Quality and quantity of RNA extracts and the correlation between gross RNA load and the Ct values in a real time RT-PCR used for the detection of SARS CoV-2

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Introduction and Objectives: Quality and quantity of the RNA extracted from nasopharyngeal or throat swabs are important to qualify RNA extracts for real time reverse transcription-PCR (rtRT-PCR) in the laboratory diagnosis of SARS-CoV-2 infection. The current study was designed to identify the characteristics of the RNA extracts and explore the relationship between gross RNA load and Ct values as well as the quality of RNA extracts on rtRT-PCR interpretations.

Methods: A total of 301 viral RNA samples from throat swabs taken from COVID-19 suspected individuals extracted using SpinStar^{TM} (89/301) and BioFlux Biospin (212/301) nucleic acid extraction kits were used for the study. There were 240/301 rtRT-PCR positive, 50/301 negative and 11/301 inconclusive (cannot interpret as positive or negative = only one target amplification is detectable with internal control) samples. Gross RNA concentration of the extracts was measured by NanoDrop 2000 Spectrophotometry and 260/280 ratio and absorption spectra given by NanoDrop were used to characterize the purity of the extracts. The correlation between the concentration of the pure extracts and the respective rtRT-PCR (Altona Diagnostics, Germany) Ct value was evaluated using Pearsons correlation at α of 5% (MiniTab 16.1.)

Results: Of the 301 RNA extracts, 215 had impurities and 86 were pure extracts. Of the 212 Bio Spin extracts, 7 (3%) were pure and 205 (97%) had impurities. Of the 89 extracted by SpinStar^{TM} 79 (89%) were pure and 10 (11%) had impurities. Of the 290/301 conclusive samples, 204 were impure and of the 11/301 inconclusive samples all were impure. 86/301 pure extracts, categorized into Ct values of 10–20, 21–30 and 31–40 coincided with average gross RNA concentration of 7.44 ng μl^{-1}, 6.21 ng μl^{-1} and 2.25 ng μl^{-1}, respectively. Gross RNA concentration showed a negative correlation with Ct values with a Pearson co-efficient of -0.39 (*p*-value <0.0001).

Conclusions: Based on the current study, the Biospin extraction kit produced higher numbers of impure extracts. Use of RNA extraction kits that produce pure RNA must be encouraged for the SARS-CoV-2 rtRT-PCR. Conclusive as well as inconclusive samples had impurities. A high gross RNA load was related to lower Ct values when pure extracts were evaluated. Purity and quantity have impact on rtRT-PCR results.

Keywords: *Purity, Quality of RNA extracts, Extraction kits, real time RT-PCR, COVID-19*

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