Chapter 13
The Detection of Tuberculosis by Loop-Mediated Isothermal Amplification (LAMP) Combined with a Lateral Flow Dipstick

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ABSTRACT

Tuberculosis (TB) is an airborne infectious disease caused by the bacterium Mycobacterium Tuberculosis (MTB) and is a persistent problem in developing countries. Present methods for its detection include normal or nested Polymerase Chain Reaction (PCR) followed by electrophoresis, real-time PCR, Ziehl-Neelsen staining, and culture assay. These techniques entail various disadvantages such as high cost, long assay time and use of toxic substances. Novel loop-mediated isothermal amplification (LAMP) permits DNA to be amplified rapidly under constant temperature. The combination of LAMP and chromatographic Lateral Flow Dipstick (LAMP-LFD) by using biotinylated LAMP amplicon hybridized with Fluorescein Isothiocyanate (FITC)-labeled probes are allowed to detect MTB without electrophoresis and interpreted within 3-5 min. LAMP-LFD is as highly sensitive as PCR-electrophoresis method. Based on its sensitivity, specificity, rapidity, cost effectiveness, ease of use, and convenience, LAMP-LFD could be suitable for use in early MTB detection.

INTRODUCTION

TB is an airborne infection caused by the bacterium *Mycobacterium tuberculosis* (MTB). World Health Organization (WHO) described that TB is a persistent difficulty in developing countries and ranks as the second leading cause of death from an infectious disease worldwide after the human immunodeficiency virus (HIV) (WHO, 2012). The pathogen is a slow-growing bacterium that desires 1-2 months for growing in a heritage; however, a fast and timely diagnosis of tuberculosis

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is absolutely vital to combat this disease. The Ziehl-Neelsen stain for direct specimen diagnosis is a conventional diagnostic device but lacks sensitivity. The checks based on PCR have shown pledge for the detection of mycobacteria in clinical samples, but this amplification process needs additional processing time, reagents and apparatus, which affect the cost of the assay (Brisson-Noel, et al., 1989; Kaewphinit, Santiwatanakul, Promptmas, & Chansiri, 2010a, 2010b; Khandekar, et al., 1994; Sritharan & Barker, 1991). Moreover, PCR investigation needs well-trained personnel.

The LAMP assay allows DNA to be amplified under isothermal condition at 60-65 °C. After LAMP, the amplified DNA is normally analyzed by gel electrophoresis, ethidium bromide staining and UV transillumination. Due to the use of several primers, LAMP generates a complex mixture of DNA amplicons of different sizes; therefore, gel analysis cannot distinguish specific and non-specific products. To avoid possible false positive results, the authenticity of LAMP products can be verified by restriction endonuclease digestion (Notomi, et al., 2000) or by hybridization to specific probes (Mori, Hirano, & Notomi, 2006). Subsequently, to further simplify and reduce the time required to develop LAMP product, a biotin-labeled oligonucleotide probe and an FITC-labeled DNA probe attached by gold-labeled anti-FITC antibody following chromatography on a LFD were developed. The techniques were effectively applied in the detection of pathogens such as shrimp infectious hypodermal and hematopoietic necrosis virus (Kiatpathomchai, Jaroenram, Arunrut, Jitrapakdee, & Flegel, 2008), shrimp Taura syndrome virus (Arunrut, Prombun, Saksmerprome, Flegel, & Kiatpathomchai, 2011) and MTB (Kaewphinit, et al., 2013a).

BACKGROUND

The name TB was probably first used by Shonlein in 1939. Its name is an older epithets including phthisis and consumption of which allude the marked wasting characteristic of advanced disease. Non-pulmonary manifestations particularly cervical lymphadenitis was known as scrofula (Grange, 1998).

TB is a disease caused by bacteria called *Mycobacterium tuberculosis* whose principal reservoir is human, and also other mycobacteria belonging to the *Mycobacterium tuberculosis* (MTB) complex such as *M. bovis* (MBV) or *M. africaum* (MAC). It is the most frequently affected to the lung but the disease has been termed as Morbud percorpus stressing that it may involve virtually any organ or system of the body. TB may, therefore, mimic many other diseases and often present a serious diagnostic challenge, especially in countries where the disease is now rare and often overlooked.

The infection is acquired by inhalation of droplet nuclei that contain tubercle bacilli from an infected person. An individual's risk of infection depends on the extent of exposure to droplet nuclei and his susceptibility to infection (Raviglione, Narain, & Kochi, 1992). Once infected with MTB, only a small proportion of individuals (about 10-12%) will develop the disease (Raviglione, et al., 1992). The incubation period of tuberculosis is highly variable and ranges from few weeks to many decades. The risk of developing the disease declines steeply with time after infection. Primary tuberculosis appears within a short period after infection and secondary TB disease due to re-activation or reinfection, normally has extended incubation period of usually (Wandwalo, 2005). In the absence of treatment, TB has a high case fatality rate, ranging from 60 to 70% for smear-positive pulmonary TB and 40 to 50% for other forms of tuberculosis, depending on the site of the disease (Raviglione, et al., 1992).

Epidemiology

WHO estimates that approximately one-third of the global community is infected with MTB. TB is among the top ten causes of global mortality and morbidity. The 196 countries reporting to WHO in 2008 notified 5.6 million new and relapse cases