**Section 2**

## Executive Summary of the Project

This should be limited to 200-250 words and include the scientific background and objectives, methodology and major findings

**Background**: Tuberculosis (TB) is a disease of poverty that contributes significantly to ill-health in developing countries. Drug resistant TB is a major challenge in controlling and prevention of TB. Therefore, early diagnosis and rapid determination of drug sensitivity is of paramount importance in eradication of TB.

**Objectives:** The study was divided into 3 main parts. The aim of the first part was to identify the rifampicin resistant gene mutations of *Mycobacterium tuberculosis* (MTB) strains in Sri Lanka (Mainly Western Province). The objective of the second part was to develop a rapid molecular method for determination of rifampicin (RIF) resistance. The aim of the third part was to determine the molecular epidemiology of RIF resistance in Western Province where the highest disease burden in Sri Lanka is reported. Each objective was achieved through several specific objectives.

**Methodology:** Five hundred and thirty four acid fast bacilli (AFB) positive sputum specimens collected from Chest Hospital- Welisara, Chest Clinic- Colombo and Prison Hospital were used for the study. Lowenstein–Jensen (L-J), Middlebrook7H9 broth and paranitrobenzoic acid incorporated L-J media were inoculated using processed and concentrated sputum. Drug susceptibility testing (DST) was carried out by using the Agar Proportion Method (APM). The Nitrate Reductase Assay (NRA) and the manual Mycobacteria Growth Indicator Tube (MGIT) were evaluated as rapid culture based DST methods. DNA was extracted from isolated *M. tuberculosis* strains. Three fragments in *rpoB* gene were PCR (polymerase chain reaction) amplified and DNA sequenced. PCR based enzyme linked immunoabsorbant assay (PCR-ELISA) was developed and evaluated for detection of RIF resistant *M. tuberculosis* isolates in Sri Lanka. Restriction fragment length polymorphism (RFLP) was carried out to investigate the molecular epidemiology of the RIF resistance.

**Findings:** Four hundred and one (401) MTB cultures were isolated form 534 AFB positive specimens. The evaluated rapid culture methods, the NRA and the manual MGIT were found to be good alternatives for conventional proportion method that spend more than 28 days for determination of RIF resistance. Two novel mutations (at codon 184 and 626) and 2 prevailing mutations (at codon 526 and 531) were identified in *rpoB* gene of MTB strains collected from Sri Lanka. The PCR-ELISA that was developed as a component of study is simple, rapid, cost effective and an accurate molecular method for determination of RIF resistance. With regard to RFLP analysis of the band pattern of the isolates, there is no clonal relationship of the RIF resistant MTB isolates retrieved from the study.