

Deteksi Molekuler dan Identifikasi *Plasmodium falciparum* Metode Real-Time Polymerase Chain Reaction (qPCR)

INTISARI

Latar Belakang: Kasus malaria di Indonesia mengalami peningkatan angka kematian pada tahun 2020. Identifikasi parasit malaria khususnya terhadap *Plasmodium falciparum* perlu dilakukan mengingat spesies ini bertanggung jawab terhadap kurang lebih 95% kematian akibat malaria. Metode deteksi molekuler, qPCR, diharapkan mampu mengatasi keterbatasan deteksi dengan pemeriksaan mikroskopis sebagai standar baku emas karena memiliki sensitivitas lebih tinggi serta dapat dilakukan dengan cepat. Dua jenis metode deteksi fluoresens yang dapat digunakan dalam qPCR yaitu SYBR Green dan metode TaqMan Probe.

Tujuan: Penelitian ini bertujuan untuk mengetahui perbedaan limit deteksi parasit *P. falciparum* dengan metode amplifikasi qPCR dengan pewarna fluoresens SYBR Green dan metode TaqMan Probe dilihat dari sensitivitas dan efisiensi reaksinya dengan menggunakan primer dan sampel DNA yang sama

Metode: Penelitian ini adalah observational deskriptif yang memberikan penjelasan atau gambaran kondisi optimal yang dibutuhkan untuk mendapatkan hasil diagnostik secara molekuler menggunakan metode *real-time* PCR. Sampel yang digunakan adalah sampel isolasi DNA kultur *P. falciparum* strain FCR3, strain 3D7, dan sampel penderita malaria Sumba Barat Daya tahun 2019 yang dilakukan amplifikasi qPCR dengan dua metode deteksi fluoresens.

Hasil: Perbandingan efisiensi reaksi qPCR deteksi parasit *P. falciparum* untuk sampel I dan II metode deteksi SYBR Green adalah 102% dan 84% dan metode TaqMan Probe adalah 105% dan 81%. Limit deteksi *P. falciparum* dengan deteksi SYBR Green adalah 100 parasit/ μ L darah dengan Nilai Ct 35,67 dan sensitivitas 53,8%, sedangkan limit deteksi metode TaqMan Probe adalah 5 parasit/ μ L darah dengan Nilai Ct 36,00 dan sensitivitas 100%.

Kesimpulan: Berdasarkan hasil penelitian ini, penggunaan deteksi fluoresens TaqMan Probe memiliki limit deteksi dan sensitivitas yang lebih baik untuk mendeteksi *Plasmodium falciparum* dibandingkan deteksi fluoresens SYBR Green.

Kata Kunci: *Plasmodium falciparum*, qPCR, SYBR Green, TaqMan Probe, limit deteksi, sensitivitas

Molecular Detection and Identification of *Plasmodium falciparum* using Real-Time Polymerase Chain Reaction (qPCR) Method

ABSTRACT

Background: Mortality rates of Malaria cases in Indonesia have increased in 2020. An identification of malaria parasites, particularly *Plasmodium falciparum*, needs to be conducted as this species is responsible for approximately 95% of malaria-related deaths. Molecular detection methods, real time PCR is expected to overcome the limitations of microscopic examination as a gold standard for its high sensitivity and rapidity. There are two types of fluorescent dyes that can be used in qPCR detection methods, namely SYBR Green and TaqMan Probe.

Objective: This study aims to determine the difference of the detection limit of *P. falciparum* parasites using qPCR amplification methods with SYBR Green and TaqMan Probe fluorescent dyes in terms of sensitivity and efficiency of a reaction using the same primers and samples.

Methods: This study is an observational descriptive study that provides an explanation or overview of the optimal conditions required to obtain molecular diagnostic results using real-time PCR methods. The samples used were isolated DNA of *P. falciparum* strain FCR3, strain 3D7 culture samples, and malaria patients in Southwest Sumba in 2019, which were subjected to qPCR amplification using two fluorescent dye methods.

Results: The efficiency comparison of qPCR reactions for the detection of *P. falciparum* parasites using SYBR Green fluorescence dye for control I and control II was 102% and 84%, respectively, while the TaqMan Probe fluorescence dye showed 105% and 81% efficiency. The detection limit of *P. falciparum* using SYBR Green fluorescence dye was 100 parasites/ μ L with a CT value of 35,67 with and sensitivity of 53.8%, whereas the detection limit using TaqMan Probe method was 5 parasites/ μ L with a CT value of 36,00 and sensitivity of 100%.

Conclusion: Based on the results of this study, the use of TaqMan Probe fluorescence has a better limit of detection and sensitivity for detecting *Plasmodium falciparum* compared to SYBR Green fluorescence dye.

Keywords: *Plasmodium falciparum*, qPCR, SYBR Green, TaqMan Probe, limit detection, sensitivity