A pooled testing approach to COVID-19 screening by real time RT-PCR

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Introduction and Objectives: Pooled testing is a cost-effective strategy to overcome the high burden on resources and increase testing capacity during pandemic times. This study was designed to analyse the effect of pooling of samples and elutes for the detection of SARS CoV-2 using real time RT-PCR (rtRT-PCR).

Methods: Twenty SARS-CoV-2 positive samples with Ct value of 25-35 and 60 known negative samples based on initial PCR results were used for this study. Ten positive and 30 negative samples were used to prepare 2-, 4- and 8-fold pooled samples prior to extraction while the rest were subjected to viral RNA extraction prior to pooling and used to prepare the same pools from elutes. Each pool contained a single positive sample together with negative samples. Each pool was duplicated, and the average Ct value was calculated. Pools were compared with the neat samples by calculating sensitivity and using paired sample *t* test with 95% confidence interval in MiniTab Version 19.

Results: The sensitivity of 2-, 4- and 8-fold pooled samples were 100%, 90% and 50%, respectively when compared with neat samples. The sensitivity of 2-, 4- and 8-fold pooled elutes remained 100% when compared with neat elutes. The mean Ct value differences (ΔCt) (±StDev.) obtained with the 2-fold pools exceeded that of the neat samples by 0.8 ± 0.57 cycles, the 4-fold pools by 2.86 ± 1.04 cycles while 8-fold pools exceeded the neat sample Ct values by 10.28 ± 2.77 cycles. Although the mean ΔCt obtained with the 2, 4 and 8-fold pools of elutes exceeded the neat elutes by 2.21 ± 0.9, 2.86 ± 1.04 and 2.92 ± 0.72 cycles respectively, the increase was consistent across all pools. The differences observed with the mean Ct values of 2-fold ($p=0.005$), 4-fold ($p =0.000$) and 8-fold ($p=0.000$) pooled samples against their neat samples were statistically significant. Similarly, the mean Ct differences observed between pooled elutes and their neat elutes were also statistically significant (p values of 2-,4- and 8-fold pooled elutes were $p<0.0001$, $p<0.0001$ and $p<0.0001$).

Conclusion: Large scale screening of asymptomatic individuals for SARS CoV-2 can be maximized with optimal use of resources by pooling 2 or 4 samples or pooling of 4 or 8 RNA extracts but the risk of getting a false negative result needs to be considered.

Keywords: *Pooled testing, COVID-19 screening capacity, real time RT-PCR*

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