Introduction
Tuberculosis is one of the oldest and most widely spread life-threatening infectious diseases of mankind. It is characterized by fever, weight loss, raised level of acute phase reactants and necrosis in lesions.

It has been estimated that every third person on this earth is infected with *M. tuberculosis*, infecting nearly 1.6 billion people of which 20 million have active disease, with eight million new cases and approximately 2-3 million deaths annually worldwide (WHO report, 2004). India alone has nearly one third of the total global burden of tuberculosis, and the disease is one of the India’s most important public health problems. Every day in India more than 20,000 people become infected with the tubercle bacillus, more than 5,000 develop the disease, and more than 1,000 die from Tuberculosis.

In India, tuberculosis kills 14 times more people than all the other tropical diseases combined, 21 times more than malaria, and 400 times more than leprosy. Every year, another 2 million people develop tuberculosis; nearly one million of them are highly infectious, sputum positive. Two such cases develop every minute. Further, in recent years there has been an upsurge in the incidence of the disease mainly due to the emergence of multidrug resistant strains of *M. tuberculosis* (MDRTB) and its association with the HIV infection leading to spread of disease in immuno-compromised and AIDS positive individuals. Globally, there are about 4.6 million cases dually infected with HIV and tuberculosis (WHO report, 2002; 2004). Tuberculosis thus, has become a major concern worldwide. If specific control measures are not taken with increased commitment and action at national and International levels, tuberculosis will claim about 35 million more lives in the next decade and
there will be about 150 million new cases of active tuberculosis. Such a vast epidemic creates challenges as it raises the demand for public health solutions. A delayed or missed diagnosis of tuberculosis is also one of the leading causes, which contribute to *M. tuberculosis* transmission.

Currently, more than 15 anti-*Mycobacterium* drugs are available for tuberculosis patients and are indispensable to prevent the progression of the disease especially those caused by MDR strains of *M. tuberculosis*. Thus, there is an urgent need to develop strategies to effectively tackle the threat of *Mycobacterium* to the mankind.

Since, the identification of *Mycobacterium* by Koch in 1882 as the causative agent of the disease, efforts have been made to develop suitable prophylactic and chemotherapeutic agent(s) for the treatment of disease. In the past, several killed and live vaccines were developed to protect against the disease. However, the only vaccine that is still in use is derived from the bovine strain of *Mycobacterium* i.e. *M. bovis*. This strain was attenuated for over thirteen years and was termed as Bacilli Calmette Gurein (BCG) vaccine (Calmette, 1927). Since then BCG vaccine has been successfully used for prophylaxis against tuberculosis. It remains to be the most wildly and most controversial of all vaccines used today. The protective efficacy of BCG vaccine has been highly variable ranges from 0-80% (Fine, 1989; Baily, 1990). Moreover, it has been observed that BCG vaccine is least effective in controlling the disease in the regions/areas where the frequency of the disease is maximum (Stanford, 1991). In addition, the BCG has been shown
to produce disseminated infection in the immunocompromised host such as AIDS patients, sometime even after thirty years of vaccination (Orme et al. 1993). Therefore, there is an urgent need to identify the protective component of *Mycobacteria* for the development of safe, stable and more effective vaccine against tuberculosis.

It is evident from several studies that resistance against tuberculosis is mediated by cellular arm of the immune system (Leveton et al., 1989; Orme et al., 1993; Turner et al., 2000; Kanaujia et al., 2004). Hence, the antigen for vaccine development should be selected on its ability to induce the cell mediated immune response. In the past, attempts have been made to investigate the immunogenic potential of different components of *Mycobacterium*. Several components viz. *Mycobacterial* cell wall, rRNA and mannosides have been evaluated for their immunoprotective potential (Mehta, 1996; Bouquet and Negre, 1923; Youmans and Youmans, 1966a; Pancholi et al., 1989; Singh and Khuller, 1993; Hetzel et al. 1998; Oliver et al., 2000; Gennaro, 2000; Leander et al., 2005; Kwasi et al., 2005).

Secretory proteins of *M. tuberculosis* appear as culture filtrate proteins (CFPs) in the culture medium in which *M. tuberculosis* is grown. Mechanism of secretion of CFPs is not clearly known. There are approximately 200 proteins found in culture filtrate of *M. tuberculosis* (Anderson, 1994; Berthet et al., 1998; Kamath et al., 1999; Sonnenberg and Bellisle, 1997; Karin et al. 1998; Kanaujia et al., 2004; Spencer et al., 2004; Young et al., 2004; Sable et al.,
Some of these proteins are associated with cells, therefore, the definition of CFP is an operational one.

Many research groups actively studied CFPs. Since many CFPs are recognized by the sera of TB patients, it has also been postulated that live attenuated *M. tuberculosis* vaccines are better than those made from heat-killed cells because during growth in the host, *M. tuberculosis* releases CFPs that stimulate host immune mechanisms (Andersen, 1994).

Many of the proteins found in the culture filtrate viz. SodA, KatG, and GlnA (glutamine synthase), do not have leader sequences that are usually involved in protein secretion, but the fact that they were released from cells early in growth suggested that this localization was physiological and not dependent on cell lysis (Daffe and Draper, 1998. Braunstein and Belisle, 2000; Brennan and Nikaido, 1995; Sonnenberg and Belisle, 1997; Tullius *et al.*, 2001; Smith Issar, 2003; Pym *et al.*, 2001). However, experiments suggest that proteins highly expressed in *M. tuberculosis* are GlnA and SodA (Piddington *et al.*, 2001; Dussurget, 2001; Harth and Horwitz, 2003). They are very stable and found in culture filtrates in early period of incubation, while less abundant intracellular proteins or those that are unstable are not found extracellularly (Molle *et al.*, 2000). These results strongly suggest that the presence of many proteins in culture filtrates, especially those with missing leader sequences, is caused by bacterial leakage or lysis.
The M. Tb secreted active proteins, which are missing in non-tuberculous Mycobacteria have proved as a promising not only for diagnostic marker, vaccine candidate but also in understanding the Mycobacterial evasion of protective immunity in susceptible individuals. Many such proteins identified and characterized from the M. TB complex include 38 KDa, 30/31KDa, 40 KDa, 42 KDa, SOD, 30 KDAMSP, 85B, ESAT-6, and CFP10 (Sonnenberg and Belisle 1997; Manca et al., 1997; Karin et al., 1998; Gennaro 2000; Silva et al., 2003; Young et al., 2004; Lanbo et al., 2004; Ayman et al., 2005). However, little information is known regarding the possible role of low molecular mass M. TB secretory proteins in immunity against tuberculosis, with emphasis on their immuno-modulatory action and the potential involvement in Mycobacterial subversion of the host immune defense.

Another important area of significant concern is the diagnosis of the disease. Although several studies have been conducted and many are still in progress for diagnosis of disease during the early stage of infection, which can distinguish M. tuberculosis infection from other atypical Mycobacteria is a challenging task worldwide.

The current methods used in clinical laboratories depend on microscopy and culture that usually takes 6-8 weeks to report negative / positive results. The isolation of organism from CSF has been disappointingly infrequent (Dingley 1979), time consuming, and lacks sensitivity (Annamma et al., 1990; Clarridge et al., 1993; Kadival et al., 1987). These methods have several limitations both in terms of its sensitivity and specificity (Garg et al., 2003). Thus the bottom line in the effective treatment still remains i. e. the early diagnosis of the
disease that could help in taking remedial measures far more early than is done at the moment. Another most commonly used procedure for diagnosis is 'Montoux test' which doesn’t make a conclusive evidence of active disease, while negative tests do not exclude it.

Several attempts have been made in the past to develop newer tools for the timely, accurate and specific diagnosis in the early stages of disease by developing the tests based on immunological and molecular methods to reduce the time of diagnosis, increased sensitivity and specificity. Although rapid culture techniques such as Radiometric liquid (BACTEC), biphasic (MB chek) culture system and Alamar blue assays have improved both the recovery rates and speed of isolation, but these systems still can not influence beside decision making (Garg et al., 2003; www.nejm.org on March 27, 2006; Dingley, 1979; Annamma et al., 1990, Clarridge et al., 1993, Kadival et al., 1987).

The introduction of fibreoptic bronchoscope has rendered lung biopsy as well as bronchial lavage and brushing a simple and safe procedure but unfortunately the equipments are not universally available (Zavala, 1975; Miro et al., 1997; Alan et al., 2002; Griffith et al., 2004).

Recently, several tests have been developed employing the r-DNA technology to develop the kits based on polymerase chain reaction (PCR) in diagnosis of tuberculosis. A number of investigators have reported the detection of specific sequence for *M. tuberculosis* directly in clinical specimen by PCR (Miller et al., 1994; Bisen et al., 2003). This technique is quite sensitive and by making
proper selection of gene sequences, it is possible to accurately establish the
diagnosis *M. tuberculosis* complex in the clinical samples. But despite its
sensitivity and specificity, this method is highly expensive e.g. one Amplicon
PCR test costs~ $15 (Bennedsen *et al*., 1996). Hence, the application of PCR
based diagnosis is the best but also very costly. Moreover, the amplification of
dead bacterial DNA and absence of amplifiable *M. tuberculosis* DNA from
blood pose a serious problem in the diagnosis (Mullis and Falloona, 1987;

Several serodignostic tests have been developed for the diagnosis of disease
(Grange and Laszlo, 1990; Gennaro, 2000; Nair *et al*., 1992; Garg *et al*., 2003;
Esther *et al*., 2004; Young *et al*., 2004; Tiwari *et al*., 2005). Many workers have
attempted to isolate the species specific antigen for use in diagnostic tests
(Manca *et al*., 1997b; Kadiwal *et al*., 1994; John *et al*., 1998; Griffin *et al*., 1991;
Daleine, 1995; Sudha *et al*., 2000; Esther *et al*., 2004; Tiwari *et al*., 2005), but
this task has proved very difficult, because of two reasons: firstly, specific
antigenic determinants often occur on the same protein molecule as shared
antigen, therefore making it very difficult to purify them by sensitive
techniques viz. affinity chromatography etc. Secondly, an antigenic
deterrinant, many a times may be shared among different species of genera
*Mycobacterium* that make the correct diagnosis of *M. tuberculosis* difficult.
Also the given determinant may be present on a range of molecules of
differing physiochemical properties. Thus preparative techniques based on
such difference (gel-filtration & ion exchange chromatography) have not
proved very useful (Grange 1988 a and b).
The rapid diagnostic test for the detection of antigen(s) in patients with tuberculosis may be the best choice for diagnosis of tuberculosis, as false positive results have been observed in antibody based diagnostic tests due to exposure of environmental Non-tuberculous *Mycobacteria* (NTM) or prior BCG vaccination (Fine 1995).

Thus the ultimate goal of biomedical research in TB around the world should be to lessen the public health burden of this disease by developing improved, specific and cost effective prophylactic diagnostic tools (Eunice *et al.* 2003) to combat the menace of the disease.

The present study was undertaken with the aim to identify the major immunoprotective and immunodiagnostic antigens of *M. tuberculosis* for the development of effective vaccine and / or diagnostic tool. The secretory protein (s) antigens of *M. tuberculosis* were identified in order to develop a rapid, cost effective, sensitive and specific test for the detection of *M. tuberculosis* antigens and / or antibodies (IgG, IgM, IgA) in patients suffering from active tuberculosis infection. The choice of specimens included the CSF for Tuberculosis Meningitis, serum for pulmonary and other extra pulmonary tuberculosis and processed tissue biopsy for extra-pulmonary tuberculosis.