SARS-COV-2 qRT-PCR CT VALUE THRESHOLD DETERMINES THE SUCCESS OF WHOLE GENOME SEQUENCING OF BIOLOGICAL SAMPLES OBTAINED FROM PATIENTS AND CADAVERS

John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, HI

Co-Authors: Tseng AC, Qin Y, Ching LLC, Yeung JW, Siu JM, Zorilla R, Salomon RS, Nerurkar VR

Introduction: Throughout the COVID-19 pandemic, real-time quantitative reverse-transcription PCR (qRT-PCR) and whole genome sequencing (WGS) have emerged as invaluable tools for detecting SARS-CoV-2 in patients and identifying new variants. Further, in academic settings, it is vital for medical students and faculty to handle cadavers that do not harbor SARS-CoV-2. However, not every biological specimen is ideal for WGS, as there are many technical and clinical variables that determine the feasibility of WGS for a specimen. qRT-PCR and WGS are costly endeavors, therefore it is essential to develop techniques that can predict the success of WGS in a specimen. The aim of this study was to evaluate the correlation between qRT-PCR Ct values and the success of WGS in respiratory swabs (ie. nasal [NS], nasopharyngeal [NPS], and oropharyngeal [OPS] swabs) and/or anal swabs (AS) collected from patients and cadavers.

Methods: NS, NPS, or OPS collected from patients confirmed as SARS-CoV-2 PCR positive were obtained from several clinical laboratories on Oahu. NS, NPS, OPS, and/or AS obtained from cadavers donated through the JABSOM Willed Body Program were tested to ensure the safety of medical students prior to anatomy labs. Following RNA extraction, the CDC 2019-nCoV qRT-PCR Diagnostic Panel, consisting of two viral targets to the nucleocapsid gene (N1 and N2), was used to detect SARS-CoV-2. For WGS, total RNA was extracted and the entire SARS-CoV-2 genome was amplified using the ARTIC Network V3 primer pools. Purified PCR products were submitted to the Advanced Studies in Genomics, Proteomics and Bioinformatics (ASGPB) facility at UH Manoa and sequenced using the Illumina MiSeq platform. Sequencing reads were mapped to the original Wuhan sequence (MN908947.3) and assembled into whole genomes using the iVar workflow. Assembled sequences were submitted to the GenBank.

Results: The average Ct value for all 24 samples amplified using qRT-PCR was 31.40, ranging from 17.15 to 40.49. The average Ct values for sequenced samples submitted and not submitted to GenBank were 24.27 (range 17.15-32.53) and 34.96 (28.10-40.49), respectively. All 52 cadaver samples tested were negative with Ct values >40.00 (40.77-44.23) for N1 and/or N2 genes or had undetermined Ct values. Out of the 24 patient samples processed for WGS, 8 samples (33%) gave high quality WGS. 100% (6/6) samples with Ct values <28.00 compared to 11% (2/18) samples with Ct values >28.01 gave high quality WGS for GenBank submission.

Conclusion: All 52 cadaver samples were clearly negative compared to patient samples. Our qRT-PCR assay had a Ct cutoff of 28.00 as patient samples that exhibited Ct values <28.00 were more likely to be submitted to GenBank than samples with Ct values >28.01. Therefore, Ct values can be used as an accurate and cost-effective parameter for prioritizing samples that can proceed for WGS. Further data analysis using GraphPad Prism is

ongoing to identify the threshold Ct value for efficient WGS for our sample population.
Acknowledgements: This research was supported by a COBRE grant (P30GM114737) from the Pacific Center for Emerging Infectious Diseases Research, a grant (P20GM103466) from the INBRE, NIGMS, and a grant (T37MD008636) from the NIMHD, NIH. We thank Dr. Jennifer Saito at the ASGPB for her expertise with WGS, and Dr. Eileen Nakano and Dr. Sandra Chang for their assistance with sample procurement. We also thank the Kaiser Permanente Clinical Laboratory, National Kidney Foundation of Hawaii and UH Clinic at Kaka'ako for providing de-identified patient samples, and the Willed Body Program at JABSOM for access to cadaver samples.