1.0. General Introduction
This thesis is a study which determines the utility and cost effectiveness of a molecular diagnostic, Polymerase Chain Reaction (PCR) compared to other routine diagnostics for diagnosis of pulmonary tuberculosis in settings of routine clinical practice. Tuberculosis is amongst the ten leading infectious causes of global mortality (Murray et al., 1997). In India incidence of tuberculosis was found to be is 168/1, 00,000 and prevalence rate was 4 per 1000 (Chakraborty, 1998).

Approximately 40% of the Indian population is infected with tuberculosis and new cases with clinically active disease continue to develop from this pool. Rapid and accurate diagnosis and treatment of active cases thus remains the most important component of the tuberculosis control programme. In resource-poor countries, like India, emphasis has historically been on diagnosis and treatment of smear-positive cases of tuberculosis as they are more severe and account for the majority of instances of transmission of infection.

A substantial percentage of cases, however, are smear negative. Delayed diagnosis of such cases has a harmful effect on individual patients, who develop more advanced disease before treatment can be initiated. In addition studies using restriction fragment length polymorphism (RFLP) analysis have indicated that smear negative cases of tuberculosis contribute to substantial transmission (Behr et al., 1999) and this effect is greater as the disease progresses.

Case-finding in India is done mainly by sputum examination of self-reporting symptomatics to the health services. Diagnosis of smear negative cases rests mainly on clinical findings and radiographs. Smear microscopy is the
cornerstone for diagnosis of tuberculosis in India (World Health Organization, 1998). It is rapid, cost-effective and does not require major infrastructural inputs (Murray et al., 1990; Rieder et al., 1998). The main shortcoming of microscopy however is the lack of sensitivity, the threshold for detection being 5,000 - 10,000 bacilli/ml (Tomam, 2004).

Culture is sensitive, provides a definitive diagnosis, and increases case detection by 30-50% (World Health Organization, 1998). The mean incubation time of 4-8 weeks, however limits its use as a first line diagnostic (Jensen, 1995). Diagnostic algorithms using combination of indirect diagnostics are thus used in developing countries for diagnosis of smear negative tuberculosis (World Health Organization, 1997).

Symptomatics are subjected to indirect and non specific clinical tests, broad spectrum antibiotics and radiological examination before being diagnosed. Symptomatic response to antibiotics does not always exclude tuberculosis, as up to 50% of smear-negative tuberculosis patients have been reported to respond to broad spectrum antibiotics (O’ Brien and Talbot, 2003).

Chest X-ray is non-specific, fails to diagnose 10%-15% of culture-positive patients, and falsely diagnoses nearly 40% of patients likely not to have active tuberculosis (Garland, 1959; Harries et al., 1997). Their interpretations have limited specificity and inter reader repeatability (Jones et al., 1997). Diagnosis is especially difficult in case of tuberculosis HIV co-infection. Overall population trends and most clinical based studies suggest that HIV positive patients have a high rate of smear negative disease. As HIV related
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Immunosuppression increases, atypical pulmonary features predominate due to other infections and smear examinations are of no use (Alpert et al., 1997).

Current methods for diagnosis are thus non-specific and non-sensitive (Foulds et al., 1998). In resource-poor countries, a compelling case can thus be made that more rapid and accurate new diagnostic tests for tuberculosis can have a substantial impact on tuberculosis control activities. Nucleic acid amplification (NAA) tests amplify target nucleic acid regions (DNA or RNA) that uniquely identify the *Mycobacterium tuberculosis* complex.

Polymerase Chain Reaction (PCR) is a method of DNA amplification characterized by its speed, sensitivity and selectivity (Mullis and Faloona, 1987; White, 1996). Polymerase chain reaction (PCR) is the most widely used NAA test. Commercial kits include the Amplicor® MTB tests (Roche Molecular Systems), the Amplified *Mycobacterium tuberculosis* Direct Test® (MTD) (Gen-Probe Inc), the LCx® kit (Abbott Laboratories), and the BD ProbeTec ET assay (BD Diagnostic Systems). In-house tests are laboratory-developed PCR assays where the investigators put together their own PCR protocols (Flores et al., 2005).

Various studies (Schirm et al., 1995; Eing et al., 1996; Aslankindeh et al., 1998; Piersimoni and Scarparo, 2003; Greco et al., 2006; Ozkutuk et al., 2006) including Indian studies have evaluated PCR and it has been shown to be a promising alternative even for developing countries (Khandekar et al., 1994; Verma et al., 1994; Dar et al., 1998; Beeral et al., 1999; Prasad et al., 2001; Tiwari et al., 2003; Chakravorty et al., 2006). Sensitivities for smear positive
specimens range from 77% to more than 95% and specificities are > 95% (Catanzaro et al., 2000; Watterson, 2000; Kaul, 2001; Soini et al., 2001; Woods, 2001; Sarmiento et al., 2003). Sensitivity for smear-negative patients has been reported to be below 90% (Sarmiento et al., 2003).

PCR has its limitations. False-positive PCR results range at rates from 0.8% to 30% (Rattan, 2000). Test performance in situations of routine clinical use are inferior to that in controlled study conditions. Various evaluation and multicentre studies have underscored the need for good laboratory practices to ensure the reliability of nucleic acid amplification tests (Noordhoek et al., 1996; Noordhoek et al., 2000).

Majority of studies have been performed using primers specific for insertion sequence IS 6110. Studies in India however have reported that 43% of strains in South India showed no/single copy of this sequence versus 8-15 copies found in developed countries (Das et al., 1995). MPB64 gene for immunogenic protein is found only in culture filtrates of \textit{M. tuberculosis} and occasional isolates of \textit{M. bovis} BCG. Various studies, including those in India have been described using primers specific for MPB64 and these primers have shown great potential especially in Indian conditions (Dar et al., 1998).

Experience with in house developed PCR tests has demonstrated overall sensitivities and specificities in the range of 70%-100%. Various in house tests with sensitivities and specificities comparable to commercial tests or even greater than commercial tests have been described (Schirm et al., 1995). Metaanalytical review of 84 in-house PCR-based studies to detect \textit{M. tuberculosis}
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in sputum samples showed a summary receiver operator characteristic (SROC) of 97%, indicating an overall high accuracy of these tests. The advantage of these tests is that they are cost effective and can be standardized according to local conditions (Flores et al., 2005).

There is an urgent need for new tests that can contribute to improving case detection and reducing diagnostic delay. The performance of new diagnostics must be evaluated in settings that reflect their intended application, and performance must be evaluated to investigate whether such new technology is cost effective and can be implemented within the existing infrastructure in high-burden countries.

PCR has a potential for diagnosis of tuberculosis especially in subsets of patients, like those with negative smears but high likelihood of disease, immunosuppressed patients in whom treatment delay can be hazardous, and patients with relative contraindications to empiric anti tuberculosis treatment. Studies have indicated that these tests may be economically feasible and clinically useful in certain settings such as centralized laboratories where a large number of specimens can be quickly and easily referred. It has been suggested that there are scenarios even in resource-poor countries, in which use of NAA, might be cost effective at certain price points (Van Cleeft et al., 2005).

1.1. Aim and Objectives

The aim of this study is to determine the comparative efficiency and cost effectiveness of PCR to other routine diagnostics for detection of *M. tuberculosis*. 
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The objectives of the study are:

1.1.1. Objective I

To determine the efficiency of routine diagnostics like microscopy and radiological examination used in the Revised National Tuberculosis Control Programme (RNTCP) for diagnosis of pulmonary tuberculosis in Pune Municipal Corporation (PMC) area.

1.1.2. Objective II

To determine the comparative efficiency of PCR (in terms of sensitivity, specificity, positive and negative predictive value) using MPB64 primers versus other routine diagnostics in the detection of *M. tuberculosis*.

1.1.3. Objective III

To determine the cost effectiveness of the Polymerase Chain Reaction (PCR) to other routine diagnostics like sputum smear microscopy, culture and radiological examination by X-ray.