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DIAGNOSTIC TESTING FOR SARS-COV-2 BY REAL TIME RT-PCR

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Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged at the end of 2019 and caused COVID-19 pandemic. This coronavirus disease pandemic demonstrated the importance of diagnostic testing in disease outbreak monitoring and control. So, reliable and accurate testing for SARS-CoV-2 was the principal prerequisite for preventing the spread of COVID-19.

Methods: Real Time RT-PCR (RT-qPCR) unquestionably represent the most reliable, rapid and sensitive method for detection of SARS-CoV-2 RNA. However, there are numerous different assays, protocols, reagents, instruments and result analysis methods in use without certified standards, standardized RNA extraction and reporting procedures. Therefore, in practice, the reliability of RT-qPCR results depends on a number of parameters that include sample collection and processing, method of RNA extraction, choice of assay, efficiency of assay, choice of instrument, analysis method as well as operator intervention.

Results: Here we present comparative analyses of the efficiency and sensitivity of 10 different amplification assays, as well as the relevance of manual RNA extractions compared to automatic one. Our results revealed that manual viral RNA extraction should be a method of choice for high sensitivity. In addition, amplification assays targeting three SARS-CoV-2 genes are much more efficient from those targeting one.

Conclusion: Unfortunately, RT-qPCR is almost exclusively used as qualitative diagnostic test for SARS-CoV-2. We think that the ideal testing regimen would involve not just qualitative detection of SARS-CoV-2 but reliable and meaningful quantitative reporting of viral load.

Key words: SARS-CoV-2; RT-qPCR; viral load

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