Evaluasi Deteksi COVID-19 Gen E dan RdRp menggunakan Sampel Saliva dengan Metode Real-Time PCR = Evaluation of COVID-19 E and RdRp Genes Detection using Saliva Samples with Real-Time PCR Method

Khalil Ahmad Aulia, author

Abstrak

Pandemi Coronavirus Disease 2019 (COVID-19) terjadi akibat cepatnya penyebaran Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) sehingga membutuhkan uji yang cepat dan tepat. Real-time polymerase chain reaction(real-time PCR) menggunakan sampel swab nasofaring merupakan standar baku emas, namun sifatnya yang invasif dan tingginya risiko aerosolisasi menjadi kekurangan sampel swab nasofaring. Saliva dinilai dapat menjadi sampel alternatif dalam uji deteksi COVID-19 karena proses koleksi yang tidak invasif, risiko aerosolisasi rendah, serta dapat dilakukan tanpa kontak langsung dengan tenaga kesehatan. Oleh karena itu, tujuan penelitian ini adalah 1) mengevaluasi potensi saliva dalam uji deteksi COVID-19 menggunakan metode real-time PCR dan gen deteksi Envelope (E) serta RNA-dependent RNA polymerase (RdRp), 2) membandingkan hasil real-time PCR sampel swab nasofaring dengan sampel saliva untuk mendapatkan nilai diagnostik, serta 3) mengetahui pengaruh penyimpanan saliva pada suhu -80°C selama tiga dan enam bulan terhadap nilai cycle threshold (Ct) dari real-time PCR yang dilakukan. Metode yang dilakukan meliputi isolasi RNA dengan QIAamp Viral RNA mini kit, kuantifikasi RNA dengan spektrofotometer, amplifikasi dengan real-time PCR, serta analisis data. Hasil real-time PCR menunjukkan bahwa gen E dan RdRp terdeteksi pada 45 dari 128 sampel dengan rentang nilai Ct 14,953—34,8509 dan rata-rata 24,98. Nilai diagnostik yang dihasilkan berupa nilai sensitivitas sebesar 87,2%, spesifisitas 95,1%, positive predictive value (PPV) 91,1%, dan negative predictive value(NPV) 92,8%. Saliva yang disimpan dalam suhu -80°C menunjukkan nilai Ct yang tidak berbeda secara signifikan setelah 3 dan 6 bulan penyimpanan. Kesimpulan penelitian adalah saliva dapat digunakan sebagai sampel alternatif dalam deteksi COVID-19 dengan metode real-time PCR dan gen deteksi E serta RdRp menggunakan sampel yang disimpan selama 3 dan 6 bulan tanpa buffer di suhu -80°C meskipun belum memenuhi kriteria standar WHO.

.....The Coronavirus Disease 2019 (COVID-19) pandemic occurred as a result of the rapid spread of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) thus requiring rapid and appropriate testing. Real-time polymerase chain reaction (real-time PCR) using nasopharyngeal swab samples is the gold standard, but its invasive nature and high risk of aerosolization is a shortage of nasopharyngeal swab samples. Saliva is considered to be an alternative sample in the COVID-19 detection test because the collection process is non-invasive, has a low risk of aerosolization, and can be done without direct contact with health workers. Therefore, the aims of this study were 1) to evaluate the potential of saliva in the COVID-19 detection test using the real-time PCR method and the Envelope (E) detection gene and RNA-dependent RNA polymerase (RdRp), 2) to compare the results of real-time PCR nasopharyngeal swab samples with saliva samples to obtain diagnostic value, and 3) to determine the effect of storing saliva at -80°C for three and six months on the cycle threshold (Ct) value of the real-time PCR performed. The methods used included RNA isolation with the QIAamp Viral RNA mini kit, RNA quantification with a

spectrophotometer, amplification with real-time PCR, and data analysis. Real-time PCR results showed that the E and RdRp genes were detected in 45 of 128 samples with a Ct value range of 14.953-34.8509 and an average of 24.98. The resulting diagnostic value was a sensitivity value of 87.2%, a specificity of 95.1%, a positive predictive value (PPV) of 91.1%, and a negative predictive value (NPV) of 92.8%. Saliva stored at -80°C showed Ct values that were not significantly different after 3 and 6 months of storage. The conclusion of the study is that saliva can be used as an alternative sample in detecting COVID-19 with the real-time PCR method and detecting E and RdRp genes using samples stored for 3 and 6 months without buffer at -80°C even though they do not meet WHO standard criteria.