Uji Set Primer-Probe Deteksi Gen N1, N2, dan RdRp dari SARS-CoV-2 menggunakan Multiplex RT-qPCR = Primer-Probe Set Test for N1, N2, and RdRp Gene from SARS-CoV-2 Detection Using Multiplex RT-qPCR

Muhammad Ikhsan, author

Abstrak

Pandemi COVID-19 bermula akibat infeksi virus SARS-CoV-2. Deteksi SARS-CoV-2 secara standar dilakukan dengan metode Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR) yang mayoritas menarget gen N dan RdRp. Produk lokal untuk mendeteksi SARS-CoV-2 belum banyak tersedia dengan gen target baru berupa N2, RdRp, dan Orf1b. Tujuan dari penelitian ini adalah mengoptimasi reaksi multiplex RT-qPCR dengan dua pasang set primer yang masing-masing menarget gen N dan RdRp dengan gen RPP30 sebagai kontrol internal pengujian. Penelitian dilakukan dengan metode berupa pembuatan kultur Escherichia coli, ekstraksi plasmid Escherichia coli, ekstraksi RNA sel HepG2, PCR, RT-PCR, RT-qPCR, dan elektroforesis. Hasil penelitian di langkah awal memberikan keberhasilan ekstraksi plasmid dan RNA. Proses PCR terhadap plasmid yang menarget gen N dan RdRp dan RT-PCR terhadap RNA yang menarget gen RPP30 memberikan kemunculan pita berukutan mendekati 100 bp setelah dielektroforesis. RT-qPCR yang dilakukan menggunakan dua pasang set primer memberikan hasil positif dengan kemunculan grafik logaritmik pada plot RT-qPCR pada masing-masing primer. Hasil penelitian dapat disimpulkan berupa RT-qPCR telah berhasil dilakukan pada dua pasang set primer yang menarget gen N dan RdRp dengan kontrol internal berupa gen RPP30.

.....COVID-19 pandemic originated in December 2020 due to SARS-CoV-2 virus infection. Standardized SARS-CoV-2 detection is examined by Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR) with N and RdRp genes as the main target. Local SARS-CoV-2 detection product is not much available with only N2, RdRp, and Orf1b as gene target. This research aims to optimize multiplex RT-qPCR with two pairs of primer set targeting N and RdRp genes with RPP30 gene as internal control. The research is done by several methods: Escherichia coli culture production and plasmid extraction, HepG2 cell RNA extraction, PCR, RT-PCR, RT-qPCR, and electrophoresis. The result of the earlier steps is successful plasmid and RNA extraction. PCR on plasmid targeting N and RdRp genes and RT-PCR on RNA targeting RPP30 gene giving results of bands appearance with size around 100 bp after electrophoresis is done. RT-qPCR of two pairs of primer set generally giving positive results with appearance of logarithmic graph on RT-qPCR plots. Research results could be concluded with RT-qPCR are done successfully using two sets of primer that targeting N and RdRp genes with RPP30 gene as internal control.