5.0. Discussion
Tuberculosis is the seventh leading cause of death, responsible for infecting approximately two billion individuals annually (Dye et al., 1999). Conventional diagnostics for tuberculosis have known limitations and their performance is suboptimal especially in the context of the HIV epidemic (Foulds and Brien, 1998; Perkins, 2000). Development of new diagnostics is thus important for case detection and control.

WHO declared tuberculosis a global public health emergency in 1993, and initiated the Tuberculosis Diagnostic Initiative in 1997, to develop new tools to diagnose tuberculosis (Word Health Organization, 1997). It is now working in collaboration with the Foundation for Innovative Diagnostics (FIND), a Bill and Melinda Gates Foundation funded initiative for the development, evaluation and demonstration of new diagnostic methods (Foundation for Innovative Diagnostics; www.finddiagnostics.org).

RNTCP has been in operation in Pune Municipal Corporation since 1995. Analysis of four year records of RNTCP in PMC indicated an overall increasing trend both in the chest symptomatics screened and tuberculosis cases diagnosed. The data indicated that though there was an 2.8 fold increase in the number of sputum smear positive cases, the increase in the smear negative cases was similar i.e. 2.5 fold, indicating that smear negative cases constituted a significant portion of the increasing tuberculous case load.

The data showed that smear negative cases accounted for more than half of the total cases diagnosed amongst the chest symptomatics. Globally rates of smear-negative pulmonary tuberculosis are rising especially in countries
with HIV and delayed diagnosis is an important cause of excess mortality in people living with HIV, especially those who have smear-negative pulmonary tuberculosis (World Health Organization, 2003).

In the RNTCP of PMC, microscopy detected two-thirds of pulmonary cases while the remaining one-third sputum smear negative symptomatics had to be screened, using the diagnostic algorithm of the RNTCP, wherein differential diagnosis of smear negative tuberculosis was done on basis of response to broad spectrum antibiotics, repeat sputum examination and X-ray chest examination.

There were 10,53,364 pulmonary cases in India in 2004, of which 4,65,354 were sputum smear positive, while 3,81,198 were smear negative cases, giving a ratio of almost 1:1 (World Health Organization, 2006). Worldwide 58% of new pulmonary cases reported by DOTS programmes were smear-positive (World Health Organization, 2006).

In a seminal study, Baily et al showed that for every 100 patients visiting a general peripheral health institution, nearly two will have chest symptoms and require a sputum examination. For ten such sputa examined, one will be sputum smear positive, and nine will require further investigations (Baily et al., 1967). In this study cost of diagnosing a smear positive case was calculated to be Rs. 54 while that of a smear negative equaled Rs. 173.

As the symptomatics approaching the centres for diagnosis increases, a comparatively greater proportion of resources are likely to be diverted to screen out sputum negative cases of pulmonary tuberculosis. In addition to the
expense, there is delay in the initiation of treatment of sputum negative cases. In our study the delay in treatment initiation for smear negative cases was found to be 7.2 ± 3.7 days. This in turn has implications in disease control, since there is transmission from smear negative patients (Behr et al., 1999) and increased patient drop out (Squire et al., 2005).

Treatment of smear-positive cases in DOTS programmes has been the basis of the tuberculosis control strategy. Studies on cost effectiveness strategies have however indicated that there is a strong economic case for treating smear-negative cases in DOTS programmes (Baltussen et al., 2005). The Stop TB Strategy now emphasizes on the timely diagnosis and treatment of all cases of tuberculosis, including smear negative cases. The balancing act between public health practices for control has reached that stage where services and treatment can be extended to smear negative cases.

There is thus an urgent need for new tests for the diagnosis of tuberculosis, which is particularly acute for smear-negative disease (Foulds and O'Brien, 1998). More attention is being paid to the impact that improving existing diagnostic might have in resource-limited settings. Studies have indicated that new diagnostics with greater than 85% sensitivity and 97% specificity could save 4,00,000 lives annually. Enhanced diagnostic techniques like rapid molecular testing are projected to reduce TB prevalence and mortality by 20% or more. Their impact is sensitive to the quality of existing diagnostic standards and the level of access to diagnostic services, but is robust across a wide range of population parameters including HIV and TB incidence (Dowdy et al., 2006).
New tools for diagnosis of active tuberculosis include newer versions of nucleic acid amplification tests, immune based assays, skin patch tests and rapid culture systems. Of all these tests NAA are studied most extensively and are available as commercial tests and also used as in house assays (Pai et al., 2006). Commercial tests are well standardized but expensive. In house tests are therefore used in developing countries as they are cheaper.

In this study, primers based on MPB64 gene of *M. tuberculosis* were used instead of the widely used IS6110 sequences. This is because many strains in India have shown a low copy number or absence of these sequences (Das et al., 1995). The aim of this thesis was thus to determine the efficiency and cost effectiveness of a low cost in-house PCR based test for diagnosis of *M. tuberculosis* which could be used in routine clinical practice.

The main strength of this study was that the PCR technique used here utilized a simple DNA extraction procedure involving few steps. The study was conducted blind and no additional sample was collected for testing for PCR. Sample was first used for microscopy and the remaining sample was used for PCR and culture. Symptomatics with different suspicion index of tuberculosis were selected i.e. highly suspect symptomatics approaching the Tuberculosis Unit, suspects who were identified by symptomatic screening by questionnaire during a morbidity survey and a control group of cases with disease other than tuberculosis.

In this study PCR was the most sensitive diagnostic (91.5%) compared to other routine diagnostics. Comparison of PCR to conventional
methods using McNemars test ($\chi^2 = 5.26, \text{df}=1, P<0.05$) showed a significant difference. The sensitivity of PCR reported here compares well to that reported from other studies performed in India (Dar et al., 1998). In addition, PCR was positive for an additional seventeen samples not diagnosed using routine diagnostics. Seven of these were later confirmed as true cases of tuberculosis. The remaining were highly suggestive of tuberculosis: one HIV positive individual with a recent history of extra pulmonary tuberculosis died; three individuals were immediate family contacts of tuberculosis cases; one had prominent cervical lymph nodes suggestive of tuberculosis and the other had previous history of tuberculosis. The population being a highly migrant population, four were lost to follow up.

Globally the fraction of the estimated number of sputum smear-positive cases detected within designated DOTS areas has remained constant at 40-50%. Unless the DOTS strategy can reach beyond traditional public health reporting systems, case detection will not rise much above 40% even when the geographical coverage of DOTS is nominally 100%. Substantial efforts are therefore needed to develop new case finding methods (Dye et al., 2003).

The overall specificity of PCR was lower (86%) compared to smear and culture. This was mainly because the highly suggestive tuberculosis suspects could not be confirmed by the gold standard. A single positive culture on LJ medium and final clinical diagnosis and treatment outcome was used as the combined gold standard. More sensitive methods including the use of automated culture systems were not used.
5.0 Discussion

Since PCR was more sensitive than the LJ culture, this may also have affected the specificity. Besides when culture is used as a gold standard in comparison studies, samples containing non-viable Mycobacteria may lead to a false positive PCR, resulting in an underestimation of the specificity of the PCR. Absence of a suitable gold standard to assess its efficiency is a major limitation of this test (Katoch, 2004)

All negative controls remained negative throughout the study and cross-contamination was not a problem. PCR did not show any cross reactivity with the two MOTT isolates, which indicate good specificity of the primers used. The MPB64 primers used in this study proved to be specific and should hold promise for the future.

PCR detected maximum cases as a single diagnostic in the shortest turnover time (less than 24 hrs) compared to other routine diagnostics. PCR was performed on a single specimen and gave result within a day. This can make the diagnostic process shorter and more patient-friendly, which may reduce dropout levels and contribute to reduced transmission.

In India several factors like the inability of health services to screen patients at first contact, poor interpersonal communication, attitude of health providers coupled with the lack of attention and support to patients are associated with health provider delay. Studies in India have found that only 22% of patients were diagnosed at the health facility where they first sought care and half of all the patients had to visit three or more health facilities before being diagnosis. Patients incurred an average cost Rs. 359 on medical consultation,
investigations, medication and travel and that health system delay was found to an average of 23 days (Rajeshwari et al., 2002). Thus costs borne by patients could be appreciably reduced by a rapid diagnostic.

In this study PCR was found to be useful in diagnosis of contacts of tuberculosis cases, and symptomatic cases who had previous recent history of tuberculosis. PCR diagnosed five contacts versus three by routine diagnostics. PCR diagnosed 17 cases with previous history of tuberculosis versus eleven by routine diagnostics combined. The significance of a positive result in these cases is not known since PCR detects both dead and viable bacilli. Further prospective studies are required to determine if they represent false positives or an early laboratory finding which predicts subsequent reactivation or early stage of disease.

Occasionally false negatives of microscopy occur due to poor quality of sputum. This study indicated that PCR can be a useful tool in those who are not able to expektorate a proper sputum sample. Out of 45 such samples, PCR was able to detect 12 positives while the routine diagnostic tests were positive in only seven. Three of the additional seven cases detected by PCR were later considered as true positives by the clinicians. In one of the subjects who had persistent chest symptoms and whose sample was available for PCR at the time of first presentation and on follow up after 6 months, a dramatic increase in bacillary load could be detected by PCR.

The major impediment to the large scale use of PCR in developing countries is its cost. Studies have indicated that PCR can be a potential cost
effective screening procedure for tuberculosis provided cost of PCR kit is brought down (Catanzaro et al., 1996). In the Indian situation, as in our study the reagent costs are substantially less as primers and reagents, are not patented and labour cost is comparatively less than that in developed countries. Considering the advantages of rapidity, and sensitivity, the cost should not be a deterrent in the adoption of molecular methods for diagnosis of tuberculosis.

PCR was 2.4 times more costly than microscopy for diagnosis of a smear positive case of tuberculosis but cheaper for diagnosis of a smear negative case of tuberculosis using the current algorithm. A recent study examined the cost-effectiveness of polymerase chain reaction versus Ziehl-Neelsen smear microscopy for diagnosis of tuberculosis in a high-burden, resource starved environment (Van Cleeff et al., 2005). The study demonstrated that costs per correctly diagnosed case were US $ 41 and $ 67 for smear microscopy and PCR, respectively. When treatment costs were included, including treatment of culture-negative cases, PCR was found to be most cost-effective at $ 382 versus $ 412. In our study PCR was found to be most cost-effective at Rs. 654 versus Rs. 811. Studies have indicated that a price reduction of PCR in the range of $6 would adjust the cost effectiveness of PCR to the same level of smear microscopy (Roos et al., 1998).

The costs of missed patients have not been included in this analysis. Though it would mean additional costs of diagnosis and treatment, substantial costs would be saved by reducing transmission. This would be of important both from the public as well as at the individual level. In this study
patient costs were not considered though patients spend a substantial amount in the form of loss of daily wages, transport costs and other costs.

Consideration of the role of new diagnostics should start with the recognition that more than one diagnostic test type is needed. This was apparent from the study as no single test could detect all cases of tuberculosis. The study shows that a molecular diagnostic like PCR can be cost effective and can be used in routine clinical settings. The probabilities of confirming a diagnosis of tuberculosis in paucibacillary cases was higher with PCR than with routine diagnostics.

The decision as to how and when to use the PCR technique should be individualized. Though several commercial NAA tests are licensed for routine testing of sputum there is uncertainty regarding how these tests should be utilized (Schulger, 2001; Barnes, 1997). Various studies recommend that these tests should be not be employed in patients with a low clinical suspicion of PTB. Test should be used to confirm the diagnosis of PTB in patients with an intermediate-to-high likelihood of disease. Despite such uncertainties, these tests have entered the realm of everyday practice in many institutions (Yee et al., 2002).

Utility of such a test in a country like India with limited resources both in terms of man power with adequate technical expertise and laboratories with technical infrastructure requires to be studied extremely carefully. To successfully implement PCR several factors like constant supply of power, water, timely procurement of PCR reagents and maintenance of equipment need to be considered.
5.0 Discussion

The reagents and primers used in this study were not imported and of local make to avoid delays and to cut costs. This work was carried out in conditions with an average of eight to nine hours power cuts per week. This background has to be taken into consideration for implementation of the test on a large scale in developing countries. However, in the future laboratories at the State level, the National Reference Laboratories in India, along with laboratories in the major referral teaching hospitals may be able to take up molecular based methods.

While PCR may not be directly applicable currently in the national programme its role in the private sector should be considered. PCR with MPB64 primers can be a useful adjunct to diagnose clinical tuberculosis. In this study it was useful for diagnosis of paucibacillary cases, patients with tuberculosis HIV co-infection, highly symptomatic contacts, and amongst symptomatics who could not expectorate sputum samples. PCR results however should be interpreted in conjunction with clinical data and routine diagnostics. A major limitation of this study was the small number of samples tested and studies with larger numbers in field conditions need to be taken up in order to validate these results.