genotype and histopathologic lesions. (*J Mol Diagn* 2021, 23: 1065–1077; <https://doi.org/10.1016/j.jmoldx.2021.05.016>)

The coronavirus disease 2019 (COVID-19) pandemic is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Coronaviruses are a family of enveloped single-strand, positive-sense RNA viruses that cause a wide spectrum of respiratory diseases. Since the initial report on this novel coronavirus in Wuhan, China,^{1–3} mortality and morbidity rapidly increased around the globe. Researchers worldwide are contributing to sequencing initiatives to try to understand how the virus is spreading. As of April 2021, up to 1,211,666 SARS-CoV-2 genomes were sequenced and

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