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Deteksi molekuler tuberkulosis paru dari sampel urine menggunakan multiplex PCR dengan gen deteksi ESAT6, IS6110, dan MPT64 dan real-time PCR dengan gen deteksi ESAT6 = Molecular detection of pulmonary tuberculosis from urine samples using multiplex PCR with ESAT-6, IS6110, and MPT64 detection genes and and real-time PCR with ESAT6 detection gene

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Abstrak

Kendala utama dalam diagnosis penyakit tuberkulosis (TB) paru di Indonesia adalah sulitnya pengeluaran sputum sebagai spesimen diagnostik dari pengidap TB paru, terutama pada kelompok anak dan lansia. Pengembangan spesimen alternatif, seperti urine, sangat dibutuhkan untuk meningkatkan notifikasi kasus TB paru pada subjek yang belum terdiagnosis secara optimal. Penelitian ini mengevaluasi potensi urine pada 60 sampel terkonfirmasi TB paru positif, sebagai studi kasus-kontrol dengan subjek yang memiliki kondisi target. Tujuan penelitian ini meliputi evaluasi multiplex polymerase chain reaction (PCR), untuk mendeteksi gen ESAT6, IS6110, dan MPT64, serta real-time PCR untuk deteksi gen ESAT6 dalam menunjang diagnosis TB paru. Selain itu, penelitian ini diharapkan dapat menggambarkan nilai sensitivitas masing-masing metode dan gen diagnostik yang digunakan. Metode penelitian meliputi preparasi sampel urine, isolasi DNA, kuantifikasi DNA, amplifikasi DNA (multiplex PCR dan real-time PCR), elektroforesis DNA, dan analisis data. Hasil evaluasi menunjukkan kemurnian total DNA yang diisolasi dari urine berdasarkan A260/A280 (Mean 3,08 SD 1,08). Evaluasi multiplex PCR dengan gen deteksi ESAT6, IS6110, dan MPT64 memberikan nilai sensitivitas sebesar 68,3% (41/60), dan real-time PCR dengan gen deteksi ESAT6 sebesar 71,67% (43/60). Nilai sensitivitas real-time PCR diperoleh dengan ketentuan limit of detection (LOD) sebesar 13,04 kopi/Î¼l. Nilai sensitivitas kedua metode tersebut menunjukkan bahwa urine dapat menjadi spesimen alternatif untuk pendeteksian Mycobacterium tuberculosis (Mtb) secara molekuler.
.....The main obstacle in the diagnosis of pulmonary tuberculosis (TB) in Indonesia is the difficulty of extracting sputum, as a diagnostic specimen for people with pulmonary TB, especially in the group of children and the elderly. The development of alternative specimens, such as urine, is urgently needed to increase the notification of pulmonary TB cases in subjects who have not been diagnosed optimally. This study evaluated the urine potency of 60 samples of confirmed positive pulmonary TB, as a case-control study with subjects with the target condition. The objectives of this study include the evaluation of multiplex polymerase chain reaction (PCR), to detect the ESAT6, IS6110, and MPT64 genes, as well as real-time PCR to detect the ESAT6 gene in supporting the diagnosis of pulmonary TB. In addition, this study is expected to describe the sensitivity value of each diagnostic method and gene used. Research methods include urine sample preparation, DNA isolation, DNA quantification, DNA amplification (multiplex PCR and real-time PCR), DNA electrophoresis, and data analysis. The evaluation results showed the total purity of DNA isolated from urine based on A260/A280 (Mean 3,08 SD 1.08). Evaluation of multiplex PCR with ESAT6, IS6110, and MPT64 detection genes gave a sensitivity value of 68,3% (41/60), and real-time PCR with ESAT6 detection genes of 71,67% (43/60). Real-time PCR sensitivity value was obtained with the limit of detection (LOD) of 13,04 copies/Î¼l. The sensitivity values of both methods indicate that urine can be an

alternative specimen for molecular detection of Mycobacterium tuberculosis.