Deteksi toxoplasma gondii dan epstein barr virus pada pasien HIV dengan gejala klinis tersangka infeksi otak menggunakan Dupleks Real-time PCR = Detection of toxoplasma gondii and epstein barr virus in HIV patient with clinical symptom suspect central nervous system infection using duplex real-time polymerase chain reactio

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Abstrak

ABSTRAK
 Lesi fokal otak merupakan komplikasi neurologi pada pasien HIV yang ditandai oleh lesi desak ruang (Space Occupying Lesion) yang membutuhkan penanganan cepat dan tepat. Di beberapa negara, lesi ini dapat disebabkan oleh toksoplasma ensefalitis dan limfoma otak primer. Lesi yang disebabkan oleh toksoplasmosis dan limfoma otak primer yang disebabkan oleh Epstein Barr virus sulit untuk dibedakan menggunakan CT scan ataupun MRI. Pemeriksaan gold standar untuk membedakan keduanya yaitu dengan biopsi otak, namun hal ini merupakan tindakan invasif dan dapat menimbulkan komplikasi. Penelitian ini bertujuan untuk memperoleh uji deteksi untuk diagnosis cepat infeksi Toxoplasma gondii dan Epstein Barr virus. Desain yang dipakai pada penelitian adalah studi eksperimental laboratorium. Uji deteksi yang dikembangkan adalah dupleks real-time PCR yang dapat mendeteksi T.gondii dan EBV atau kombinasi keduanya dalam satu reaksi pada sampel pasien HIV dengan gejala klinis tersangka infeksi otak. Tahap pertama dilakukan optimasi dupleks real-time PCR meliputi suhu annealing, konsentrasi primer dan probe, uji volume elusi dan volume cetakan. Penentuan ambang batas deteksi dilakukan untuk mengukur minimal T.gondii dan EBV yang dapat dideteksi. Reaksi silang untuk mengetahui spesifisitas teknik dilakukan menggunakan bakteri dan virus sebagai berikut Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Mycobacterium tuberculosis H37Rv, Candida spp, Cytomegalo virus, Herpes zoster virus, dan Varicella zoster virus. Dupleks real-time PCR yang telah optimal diaplikasi pada sampel pasien. Sampel yang digunakan adalah darah dan cairan serebrospinal dari pasien HIV dengan gejala klinis infeksi otak yang dirawat di bagian neurologi RSCM. Hasil optimasi dupleks real-time PCR diperoleh suhu annealing untuk T.gondii dan EBV 58°C, konsentrasi primer forward dan reverse untuk T.gondii dan EBV adalah 0,2 µM, konsentrasi probe T.gondii 0,4µM, konsentrasi probe EBV 0,2 µM. Deteksi ambang batas minimal DNA untuk T.gondii 5,68 copy /ml, sedangkan EBV 1,31 copy/ml. Uji yang dikembangkan pada penelitian ini termasuk uji yang sensitif dibandingkan hasil penelitian lain. Uji reaksi silang primer dan probe dupleks real-time PCR terhadap beberapa bakteri dan virus lain, menunjukkan tidak bereaksi silang dengan primer dan probe yang digunakan untuk mendeteksi T.gondii dan EBV. Hasil pemeriksaan dupleks real-time PCR pada sampel darah diperoleh 16% positif T.gondii, 40% positif Epstein Barr virus, sebanyak 16% positif Epstein Barr virus dan T.gondii dan pada sampel cairan serebrospinal diperoleh hasil 20% positif T.gondii, sebanyak 28% positif Epstein Barr virus dan 4% positif terhadap Epstein Barr Virus dan T.gondii. ABSTRACT
 Focal brain lesion is neurology complication in HIV that marked with Space Occupying Lesion (SOL), that need rapid and effective handling. In most country, this lesion could be cause by encephalitis toxoplasma and Primary Central Nervous System Lymphoma that related to Epstein Barr virus infection that was difficult to distinguished using CT scan or MRI. Gold standard to distinguished was brain biopsy, but this examination

was invasive procedure that cause complication. Therefore, we need a reliable and rapid examination to distinguished it. This study aimed to get detection for rapid diagnosis of T.gondii and EBV infection. This study was an experimental laboratory. First step was optimation of dupleks real-time PCR include annealing temperature, primer andprobe consentration, elution volume and template volume. Minimal detection of DNA to measured minimal T.gondii and EBV that could be detected. Cross reaction to know technique spesivisity using bacterial and virus Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Mycobacterium tuberculosis H37Rv, Candida spp, Cytomegalo virus, Herpes zoster virus, and Varicella zoster virus. Dupleks real-time PCR has been optimally applied to patient. The sample from blood and cerebrospinal fluid of HIV patients who admitted in the neurology department of RSCM then examined to duplex real-time PCR to detect T.gondii and EBV. The optimation of duplex real-time PCR, the annealing temperature for T.gondii and EBV were 58°C, consentration of primer forward and reverse for T.gondii and EBV were 0,2 µM, consentration of probe for T.gondii was 0,4µM and EBV was 0,2µM.. Minimal DNA detection for T.gondii was 5,68 copy/ml and EBV was 1,31 copy /ml. This study was sensitive like the others. Spesivisity technique of real-time PCR, there was not cross reaction between another bacteria and virus that used as primer and probe for T.gondii and EBV. From the results of the duplex real-time PCR on blood samples, 16 % was positive T.gondii, 40% Epstein Barr virus, and 16% were positive Epstein Barr virus and T.gondii and from cerebrospinal fluid samples 20% was positive T.gondii, 28% was positive Epstein Barr virus and 4% were positive for Epstein Barr Virus and T.gondii.;Focal brain lesion is neurology complication in HIV that marked with Space Occupying Lesion (SOL), that need rapid and effective handling. In most country, this lesion could be cause by encephalitis toxoplasma and Primary Central Nervous System Lymphoma that related to Epstein Barr virus infection that was difficult to distinguished using CT scan or MRI. Gold standard to distinguished was brain biopsy, but this examination was invasive procedure that cause complication. Therefore, we need a reliable and rapid examination to distinguished it. This study aimed to get detection for rapid diagnosis of T.gondii and EBV infection. This study was an experimental laboratory. First step was optimation of dupleks real-time PCR include annealing temperature, primer andprobe consentration, elution volume and template volume. Minimal detection of DNA to measured minimal T.gondii and EBV that could be detected. Cross reaction to know technique spesivisity using bacterial and virus Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Mycobacterium tuberculosis H37Rv, Candida spp, Cytomegalo virus, Herpes zoster virus, and Varicella zoster virus. Dupleks real-time PCR has been optimally applied to patient. The sample from blood and cerebrospinal fluid of HIV patients who admitted in the neurology department of RSCM then examined to duplex real-time PCR to detect T.gondii and EBV. The optimation of duplex real-time PCR, the annealing temperature for T.gondii and EBV were 58°C, consentration of primer forward and reverse for T.gondii and EBV were 0,2 µM, consentration of probe for T.gondii was 0,4µM and EBV was 0,2µM.. Minimal DNA detection for T.gondii was 5,68 copy/ml and EBV was 1,31 copy /ml. This study was sensitive like the others. Spesivisity technique of real-time PCR, there was not cross reaction between another bacteria and virus that used as primer and probe for T.gondii and EBV. From the results of the duplex real-time PCR on blood samples, 16 % was positive T.gondii, 40% Epstein Barr virus, and 16% were positive Epstein Barr virus and T.gondii and from cerebrospinal fluid samples 20% was positive T.gondii, 28% was positive Epstein Barr virus and 4% were positive for Epstein Barr Virus and T.gondii.;Focal brain lesion is neurology complication in HIV that marked with Space Occupying Lesion (SOL), that need rapid and effective handling. In most country, this lesion could be cause by encephalitis toxoplasma and Primary

Central Nervous System Lymphoma that related to Epstein Barr virus infection that was difficult to distinguished using CT scan or MRI. Gold standard to distinguished was brain biopsy, but this examination was invasive procedure that cause complication. Therefore, we need a reliable and rapid examination to distinguished it. This study aimed to get detection for rapid diagnosis of T.gondii and EBV infection. This study was an experimental laboratory. First step was optimation of dupleks real-time PCR include annealing temperature, primer andprobe consentration, elution volume and template volume. Minimal detection of DNA to measured minimal T.gondii and EBV that could be detected. Cross reaction to know technique spesivisity using bacterial and virus Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Mycobacterium tuberculosis H37Rv, Candida spp, Cytomegalo virus, Herpes zoster virus, and Varicella zoster virus. Dupleks real-time PCR has been optimally applied to patient. The sample from blood and cerebrospinal fluid of HIV patients who admitted in the neurology department of RSCM then examined to duplex real-time PCR to detect T.gondii and EBV. The optimation of duplex real-time PCR, the annealing temperature for T.gondii and EBV were 58°C, consentration of primer forward and reverse for T.gondii and EBV were 0,2 µM, consentration of probe for T.gondii was 0,4µM and EBV was 0,2µM.. Minimal DNA detection for T.gondii was 5,68 copy/ml and EBV was 1,31 copy /ml. This study was sensitive like the others. Spesivisity technique of real-time PCR, there was not cross reaction between another bacteria and virus that used as primer and probe for T.gondii and EBV. From the results of the duplex real-time PCR on blood samples, 16 % was positive T.gondii, 40% Epstein Barr virus, and 16% were positive Epstein Barr virus and T.gondii and from cerebrospinal fluid samples 20% was positive T.gondii, 28% was positive Epstein Barr virus and 4% were positive for Epstein Barr Virus and T.gondii.