Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work

Supplement to:

Halvatsiotis P, Kotanidou A, Tzannis K, et al. Demographic and Clinical Features of Critically Ill Patients with COVID-19 in Greece: The Burden of Diabetes and Obesity

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METHODS

ASSAY COLLECTION AND ANALYSIS

Laboratory confirmation for SARS-2-CoV-2 was defined by a positive result of real-time reverse transcriptase-polymerase chain reaction (Real-time PCR) method (VIASURE Sars-CoV-2) using as specimen nasal or nasopharyngeal swamp samples obtained from all patients at admission to the hospital. The detection is done in one step real time RT format where the reverse transcription and the subsequent amplification of specific target sequence occur in the same reaction well. The isolated RNA target is transcribed generating complementary DNA by reverse transcriptase which is followed by the amplification of a conserved region of ORFJab and N genes for SARS-CoV-2 using specific primers and a fluorescent-labeled probe. The use of positive and negative controls in each run, validate the reaction by checking the absence of signal in the negative control well and the presence of signal for SARS-CoV-2 in the positive control well. Check Internal Control signal to verify the correct functioning of the amplification mix. A sample is considered positive if the Ct value obtained is less than 38and the internal control shows or not an amplification signal. Sometimes, the detection of internal control is not necessary because a high copy number of target can cause preferential amplification of target-specific nucleic acids. A sample is considered negative, if the sample shows no amplification signal in the detection system but the internal control is positive. An inhibition of the PCR reaction can be excluded by the amplification of internal control. VIASURE SARS-CoV-2 Real Time PCR Detection Kit has a detection limit of ≥10 RNA copies per reaction for ORF1ab and N genes[1].

Reference

1. Victor M Codman, Olfert Landt, Marco Kaiser, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill.* 2020Jan23; 25(3): 2000045. doi:10.2807/1560-7917.ES.2020.25.3.2000045