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A NOVEL MOLECULAR ASSAY FOR DIAGNOSIS OF TUBERCULOSIS: PROBLEMS IN ESTABLISHMENT

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Tuberculosis (TB), an infectious disease caused by *Mycobacterium tuberculosis* (MTB), is one of the deadliest diseases in the world and remains a burden to Sri Lanka. Lack of a rapid, sensitive and accurate diagnostic method for the disease in its early stage is the main obstacle to its control throughout the world. Current TB diagnostic methods in practice have their own limitations in sensitivity, specificity, rapidity and cost. Therefore, a low cost, less sophisticated TB diagnostic method with high sensitivity and specificity is crucial for proper TB control, particularly in developing countries such as Sri Lanka. The objective of this study was to establish a novel nucleic acid amplification method named loop mediated isothermal amplification (LAMP) for the diagnosis of TB. This method specifically amplifies a DNA sequence very rapidly at a low cost, under limited resources, making it a more suitable disease diagnostic method for developing countries like Sri Lanka.

Pathogen specific DNA sequences in culture isolates prepared from sputum, which were collected from patients with suspected TB, at Chest Hospital Welisara, Sri Lanka were amplified by three separate LAMP reactions in three different laboratories. One assay was specific for 16S ribosomal RNA (16S rRNA) gene in genus *Mycobacterium*, and could detect the pathogen up to the genus level. The other two assays contained MTB specific primers targeting *rimM* and *gyrB* gene sequences in MTB, which enabled detection up to the species level. Each parameter, which is thought to affect the LAMP assay, was varied to establish standards to the assay. The detection of false positives, based on the colour change of SYBR Green dye (Sigma), was unavoidable throughout the study in all laboratories. However, it was established that the false positives are not due to contamination with MTB by carrying out restriction digestion of LAMP amplicons in false positive reactions. The sequencing results of amplicons in both MTB positive sample and a negative control further suggested that the false positives could be due to a self amplification of the LAMP primers.

Therefore, the LAMP assay could be recommended for the diagnosis of TB if the detection is carried out using restriction digestion of the LAMP amplicons. However, since this reaction is more expensive and time consuming, further refining of the method is necessary before it can be validated as a fast and cost effective method for TB diagnosis.

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