# Optimasi real time polymerase chain reaction untuk deteksi cytomegalovirus pada sampel plasma, urin, dan liquour cerebrospinal pasien Human Immunodeficiency Virus dengan tersangka infeksi otak. = Optimization of real time polymerase chain reaction for detection of cytomegalovirus in plasma, urine, and liquor cerebrospinal samples from Human Immunodeficiency Virus positive patients with suspected central nervous system infections

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Abstrak

ABSTRAK<br> Latar belakang : Cytomegalovirus (CMV) merupakan salah satu infeksi oportunistik
pada pasien dengan sindrom immunodefisiensi (AIDS). Gejala klinis dan CT scan
tidak dapat menegakkan diagnosa definitif ensefalitis CMV. Oleh karena itu
diperlukan uji alternatif untuk menegakkan diagnosis infeksi CMV pada pasien HIV
dengan infeksi otak. Salah satu uji yang sensitif dan spesifik adalah Real Time
Polymerase Chain Reaction (rPCR).
Tujuan : Mendapatkan uji deteksi molekular CMV pada pasien HIV dengan
tersangka infeksi otak.
Metode : Penelitian dilakukan dalam 3 tahap. Tahap 1 adalah optimasi konsentrasi
primer, probe, suhu annealing, volume elusi ekstraksi DNA, dan volume cetakan.
Tahap 2 adalah uji spesifisitas (reaksi silang) dan uji sensitivitas (ambang batas
deteksi DNA) rPCR dan tahap 3 adalah penerapan uji rPCR yang sudah dioptimasi
terhadap sampel plasma, urin, dan LCS.
Hasil : Kondisi optimal uji rPCR telah diperoleh dengan konsentrasi primer dan
probe 0,1 μM, dengan kondisi suhu reaksi rPCR: aktivasi enzim pada 950C selama 3
menit; 45 siklus pada 950C selama 15 detik (denaturasi) dan 560C selama 1 menit
(annealing dan ekstensi). Volume elusi ekstraksi DNA yang optimal untuk ketiga
jenis sampel (LCS, plasma dan urin) adalah 40 μL, dan volume cetakan rPCR untuk
LCS, plasma, dan urin, masing-masing adalah 5, 4, dan 3 μL. Uji rPCR mampu
mendeteksi DNA pada 50.000 jumlah kopi/mL dan tidak bereaksi silang dengan
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus,
Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Mycobacterium
tuberculosis, Candida spp, Toxoplasma gondii, EBV,HSV,dan VZV. Penerapan uji
rPCR pada sampel klinis memberikan hasil negatif pada semua sampel LCS, 72,22%
positif pada sampel plasma, dan 72,22% positif pada sampel urin.
Kesimpulan: Telah dilakukan optimasi uji rPCR dengan minimal deteksi DNA
CMV 50.000 jumlah kopi/mL dan tidak bereaksi silang dengan mikroorganisme yang
berpotensi menyebabkan positif palsu (false positive).ABSTRACT<br> Background:
Cytomegalovirus (CMV) is one of opportunistic infections in patients

with Aquired Immunodeficiency Syndrome (AIDS). Clinical manifestations are not typical, and CT scans can not define encephalitis CMV specifically. Therefore, it is important to apply an alternative assay for sensitive and specific detection of CMV infection in HIV patients with suspected central nervous system (CNS) infections. One of the assays is real time polymerase chain reaction (rPCR).
Objective: To obtain a molecular assay for detection of CMV in HIV patients with suspect CNS infections.
Methods: This study was conducted in three phases. The first is optimization of concentrations of primers, probe, annealing temperature, final elution of DNA extraction, and volume of PCR template. The second is determinations of sensitivity (minimal detection of DNA) and specificity (cross-reaction) of the optimized rPCR, and the third is application of the rPCR for clinical samples of plasma, urine, and liquor cerebrospinal (LCS).
Results: The rPCR reaction showed optimal concentrations of primers and probe at 0.1 μM, with thermal cycler: 950C for 3 min (enzyme activation), followed by 45 cycles of 950C for 15 sec (denaturation) and 560C for 1 min (annealing and extension). Final elution of DNA extraction was 40 μL and volume of PCR templates for urine, plasma, and LCS was 3, 4, and 5 μL, respectively. The rPCR had minimal detection of DNA at 50,000 copies/mL and was not cross-reacted with Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Mycobacterium tuberculosis, Candida spp, Toxoplasma gondii, Epstein-Bar Virus (EBV), Herpes Simplex Virus (HSV) and Varicella Zoster Virus (VZV). Application of rPCR for clinical samples showed that the rPCR yielded 72.22% positive for plasma or urine, and negative for all LCS samples.
Conclusion: The rPCR has been optimized in this study with minimal DNA detection at 50,000 copies/mL and was not cross-reacted with other microorganisms that are potential to cause false positive results.;Background: Cytomegalovirus (CMV) is one of opportunistic infections in patients
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