Evaluating the use of loop-mediated isothermal amplification (LAMP) method for detection of Mycobacterium tuberculosis in Indonesian clinical isolates

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Abstrak

Latar belakang: Metode loop-mediated isothermal amplification (LAMP) merupakan metode sederhana yang dapat mengamplifikasi DNA/RNA menggunakan empat sampai dengan enam primer dalam bentuk ?pasangan? dari sekuens conserved gen. Penelitian ini bertujuan untuk mengoptimasi LAMP dalam menegakkan diagnosis kasus TB di Indonesia.
Metode: Setelah uji optimasi, metode LAMP kemudian diujikan pada 122 DNA Mycobacterium tuberculosis (Mtb) sampel tersimpan, yang merupakan spesimen sputum pasien TB dengan BTA positif yang dikumpulkan dari 13 provinsi di Indonesia pada tahun 2008 untuk studi genotipe dan merupakan koleksi Pusat Biomedis dan Teknologi Dasar Kesehatan (PBTDK), Balitbangkes. Uji optimasi meliputi uji sensitifitas dan uji spesifisitas sejumlah pasangan primer LAMP terhadap larutan serial DNA Mtb H37Rv dan 12 spesies Mycobacteria. Uji LAMP dilakukan menggunakan tiga jenis instrumen yaitu LAMP turbidemeter, pelat pemanas dan penangas air. Hasil pengujian beberapa pasang primer dan instrument ini kemudian diterapkan untuk uji LAMP pada isolat spesimen klinik Indonesia, yaitu menggunakan pasangan primer dari gen gyrB, Hasil amplifikasi dideteksi dengan lampu UV.
Hasil: Uji sensitivitas menunjukkan bahwa pasangan primer gen 16S rRNA dan gyrB memberikan hasil terbaik yaitu mampu mendeteksi 10.0 fg - 1.0 pg genomik DNA Mtb H37Rv. Uji spesifisitas menunjukkan bahwa pasangan primer gen gyrB merupakan pasangan primer paling spesifik. Hasil pengujian pasangan primer gyrB pada isolat klinis Indonesia didapatkan positivity rate 94,2% (114/121).
Kesimpulan: Metode LAMP berpotensi untuk digunakan dalam diagnosis kasus TB di Indonesia.
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Abstract
Background: Loop-mediated isothermal amplification (LAMP) is a method already claimed as a simple technique to amplify DNA/ RNA using four to six primers as ?a set? from conserved sequence of target gene. In this study we optimize the use of LAMP for detection of Mycobacterium tuberculosis in clinical isolates from Indonesia.
Methods: Procedures to perform LAMP were optimized, then the method was applied to 122 archieved samples of DNA?s Mtb from clinical TB patients with Acid Fast Bacilli (AFB) smears positive. The samples were obtained in 2008 from 13 provinces in Indonesia for genotyping study, which then become collections of Center for Biomedical and Basic Technology of Health (CBBTH), NIHRD Indonesia. The optimization tests include sensitivity and specificity tests of several sets primers, which were evaluated using 10-fold serially diluted DNA of Mtb H37Rv and 12 species of Mycobacteria. Three equipments consisted of LAMP turbidimeter, heating block and water bath were compared for its ability in DNA amplification. Detection of M. tuberculosis from clinical isolates used set primers specific for gyrB gene, amplicon was detected with UV fluorescence system.
Results: The results showed that the highest sensitivity was obtained using the set primers specific for 16S

rRNA and gyrB which could detect 10.0 fg to 1.0 pg genomic DNA of Mtb H37Rv. The set primers specific for gyrB gene was the most specific primers. Application of LAMP using gyrB set primers on Indonesian clinical isolates showed 94.2% (114/121) positivity rate.
Conclusion: LAMP method is potentially used in TB diagnosis in Indonesia.