ORT_25 - Evaluation of SARS-COV-2 active replication using RT-QPCR to quantify viral subgenomic RNA

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Introduction: SARS-CoV-2 virus is a non-segmented positive-sense, single-stranded ribonucleic acid (RNA) viruses packaged in helical nucleocapsids. During the intracellular life cycle, coronaviruses express and replicate their genomic RNA to produce full-length copies that are incorporated into newly produced viral particles. The replication occurs from the transcription of negative strain RNA intermediates, which is a template for the positive genomic strain RNA (gRNA) and subgenomic RNAs (sgRNA) synthesis. sgRNAs have been used as a possible marker of active SARS-CoV-2 replication since they are the infectious viral particles proteins precursor.

Objectives: Thus, this study aims to use the RT-qPCR technique for sgRNA detection and quantification and its intermediate negative RNAs, to evaluate viral replication in Betapropiolactona inactivated virus samples, as strategies for immunobiologicals development.

Methodology: For this purpose, inactivated virus, subjected to five serial blind passages (BP), were amplified by the RT-qPCR using oligonucleotides targeting genomic envelope (ENV), subgenomic RNA (sgRNA) and intermediate RNAs (ENV negative strain) genes.

Results: Through five serial BP, all SARS-CoV-2 PCR targets have been detected in positive controls. Viral titer, among these samples, remained similar all over the passages, regardless the detected target. Considering inactivated viral samples, this fact was also observed for ENV and ENV negative strain gene targets, ranging 8.66 Log10 copies/mL in BP1 to 3.64 Log10 copies/mL in BP5. In contrast, low titers of sgRNA were only detected in initial passages (5.84 Log10 copies/mL in BP1 and 4.02 Log10 copies/mL in BP2), comparing to gRNA titers. After the second passage, sgRNA was no longer detected in the inactivated SARS-CoV-2 virus samples.

Conclusion: These results indicate successful SARS-CoV-2 virus inactivation due to sgRNA titers decreasing in serial blind passages. Thus, sgRNA monitoring by RT-qPCR can be an alternative tool to confirm viral inactivation, reducing time and allowing the rapid use of inactivated viruses in the immunobiological development. However, further experiments must be carried out to follow up SARS-CoV-2 replication and detection of these targets over time.

Keywords: SARS-CoV-2, RT-qPCR, sgRNA, Viruses Inativation