Deteksi dini dan cepat infeksi dengue dengan menggunakan in house multiplek realtime RRT-PCR berbasis SYBR green untuk diagnosis dini dan cepat infeksi dengue= Early and rapid detection dengue infection with in house multiplex RRT-PCR based on SYBR green

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Abstrak

[ABSTRAK
Virus dengue (DENV) adalah penyebab penyakit infeksi yang endemik di 100 negara di dunia. DENV dapat menyebabkan infeksi primer dan sekunder. Infeksi sekunder diperkirakan lebih berat dan akan menyebabkan menjadi Demam Berdarah Dengue (DBD) dan Sindrom Renjatan Dengue (SRD). Penatalaksanaan yang lebih dini dan tepat akan membantu mengurangi terjadinya kasus berat seperti DBD dan SRD. Teknik diagnostik yang dikembangkan belum ada yang dapat mendeteksi secara cepat dan tepat pada awal infeksi terutama untuk virus dengue strain Indonesia. Tujuan penelitian ini untuk mendapatkan kondisi yang optimal untuk deteksi DENV dan analisis validasi tehnik in house multiplek realtime Reverse Transcriptase-Polimerase Chain Reaction (rRT-PCR) sehingga dapat digunakan untuk deteksi dini dan cepat infeksi DENV. Rancangan penelitian ini adalah penelitian eksperimental laboratorium. Strain standar DENV diisolasi dengan kit Roche® dan spesimen diekstraksi dengan kit Qiagen®. Strain standar DENV digunakan untuk optimasi suhu annealing dan konsentrasi primer masing-masing serotipe DENV dengan metode in house multiplek rRT-PCR berbasis SYBR green. Sebagai pembanding digunakan RT-PCR konvensional dengan menggunakan primer di daerah C-PrM. Primer in house multiplek rRT-PCR didesain di daerah envelope pada masing masing serotipe. Analisis limit of detection (LOD) dilakukan dengan pengenceran titer virus 105, 104, 103, 102, 10 dan 1 FFU/ml (in house multiplek rRT-PCR) pada keempat serotipe. Hasil in house multiplek rRT-PCR dibandingkan dengan hasil RT-PCR konvensional Lanciotti pada pasien dengue yang telah masuk dalam kriteria inklusi dan eksklusi. Suhu annealing optimal didapatkan pada suhu 58oC sedangkan konsentrasi optimal masing-masing primer untuk in house multiplek rRT-PCR adalah 4 pmol. LOD RNA pada DENV-1, DENV-2, DENV-3, DENV-4 adalah 1 FFU/ml, 10 FFU/ml, 104 FFU/ml dan 1 FFU/ml. In house multiplek rRT-PCR dibandingkan RT-PCR konvensional mempunyai sensitivitas sebesar 100%, spesifisitas 94,2 %, nilai prediksi positif 85,7% dan nilai prediksi negatif 100 %. In house multiplek rRT-PCR berbasis SYBR green merupakan metode yang dini, cepat dan tepat untuk deteksi DENV pada awal infeksi dengan sensitivitas dan spesifisitas yang baik sebagai metode diagnostik infeksi dengue dimasa mendatang.
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ABSTRACT
Dengue virus (DENV) is an infectious disease that is endemic in 100 countries in the World. Dengue virus infections can cause primary and secondary. Secondary infection is estimated to be more severe DHF and SDD. Developed diagnostic technique that no one has been able to quickly and accurately detect the infection early, especially for the Indonesian strain of dengue virus. The

purpose of this study is to obtain optimal conditions for the detection of dengue infection and analysis techniques in-house validation of multiplex real-time Reverse Transcriptase-Polymerase Chain Reaction (rRT-PCR) for the Indonesian strain of dengue virus. Design of this study is an experimental research laboratory. Standard strains of dengue virus was isolated with a kit Roche® and the specimen was extracted with Qiagen® kit. Standard strains of dengue virus is used for optimization primer annealing temperature and the concentration primers of each serotype dengue virus by multiplex rRT-PCR method based on SYBR green. Primers for RT-PCR conventional based lanciotii et al while rRT-PCR primer was designed in the envelope gen at each serotype. Limit of detection (LOD) by diluting the virus titer 105, 104, 103, 102 FFU /ml performed by rRTPCR in all four serotypes.The results of multiplex rRT-PCR compared with results of conventional RT-PCR in patients with dengue lanciotti superbly into the criteria inclusion and exclusion. Optimal annealing temperature results obtained at a temperature of 58oC and optimal primer concentration of 4 pmol of each primer for 25 ul total reaction. LOD RNA in DENV-1, DENV-2, DENV-3 and DENV-4 at titer of 1 FFU / ml, 10 FFU/ml, 104 FFU/ml and 1 FFU/ml. .In house multiplex rRT-PCR compared with RT-PCR has a sensitivity of 100%, specificity 95,2%, positive predictive value 85,7% and negative predictive value of 100%. In-house multiplex rRT-PCR with SYBR green-based research is a method that is rapid and precise detection of dengue virus in early infection with good sensitivity and specificity compared to RT-PCR as a diagnostic method in the future dengue infection.;Dengue virus (DENV) is an infectious disease that is endemic in 100 countries in the World.

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