

UNIVERSITI TEKNOLOGI MARA

**DEVELOPMENT OF ON-SITE
DETECTION DEVICE FOR
PATHOGENIC *LEPTOSPIRA* SPP. IN
STREAM WATER**

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ABSTRACT

Leptospirosis is a disease caused by bacteria *Leptospira interrogans*. The number of leptospirosis occurrence is increasing, especially in a tropical climate country like Malaysia. Lack of awareness towards this disease can be associated with insufficient information of *Leptospira* distribution in the environment, especially at the recreational stream water. This study describes the optimization of Loop-mediated isothermal amplification targeting *LipL32* gene specific to pathogenic *Leptospira* (LipL32-LAMP). The successfully optimized LipL32-LAMP assay further was integrated into the design, fabrication and experimental verification of a portable on-site detection device for pathogenic *Leptospira* at recreational stream water. The device consists of three different parts and processes, the filtration and entrapment systems, the thermal lysis and DNA collection system and at the end of the process the LipL32-LAMP assay was used for specific detection of pathogenic *Leptospira*. In this study, the *LipL32* gene of pathogenic *Leptospira* was successfully detected by the optimized LipL32-LAMP assay in 10-20 minutes of incubation at 65°C, shown by the changes in color of the reaction from orange to pink. The portable on-site detection device for pathogenic *Leptospira* was successfully developed and demonstrated on-site with verification in laboratory by LipL32-PCR. The total detection time from sample filtration to LipL32 detection was 30-45 minutes. A year monitoring study on the occurrence of pathogenic *Leptospira* concludes that the occurrence *Leptospira* in stream water have a strong relationship with rainfall. Statistical analysis revealed that the occurrence of *Leptospira* in stream water was higher during dry season and lower or non-detected during rainy season. In conclusion, this study has successfully developed a portable detection device for pathogenic *Leptospira* spp. in waterfalls that have novelty in the portability of the design, sample processing and analysis of *Leptospira* in stream water. The application of this device has huge potential for prevention of Leptospirosis infection in recreational area especially in the tropical climate country where infectious *Leptospira* is a great concern.

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CHAPTER ONE

INTRODUCTION

1.1 Research Background

Worldwide, the popularity of recreational activities which involve contact with water has grown. Moreover, ease of travel has altered the public use of water for recreational purposes. The adverse impacts of recreational use of freshwater environments upon the health of users must be weighed against the enormous benefits to health and well-being. Recreational area always associated with the use of this environment for rest, relaxation and exercise. In Malaysia, in addition to being the focus for leisure, recreational area is also a focus for family activities like picnics and grills and other activities such as camping group of students.

Contaminated water with pathogenic *Leptospira* will pose a hazardous threat to deadly disease infections if not detected early. The case of death from Leptospirosis is a great concern for a tropical country like Malaysia. The warm and humid climate throughout the year in Malaysia is a favour of *Leptospira* leading to longer survival. Any water bodies such as streams, stagnant water, bogs, ponds and puddles are natural habitats for *Leptospira*. The survival of this bacteria mostly depends on high humidity with neutral pH (6-7.5). In addition to natural habitats, the presence of *Leptospira* in the water is also due to the excreted of urine of *Leptospira* infected animals. Humans are infected either directly through contact with infected urine or indirectly through contaminated water or soil. The bacteria enter the body through wounds and abrasions of the skin and through the mucosal surface of the mouth, nose and conjunctiva.

In recent years, the case of Leptospirosis that occurred after exposure to recreational activities in the waterfall area seemed like no solution. Most cases are often critical or delayed, leading to a lot of loss of life. So many of these unwanted events should be a signal that detailed investigations to prevent the occurrence of an infection should be implemented. Action should be taken to reduce the *Leptospira* infection during recreational water activities.