Chapter 1

Introduction

1.1 Background

Dengue virus (DENV), a member of the *Flaviviridae* family, is a significant global health threat and the causative agent of Dengue Haemorrhagic Fever (DHF), a severe and potentially fatal disease. Its prevalence is most pronounced in tropical and subtropical regions, with countries like Indonesia in Southeast Asia bearing a substantial portion of the global burden. The World Health Organization (WHO) has classified Indonesia as a hyperendemic country for dengue, where the disease places a severe strain on both the healthcare infrastructure and the economy due to high hospitalization rates and outbreak management costs (Harapan et al., 2019). In this context, the early and accurate diagnosis of DENV is a critical public health intervention. It is essential for initiating timely clinical management, effectively reducing morbidity and mortality, and enabling rapid public health responses to contain outbreaks.

To combat this threat, the WHO endorses several diagnostic methods, with Reverse Transcription Polymerase Chain Reaction (RT-PCR) and Enzyme-Linked Immunosorbent Assay (ELISA) being the most established. RT-PCR is considered the gold standard for early detection due to its high sensitivity and specificity in identifying the viral RNA (Guzman & Harris, 2015). Conversely, ELISA, which detects the viral NS1 antigen or anti-DENV antibodies, offers a more accessible and cost-effective alternative. However, these methods possess significant limitations. RT-PCR requires sophisticated, expensive thermal cycling equipment, as stable power supply, and highly trained personnel, confining its use to well-equipped central laboratories. While ELISA is less technically demanding, its sensitivity, particularly for NS1, can be variable which could lead to false negatives (Ghosh et al., 2022). These limitations are acutely felt in a vast archipelago nation like Indonesia, where geographic dispersion, limited infrastructure, and resource constraints in rural and remote areas severely hinder the accessibility and timeliness of accurate diagnosis (Nurfadillah Alwi et al., 2025).

In response to these diagnostic challenges, Reverse Transcription Loop-mediated Isothermal Amplification (RT-LAMP) has emerged as a highly promising molecular alternative. The technique involves the conversion of viral RNA into complementary DNA (cDNA) followed by rapid, auto-cycling amplification under isothermal conditions using a strand-displacing DNA polymerase and four to six specially designed primer that recognize distinct regions of the target gene (Notomi et al., 2015). This mechanism confers significant advantages, including operational simplicity, rapid reaction times (often 15-60 minutes), and high tolerance to inhibitors commonly found in clinical samples. Crucially, RT-LAMP eliminates the need for complex thermal cyclers, making it suitable for field-deployable and point of care applications (Mautner et al., 2020). Results can be visualized through simple colorimetric changes or fluorescence, detectable with the naked eye or basic equipment, further enhancing its utility in low-resource settings (Grammatikos et al., 2023)

Despite the demonstrated potential of RT-LAMP for pathogen detection, its widespread adoption for dengue diagnosis, particularly in an Indonesia context, is hindered by a critical research gap. Many existing RT-LAMP assays require further optimization and robust validation to achieve performance metrics comparable to the gold standard RT-qPCR. Key variables such as primer design for high specificity across all four DENV serotypes, incubation time and temperature, and reagent concentrations significantly impact the assay's sensitivity, specificity, and reliability (Grammatikos et al., 2023). Therefore, this research aims to directly address this gap by systematically developing and optimizing a robust RT-LAMP assay specifically for DENV detection. The study will include primer design, optimize reaction conditions, and perform a validation against RT-qPCR with reference strain viruses and clinical samples. The expected outcome is a highly sensitive, specific, rapid, and cost-effective diagnostic tool that is readily deployable in resource limited settings across Indonesia, with the ultimate goal of improving early diagnosis, clinical management, and dengue surveillance outcomes.

1.2 Objective

1. To develop and optimize the RT-LAMP assay protocol by determining the optimal isothermal amplification temperature.

2. To analytically validate the optimized RT-LAMP assay by evaluating its sensitivity, specificity against other arboviruses, and cross-reactivity between DENV serotypes.

3. To assess clinical applicability of RT-LAMP assay by testing its performance with clinical confirmed patient samples

1.3 Hypothesis

H_o: The optimized RT-LAMP assay demonstrates no significant difference in sensitivity, specificity, and clinical detection to identify dengue virus in laboratory and clinical samples

H_1: The optimized RT-LAMP assay demonstrates a good result in sensitivity, specificity, and clinical detection capability for identifying dengue virus.