Diagnosis of tuberculosis is different in children. The diagnosis of active tuberculosis in adults is mainly bacteriologic, in children it is usually epidemiologic and indirect. In the absence of a positive culture, recent exposure to an adult with an active disease is the strongest evidence. Indirect diagnostic techniques such as physical examination, tuberculin test, X-rays and CSF picture offer supportive information. The conventional modalities of diagnosing TBM and the development of immunodiagnostics for TBM are reviewed.

CLINICAL FEATURES:

Neurotuberculosis occurs as a consequence of lymphohemato-genous spread of tubercle bacilli during the primary infection. TBM is most common in young children. Initial signs are non-specific including fever, personality changes, listlessness or irritability and loss of developmental milestones. As the meningitis worsens, the flow of CSF is interrupted and signs of raised intracranial pressure develop - cranial nerve palsies, vomiting, lethargy and convulsions. With further progression coma and alterations in cardiac and respiratory functions ensue. Diagnosis in the early stages of TBM is usually missed unless suspected.
The importance of adequate history and contact tracing cannot be overemphasised and a lumbar puncture is mandatory to make or break the diagnosis. But unfortunately atypical presentations are not so uncommon (UDANI, 1974). A history of contact with tuberculosis, though emphasised by KILPATRICK et al (1986) was obtained in only 10, 19, 20 and 38% of patients in the Indian studies (RAMACHANDRAN, 1966; MAGOTRA, 1974; BENEKAPPA, 1983; SHARMA, 1994). Clinical suspicion is strengthened by the detection of choroidal tubercles in the optic fundus but the detection rate is as low as 1-2%, unless meningitis is associated with miliary tuberculosis (VARMA, 1966). In his series of 100 patients with culture proven TBM, KILPATRICK et al could not find any patient with choroidal tubercles (KILPATRICK, 1986). Failure of clinical improvement within 48 hours of initiation of presumptive pyogenic meningitis treatment used as one of the clinical criterion for diagnosis of TBM by Girgis et al is only conjectural (GIRGIS, 1991). The manifestations of TBM are protean and may continue for months with poor localising signs.

The following manifestations in a child should lead to a suspicion of TBM (UDANI, 1974).

(i) The classical clinical triad of symptoms of fever, apathy and vomiting particularly when they are persistent. These children have also anorexia and constipation.
(ii) Persistent pyrexia with doubtful meningeal signs, or evidence of cerebral irritation or disturbances of behaviour like irritability alternating with apathy, anxious or frightened look, excessive cry or a peculiar shrill cry.

(iii) Fever or vague illness following measles, whooping cough or head injury.

(iv) Irregular fever, usually of a few days duration followed by convulsions with or without neurological deficit.

(v) Fever, vomiting and drowsiness.

(vi) Persistent headache in an older child or persistent unexplained vomiting in a younger child.

(vii) Meningeal or neurologic signs following an acute or subacute or chronic or vague illness.

(viii) Suspicious head enlargement or boggy fontanelle with or without history of trauma.

(ix) Typhoid like temperature with mild meningeal signs.

(x) Onset of any of the above symptoms or signs in a child found to be in contact with an open case of chronic pulmonary tuberculosis.
It is thus evident that diagnosis of TBM in children is not always easy and additional indirect confirmation based on tuberculin test, radiology and CSF picture are required.

**TUBERCULIN TEST:**

The intradermal tuberculin test using Old Tuberculin was described by CHARLES MANTOUX in 1908. Tuberculin test was the first immunodiagnostic test. One must distinguish tuberculin test when used as an epidemiologic tool from that as a diagnostic tool. In epidemiologic surveys there is validity in considering weak reactions or strong reactions as false positives or cross-over reactions from infections with Non-Tuberculous Mycobacteria (NTM). In critically ill or malnourished children with TBM the skin reactivity to tuberculin can be temporarily depressed to the point where there is no reaction. If it is repeated some months later when the general condition of the patient has improved, it will usually be positive.

A positive tuberculin skin test is the hallmark of primary infection. A positive response results in induration within 72 hours associated with migration of activated lymphocytes and macrophages to the site of infection. To use the test effectively one must be familiar with its limitations and variations.
In older children and adults, a wheal of less than 5 mm is regarded as insignificant, 6-9 mm likely to be associated with infection by NTM and greater than 10 mm indicative of infection by *M. tuberculosis* unless the person has received BCG. After BCG, the tuberculin response is usually less than 10-15 mm. In infants and young children with clinical evidence suggestive of tuberculosis, those with malnutrition or in close contact with a case, an intermediate reaction of 6-9 mm may be significant.

A negative tuberculin test does not rule out the presence of active tuberculosis. False negative response may be present in up to 20-50% of patients suffering from tuberculosis (UDANI, 1980). The various reasons include (a) loss of potency of PPD due to improper storage, contamination, improper administration (i.e., leakage from site of injection, (b) factors interfering with lymphocytic activation and delayed hypersensitivity reaction, e.g. viral infections, like measles, HIV, influenza.

False positive tuberculin reaction can occur due to repeated skin testing with PPD, prior BCG vaccination. Positive tuberculin following BCG rarely exceeds 10 mm and any reaction exceeding 10 mm should be considered as indicative of presence of tubercular infection.
The key question related to tuberculin testing is what amount of induration should be considered significant, as a true indication of TB infection. The cut-off point for induration and its degree of overlap in children with and without known tuberculous infection vary with the characteristics of the population tested. In areas with high incidence of tuberculosis, regardless of the presence of environmental NTM, the cut-off point should be 12 mm.

Positive tuberculin test was reported in 41% and in 25-50% of children with TBM from studies in Bangalore and Bombay respectively (BENAKAPPA, 1983; UDANI, 1991). The reported tuberculin negativity in studies on TBM in children varied from 20-60% (DONALD, 1990; UDANI, 1980; STEINER, 1973).

In children with TBM, a positive tuberculin test if obtained may strengthen the suspicion.

RADIOLOGIC EVALUATION:

There is no single pulmonary radiological change that is pathognomonic of tuberculosis. Certain changes have come to be associated with proven cases.
The radiological features compatible with the diagnosis of primary tuberculosis include hilar adenopathy (79%), consolidation (29%), parenchymal infiltration (28%), pleural effusion (12%), miliary mottling (10%), segmental collapse (3%) (ADERELE, 1980).

Positive chest X-ray suggesting intrathoracic tuberculosis was reported in 41% children and 46% of children with TBM from studies in Bangalore and Bombay respectively (BENEKAPPA, 1983; UDANI, 1974). Non-specific findings and normal chest X-ray are reported in 27 and 29% of children with TBM by ALTINBASAK et al (1994). Association of extrathoracic tuberculosis with TBM is seen in less than 1% of cases (BENEKAPPA, 1983).

Calcification in skull/sutural diastasis or silver beaten appearance due to increased intracranial pressure offer supportive evidence for tuberculosis. Cranial CT examination is useful in detecting tuberculomas and is not very helpful in diagnosing TBM.

Dilated ventricles (90%), parenchymal involvement (25%), basilar meningitis (51.9%), foci of infarcts/hypodense mass and ring enhancement on contrast, solitary or multiple tuberculomata are other helpful findings in CT examination for neurotuberculosis. (ALTINBASAK, 1994).
Roentgenographic diagnosis, though with low specificity, is useful to strengthen the diagnosis in neurotuberculosis.

**CEREBROSPINAL FLUID PROFILE**

The classical CSF findings in children with TBM are well known and include a clear CSF, a relatively low cell count ($< 400 \times 10^6 / l$) with an excess of lymphocytes, an accompanying rise in the protein concentration (50-200 mg/dl) and a fall in the glucose concentration (20-50 mg/dl). Less well known, but none the less well documented, is the occurrence of a polymorphonuclear predominance with a cell count of up to $200 \times 10^6 / l$ in a small percentage of cases (KENNEDY, 1979; LINCOLN, 1960).

Hazy fluid simulating pyogenic meningitis, 'cobweb' formation and haemorrhagic CSF due to vasculitis are also known in this condition.

Typical, atypical and normal CSF pictures were obtained in 91, 6, and 3% of TBM patients by BENKAPPA et al (1983) whereas UDANI et al (1974) found 73% of CSF with classical findings and 11% and 16% of CSF with atypical and normal picture. In some of his cases, CSF remained normal till the end though the diagnosis was proved at autopsy.

The type of cellular response in CSF and the trend of changes in cell count are noteworthy in TBM. Maximum variability of cells in the initial stages of the illness and persistence
of polymorphs in the later stages, monocyte-macrophage cells (large cell with indistinct cell margin, abundant cytoplasm and elongated irregular nucleus) have been noted in majority of patients (JEREN, 1982). Neutrophils predominated (60-80%) in the first 10 days of illness, the mononuclear cells such as lymphocytes, lymphoid cells, monocytoid cells and macrophages replaced them. Reticulomonocytes and reticulohistiocytic cells were also noted.

The trend in cellular count was evaluated in childhood TBM over a period of 4 weeks by DONALD et al (1991). Uninterrupted decline (37%), fluctuating downward trend (43%) and fluctuating upward trend (21%) were noted in his study.

Detailed cytologic analysis of CSF sediment, following the dynamics of cellular modifications, can suggest a diagnosis of TBM even when the clinical picture and the biochemical findings of the CSF are atypical.

The rise in protein concentration (50-200 mg/dl) accompanies the cellular changes. Xanthochromic fluids with increased protein, normal sugar were found in 2% of cases (UDANI, 1974). Normal CSF glucose cannot be taken to exclude TBM and 40% of children had an initial normal CSF glucose (DONALD, 1991). The value of persistent low glucose levels in strengthening the diagnosis of TBM had been emphasised in studies from
India, Egypt and South Africa (RAO, 1959; KILPATRICK, 1986; DONALD, 1981). CSF glucose values, when persistently low indicated poor outcome. RAO et al noted CSF glucose levels of less than 20 mg% in 50% of fatal cases.

There is little difficulty in the majority of cases in recognising the CSF changes accompanying TBM in children and low glucose value is a dependable diagnostic parameter.

ISOLATION OF _M._TUBERCULOSIS FROM CSF

Smear and Cultures:

Demonstration of Acid Fast Bacilli (AFB) in smear/culture is the definite diagnostic aid and is the Gold standard for evaluating other diagnostic parameters. But smears of CSF for AFB are usually negative.

In a series of 100 patients AFB were demonstrated in smear in only 2% of cases by KILPATRICK et al (1986). None of the 125 samples studied by BENAKAPPA et al including those which were positive by culture were smear positive (1983). Similar experience was reported by CHANDRAMUKI et al who studied 105 children with TBM (1990). TANDON in an exhaustive study of TBM in adults isolated bacilli only in 2% of cases (1988). However, JOISHY and SANT reported 20% positivity in their CSF smears of children with TBM (1974).
These reports show that the yield of tubercle bacilli is very low in CSF even at well established centres in India. However, in developed countries AFB were identified by smear of CSF in 10-40% of cases in most series (MOLAVI, 1985). They suggested that examining second and third CSF specimens added substantial number of positives.

Cultures of _M. tuberculosis_ do not have a high yield rate. There is a wide percentage of isolation of tubercle bacillus from CSF, from 3-97%. THAPAR reported 3% isolation by ordinary centrifugation technique, 38% by alcoholic precipitation from CSF and 49-60% by floatation hydrocarbon technique (THAPAR, 1979). Culture positivity of 49 and 52% for tubercle bacillus in CSF were reported by BENKAPPA (1983) and GIRGIS (1991) respectively. Though only 52% of initial cultures were positive, the positivity for culture increased to 83% on examining second and third CSF specimens (KENNEDY and FALON, 1979). From time to time, there can be a failure of isolation in CSF in children, as hypersensitivity plays a predominant role in the pathogenesis of TBM in them.

The culture media used for growth of _M. tuberculosis_ are non-selective and selective media. The former includes those that are egg-based (Lowenstein-Jensen), agar based (Middlebrook and Cohn), or liquid (Dubos, 7 Hq Broth). Selective media contain antimicrobial agents and include modifications of Lowenstein-Jensen
medium (Gruft modification, 7H10, 7H11 media). Liquid medium with the same characteristics as selective 7H11 medium was introduced with radiolabelled $^{14}$C fatty acid component (7H12 broth). Growth is detected radiometrically by measuring release of CO$_2$ (BACTEC). The system reduces time to recover mycobacteria by one to weeks. These newer systems are used in mycobacterial reference laboratories for sputum only and little information is available about BACTEC methodology for gastric aspirates or CSF specimens.

To summarise the present status of conventional modalities for diagnosing TBM in children.

* **TUBERCULIN** induration and percentage positivity are independent of both prior BCG vaccination and a positive family history of tuberculosis. Even among BCG vaccinated children, a positive tuberculin reaction of 10 mm or more is a definitive indicator of natural infection with *M. tuberculosis*. However, the association of post-measles immunosuppression state and protein-energy malnutrition (PEM) in children with TBM make the interpretation of negative tuberculin test difficult.

* **ROENTGENOGRAPHIC DIAGNOSIS**, though with low specificity is helpful in presumptive diagnosis. The difficulties in differential diagnosis, evaluation of the activity of the tuberculous process and conflicting interpretations of the abnormalities are important drawbacks of this diagnostic tool.
* CSF CYTOLOGY is an useful adjunct in the diagnosis. The variable cellular response, depending on the stage of illness, neutrophilia in CSF with high pleocytosis, difficulties in classifying lymphoid, reticulohistiocytic, monocytoid type of cells may cause confusion and errors in interpretation.

* CSF BIOCHEMISTRY - The occurrence of protein and sugar values falling either within the range of those seen during viral or pyogenic meningitis must be remembered. Low CSF sugar is a dependable diagnostic parameter in TBM. However, protein levels have more value for prognosis.

* CSF CULTURE for M.tuberculosis: The organism are few in CSF of TBM cases. Mycobacteria were identified by smear of CSF in 10-40% of cases in most series and by culture in 45-90% of cases (KENNEDY, 1979). The diagnostic yield of CSF smear and culture in Indian studies has generally been lower.
NEWER DEVELOPMENTS FOR THE RAPID DIAGNOSIS OF TBM

The newer tests include Adenosine Deaminase Assay (ADA), Bromide Partition Test, Identification of mycobacterial products like 3-2'(ketoheptyl) indoline, tuberculostearic acid and new gene amplification technology such as Polymerase Chain Reaction (PCR).

Adenosine Deaminase (ADA) Enzyme Assay:

ADA is an enzyme which catalyses the purine catabolic pathway and its principal biological activity is detected in T lymphocytes. Increased levels of ADA have been observed in the plasma of patients with infections which elicit an active participation of cell mediated response (GALANTI, 1981). The usefulness of estimation of ADA in serous fluids like pleural and peritoneal fluids of tuberculous patients was confirmed by RIBERA et al (1987). COOVADIA et al (1986) found the ADA assay to have a sensitivity and specificity of 73% and 71% using a level of 10 U/L as the cut-off point in TBM cases. A considerable overlap between the ADA values in children with TBM, pyogenic meningitis and viral aseptic meningitis was reported by MALAN et al (1984). No valid conclusion can be arrived at as the ADA levels did not correlate with the tuberculin reactor status of the patients.
Radioactive Bromide Partition Test:

Radioactive Bromide $^{82}$Br is administered in a dose of 0.6-0.8 microcurie/kg weight suspended in isotonic saline either intravenously or orally and after equilibration periods of 24 and 48 hours respectively, specimens of serum and lumbar CSF are collected for measurement of $^{82}$Br content. In normal subjects with an intact blood-brain barrier the $^{82}$Br partition ratio (Serum : CSF ratio) is 2.5-3.5 with a mean of 3.1 (TAYLOR, 1954), this concentration gradient being maintained by the active transport system for $^{82}$Br in the choroid plexus. In TBM, probably due to the alteration in the blood-brain barrier, this ratio falls to less than 1.6 (COOVADIA, 1986).

WIGGELINKHUZEN et al (1980) have reported a sensitivity of 94% and specificity of 88% while COOVADIA (1986) reported sensitivity and specificity of 92% each. The only Indian study by BARUCHA (1980) found a ratio of 1.6-1.9 to be consistent with active TBM.

VON WENZEL et al (1989) studied the use of $^{99}$TC for the partition test in the place of $^{82}$Br. The result of technetium partition test compared favourably to $^{82}$Br partition test. They advocated the use of $^{99}$TC-Diethylene triamine penta-acetic acid partition test because of lower radiation dose and possibility of concomitant brain scintigraphy.
Biochemical Markers:

Levels of lactate dehydrogenase, Isocitrate dehydrogenase (SHARMA, 1980) and Gamma-aminobutyric acid (GABA) levels (DAVE, 1986) were used to diagnose TBM but these tests are too non-specific.

Using mass spectrographic technique, mycobacterial metabolites like 3-(2' ketoheptyl) indolic acid was detected in 4 out of 5 TBM patients (BROOKS, 1977). No further studies are available.

Using gas chromatography / mass spectroscopy tuberculostearic acid was identified in a single patient with TBM (MARDH, 1983).

Chromatographic techniques based on making cell wall lipid profiles unique to mycobacterial cell wall/membranes are newer developments. Mycolic acid analysis by high performance liquid chromatography (HPLC) is also available. This method has been proved to be reliable, rapid easy to perform and less costly than other identification methods. (THIBERT, 1993).

Diagnosis by Polymerase Chain Reaction (PCR):

PCR, a comparatively simple technique, invented by KARY MULLER comprises replication of specific target DNA (e.g. that extracted from CSF) until there is sufficient to detect by
standard hybridisation techniques. This replication is achieved by using synthetic oligonucleotide primers which are complementary to each strand of the target double stranded sequence. PCR is highly specific and can detect as low as two bacilli in the sample. The most commonly used DNA target for amplification is a 36 base-pair repetitive sequence from *M. tuberculosis* genome.

**Nucleic Acid Probes:**

Several investigators have developed DNA probes complementary to specific ribosomal RNA sequences of either mycobacterial genus or particular mycobacterial species such as *M. tuberculosis*, *M. avium*, *M. intracellulare* (ROBERTS, 1987). Though theoretically possible, attempts to use nucleic acid probes directly on clinical specimens have not been uniformly successful. At present all probes require the use of radioisotopes and this fact makes them technologically beyond the reach of developing countries.

Bromide partition tests, measurements of adenosine deaminase, tuberculostearic acid offer promise but they may not have very high specificity that is necessary. Initial studies have shown that PCR is at least as sensitive as culture in diagnosing tuberculosis but even the smallest amount of contaminating DNA can be amplified, with misleading results.
Despite the efforts by a number of investigators to develop new, rapid methods for diagnosing TBM, smear examination and culture remains the principal diagnostic method even today.

Detection of specific antigens seem most likely to yield rapid and simple diagnostic tests of very high specificity. The need for research in this direction is great.

RATIONALE BEHIND THE USE OF IMMUNOLOGICAL METHODS FOR DIAGNOSIS OF NEUROTUBERCULOSIS

Mycobacteria are known to be strong stimulators of T and B cell mediated immunity. They have been traditionally used as adjuvants to boost immune response to a variety of antigens in man and animals. The use of BCG for anti-tumor immunity and the use of Freund's adjuvant are prime examples of their potency.

\textit{M. tuberculosis} induces strong T-cell responses and antibody synthesis in man which are manifested by the tuberculin skin test reactivity and the presence of significant anti-mycobacterial antibodies in tuberculous serum. Since man is exposed to mycobacteria of the environment, in particular, antibodies to mycobacteria occur universally in human serum. Tuberculous sera thus contain antibodies specific to \textit{M. tuberculosis} as well as those which react with many antigens of mycobacteria shared by other bacteria.
Immunologically "privileged" sites like CNS compartment are however, free from such "natural exposure" antibodies so that an infection in such sites may perhaps be detected with much more ease.

There is antigen specific, localised immunologic response detectable in the cerebrospinal fluid in TBM, thus suggesting an immunologic compartmentalisation of the CNS. In other words, antibodies present in the CSF result from the synthesis by the activated lymphocytes, plasma cells present in the meningeal exudate and not from passive diffusion from serum. A greater purified protein derivative (PPD) induced proliferative response of cerebrospinal fluid lymphocytes than of peripheral blood lymphocytes was shown in patients with TBM (PLOUFF, 1979; KINMAN, 1981). Such a response was also greater when compared with CSF lymphocytes in aseptic meningitis (MALASHKHIA, 1976). Local synthesis of anti-PPD immunoglobulins, antibodies to 42 KDa protein have been convincingly demonstrated (KALISH, 1983; VAN VOOREN, 1989). Hence it follows as a corollary that BCG vaccination and tuberculous infection elsewhere in the body will not lead to the appearance of antibodies to M.tuberculosis in CSF.

**Mycobacterial Antigens:**

During an infection of CNS with M.tuberculosis many secretory and/or degradative products of the bacilli occur in CSF.
While the presence of antibodies generally indicates infection with *M. tuberculosis*, the presence of antigens of *M. tuberculosis* invariably indicates active infection.

Purification and isolation of species-specific antigens from *M. tuberculosis* to serve as reliable diagnostic agents has been the goal of many investigators. Polypeptides have been purified on combined selective physicochemical and immunologic procedures including precipitation, gel filtration, polyacrylamide gel electrophoresis and affinity chromatography. Isolation of specific small polypeptides has been reported by several authors (Table 2). Investigations on these were directed towards measuring specific delayed hypersensitivity rather than their diagnostic potential.

**TABLE 2**  
SMALL MYCOBACTERIAL PROTEINS AND PEPTIDES

<table>
<thead>
<tr>
<th>Reference</th>
<th>Species used in investigation</th>
<th>Source of antigen</th>
<th>Molecular wt of antigen(s) (KDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yamamura et al (1968)</td>
<td><em>M. tuberculosis</em></td>
<td>Cell wall</td>
<td>5-10</td>
</tr>
<tr>
<td>Stottmeier et al (1969)</td>
<td><em>M. tuberculosis</em></td>
<td>Press extract</td>
<td>4-10</td>
</tr>
<tr>
<td>Kuwabara (1975)</td>
<td><em>M. tuberculosis</em></td>
<td>Heated cells</td>
<td>9.7</td>
</tr>
<tr>
<td>Gupta and Lundi (1978)</td>
<td><em>M. tuberculosis</em></td>
<td>Hydrolysed PPD3,12</td>
<td></td>
</tr>
<tr>
<td>Harpur (1981)</td>
<td><em>M. bovis</em></td>
<td>Heat denatured 8,12</td>
<td></td>
</tr>
<tr>
<td>Ma and Daniel (1983)</td>
<td><em>M. tuberculosis</em></td>
<td>PPD</td>
<td>8</td>
</tr>
<tr>
<td>Kiyotani et al (1983)</td>
<td><em>M. tuberculosis</em></td>
<td>Culture filtrate 8,12</td>
<td></td>
</tr>
<tr>
<td>Krambovitis (1986)</td>
<td><em>M. tuberculosis</em></td>
<td>Plasma memb- rane 5,6,8,12</td>
<td></td>
</tr>
</tbody>
</table>
On review of mycobacterial antigens one is struck by lack of precise knowledge on the identity and biological significance of the antigens of tubercle bacillus. The major groups of antigens from \textit{M. tuberculosis} (Table 3) are (i) \textbf{EXCRETED PROTEINS} - produced in large quantities of \textit{M. tuberculosis} during the first few days of culture, (ii) \textbf{SECRETED PROTEINS} of the outer cell wall gradually released during growth of the bacillus. The concentration of these antigens steadily increase during culture period. (iii) \textbf{CYTOPLASMIC ANTIGENS} released from dead bacteria during late logarithmic phase of growth. The appearance of these proteins correlate well with the massive release of isocitrate dehydrogenase indicating that substantial autolysis has occurred.

\begin{table}[h]
\centering
\caption{\textbf{MAJOR GROUPS OF ANTIGENS FROM \textit{M. TUBERCULOSIS}}}
\begin{tabular}{lll}
\hline
Group & Release into filtrate & Molecular masses of reactive antigens (KDa) \\
\hline
1. & Early (Day 2-4) & 24 \\
& & 70-72 \\
2. & Gradual (Throughout) & 17-19 \\
& & 31-32 \\
& & 82,170 \\
3. & Late (Day 5 - Maximum Day 5) & 60-66 \\
& & 64-66 \\
\hline
\end{tabular}
\end{table}

\textit{ANDERSEN} (1991)
The actively secreted proteins of *M. tuberculosis* are of current interest and are intensely studied in several groups. NAGAI et al (1981) isolated two distinct groups of proteins being actively secreted from the mycobacterial cells or appearing later in culture fluids as a result of the release of soluble proteins from cytosol after lysis of bacteria. The quantity of antigen was affected by the time of harvest of culture and denaturation resulted in loss of some determinants. The stage of growth of the organism can affect the antigenic quality of the extract and non-replicating cells contained antigens not present in replicating cells.

Mycobacterial cell wall (perhaps not cytoplasmic) polysaccharides, proteins and peptides all have been shown to be antigenic in some circumstances. Arabinogalactan and Arabinomannan are excellent antigens. These components of tubercle bacillus may also suppress immune reactivity in the host. Lipoarabinomannan (LAM), a glycolipid peculiar to mycobacteria, has been shown to inhibit the processing of antigen for presentation and thereby activation of the relevant T-cells (MORENO, 1988). The polysaccharide side chains of LAM antigen are responsible for the surface antigen specificity and they are principally non-specific antigens with limited diagnostic significance. Antibodies evoked by peptido-glycolipids cross react strongly with mycosides from nonpathogenic mycobacteria.
Thus the essential question is to what extent the antigens identified are immunogenic and if immune responses to these proteins are of importance for development of clinical symptoms or complications during the disease. Immune responses to these components of bacilli and feasibility of detecting them for diagnostic purposes at an early phase of the disease need to be studied. However, it is not known whether \textit{M.tuberculosis} produce the same antigens in culture as in infection (let alone in CSF) and how to get the right antigens.

\textbf{Cross Reactivity with other Bacteria:}

There is sharing of some of the antigens in the rapidly growing mycobacterial species with nocardia, corynebacterium and \textit{Listeria} (STANFORD, 1974). This intergeneric sharing of antigenic components with each other and with a number of other taxonomically unrelated species is probably responsible for the lack of specificity of serological tests of tuberculosis. In frequent observations antibodies in sera from normal humans and animals have often been referred to as non-specific or natural antibodies. There is evidence of antigenic determinants shared between gram negative and positive organisms as well as mycobacteria. The binding of antigens from bacteria to normal sera was demonstrated to antigen binding portion of IgG indicating that the binding was a true immunological reaction. The antigenic determinant - a major arabinose
side chain is probably shared by all species of mycobacteria, nocardia and corynebacterium as well.

At a WHO workshop mycobacterial antigens were identified using monoclonal antibodies raised using the mouse hybridoma technique (IVANYI, 1988). 7 distinct antigens, 71 KDa, 65 KDa, 38 KDa, 23 KDa, 19 KDa, 14 KDa and 12 KDa were found of which only 38 KDa and 14 KDa carried epitopes unique to tubercle bacilli.

Genomic library has been created by inserting the genes of *M. tuberculosis* and BCG into the lambda phage of *E.coli* (YOUNG, RA, 1985). This approach provides a novel source of mycobacterial antigens. Genetic cloning has led to the identification of other proteins with epitopes restricted to the tubercle bacilli like 36 KDa, 30-32 KDa, 28 and 23 KDa proteins which are yet to be evaluated in detail.

**HEAT SHOCK PROTEINS AND M. TUBERCULOSIS ANTIGENS:**

Sequence analysis of several prominent antigens of *M. tuberculosis* has identified them as members of highly conserved Heat Shock Protein families related to the *E.coli* proteins Dnak, Groel, Groes (SHINNICK, 1989). These proteins did not co-migrate with known antigens during two dimensional gel electrophoresis (YOUNG, 1991).
Under mild heat shock conditions (37-42°C) strong induction of 70 and 65 KDa proteins was evident while under more severe temperature stress (37-48°C) the 65 KDa band was very much less prominent and additional protein bands were strongly induced (90, 20 and 15 KDa). It is possible that these variations reflect some heterogeneity in heat shock protein synthesis and temperature sensitivity of mycobacteria at different stages of their growth cycles. It was recognised at an early date that the antigenic composition of filtrate of cultures grown under differing conditions differed significantly, with the differences being chiefly quantitative than qualitative. CASTELLNUOVO and coworkers (1964) felt that the antigenic composition of culture filtrates was determined principally by the age of culture at the time of filtration.

Proteins which play a key role in host-parasite interactions are coregulated at the transcriptional level in response to environmental signals encountered during an infection. The mechanism by which the change in temperature is sensed by the organism and the pathway involved in alteration of level of particular transcription factors remain to be clearly defined.

Verbon characterised 16 KDa protein (often referred to as 14 K protein) of _M. tuberculosis_. This has homology with various antigens belonging to the alpha-crystalline family of low molecular weight heat shock proteins. The 16 K protein has been used for
the identification of mycobacteria from early cultures. Promising results have been produced in an ELISA test in which 16 K protein was used as an antigen and in a competition assay using Mo Ab TB$_{68}$ against the 16 K protein. These proteins are believed to play a part in antigen processing and presentation. They function in normal cells by binding to unfolded proteins and facilitating their transport across lipid layers (VERBON, 1992). 12 KD protein with sequence similarity to heat shock proteins has been reported (NAVALKAR, 1993).

Virtually all of the proteins isolated from _M._tuberculosis appear to possess cross-reactive components limiting their potential as diagnostic agents. As protein molecules had several antigenic determinants, there was search for a single species specific antigen of _M._tuberculosis. Individual antigenic determinants or epitopes unique to _M._tuberculosis have been identified by monoclonal antibodies (ENGENS, 1986).

The levels of antibodies to such epitopes differ according to the nature of tuberculous infection and the immune response to them varies from person to person (BOTHAMLEY, 1989). T-cells recognise _M._tuberculosis antigens when they are presented in association with the major histocompatibility antigens. Class I molecules (HLA- A, B and C) present antigens to CD8 + T-cells and Class II molecules (HLA - DR, DP, DQ) present antigens to
CD4 + T-cells. There is growing evidence that Class II molecules may determine the tuberculin reactivity and susceptibility to tuberculosis (VAN EDEN, 1983). The different antibody responses might be attributed to multifunctional cells at different stages of maturation (BOTHAMLEY, 1989).

The genetic difference in the immune response, the repertoire of recognisable mycobacterial antigens, sharing of antigenic determinants between mycobacteria and other organisms and polyclonal stimulation of B cells by infections unrelated to mycobacteria make the development and interpretation of immunodiagnostic tests difficult.

DETECTION OF MYCOBACTERIAL ANTIGEN:

As the quantity of antigen in CSF sample to be tested is minute, sensitive procedures like Radio-Immuno-Assay (RIA) or ELISA have to be used. RIA was reported to be able to detect $1 \times 10^3$ tubercle bacilli or the equivalent of 1 ng of sonicated antigen. ELISA system was shown to be more sensitive in detecting mycobacterial antigen in CSF than RIA.

Many Monoclonal Antibodies (Mo Ab) to M. tuberculosis have been produced and genes for many of the antigens have been cloned (IVANYI, 1988; KADIVAL and CHAPRAS, 1987). However, most
epitopes and antigenic determinants on _M. tuberculosis_ are shared with other mycobacteria, so that only a small number of monoclonal antibodies are _M. tuberculosis_ specific (TB 23, 68, 71, 78).

**STANDARDISATION OF **_**M. TUBERCULOSIS **ANTIGENS:**

As protein molecules had several antigenic determinants, there was search for a single species specific antigen of _M. tuberculosis_. Advanced fractionation methods to separate antigens by ion-exchange chromatography did not yield pure antigens. Acrylamide gel electrophoresis was found valuable to isolate small amounts of antigen specific for _M. tuberculosis_. Isoelectric focussing and affinity chromatography have also been used for isolation and standardisation of _M. tuberculosis_ antigens.

**ANTIGEN 5:**

This is a purified antigen obtained from cell free culture filtrate of _H_37 _RV_ strain by immunosorbent affinity chromatography (DANIEL, 1978). That it is a cytoplasmic (soluble) antigen is borne out of the fact that it is composed of aminoacids rich in aspartic acid and not containing muramic acid or diaminopimelic acid of the mycobacterial cell wall. By SDS-PAGE, it has been identified as a 38 KDa protein. A double band may be observed near 38-39 KDa in SDS-PAGE. The high molecular variant corresponded to the unprocessed protein or to a protein with a conjugated lipid tail.
Physicochemically purified antigen contained 25% carbohydrate. The role of carbohydrate residues associated with 38 KDa protein in antigenicity remains to be elucidated.

This antigen is actively secreted with low efficiency and with a major part of the protein associated with the bacterial surface. *M. tuberculosis* antigen 5 was present in the culture filtrate of 6 strains of *M. tuberculosis* and 4 strains of *M. bovis* and not in other mycobacterial strains. (DANIEL, 1979).

This protein corresponded to the 38 KDa protein, antigen 78 in CIE reference system and antigen 5 in the U.S.-Japan reference system and was defined by the monoclonal antibodies TB<sub>71</sub> and TB<sub>72</sub>. Studies on purified antigen 5 indicate that insufficient sensitivity represents an important problem. 20-30% of tuberculous patients were negative for antibodies to antigen 78 and markedly positive responses were found in 4 of 23 healthy PPD-positive controls.

19 KDa Protein:

Major membrane protein (MMP) of 19 KDa was isolated and characterised by LEE (1992). The sequence of this protein differed from another 19 KDa protein, a lipoprotein, described by YOUNG et al (1985). However, some similarities with low molecular weight heat shock proteins were observed.
14 KDa Protein:

PARRA CA (1991) cloned the 14 KDa protein from *M. tuberculosis* and called it MTP 40. B and T cell epitopes within this protein were identified. The whole antigen or some of the peptides derived from it are candidates for diagnostic studies.

Thus in literature one comes across only a few antigens of *M. tuberculosis* which have been purified to homogeneity like Antigen 5, monoclonal antibody based affinity purified antigens like 38 KDa Ag, 19 KDa, 14 KDa. These antigens have been tested in a limited number of experiments to explore their diagnostic potential. The merits and demerits of these in various diagnostic assays have not been exhaustively worked out. So far there is no single antigen which has been extensively tested and universally recommended for use in tuberculosis especially in childhood neurotuberculosis.

IMMUNODIAGNOSTIC TESTS:

Immunodiagnostic tests for tuberculosis use three basic approaches - (a) Measurement of the ability of specific *M. tuberculosis* antibodies to detect antigen present in the sputum, blood, CSF or urine, (b) Measurement of the ability of antibodies of *M. tuberculosis* in the serum of patients to compete with a labelled monoclonal antibody in binding to *M. tuberculosis* antigens.
and (c) Measurement of binding of patients' immunoglobulin antibodies to *M. tuberculosis* specific purified or recombinant antigens and epitopes using ELISA or comparable assays.

The specificity of the immunodiagnostic tests is affected by the type of antigens used in the various studies - (i) crude bacillary antigens like ultrasonicates of *H₃₇RV* strain, *M. bovis* BCG, mycobacterial saline extract, (ii) Purified protein derivative, (iii) Purified and semi-purified mycobacterial antigens like soluble proteins of *M. tuberculosis* carrying specific epitopes of 14 KDa, 19 KDa, 38 KDa.

**ANTIBODY ASSAYS OF CSF:**

Table 4 summarises the studies in CSF where *M. tuberculosis* reactive antibodies were assayed to diagnose TBM. Sensitivity in various studies was reported ranging 53-100% while specificity ranged 70-100%.

KALISH et al (1983) analysed the CSF of 3 patients with culture proven TBM and CSF of 33 control patients for IgG antibody to Purified Protein Derivative (PPD) by an ELISA technique. All the control CSF were negative and all 3 patients had positive results. Results of serial evaluation of anti-PPD IgG immunoglobulins performance in a single patient was in agreement with the immunological compartmentalisation of CNS in TBM.
<table>
<thead>
<tr>
<th>RESEARCHER</th>
<th>YEAR</th>
<th>METHOD</th>
<th>ANTIBODY AGAINST</th>
<th>SENSITIVITY %</th>
<th>SPECIFICITY %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kalish et al</td>
<td>1983</td>
<td>ELISA</td>
<td>IgG Anti PPD</td>
<td>100 (n=33)</td>
<td>100 (n=33)</td>
</tr>
<tr>
<td>Hernandez et al</td>
<td>1984</td>
<td>ELISA</td>
<td>IgG, IgM to BCG</td>
<td>100 (n=20)</td>
<td>100 (n=80)</td>
</tr>
<tr>
<td>Chandramuki et al</td>
<td>1985</td>
<td>RIA</td>
<td>M. tb Sol. Extract</td>
<td>68 (n=89)</td>
<td>90 (n=127)</td>
</tr>
<tr>
<td>Coovadia et al Durban, S.Africa</td>
<td>1986</td>
<td>ELISA</td>
<td>Antigen 5</td>
<td>53 (1:20 d11) 90 (1:20)</td>
<td>40 (1:40 d11) 94 (1:40)</td>
</tr>
<tr>
<td>Watt et al</td>
<td>1988</td>
<td>ELISA</td>
<td>Sonicate Antigen</td>
<td>52 (n=29)</td>
<td>96 (n=98)</td>
</tr>
<tr>
<td>Dole, Maniar et al Bombay, India</td>
<td>1989</td>
<td>ELISA</td>
<td>MSE Antigen</td>
<td>86 (n=50)</td>
<td>95 (n=98)</td>
</tr>
<tr>
<td>Chandramuki et al Bangalore, India</td>
<td>1989</td>
<td>ELISA</td>
<td>Sol. Extracts, LAM, 14, 19, 38 KDa antigen</td>
<td>61 (n=26)</td>
<td>100 (n=98)</td>
</tr>
<tr>
<td>Pounyvarin et al Bangkok, Thailand</td>
<td>1990</td>
<td>ELISA</td>
<td>IgG Anti PPD</td>
<td>82 (n=26)</td>
<td>70 (n=30)</td>
</tr>
<tr>
<td>Mathai et al</td>
<td>1990</td>
<td>ELISA</td>
<td>Antigen 5</td>
<td>84 (1:40 d11) 92 (1:40 d11)</td>
<td>75 (1:80 d11) 100 (1:80 d11) (n=40) 90 (n=42)</td>
</tr>
<tr>
<td>Mathai et al</td>
<td>1990</td>
<td>ELISA</td>
<td>IgG Anti PPD</td>
<td>82 (n=17)</td>
<td>70 (n=55)</td>
</tr>
<tr>
<td>Maniar &amp; Joshi Bombay, India</td>
<td>1990</td>
<td>ELISA</td>
<td>MSE Antigen</td>
<td>97 (n=44)</td>
<td>95 (n=72)</td>
</tr>
<tr>
<td>Radhakrishnan et al Trivandrum, India</td>
<td>1990</td>
<td>ELISA</td>
<td>Antigen 5</td>
<td>60 (n=70)</td>
<td>100 (n=70)</td>
</tr>
<tr>
<td>Radhakrishnan et al Trivandrum, India</td>
<td>1991</td>
<td>Dot-Iba</td>
<td>Antigen 5</td>
<td>77 (n=70)</td>
<td>100 (n=100)</td>
</tr>
<tr>
<td>Mathai et al</td>
<td>1991</td>
<td>Dot-Iba</td>
<td>Antigen 5</td>
<td>70 (n=40)</td>
<td>100 (n=100)</td>
</tr>
<tr>
<td>Sarala &amp; Raja Madras, India</td>
<td>1991</td>
<td>ELISA</td>
<td>Antigen 5</td>
<td>70-80 (n=28) 90-95 (n=12)</td>
<td></td>
</tr>
<tr>
<td>Park, Lee et al Seoul, Korea</td>
<td>1993</td>
<td>ELISA</td>
<td>PPD, LAM</td>
<td>59 (n=27)</td>
<td>93 (n=78)</td>
</tr>
<tr>
<td>Srivastava et al New Delhi, India</td>
<td>1994</td>
<td>ELISA</td>
<td>Sonicate Antigen</td>
<td>90 (n=60)</td>
<td>100 (n=58)</td>
</tr>
</tbody>
</table>
The study by HERNANDEZ et al (1984) from Mexico is significant in that all TBM patients were below 16 years of age. They analysed the CSF for anti-BCG antibodies using an ELISA system to detect both IgG as well as IgM antibodies. All the 20 samples with bacteriological diagnosis of TBM were positive when tested during the second or third week of illness while all the 80 control samples (n=31 pyogenic meningitis, n=20 viral meningitis, n=29 normal controls) tested were negative.

PARK (1993) in a prospective study from Korea on 27 patients with TBM and 78 controls used PPD and LAM antigens. The assay of IgG reactivity to LAM antigen was found useful for the early diagnosis of TBM and superior to PDD (Sensitivity 85% for LAM antigen Vs 59% for PPD).

Antibody responses to BCG, PPD and \( H_{37} \) RV culture filtrate were neither specific nor sensitive for the diagnosis of TBM as noted by SARALA et al (1991). Though the sample size analysed by them was small, they concluded that in endemic areas only antigen detection assays using monoclonal antibody probes may be useful.

**ANTIGEN ASSAYS OF CSF:**

In Table 5, studies undertaken to detect *M. tuberculosis* derived antigens in CSF have been summarised. It is obvious that
<table>
<thead>
<tr>
<th>Sl.</th>
<th>RESEARCHER</th>
<th>YEAR</th>
<th>METHOD</th>
<th>ANTIBODY DETECTED</th>
<th>SENSITIVITY %</th>
<th>SPECIFICITY %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sada et al, Mexico</td>
<td>1983</td>
<td>ELISA</td>
<td>BCG Antibody</td>
<td>81 (n=16)</td>
<td>95 (n=22)</td>
</tr>
<tr>
<td>2</td>
<td>Bal et al, Bombay, India</td>
<td>1983</td>
<td>ELISA</td>
<td>Sonicate Antigen of <em>M. tuberculosis</em></td>
<td>100 (n=8)</td>
<td>97 (n=33)</td>
</tr>
<tr>
<td>3</td>
<td>Krambovitis et al, United Kingdom</td>
<td>1985</td>
<td>Latex</td>
<td>Plasma membrane Antigen of <em>M. tuberculosis</em></td>
<td>94 (n=18)</td>
<td>99 (n=134)</td>
</tr>
<tr>
<td>4</td>
<td>Chandramuki et al, Bangalore, India</td>
<td>1985</td>
<td>RPHA</td>
<td>Cell wall antigen of <em>M. tuberculosis</em> MY-4</td>
<td>77 (n=89)</td>
<td>95 (n=127)</td>
</tr>
<tr>
<td>5</td>
<td>Kadival et al, Bombay, India</td>
<td>1986</td>
<td>ELISA</td>
<td>Sonicate antigen of <em>M. tuberculosis</em></td>
<td>75 (untreated)100 (n=52) TBM n=24</td>
<td>35.7 (treated TBM n=14)</td>
</tr>
<tr>
<td>6</td>
<td>Kadival et al, Bombay, India</td>
<td>1987</td>
<td>RIA</td>
<td>Sonicate antigen of <em>M. tuberculosis</em></td>
<td>79 (untreated 100 (n=56) TBM n=19)</td>
<td>10 (treated TBM n=17)</td>
</tr>
<tr>
<td>7</td>
<td>Wu et al, Taiwan, China</td>
<td>1989</td>
<td>ELISA</td>
<td>Antibodies against <em>M. bovis</em></td>
<td>100 (n=5)</td>
<td>100 (n=140)</td>
</tr>
<tr>
<td>8</td>
<td>Vaidya &amp; Wagle, Bombay, India</td>
<td>1990</td>
<td>ELISA</td>
<td>38 KDa Antigen</td>
<td>82 (n=300)</td>
<td>100 (n=150)</td>
</tr>
<tr>
<td>9</td>
<td>Radhakrishnan &amp; Mathai, Trivandrum, India</td>
<td>1990</td>
<td>ELISA</td>
<td>Antigen 5</td>
<td>67.8 (n=56)</td>
<td>100 (n=70)</td>
</tr>
<tr>
<td>10</td>
<td>Radhakrishnan &amp; Mathai, Trivandrum, India</td>
<td>1990</td>
<td>ELISA</td>
<td>Antigen 5</td>
<td>65 (n=40)</td>
<td>100 (n=42)</td>
</tr>
<tr>
<td>11</td>
<td>Radhakrishnan &amp; Mathai, Trivandrum, India</td>
<td>1991</td>
<td>ELISA</td>
<td>Antigen 5</td>
<td>77.5 (n=40)</td>
<td>100 (n=40)</td>
</tr>
<tr>
<td>12</td>
<td>Radhakrishnan &amp; Mathai, Trivandrum, India</td>
<td>1991</td>
<td>Dot-Iba</td>
<td>Antigen 5</td>
<td>49 (n=70)</td>
<td>100 (n=100)</td>
</tr>
<tr>
<td>13</td>
<td>Desai T &amp; Gogte A, Bombay, India</td>
<td>1993</td>
<td>ELISA</td>
<td>Sonicate Antigen of <em>M. tuberculosis</em></td>
<td>80 (n=148)</td>
<td>100 (n=278)</td>
</tr>
</tbody>
</table>
even in antigen detection methods, necessary levels of sensitivity and specificity have not yet been attained.

SADA et al (1983) were the first research workers to use ELISA to detect mycobacterial antigens in CSF. They used antibody against BCG to coat the microtiter wells as it had been shown that \textit{M. tuberculosis} and BCG have the same surface antigens. This assay was positive in 4 patients with confirmed TBM and 9 of 12 with probable TBM. One patient with cryptococcal meningitis gave a false positive result but the assay was negative in the remaining 9 meningitis cases other than TBM and in 11 control subjects.

BAL et al (1983) by an ELISA technique estimated the sonicate antigen of \textit{M. tuberculosis} in the CSF samples of 9 patients with clinical TBM and 32 non-TBM cases. The reported specificity of 97\% and sensitivity of 100\% in their study is in contrast to other studies with sensitivity ranging from 48\%-95\% (Table 5). However, the specificity range in other studies was comparable 95-100\%. False positive result to \textit{M. tuberculosis} plasma membrane antigen in CSF was seen in a patient with Hemophilus influenzae meningitis (KRAMBOVITIS, 1984). Persistence of mycobacterial antigen was noted in majority of cases even after 4 weeks of treatment (GOGATE, 1993).

\textbf{End of Literature Review.....}
The foregoing literature survey indicate that substantial progress has been made in the development and application of immunodiagnostic methods for the early detection of TBM. In order to apply these strategies in practicality, the INTERNATIONAL ATOMIC ENERGY AGENCY (IAEA), VIENNA, AUSTRIA sponsored a multi-centred double blind trial between 1989-1991. This double blind trial involved the detection of mycobacterial antigen as well as antibody in CSF from TBM patients and controls (Next page - Table 6) by methods established by six laboratories around the world. The investigators who participated in this study have adequately published their methods as cited in literature section. The following table illustrates that despite a wide variety of methods obviously used by these investigators, a concurrent sensitivity and specificity levels of more than 90% was not obtained in any laboratory, either in the antigen assay or antibody assay.

The reasons for failure of these methods in double blind trials could be many, but it appears that two major inherent problems are the need of specific antigen for detecting antibodies and a specific antibody probe for detecting antigen. Indeed in many of the studies cited in Tables 4 and 5, crude bacillary antigens were used.

Some studies have used Antigen 5, 38 KDa (these are reputed to be the same antigen) or 14 KDa which have been found to be
<table>
<thead>
<tr>
<th>ASSAY</th>
<th>LABORATORY</th>
<th>SENSITIVITY</th>
<th>SPECIFICITY</th>
<th>PREDICTIVE VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANTIGEN</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>75</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>27</td>
<td>92</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>28</td>
<td>88</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>69</td>
<td>68</td>
<td>68</td>
</tr>
<tr>
<td>ANTIBODY</td>
<td>1</td>
<td>80</td>
<td>60</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>71</td>
<td>80</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>33</td>
<td>95</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>39</td>
<td>93</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = Not done

NOTE: 50-60 CSF samples from TBM, pyogenic meningitis and other disorders were sent out to each of the six laboratories and coded independently. They were assayed by methods standardised in each of these laboratories. Results were sent to International Atomic Energy Agency (IAEA) where they were decoded and analysed as above.

It is not clear however if any lab used an antigen and antibody assay concurrently on any sample of CSF.
restricted largely to *M. tuberculosis* strains. However, specific antibody reactivity to these proteins were also not sensitive enough in their assays to yield a practical method. Thus Chandramukhi et al showed 100% specificity but sensitivity of 61% with 14 KDa antigen. On the other hand VAIDYA and WAGLE (1990) used a polyclonal specific antibody to detect the 38 KDa antigen in CSF and found a specificity of 100% and a sensitivity of 82%. The later study would have been very valuable had it not been that Antigen 5, has not shown similar levels of sensitivity and specificity in ELISA based on antibody or antigen assay of CSF as in many other studies (Refer ELISA literature Tables 4 and 5).

The purpose of illustrating the IAEA data (see note of communication from Bhaba Atomic Research Centre, BARC, Bombay, Appendix-I) is to emphasise that despite claims of high levels of sensitivity and specificity by different investigators in literature, their published data has not been vindicated.

This observation forms the basis of one part of the present investigation where it is attempted to answer the apparent inadequacy of the current day immunodiagnostic methods to achieve satisfactory levels of sensitivity and specificity, even when apparently specific antigens and/or antibodies were used.

In the present study a new 17 KDa protein antigen was purified from *M. tuberculosis* (H<sub>37</sub> RV) whole cell sonicates and was
identified as being specific for \textit{M. tuberculosis}. Using this immunodominant protein antigen, assays were performed in CSF of children diagnosed as TBM for antibodies and antigens using standard ELISA. This extent to which this approach could reinforce the diagnosis of TBM in children was evaluated.
OBJECTIVES

1. To study on a prospective basis the usefulness of conventional clinical and laboratory parameters in the diagnosis of Tuberculous meningitis (TBM) among children aged 6 months - 12 years.

2. To evaluate the diagnostic efficiency of simultaneous detection of

a) Antibody in cerebrospinal fluid (CSF) reactive to 17-kDa specific antigen of \textit{M. tuberculosis}.

b) 17-kDa antigen in CSF.

SCOPE OF THE STUDY

The scope is limited to certain aspects of immunodiagnosis in the CSF of children with TBM.

I. Sensitivity and specificity levels of immunoassays optimal for detection of TBM in early stages can probably be derived by

a) Identification of \textit{M. tuberculosis} antigen(s) which react specifically with antibodies present in all TBM cases.

b) Identification of \textit{M. tuberculosis} specific monoclonal antibody probe(s) which reacts with \textit{M. tuberculosis} derived antigen in all TBM samples.
These concepts assume that *M. tuberculosis* specific antibodies and antigens occur among all cases of TBM, although the pathogenesis of TB in man may not result in such events facilitating early diagnosis. Nevertheless use of *M. tuberculosis* specific antigens and antibody probes in antibody and antigen detection methods may enhance the diagnostic efficiency for early stages of TBM. The present study was therefore undertaken in this direction.

II. The International Atomic Energy Agency (IAEA) sponsored a multicentric double blind study on Immunodiagnostic methods in TBM between the years 1989-1991. Even when apparently specific antigen or antibodies were used in the study, the antigen and antibody titrations did not live up to the expectations as claimed by the investigators earlier in the literature. This observation forms the basis of another aspect of our investigation to detect any possible factor influencing such assays.

Confining the scope of the study to the above aspects, the steps envisaged are:-

A. Identification and use of a specific antigen for ELISA:

Initial phase of the study focussed on identifying *M. tuberculosis* specific proteins. An immunodominant 17-kDa *M. tuberculosis* specific antigen was purified from H₃⁷ RV whole cell sonicates. This protein antigen detected antibody from among the
specimens of pulmonary and extrapulmonary tuberculosis with high levels of sensitivity and specificity. The 17-kDa antigen and a polyclonal antibody reactive with 17-kDa antigen developed and standardized, was used in the study.

B. Study of children with TB meningitis and controls:

No attempt has been made by earlier investigators to assay an antigen and its corresponding antibody concurrently on any sample of CSF in childhood TBM. Such an attempt would be made in this study and its implications for diagnosis of TBM in the early stages analysed.

An estimated number of 90 children aged 6 months -12 years would be recruited into the study as TBM based on conventional clinical and laboratory parameters. The parameters include (a) clinical features, (b) tuberculin testing, (c) supportive radiologic changes, (d) CSF cellular response and (e) CSF protein and sugar levels at admission.

* The CSF smear for AFB and culture for *M. tuberculosis* would be done to confirm the presence or absence of organism in the CSF.

* Standardisation of ELISA technique for detecting 17-kDa antigen and antibody in the CSF would be done. CSF samples would be coded till assay of antigen and antibody completed.
Specificity evaluation of 17-kDa antigen-antibody would be done on CONTROLS which include (i) children with CSF-culture proven bacterial meningitis and (ii) children with normal CSF admitted for seizure disorders (Total estimated at 90). The coded CSF samples would be assayed for antigen and antibody.

Comparison of conventional criteria with antigen antibody assay results:

The children would be grouped as proven or suspect cases of TBM based on positive or negative CSF culture for *M. tuberculosis* after decoding. The sensitivity, specificity, positive predictive value and diagnostic efficiency of conventional parameters would be compared against those of antigen and antibody in the proven group. The extent to which the immunoassay of CSF at admission would be useful in establishing the diagnosis of TBM in the suspect group would be studied in relation to the conventional parameters.

Clinical stage of TBM and assay results:

The CSF antigen and antibody results in the two groups (proven and suspect) would be studied in relation to the clinical staging of the children at admission. The usefulness of the assay procedure for diagnosis of TBM in early stage (Stage I) and any factor influencing the sensitivity of the assay procedure would be looked into.