Chapter-5

Discussion
DISCUSSION

Tuberculosis (TB) remains a major global health problem. It causes ill-health among millions of people each year and ranks as the second leading cause of death from an infectious disease worldwide. India is the highest TB burden country, and it is estimated that about 40% of the Indian population is infected with TB bacteria, the vast majority of whom have latent rather than active TB (WHO, 2013). Situation has further worsened due to increasing prevalence of HIV infection and emergence of MDR-TB/ XDRTB all over the world (Shah et al., 2007). TB manifests clinically as pulmonary (PTB) or extra-pulmonary tuberculosis (EPTB), with the former being more common. EPTB is a significant health dilemma in both developed and developing countries (Cailhol et al., 2005). In India, 10 to 15% of TB cases are estimated to be cases of EPTB (which affects mainly the lymph nodes, meninges, kidney, spine, and growing ends of the bones), with a 25 to 50% case mortality rate within months. The major challenge in the diagnosis of EPTB is the frequently atypical clinical presentation stimulating other inflammatory and neoplastic conditions, which frequently results in a delay or deprivation of treatment. Therefore, a high index of suspicion is necessary to make an early diagnosis, and quite often, more than one procedure is necessary for the confirmation of the diagnosis. In lower-income countries, the lack of diagnostic infrastructure substantially aggravates the problem (Agrawal et al., 2005). Conventional methods have a very low sensitivity in the diagnosis of extra-pulmonary clinical specimens. The diagnosis of smear-positive PTB has been considerably established, but the diagnosis of smear negative PTB, TB–HIV co-infection and EPTB poses serious challenges (Golden & Vikram, 2005; Chang, 2007).
For the smear to be AFB positive, the sample should contain at least 10000 bacilli/ml. LJ culture is still considered to be the gold standard, but 10-100 viable bacilli are mandatory for culture positivity. Moreover, long period of time (of about 6-8 wk) is required for positive reports, hence most clinical and therapeutic decisions cannot be made (Prasad et al., 2001).

Automated liquid culture systems such as BacT/ ALERT MP (bioMerieux Inc, Durham, NC, USA) and BD BACTEC MGIT (Becton Dickinson, Sparks, MD, USA) are currently considered the gold-standard approach for isolating mycobacteria. Meta-analyses have shown that liquid systems are more sensitive for detection of mycobacteria and may increase the case yield by 10% compared with solid media (Cruciani et al., 2004; Dinnes et al., 2007). They also reduce the delays in obtaining results to days rather than weeks. However, liquid systems are prone to contamination and require stringent quality assurance systems and training standards. In addition, they are more expensive and require equipment investments (Ngamlert et al., 2009).

NAA tests have appeared with the goal of enabling clinicians to make a rapid and accurate diagnosis. PCR is the most excellent known NAA test. It amplifies target nucleic acid regions that uniquely identify the *M. tuberculosis* complex. Several *M. tuberculosis* specific target DNA sequences have been tried so far for the diagnosis of pulmonary and extra-pulmonary tuberculosis by PCR and various other genotypic methods (Tiwari et al., 2003). These include gene coding for the *IS6110* insertion sequences, 65 kDa heat shock protein (HSP), gene coding for 38 kDa, 85B antigen and 16S rRNA. The only repetitive target useful for a NAA in tuberculosis,
which is so far available, is an insertion sequence designated IS6110 (Theirry et al., 1990). IS6110 specific for the *M. tuberculosis* complex (*M. tuberculosis*, *M. africanum*, *M. bovis*, *M. microti* and *M. canetti*) generally occurs in 1-20 copies per cell, making it an ideal target for amplification (Negi et al., 2007).

In the present study, we investigated on improving the diagnostics of TB in paucibacillary diseases. We compared the conventional diagnostic modalities: smear microscopy, culture on solid and liquid media with PCR targeting the IS6110 elements, for diagnosis of MTB infection. Another objective was to assess which sample, blood or specific site sample of TB patient had better diagnostic value. The study was set in the SMS & Allied Hospital, Jaipur on suspected cases of EPTB (n=655) whose specimen were received for routine diagnosis. Majority of these suspected cases [433(66.10%)] were in the 21-40 years age group. Several studies have reported that young adults are at a high risk for development of TB infection; both PTB & EPTB (Shukla et al., 2004; WHO 2013; RNTCP 2013).

**Comparison of the positivity obtained by the methods**

In our study, overall positivity by AFB smear microscopy after concentration by modified Petroff’s method was 3.96% (26/655). This is in agreement with the studies reporting smear positivity in extra-pulmonary samples after processing with modified Petroff’s method; 3.85% (Munir et al., 2008), 3.9% (Chakravorty et al., 2005), 3.97% (Munir et al., 2010) but lower than some studies like, 5 % by Ukwaja et al., (2011), 8.3% by Bungar et al., (2013), 11% by Hillemann et al., (2006), 15.9% by Deribew et al., (2011), 19% by Kumar et al., (2014), 20.6% Huang byet al., (2001), 28% by Yassin et al., (2006) & 28.3% by Tessema et al., (2009).
In the present study, the positivity by **LJ solid culture** and **Middle Brook 7H9 culture** were 6.26% (41/655) and 10.22% (67/655) respectively. Recent Indian studies have reported the positivity by LJ culture as 1.9% (Rijal et al., 2004), 3.37% (Makeshkumar et al., 2014), 6.06% (Bunger et al., 2013), 13% (Rodrigues et al., 2002; Siddiqui et al., 2013), 21.18% (Munir et al., 2008; Iqbal et al., 2011), 24.5% (Salian et al., 1998), 34.2% (Shukla et al., 2011), 45% (Maurya et al., 2011) in extra-pulmonary specimen.

The use of liquid culture significantly increased (P<0.0001; CI 95%) the positivity for *Mycobacterium sps* in these extra-pulmonary specimens. The liquid culture has been found to be more useful for detection of mycobacteria in paucibacillary specimens where LJ media gives very scanty or no growth (Hilleman et al., 2006). In a study by Palacios et al., (1996), 77.7% of the samples were positive for culture in liquid media. The positivity by culture on LJ medium was 27.7% in the same samples. Liquid culture positivity for EPTB samples with a positivity of 59.7% to 87.2% on LJ medium has been reported to be very high ranging from 80 to 100% (Hanna et al., 1999; Alcaide et al., 2000; Lu D et al., 2002).

12 isolates grown on LJ & MB media were identified as NTM on further biochemical analysis. Out of these 12 NTM, 7/12 (58.33%) were isolated from genital samples (EB and MB), 2/12 (16.66%) from peritoneal fluid, 1/12 (8.33%) was from gastric aspirates, 1/12 (8.33%) was from CSF, 1/12 (8.33%) were from pleural fluid.

The overall positivity of **PCR** was higher than other conventional methods (28.54%). Several studies have reported increased positivity by PCR targeting
IS6110 in specimens of EPTB (Lahiri *et al.*, 2001; Sekar *et al.*, 2008). Various authors have reported PCR positivity as 26.96% (Makshkumar *et al.*, 2012), 62% (Tiwari *et al.*, 2003), 63% (Sekar *et al.*, 2008), 65% (Kumar *et al.*, 2013), 73% (Negi *et al.*, 2005), 74.2% (Shukla *et al.*, 2011), 92.1% (Ogusku *et al.*, 2004).

**Comparison of sensitivity and specificity of various methods against LJ culture**

The sensitivity and specificity of AFB smear microscopy was low when compared to culture based methods. AFB smear microscopy has shown 10.34% sensitivity (with 95% confidential interval (CI) 24.1; 94) and 96.33% specificity (with 95% CI, 67.1; 80.6). Overall positive and negative predictive value of ZN staining was observed as 11.54% (with 95% CI, 2.7; 20.8) and 95.87% (with 95% CI, 93.9; 99.7). The sensitivity of microscopy depends on the clinical presentation and more than 10,000 bacilli per milliliter are necessary for secure microscopic positivity (Levy *et al.*, 1989). Munir *et al.*, 2008 have reported sensitivity of AFB smear microscopy as 20.84% and specificity 100%, PPV of 100%, NPV of 84%. Recently, Maurya *et al.*, 2012 have reported 13.7% sensitivity; 100% specificity for AFB smear microscopy. There are various reports regarding the sensitivity of ZN smear for extra-pulmonary specimen ranging from as low as 0% to as high as 75% (Kamboj *et al.*, 1994). In some studies it has been shown that this technique has a low sensitivity, 22-43% for a single smear (Toman *et al.*, 2005) and up to 60% under optimal conditions (Siddiqi *et al.*, 2003; Apers *et al.*, 2003). This limitation has been reported to be due to inadequacy and paucibacillary nature of specimen (Tiwari *et al.*, 2003; Yam *et al.*, 2006). Sensitivity is even more reduced if samples are of poor quality, which is often the case in children and HIV co-infected patients (Corbett *et al.*, 2003; Getahun *et al.*, 2007). In this study, 18/26 (69.23%) specimens found to be
positive by concentrated smear microscopy were negative on LJ. Similarly, 9/26 (34.61%) samples that were AFB smear positive were negative on MB liquid culture. Overall 7/26 (26.92%) AFB smear positive samples were negative by both culture methods. This could be due to the presence of non-viable mycobacteria in the samples as 4 subjects had a recent history of anti-tubercular treatment.

In our observation, sensitivity of MB7H9 liquid culture was 44.83% (CI; 95%) and specificity was 91.37% (CI; 95%) with a PPV= 19.40 % (CI 95%), NPV = 97.28 % (CI 95%). Bhargava et al., (2000) reported 62.9% sensitivity of MB7H9 broth culture when compared with LJ medium.

An in vitro amplification using IS6110 as a target sequence for detection of mycobacterial DNA from clinical samples has previously shown wide variation in specificity and sensitivity (Myint et al., 2002; Tiwari et al., 2003; Bhattacharya et al., 2003; DeBiasi et al., 2004). In our study, IS6110 PCR has shown 86.21% sensitivity (CI; 95%) and 74.12% specificity (CI; 95%). Overall positive and negative predictive value of IS6110 PCR was observed as 13.37% per cent (CI; 95%) and 99.15% (CI; 95%). Previous workers have reported the sensitivity and specificity of IS6110 PCR in EPTB samples as 87.5% and 100% (Chakravorthy et al., 2005), 89.5% and 86.1% (Pahwa et al., 2005), 57% and 100% (Prakash et al., 2012), 70% and 100% (Siddqui et al., 2013) respectively. In a recent study by Makeshkumar et al., (2012), IS6110 PCR had shown 66.66 % sensitivity (CI; 95%) and 74.41% specificity (CI; 95%) & overall positive and negative predictive value was observed as 8.33 % (CI; 95%) and 98.46% (CI; 95%). Zakham et al., (2012) reported high sensitivity (92.4%) and specificity (98.0%) of IS6110 PCR. The
positive and negative predictive values were 96.4% and 95.3% respectively. Gholoobi et al., (2014) reported the sensitivity of IS6110/buffer PCR as 58.33%, with specificity of 77.78%; the positive and negative predictive values were 100% and 78.26%, respectively.

Comparison of sensitivity of IS6110 PCR with conventional techniques

Several studies have documented the use of the IS6110 for the direct diagnosis of TB with reliable results comparing to traditional techniques (Ani et al., 2009; Abraham et al., 2012). By the use of PCR test we were able to detect *M. tuberculosis* in 67.21% smear negative samples which were positive by either of the culture methods. Similar finding were reported by Shukla et al., (2011) who found 60% positivity in smear negative samples which were positive by either of the culture methods.

The 5 sample where AFB smear and PCR were negative but culture was positive could be due to presence of PCR inhibiting substances in the samples and low bacterial load as supported by (Negi et al., 2005).

All the smear-positive samples were positive either by culture or PCR, 31/83 (37.34%) samples which were positive by either culture methods turned out to be negative by PCR. 12/31(38.70%) were identified as NTM identified as non-tubercular mycobacteria (NTM) by biochemical tests. The rest of the PCR negative samples may be due to presence of PCR inhibitors or improper appropportioning of sample or absence/fewer copies of target sequence IS6110 in some MTB strains. Das et al., (1995) have reported 40% of MTB from clinical isolates from South India with single or no copy of this target element. Absence or the presence of fewer copies of target
sequence in some strains of *M. tuberculosis* has already been reported (Fomukong *et al.*, 1994; Dale *et al.*, 1997; El Baghdadi *et al.*, 1997; Aqasino *et al.*, 1998).

The primers used by us were specific only for *Mycobacterium tuberculosis* so NTMs were not detected by it. Now with rising incidence of infections due to NTM it is important to also include primers which are genus specific. Contamination was observed in 27/655 (4.12%) samples which were considered negative by culture, 5 of these were found to be PCR positive. The *sensitivity and specificity* values obtained in the study indicates that the nucleic acid amplification technique offers many advantages over conventional methods and appears to be a useful diagnostic tool in detecting *M. tuberculosis* in clinical samples.

**Mean TAT & cost effectiveness of the methods**

AFB smear microscopy is inexpensive, simple and people can be trained to do it relatively quickly and easily. In addition the results are available within hours. But the major drawback is its poor sensitivity. Time taken for detection of *M. tuberculosis* by PCR was less than one day, compared to conventional methods of culture requiring 3–8 weeks for solid culture and 10–14 days for liquid culture which is a consequence of the long doubling time of *M. tuberculosis* (Negi *et al.*, 2005; Oberoi *et al.*, 2007). Additionally, culture methods are technically demanding, require implementation of bio-safety practices and equipment to prevent inadvertent infection of laboratory personnel and have relatively high per-test prices. In spite of the price differences, the sensitivity and short turn-around time of PCR outweighs its costs. While cost effectiveness and affordability are significant in the choice of diagnostic methods, especially in resource-limited settings, the cost of losses in terms of life is much more expensive.
Comparison of methods for detection of Female Genital Tuberculosis (FGTB)

319/655 (46%) of specimen included in this study were from suspects of Female Genital Tuberculosis (FGTB). These included Endometrium Biopsies (n=303) & Menstrual Blood (n=16). TB of the female genital tract is nearly always secondary to a focus elsewhere in the body (Gatongi et al., 2005). Reportedly, about 9% of all extra-pulmonary tuberculosis cases are genital tract TB. It is the root cause in 5-16% of case of infertility among Indian women (Shrivastava et al., 2014). Various Indian studies have shown that tuberculous endometritis and salpingitis account for 4-9% of all infertility cases (Thangapah et al., 2012).

Although genital TB can occur in any age group, the majority of patients are in the reproductive age group. In our study also 272/319 (85.26%) FGTB suspects were aged between 21-40 yr. High prevalence of FGTB amongst women in the reproductive age group has been described by several workers (Mondal et al., 2009; Thangappah et al., 2011; Jindal et al., 2012; Shrivastava et al., 2014). Genital TB may be asymptomatic and the majority of women are diagnosed during investigations for infertility (Goel et al., 2013, Shrivastava et al., 2014). In the present study also infertility was the most common presenting symptom in suspects of FGTB (222/319 (69.59%)); these included 170 (53.29%) suspects with primary infertility, 52 (16.30%) with secondary infertility. Primary and secondary infertility have been reported as the most common presenting symptom in GTB (Qureshi et al., 2001; Gurgan et al., 2004). Additionally, menstrual irregularities, dyspareunia, abdominal pain have also been reported to be associated with FGTB (Ekpenyong et al., 2013). Other problems reported in our study were: menstrual disturbances 35/303 (11.55%), abdominal pain 35/303 (11.55%), history of abortion 23/303 (7.5%) & ectopic
pregnancies 10/303 (3.30%). Bose et al., (2011) stated that common clinical presentations associated with GTB include oligomenorrhoea (54%), amenorrhoea (14%), menorrhagia (19%), abdominal pain (42.5%), dyspareunia (5-12%) and dysmenorrhoea (12-30%).

This patient to patient variation in the clinical presentation of the disease confounded by diverse results on imaging, laparoscopy, histopathology and various bacteriological and serological tests, poses a challenge to the early diagnosis of FGTB. Timely diagnosis and treatment of the disease may help in favorable results before extensive genital damage occurs. A definite diagnosis can be made by positive mycobacterium smear and culture by demonstrating specific histopathological lesion in the specimen. However, these conventional methods have low detection rates and limitations as GTB is paucibacillary. In our study, AFB were observed in 23/319 (7.21%) specimen from FGTB suspects. This was higher than smear positivity in genital samples reported in a recent study from our center i.e. 2.7% (Malhotra et al., 2012) which correlated well with other studies done like 0.4% (Agarwal & Gupta, 1993); 0.66% (Khaled et al., 2013); 1.23% (Misra et al., 1996); 1.6% (Bhanu et al., 2005); 0% (Rijal et al., 2004) & 0.45% Shrivastava et al., 2014. However, similar results have been obtained by Thangapah et al., 2011 who reported that 8.3% genital samples revealed AFB on smear examination & Namavar et al., 2001 who reported AFB in tissue biopsies in 12.19 % of cases.

In the present study, 23/319 (7.21%) specimens yielded growth on LJ slants, 43/319(13.47%) on MB7H9 broth. Rate of detection by culture was found to be higher in our study (7.21%) in comparison to others, reported as 3.2% by Bhanu et
al., (2005), 3.3–10.6% by Manjunath et al., (1991); Roy et al., (1993); Srivastava et al., (1997) 0.66% by Khaled et al., (2013); and lower than 10.7%-80% (Gracia et al., 1994); 18.46% by (Rozati et al., 2006), 19.53% by (Pingle et al., 2014), 30.95% by (Hemal et al., 2000), 48.5% by (Negi et al., 2005). In the present study, LJ culture was positive in 7.21% samples whereas it was 1.83%, 4%, 4.6% 5.6%, 13.6%, in recent Indian studies by Goel et al., (2013), Khanna & Aggarwal (2011), Kumar et al.,(2008), Thangappah et al., (2011), Mani et al., (2003), respectively. Kohli et al., (2011) could not diagnose any patient on LJ culture. The use of liquid culture doubled the rate of detection of Mtb from these genital specimens. Previous study by Malhotra et al., (2012) reported 8.28% positivity by MB7H9 broth culture in genital samples. The liquid culture has been reported to be advantageous than solid media for detection of Mtb (Prasad et al., 2012; Goel et al., 2013).

In our study PCR was found to be most efficient in diagnosing FGTB. 79/319 (24.76%) specimen were PCR positive. It was found to be most sensitive (89.04%) method among all the four methods used. In our study it was around 8 fold more sensitive than the smear examination and 2-fold more sensitive than the liquid culture and 4-fold more sensitive than the solid culture. Bhanu et al., (2005) had reported 14-fold more sensitivity of the PCR in comparison to smear examination. Malhotra et al., (2012) reported IS6110 PCR to be 9 times more sensitive than smear examination & 3 times more sensitive than MB7H9 broth culture. Shrivastava et al., (2014) reported IS6110 PCR to be 8 times more sensitive than LJ culture for detection of MTB.
Discussion

PCR positivity in our study (24.76%) is lower than 6.3% by Leonardo et al., (1997), 8.69% by Pingle et al., (2014), 13% Kohli et al., (2011) & 14.6% Prasad et al., (2012). Similar PCR positivity in genital samples has been reported as 22.2% (Goel et al., 2013), 22.5% (Gupta et al., 2007), 23.78% (Malhotra et al., 2012) & 26% (Khanna & Aggarwal 2011). PCR positivity was lower than 32.1% by Baxi et al. (2011), 36.7% by Thangappah et al., (2011), 38.9% by Kumar et al., (2008), 43.07% by Rozati et al., (2006) 53.3% by Bhanu et al., (2005), 54.5% by Jindal et al., (2010), 74.4% by Negi et al., (2005), 80.95% by Hemal et al.,(2000). Variations in PCR positivity observed by workers around the world due to bacillary load in the sample, method of DNA extraction/PCR amplification used & the primer used. Irrespective of the above PCR has been found to be advantageous for early detection of FGTB as compared with conventional microbiological techniques.

In this study, in 4.29 % (13/655) patients had definite past history of tuberculosis and they were treated with anti tuberculous treatment (ATT) 2-15 years earlier. In all the thirteen cases, either one or more of the other diagnostic parameters were positive; therefore, a definite past history of tuberculosis was taken as one of the parameters to arrive at a diagnostic criterion to suspect genital tuberculosis.

Comparison of methods for detection of tuberculosis meningitis (TBM)

254/655 (39%) specimen included in this study were from suspects of TB meningitis (TBM). 52/254 (20.39%) suspects were 15 yrs of age. TBM occurs in 7%-12% of tuberculosis patients in our country (Shanker et al, 1991). It accounts for 20%-45% of all types of tuberculosis among children, when compared with only 2.9%-5.9% of adult tuberculosis (Mahadevan et al., 2005; Principi & Esposito, 2012).
The poor sensitivity of standard laboratory tests is a major obstacle in diagnosing tuberculosis in children, because the organism load is generally much lower than that in adults. Another challenge is the lack of characteristic symptoms in many children, compared with those in adults. Symptoms of *M. tuberculosis* infection are often nonspecific or absent in affected children (Khan *et al.*, 1995). Diagnostic uncertainty arises commonly in patients who present with a few days of headache, fever, and neck-stiffness; undefined treatment in the community; a low concentration of glucose in cerebrospinal fluid (<50% of that in blood), and neutrophils and lymphocytes in the cerebrospinal fluid.

In the present study fever 158(61.02%) was the most common presenting symptom in suspects of TBM followed by headache (12.99%), seizure (6.69%) & paralysis (5.51%). The above have been described to be common presenting symptoms in suspects of TBM (Swartz *et al.*, 2011; Marx *et al.*, 2011).

Although several biochemical characteristics (adenosine deaminase activity (ADA), protein level, glucose level, lymphocytic pleocytosis etc) are used for diagnosis of TBM but a definite diagnosis can be made after a lumbar puncture (LP) in a patient with signs and symptoms of central nervous system (CNS) disease, acid-fast bacilli (AFB) are seen and/or *M. tuberculosis* is detected by molecular methods and/or cultured in cerebrospinal fluid (CSF) (Principi & Esposito, 2012). In our study AFB were observed in 1/254 (0.39%) samples. AFB smear positivity has been reported as 0% (Rijal *et al.*, 2004; Mahadevan *et al.*, 2005; Makeshkumar *et al.*, 2012; Siddiqui *et al.*, 2013). Others report that only 5% to 20% of patients have Ziehl-Nielsen positive result (Sekar *et al.*, 2008; Thakur *et al.*, 2010). This
paucibacillary nature of the specimen necessitates the use of other methods for confirmation of TB in CSF samples.

19/254 (7.48%) CSF samples were positive by either of the culture methods. The **LJ culture** positivity in this study was 4.72 % (12/254) and **MB7H9 culture** positivity was 4.33% (11/254). Previously Sekar *et al.*, 2008 reported 6.52% LJ culture positivity in CSF samples. Recent study by Makeshkumar *et al.*, (2012) reported culture positivity as 0%. Siddqui *et al.*, (2013) have reported 5.4% positivity in CSF samples by both solid and liquid culture. 1/19 (5.26%) culture positive isolate turned out to be NTM on further biochemical analysis. Meningitis due to NTM is rare; however, infections due to NTM are especially common in immunocompromised patients (Jesudason, 2005).

Many authors have reported **PCR** as more sensitive technique for the detection of mycobacteria in both suspects & clinically/radio-logically confirmed cases of TBM. In our study the positivity by PCR was 35.82% (91/254) which significantly correlates with recent study by Makeshkumar *et al.*, (2014) reporting 36% (9/25) positivity in CSF samples. In a study by Deshpande *et al.*, 2007, the IS6110 PCR assay detected the presence of *M. tuberculosis* DNA in 91.4% (32/35) cases of confirmed TBM and in 62.5% (10/16) cases of clinically diagnosed TBM which were negative for mycobacterial culture, but had a clinical index of suspicion for TBM. Iqbal *et al.*, 2010 reported 60% (18/30) PCR positivity in CSF samples with strong clinical & radiological evidences of TBM. Sekar *et al.*, (2008) & Siddqui *et al.*, (2013) observed 73.91% (34/46) & 75.7% (26/37) PCR positivity in similar cases. PCR, therefore, shows great potential as a rapid diagnostic test for
tuberculous meningitis particularly for cases of smear and/or culture negative TBM (Sharma et al., 2010).

Comparison of methods for detection of mycobacteria from other body fluids & aspirates

In our study 0/41(0%) of the peritoneal fluids found were determine to be positive by microscopy, 3/41(7.3%) by LJ culture, 7/41(17.07%) by Middlebrook 7H9 broth, 7/41(17.07%) by PCR. Several studies reported low detection rate by smear in extra pulmonary tuberculosis. Many authors have found AFB positivity in peritoneal fluids as 0% (Rijal et al., 2004), 5.4% (Therese et al., 2005), 10% (Cagatay et al., 2004).

A definitive diagnosis of tuberculous pleurisy usually requires mycobacterial culture of pleural fluid, pleural biopsy or other diagnostic tests. In our study 2/18(11.11%) pleural fluids received were positive by microscopy, 1/18(5.5%) on LJ culture, 2/18 (11.11%) on MB 7H9 broth & 4/18(22.22%) by PCR. Previous studies have reported AFB smear positivity as 0% (Rijal et al., 2004); 3.8% (Hasaneen et al., 2003); 9.3% (Fain et al., 2000) 12.5% (Chakravorty et al., 2005); 33.3% (Cagatay et al., 2004) & 35.2 % (Nassaji et al., 2014). Several studies have reported improved sensitivity of PCR over conventional methods. Sekar et al., (2008) reported 12.5% positivity by smear, 0 % by LJ & 45.83% by PCR in pleural specimens. Maurya et al., (2011) observed 15.5% positivity by smear, 13.2% by automated liquid culture & 12.1% by PCR. Siddiqui et al., (2013) found 8.3% by positivity by smear, 25% by LJ, 25% by liquid culture, 75% by PCR. Mradula Singh et al., (2013) reported 3.1% positivity by smear, 11.53% by LJ culture, and 62.01%
Discussion

by IS6110 PCR in pleural fluid. According to Makeshkumar et al., (2014), 0% & 22.22% pleural fluids were determined to be positive by smear/LJ culture & PCR respectively.

In our study none (0%) of the pus samples were positive by microscopy, 1/6 (16.66%) was positive by LJ culture, 2/6 (33.33%) on MB 7H9 broth & 2/6 (33.33%) by PCR. Previous studies have reported AFB smear positivity as 0% (Singh et al., 2013), 4.2% (Rijal et al., 2004), 8.4% (Maurya et al., 2011) & 14.28% (Makeshumar et al., 2014) in pus specimen. Similarly, culture positivity has been reported as 2.90% (Singh et al., 2013), 8.4% (Maurya et al., 2011) & 14.28% (Makeshkumar et al., 2014) whereas PCR positivity has been reported as 7.8% & 42.85% & 77.41% by Maurya et al., 2011 & Makeshkumar et al., 2014, Mradula Singh et al., 2013 respectively.

None of the synovial fluid specimen included in this study were found to be positive by smear and/or culture. 1/5 (20%) specimen was positive by PCR. Sekar et al., (2008) reported 100% PCR positivity in 2 synovial fluid samples which were negative for AFB smear microscopy. Maurya et al., (2011) observed positivity by smear, automated liquid culture & PCR as 2.8%, 2.6% & 3.1% respectively in synovial fluids. In another study by Siddqui et al., (2013), 50% smear and/or culture negative synovial fluids were found positive by PCR. Contrary to the above findings, Makeshkumar et al., (2014) failed to detect MTB by PCR in any of the 6 synovial fluids received. 1/61 (16.66%) of these samples were positive by smear & culture.
1/4 (25%) ascitic fluid was positive by PCR. None of the four was positive by smear and/or culture. Sekar et al., (2008), Maurya et al., (2011) Siddqui et al., (2013) & Makeshkumar et al., (2014) have reported smear positivity as 19.51%, 2.8%, 3.4% & 0%; culture positivity as 15.38%, 4.4%, 17.4% & 0% and PCR positivity as 60.97%, 4.8%, 65.5%, 27.11% respectively in ascetic fluids.

However, in case of gastric aspirates MB7H9 broth culture (66.66% positivity) was more efficient in than PCR (33.33% positivity). Maurya et al., (2011) have also reported better performance of liquid culture methods (7.5%) than PCR (7.2%) in detection of mycobacteria in gastric aspirates. The total no. of other specimen like pericardial fluid & lymph node aspirate was very less in the study and so it cannot be commented which of the above methods was more suitable for detection of MTB.

Utility of TB PCR from blood samples

*Mycobacterium tuberculosis* blood stream infection was described within a few decades of the discovery of the tubercle bacillus (Crump et al., 2011). Early recognition and treatment are likely to be important to avoid death. The second part of this study was designed to evaluate the utility of peripheral blood samples in the diagnosis of tuberculosis. The PCR results on different samples obtained from specific-site according to the disease were compared with blood PCR and blood culture on Myco/F lytic medium. Our main objective to assess which sample, blood or specific site sample of the TB patient was better in terms of diagnostic value.

A total of 80 blood samples were collected from known patients of pulmonary (n=28), extra-pulmonary (n=40) & HIV-TB (n=12) co-infection
(diagnosed by PCR of site specific sample). Out of these 80 samples none was found to be positive on Myco/F Lytic medium. Although the incremental value of mycobacterial blood culture for the diagnosis of disseminated tuberculosis has been both recognized and debated (McDonald et al., 2000; Crump et al., 2003; Monkongdee et al., 2009), several studies have concluded that mycobacterial blood cultures add little value to the conventional diagnostic work-up (Esteban et al., 2001; von Gottberg et al., 2001) Contrary to our results, a previous study by Hanscheid, et al., 2005 has reported that blood cultures can be a useful tool for cultural confirmation of tuberculosis in HIV-infected patients in Portugal. The possible reason for this observation could be that the no. of HIV-TB suspects taken into account in this study was very low.

Ahmed et al., 1998 & Khan et al., 2006 have stated that tests based on blood PCR have shown promise for the detection of mycobacteria in clinical samples. Amongst the blood samples collected from 28 PTB patients, positive for AFB smear & site-specific PCR; 5 (17.85%) were positive by blood PCR. Of the 40 EPTB patients only 3 (7.5%) were positive by blood PCR. A previous study by Iqbal et al., (2010) reported that 1/61(1.63%) PCR positive PTB patient & 2/77(2.59%) EPTB patients were found positive by PCR on blood. All 12 HIV-TB co-infected subjects were negative for blood PCR. Earlier studies with blood-based PCR assays in humans have suggested that PCR with peripheral blood mononuclear cells for the diagnosis of tuberculosis may be useful only in those patients who are substantially immune-compromised (Khan et al., 2006). However, no such correlation has been found in our study. It appears that more data are required to determine the effectiveness of the blood-based PCR assay for the diagnosis of tuberculosis, especially in immuno-competent patients (Ahmed et al., 1998).
According to our findings it is clear that specific site sampling from TB patients according to disease plays a vital role in the diagnosis of extra-pulmonary tuberculosis by PCR. Percentage positivity of blood samples by PCR was only 10% in samples positive for site specific PCR. These results confirm that for accurate diagnosis of TB cases by PCR, specific site sampling is significant. Therefore, the idea of TB diagnosis by PCR on blood samples should be discouraged. These findings also illustrate that the application of PCR to the diagnosis of tuberculosis has the potential to resolve one of the foremost challenges faced by a clinician and the diagnostic laboratories.