Optimasi dan uji awal tes molekuler untuk deteksi dan identifikasi mycobacteria secara langsung pada sputum pasien terduga infeksi nontuberculous mycobacteria paru = Optimization and preliminary molecular test for direct detection and identification of mycobacteria in sputum of patient suspected of pulmonary nontuberculous mycobacteria infection

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Abstrak

Latar Belakang: Ada sekitar 50 spesies Nontuberculous mycobacteria (NTM) berpotensi patogen di manusia, yang menyebabkan infeksi tersering di paru. Pemeriksaan mikrobiologi berbasis molekuler diperlukan dalam mendiagnosis infeksi NTM paru. Oleh karena itu perlu dilakukan uji awal untuk deteksi mycobacteria dari spesimen klinis secara langsung dengan metode molekuler yaitu real-time PCR dan sekuensing DNA untuk identifikasi spesies mycobacteria serta mengaplikasikan pada kit PaxView® TB/NTM MPCR-ULFA. Tujuan: Melakukan optimasi berbasis molekuler untuk deteksi dan identifikasi Mycobacteria secara langsung pada sputum pasien terduga infeksi NTM paru. Metode: Studi dekskriptif dan eksperimental laboratorium dengan melakukan optimasi suhu penempelan, reaksi silang, ambang batas deteksi DNA, dan penerapan real-time PCR berbasis SYBR Green yang telah dioptimasi dan PaxView® TB/NTM MPCR-ULFA pada hasil sputum pasien terduga infeksi NTM paru. Hasil: Dua hasil positif dari 30 sampel sputum pada real-time PCR mycobacterium dan hasil sekuensing adalah Mycobacterium tuberculosis, menunjukkan adanya discordant hasil dengan real-time PCR MTB. Pada kit PaxView® TB/NTM MPCR-ULFA didapatkan 16 hasil positif MTB dan tidak ditemukan NTM. Kesimpulan: Terdapat discordance pada dua sampel hasil penerapan uji awal real-time PCR mycobacterium dengan sekuensing DNA, yang diduga NTM tetapi hasilnya M. tuberculosis. Perlunya dilakukan evaluasi lebih lanjut real-time PCR berbasis SYBR Green.

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Background: There are approximately fifty species of Nontuberculous mycobacteria (NTM) are potentially pathogenic in humans, the infection is most common in the lungs. Molecular-based microbiological examination is needed in diagnosing pulmonary NTM infection. Therefore, it is necessary to preliminary test the detection of mycobacteria from clinical specimens directly by molecular methods, namely real-time PCR and DNA sequencing to identify mycobacteria species and apply to the Pax-ULFA PaxView® TB/NTM kit. Aims: To perform Molecular-based optimization for the detection and identification of mycobacteria directly in sputum patient suspected of pulmonary NTM infection. Method: A descriptive and experimental laboratory study, to optimize the annealing temperature, determination of minimal detection of DNA, cross reaction of optimized real-time PCR based on SYBR- Green and applied sputum from patients suspected of NTM pulmonary infection to real-time PCR and PaxView® TB/NTM MPCR-ULFA. Results: Two positive results from 30 sputum samples on real-time PCR mycobacterium and sequencing results were MTB, the results discordant with real-time PCR MTB. In the PaxView® TB/NTM MPCR-ULFA, 16 positive MTB results were obatined and no NTM was found. Conclusion: There was discordance in two sample of real-time PCR mycobacterium spp. with DNA sequencing, which is thought to be NTM but the

result is M. tuberculosis. The need for further evaluation of real-time PCR based Mikrobiologi Klinik on SYBR Green.