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DETERMINATION OF THE SENSITIVITY OF PCR BASED NON-RADIOLABELED HYBRIDIZATION FOR THE DETECTION OF *Mycobacterium tuberculosis*

A PROJECT REPORT PRESENTED BY

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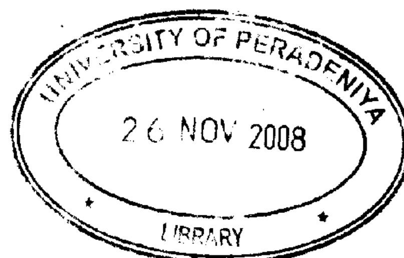

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ABSTRACT

DETERMINATION OF THE SENSITIVITY OF PCR BASED NON-RADIOLABELED HYBRIDIZATION FOR THE DETECTION OF *Mycobacterium tuberculosis.*

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One of the latest advances in disease diagnosis is the utilization of molecular biology tools. The important discovery of DNA amplification by Polymerase Chain Reaction (PCR) has changed the entire approach to the detection of many pathogens because of the advantages it has over the conventional methods such as diagnosis based on clinical features, microscopy results, culturing and serologic testing.

In house PCR was performed to amplify a minute DNA amount which was extracted from *Mycobacterium tuberculosis* cultures. A 123bp DNA fragment in the IS 6110 was amplified and subjected to agarose gel electrophoresis and hybridization. This method is capable of detecting a specific DNA fragment in MTB genome. In house PCRs are capable of identifying MTB in clinical samples with a higher degree of sensitivity, specificity and accuracy. The objective of this study was to determine the sensitivity of a PCR based non radio active labeled hybridization to detect MTB.

Firstly DNA was extracted and quantified according to Ethidium bromide fluorescence quantification method which is useful in the quantification of minute amounts of DNA, although it is a laborious method and lacks reproducibility. A dilution series was then prepared with quantified MTB DNA and the number of bacteria in each dilution was calculated according to the amount of DNA using following equation;

$$\text{Number of MTB genomes in a dilution} = \frac{\text{Optical Density (OD)} \times 4.74 \times 10^{16}\ \text{bp}}{4411529\ \text{bp}}$$

The sensitivity of the PCR based agarose gel electrophoresis was determined to be 23297 bacterial genomes per micro liter of the concentrated MTB DNA extract. Subsequently, each PCR product was subjected to hybridization by slot blotting and its sensitivity was determined. The sensitivity of the PCR based non-radio labeled hybridization was 2330 bacterial genomes per micro liter of the concentrated MTB DNA extract, which is 10 times more sensitive than the PCR based agarose gel electrophoresis. In a situation where the interpretation of test result is made difficult due to the lack of sensitivity in the PCR based Agarose gel electrophoresis, the PCR based hybridization technique can be used.