CONCURRENT SESSION 2 – COVID-19 RESEARCH EFFORTS

Development of Rapid Viability RT-PCR (RV-RT-PCR) Method for Detection of Infectious SARS-CoV-2

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The ongoing COVID-19 pandemic caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) continues to consume many lives worldwide. One of the potential ways this virus can spread is through direct contact with an infectious person or with a contaminated article or surface. Although transmission of SARS-CoV-2 via surfaces is not thought to be the primary way the virus spreads, one could get infected by touching the virus contaminated article or surface and then touching one's own mouth, nose, or eyes. Along with symptomatic patients, pre-symptomatic, asymptomatic, and convalescence phase carriers of SARS-CoV-2 can shed the virus. The environment and surfaces surrounding such virus carriers can get contaminated by droplets (coughs, sneezes, and other exhalations) and/or surface contact in healthcare and non-healthcare settings. Depending on the material, type of surface, and environmental conditions used in experimental studies, surface stability of SARS-CoV-2 virus has been reported from a few hours to days and even up to 28 days in some conditions. To expedite studies on understanding potential surface transmission of the virus and to aid environmental epidemiological investigations, we developed a rapid viability reverse transcriptase PCR (RV-RT-PCR) method that detects viable (infectious) SARS-CoV-2 from swab samples in <1 day compared to several days required by current gold-standard cell-culture-based methods. The method integrates cell-culture-based viral enrichment in a 96-well plate format with gene-specific RT-PCR-based analysis before and after sample incubation to determine the cycle threshold (CT) difference (ΔCT). An algorithm based on $\Delta CT \geq 6$ representing ~ 2-log or more increase in SARS-CoV-2 RNA following enrichment determines the presence of infectious virus. The RV-RT-PCR method with 2-hr viral infection and 9-hr post-infection incubation periods includes ultrafiltration to concentrate virions, resulting in detection of <50 SARS-CoV-2 virions in swab samples in 17 hours (for a batch of 12 swabs), compared to days typically required by the cell-culture based method. The SARS-CoV-2 RV-RT-PCR method may also be useful in clinical sample analysis and antiviral drug testing, and could serve as a model for developing rapid methods for other viruses of concern.