

## PENGEMBANGAN *LOOP MEDIATED ISOTHERMAL AMPLIFICATION* (LAMP) UNTUK DETEKSI *MULTI DRUG RESISTANT TUBERCULOSIS* (MDR-TB) DENGAN MARKER *rpoB* S450W DAN *katG* S315T

Dian Ritma Setyorini  
23/524836/PMU/11534

### INTISARI

Indonesia diketahui memiliki jumlah kasus infeksi *Mycobacterium tuberculosis* tertinggi ke-2 di dunia setelah India. Bersamaan dengan meningkatnya jumlah kasus tuberkulosis, kemunculan resistensi terhadap obat antibiotik juga semakin tinggi sehingga diperlukan strategi medikasi yang tepat. Sifat resistensi tuberkulosis terhadap antibiotik lini pertama seperti rifampicin dan isoniazid disebut sebagai *multi drug resistant tuberculosis* (MDR-TB). Resistensi disebabkan oleh mutasi pada *coding region* berbagai gen pengkode protein fungsional sehingga menyebabkan penurunan afinitas ikatan protein terhadap antibiotik. *Single nucleotide polymorphism* (SNP) pada gen *rpoB*, *katG*, *inhA*, *embB*, dan *pncA* telah banyak diasosiasikan dengan sifat resistensi tinggi sehingga dapat digunakan sebagai biomarker dalam metode diagnostik molekular. *Loop mediated isothermal amplification* (LAMP) telah banyak digunakan dalam pengembangan deteksi penyakit infeksius, termasuk tuberkulosis. Penggunaan LAMP yang mudah, cepat, akurat, dan terjangkau menjadikannya sebagai kandidat *rapid diagnostic tools* yang potensial untuk deteksi MDR-TB. Penelitian ini bertujuan untuk mengembangkan set primer LAMP yang didesain untuk mengamplifikasi marker potensial MDR-TB. Identifikasi genotipik mutasi menggunakan sanger sequencing pada sampel DNA tuberkulosis asal Padang dan Yogyakarta menggunakan 5 gen target. Optimasi LAMP dilakukan secara *colorimetric* dan *real-time*, sedangkan performa pengujian ditinjau berdasarkan *limit of detection* (LOD). Hasil menunjukkan 2 marker potensial yang dapat digunakan sebagai set primer LAMP, yaitu S450W *rpoB* (RM2) dan S315T *katG* (RM4). Limit deteksi S450W *rpoB colorimetric* pada 58.100 copy/test dan *real-time* pada 581 copy/test. Limit deteksi S315T *katG colorimetric* pada 539.900copy/test, sedangkan *real-time* pada 53.990 copy/test. *Threshold* G/R ratio untuk *colorimetric* LAMP ditentukan pada nilai 0,7 untuk diferensiasi hasil positif dan negatif.

**Kata kunci:** *Mycobacterium tuberculosis*, *loop mediated isothermal amplification*, *rpoB*, *katG*, limit deteksi

## DEVELOPMENT OF LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) FOR MULTI DRUG RESISTANT TUBERCULOSIS (MDR-TB) DETECTION USING *rpoB* S450W AND *katG* S315T MARKERS

Dian Ritma Setyorini  
23/524836/PMU/11534

### ABSTRACT

Indonesia is the 2nd highest number of *Mycobacterium tuberculosis* infection cases in the world after India. Along with the increasing number of tuberculosis cases, the emergence of resistance against antibiotic drugs is also getting higher, indicates the appropriate medication strategies are needed. The resistant traits of tuberculosis against first-line antibiotics such as rifampicin and isoniazid is referred to as multi drug resistant tuberculosis (MDR-TB). Resistance is caused by mutations in the coding regions of various functional protein-coding genes that lead to a decrease of protein-antibiotic binding affinity. Single nucleotide polymorphism (SNP) in *rpoB*, *katG*, *inhA*, *embB*, and *pncA* gene has been known to be associated with high resistance properties, indicates its potential as biomarker in molecular diagnostic methods. Loop mediated isothermal amplification (LAMP) has been widely used as detection tools of several infectious diseases, including tuberculosis. The easy, fast, accurate, and affordable use of LAMP makes it a potential candidate for rapid diagnostic tools for MDR-TB detection. This study aims to develop LAMP primers designed to amplify potential markers of MDR-TB. Genotypic identification of mutations conducted by sanger sequencing on clinical DNA samples from Padang and Yogyakarta using 5 target genes. LAMP optimization include *colorimetric* and *real-time*, while test performance is reviewed based on limit of detection (LOD). The results showed 2 potential markers that could be used as LAMP primer sets, S450W *rpoB* (RM2) and S315T *katG* (RM4). The detection limit of the S450W *rpoB colorimetric* is 58,100 copy/test and *real-time* at 581 copy/test. The detection limit of the S315T *katG colorimetric* is 539,900 copy/test, while the *real-time* is at 53,990 copy/test. The threshold value for G/R ratio in *colorimetric* LAMP is determined at a value of 0.7 for the differentiation of positive and negative results.

**Keywords:** *Mycobacterium tuberculosis*, loop mediated isothermal amplification, *rpoB*, *katG*, detection limit