DISCUSSION

TB is one of the oldest known infectious diseases of the world with great public health implications. TB manifests as pulmonary (PTB) and extra pulmonary (EPTB). Routine diagnosis of PTB is done by AFB smear microscopy and culture. Diagnosis of extra-pulmonary TB is difficult as it is paucibacillary, and as a result, the sensitivities of the AFB smear and culture techniques are poor, and diagnosis of EPTB remains a challenge worldwide (Cailhol et al., 2005). In India, 10 to 15% of TB cases are estimated to be EPTB cases, primarily affecting the meninges, kidneys, spine, lymph nodes, and the growing ends of the bones. The case mortality rate is 25 to 50% within months (Agrawal et al., 2005). Other sites that may be involved include pleura, peritoneum, pericardium, liver, gastro-intestinal tract, and genito-urinary tract.

Female genital TB is a form of extra-pulmonary TB which affects the female genital organs. Fallopian tubes are the most commonly affected (90%), followed by the endometrium (50%), and the ovaries (10–30%) in FGTB (Rana et al., 2011). Females of the reproductive age group are affected by female genital tuberculosis. The disease frequently remains unnoticed even in most of the tertiary health care set ups particularly in developing countries and its true incidence remains unknown. This poses diagnostic challenges mainly because the initial symptoms of the disease are usually not characteristic of the disease (Varma 1991).

FGTB is an important cause of infertility, being an etiological factor in 5-16% of all infertile cases in India (Arora et al., 2003; Bapna Net al., 2005; Gatongi et al., 2005). Diagnosis at an early stage would help the clinician in giving appropriate treatment, thereby reducing the morbidity.

The most significant problem associated with FGTB is that it remains largely undiagnosed due to two reasons: i) patients usually do not exhibit any symptoms, and ii) there is a paucity of investigations (Malhotra et al., 2012). As a result, the disease is either not diagnosed, or diagnosed at an advanced stage, and at this stage the prognosis for fertility is poor despite treatment (Sharma et al., 2007, 2008).
Patient to patient variation in clinical presentation of the disease, confounded by varied results on imaging, laparoscopy, histopathology, and various bacteriological and serological tests, pose a challenge to the early diagnosis of FGTB. Timely diagnosis and treatment of the disease may help in preventing extensive genital damage.

Diagnosis can be confirmed by AFB smear, culture, PCR, or histopathology. Conventional methods have poor sensitivity, particularly in paucibacillary disease. In recent years, there have been developments in culture, as well as in molecular methods based on different genes such as IS6110, MPB64, ESAT-6, hupB DNA, 38 kDa, and 65kDa (Eisenach et al., 1991; Bhanu et al., 2005; Rozati et al., 2006; Kumar et al., 2008; Sharma et al., 2010). These methods are now being used more frequently, and therefore, various options are now available for confirming the diagnosis of TB.

Importantly, there is a need to compare the efficiencies of various available methods, particularly the PCR based methods. IS6110 based PCR has been extensively used for diagnosis of TB, but it has its limitations as many strains from India have either no copy or low copy number of the sequence resulting in a false negative test result. Therefore, there is a need to evaluate the diagnostic accuracy of other PCR based methods in order to find the optimum PCR strategy for the diagnosis of GTB.

Notably, the success of these tests depends on the nature and quality of the clinical specimen, particularly in cases of tuberculosis occurring at difficult anatomical sites such as female genitalia. Therefore, in the present study, we investigated potential novel strategies for the diagnostics of TB in paucibacillary disease such as female genital tuberculosis.

We compared conventional diagnostic modalities: smear microscopy, culture on solid and liquid media, with PCR targeting the IS6110, MPB64 and ESAT-6 genes, for diagnosis of MTB infection. The study was conducted at the Mycobacteriology and Advanced Research laboratory, Department of Microbiology,
Discussion

SMS Medical College, Jaipur. 300 suspected cases of FGTB, whose specimen were received in the laboratory for diagnosis of TB, were utilized in this study. Patients enrolled in the study were 18-45 years old. The median age of presentation was 27 years with a standard deviation of 5.63 years. Majority of the suspected cases in our study [225 (75%)] were 18-30 years of age, suggesting that genital tuberculosis affects women who are relatively young. This is in agreement with earlier studies (Bhanu et al., 2005; Sharma et al., 2010; Kulshrestha et al., 2011). The occurrence of genital tuberculosis in young patients can be explained by the fact that after puberty, the blood supply to the genital organs is increased, resulting in more bacilli reaching the site and causing infection. In such cases, even dormant bacteria can get reactivated and cause infection. This leads to a higher incidence of genital tuberculosis, leading to infertility, among young women.

Although the smear microscopy technique has very poor sensitivity, it remains a specific and economical mode of diagnosis, with results available immediately. Also, it is very simple and easy to perform and interpret, thus making it the technique that is most frequently chosen. In our study, 2.33% (7/300) cases were reported to be positive by AFB smear microscopy. This low positivity rate is due to the fact that FGTB is a paucibacillary disease, and AFB smear has low sensitivity (detects >10^4 bacilli/ml). Earlier studies have reported similar findings (Misra et al., 1996; Namavar et al., 2001; Abebe et al., 2004; Bhanu et al., 2005; Rozati et al., 2006; Rana et al., 2011; Kulshrestha et al., 2011). Therefore, AFB smear alone is not an adequate criterion, and must be followed by culture which additionally helps to determine the species of Mycobacteria involved (Wolinsky 1979).

In our study, culture was positive in 7.33% (22/300) cases. Previous studies have reported similar findings with regard to low sensitivity of the culture technique in diagnosis of FGTB (Chhabra et al., 1986; Deepjyoti et al., 1990; Manjari et al., 1995; Abebe et al., 2004; Bhanu et al., 2005; Rozati et al., 2006; Rana et al., 2011; Kulshrestha et al., 2011). This is due to the fact that FGTB is a paucibacillary disease, as only few bacteria are present in the endometrium sample (Bhanu et al., 2005). Moreover, it has been reported that growth may be inhibited by the presence of some inhibitors in the sample (Soltys 1953). LJ culture plays an important role in
distinguishing tubercular and non tubercular Mycobacteria (Wolinsky 1979). However, this technique renders positive results only if >100 bacilli/ml are present in the sample. Another limitation of the culture method is that it takes 6-8 weeks to give a positive result thereby limiting its clinical utility (Prasad et al., 2001).

BacT/ ALERT 3D (BioMerieux Inc, Durham, NC, USA) and MGIT 960 (Becton Dickinson, Sparks, MD, USA) are automated liquid culture systems that are considered to be the gold-standard for the culture of Mycobacteria. It has been shown that liquid media have a greater sensitivity for the isolation of Mycobacteria in comparison to solid media, and can give a 10% higher yield (Cruciani et al., 2004; Dinnes et al., 2007). In our study, liquid culture was positive in 9.67% (29/300) cases as compared to 7.33% (22/300) by culture on solid media. The use of liquid culture also reduces the delays in obtaining results. However, with liquid systems strict precautions have to be taken to prevent contamination. Moreover, the risk of infection is high. Liquid systems also require skilled personnel and specialized lab infrastructure such as a BSL 3 lab for biosafety reasons. Hence they are more expensive, with a substantial equipment investment requirement (Namlert et al., 2009).

PCR based tests amplify Mycobacterial nucleic acid, and are very sensitive, specific, and rapid. Several studies have highlighted the significance of PCR in the rapid diagnosis of TB of the genital tract (Hashimoto et al., 1994; Ferrara et al., 1999; Vishnevskiaia, 2000; Baum et al., 2001; Negi et al., 2005). Various PCR systems have been designed to amplify specific regions of the Mycobacterial genome. In previous studies, authors have successfully utilized target sequences such as IS6110, MPB64, ESAT-6, hupB DNA, 38 kDa, and 65kDa genes for the detection of MTB in clinical samples (Eisenach et al., 1991; Bhanu et al., 2005; Rozati et al., 2006; Kumar et al., 2008; Sharma et al., 2010). Among these, IS6110 is the most commonly used target for the diagnosis of TB, but the repetitive element (insertion sequence) is present in very low copy numbers among Indians. Therefore, for our study, we chose other targets, specifically MPB64 and ESAT-6 genes, in order to avoid missing true positive cases.
PCR offers many advantages. In comparison to culture, it is rapid, sensitive, and specific. The PCR method produces results within 4-5 hours, while the culture technique requires a few weeks and has a lower detection rate (Agarwal et al., 1993; Misra et al., 1996; Sharma et al., 2010). However, owing to the high sensitivity of PCR, false positivity becomes an issue (Kwok et al., 1989). Results from our study showed that PCR may be considered a relatively better diagnostic gold standard for efficient diagnosis of GTB owing to its high sensitivity and specificity.

**Comparison of the Positivity Obtained by the Methods**

In our study, overall positivity by AFB smear microscopy, after decontamination by modified Petroff’s method, was 2.33% (7/300). This is similar to observations by other authors who have reported AFB positivity after processing with modified Petroff’s method to be: 2.50% (Khosravi et al., 2010), 2.53% (Sankar et al., 2013), 2.7% (Malhotra et al., 2012), but lower than some studies like: 3.85% (Munir et al., 2008), 3.9% (Chakravorty et al., 2005), 3.97% (Munir et al., 2008), 5% by Ukwaja et al. (2011), 8.3% by Bunger 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), and 28.3% by Tessema et al. (2009). Some studies have reported even lower positivity by AFB smear microscopy than our study: 0.18% (Goel et al., 2013), and 0.45% (Shrivastava I et al., 2014).

In the present study, the positivity by LJ solid culture and Middle Brook 7H9 culture were 7.33% (22/300) and 9.67% (29/300), respectively. Recent Indian studies have reported variable positivity by LJ culture: 1.9% (Rijal et al., 2004), 3.08% (Shrivastava G et al., 2014), 3.37% (Makeshkumar et al., 2014), 5.5% (Shrivastava I et al., 2014), 6.06% (Bunger et al., 2013), 8.28% (Malhotra et al., 2012), 12.0% (Abebe et al., 2004), 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), and 45% (Maurya et al., 2011) in EP specimen.

The liquid culture method has been found to be more useful in the detection of Mycobacteria in paucibacillary specimens, where LJ media gives very scanty, or no growth (Hilleman et al., 2006). Similarly, in our study, liquid culture was positive
Discussion

in 9.67% (29/300) cases, as compared to 7.33% (22/300) by culture on solid media. In a study by Palacios et al., (1996), 77.7% of the samples were positive by culture in liquid media. The positivity by culture on LJ medium was 27.7% in the same samples. For EPTB samples with a positivity of 59.7% to 87.2% on LJ medium, the positivity by liquid culture has been reported to be very high, usually within the range of 80 to 100% (Hanna et al., 1999; Alcaide et al., 2000; Lu D et al., 2002).

In our study, 4/31 (12.9%) isolates grown on LJ and MB media were identified as NTM on further biochemical analysis. Malhotra et al. (2012) reported 6/46 (13.04%) NTMs among culture positive specimens.

In our study, the positivity by PCR was higher than by other conventional methods. Out of the 300 samples received, a total of 33.33% (100/300) were positive by at least one of the PCR methods used. Of these 100 samples, 49% (49/100) were positive by all the three PCR assays. 29.67% (89/300) were positive by IS6110, 24.67% (74/300) were positive by MPB64, and 20% (60/300) were positive by ESAT-6.

Several studies have reported high positivity by IS6110 based PCR in EP samples (Lahiri et al., 2001; Sekar et al., 2008). Various authors have reported PCR positivity as 23.78% (Malhotra et al., 2012), 26.96% (Makeshkumar et al., 2012), 36.7% (Thangappah et al., 2008), 38.5% (Shrivastava I et al., 2014), 50.66% (Shrivastava G et al., 2014), 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), and 92.1% (Ogusku et al., 2004).

In order to avoid missing true positive cases, some authors have used PCR targeting genes other than IS6110, like MPB64, ESAT-6, hupB DNA, 38 kDa, and 65kDa genes for the diagnosis of EPTB (Bhanu et al., 2005; Rozati et al., 2006; Kumar et al., 2008; Sharma et al., 2010). In our study, we used two additional target genes i.e. MPB64 and ESAT-6. 24.67% (74/300) cases were found to be positive by MPB64, while 20% (60/300) cases were positive by ESAT-6. Out of all the PCR positive samples, 17% (17/100) were positive by IS6110 PCR alone, 9% (9/100)
were positive by \textit{MPB64} PCR alone, while there was no additional pick up by \textit{ESAT-6} based PCR. 14\% (14/100) were found to be positive by a combination of \textit{IS6110} and \textit{MPB64} PCR, 14\% (14/100) by a combination of \textit{IS6110} and \textit{ESAT-6}, 9\% (9/100) by a combination of \textit{IS6110} and \textit{ESAT-6}, and 2\% (2/100) by a combination of \textit{MPB64} and \textit{ESAT-6}. 11\% (11/100) samples were positive by \textit{MPB64} but negative by \textit{IS6110}, 2\% (2/100) were positive by \textit{ESAT-6} but negative by \textit{IS6110}, and 2\% (2/100) were positive by both \textit{ESAT-6} and \textit{MPB64} but negative by \textit{IS6110}. 11\% (11/100) samples were missed by \textit{IS6110} PCR i.e., these were positive by either \textit{ESAT-6} or \textit{MPB64} but were negative by \textit{IS6110} PCR. 26\% (26/100) were positive by \textit{IS6110} but negative by \textit{MPB64}, 9\% (9/100) were positive by \textit{ESAT-6} but negative by \textit{MPB64}, 9\% (9/100) were positive by both \textit{ESAT-6} and \textit{IS6110} but negative by \textit{MPB64}. 26\% (26/100) samples were missed by \textit{MPB64} PCR i.e., these were positive by either \textit{ESAT-6} or \textit{IS6110} but negative by \textit{MPB64} PCR. 31\% (31/100) were positive by \textit{IS6110} but negative by \textit{ESAT-6}, 23\% (23/100) were positive by \textit{MPB64} but negative by \textit{ESAT-6}, 14\% (14/100) were positive by both \textit{MPB64} and \textit{IS6110} but negative by \textit{ESAT-6}. 40\% (40/100) samples were missed by \textit{ESAT-6} i.e., these were positive by either \textit{MPB64} or \textit{IS6110} but were negative by \textit{ESAT-6} PCR.

Some authors have used \textit{MPB64} as a gene target for PCR amplification. These authors have reported a positivity rate of 23.30\% (Baveja \textit{et al.}, 2011), 26.50\% (Manjunath \textit{et al.}, 1991), 42.57\% (Bhanothu \textit{et al.}, 2014), and 56\% (Bhanu \textit{et al.}, 2005).

Rozati \textit{et al.} (2006) used the \textit{ESAT-6} gene as a target for PCR in their study, and compared results with outcomes from other conventional methods for identification of Mycobacteria. The authors reported a positivity rate of 43.1 \% by \textit{ESAT-6} PCR amplification, and concluded that PCR is the method of choice for identifying infection in FGTB samples. In our study, we used \textit{ESAT-6} gene amplification, and found a positivity rate of 20\% (60/300).

Comparison of various studies on female genital tuberculosis is given in Table 15.
Table-15: Studies Showing Positive Results in AFB Smear, Culture, PCR and Histopathology in Various Studies on Female Genital Tuberculosis

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Author</th>
<th>Year</th>
<th>Place</th>
<th>Sample Size</th>
<th>Smear</th>
<th>Culture (Positivity Rate %)</th>
<th>PCR (Positivity Rate %)</th>
<th>Gene</th>
<th>Histopathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Present study</td>
<td>2016</td>
<td>Jaipur</td>
<td>300</td>
<td>2.33</td>
<td>7.33(LJ), 9.67(MB)</td>
<td>29.67, 24.67, 20</td>
<td>IS6110, MPB64 &amp; ESAT-6</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Manjunath et al.</td>
<td>1991</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Abebe et al.</td>
<td>2004</td>
<td>Ethiopia</td>
<td>25</td>
<td>4</td>
<td>12</td>
<td>48</td>
<td>MPB64</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>Bhanu et al.</td>
<td>2005</td>
<td>Delhi</td>
<td>25</td>
<td>1.6</td>
<td>3.2</td>
<td>56</td>
<td>MPB64</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Rozati et al.</td>
<td>2006</td>
<td>Hyderabad</td>
<td>65</td>
<td>5.2</td>
<td>7.8</td>
<td>43.1</td>
<td>ESAT-6</td>
<td>11.05</td>
</tr>
<tr>
<td>6</td>
<td>Thangappah et al.</td>
<td>2008</td>
<td>Chennai</td>
<td>72</td>
<td>8.3</td>
<td>5.6</td>
<td>36.7 (18/49)</td>
<td>IS6110</td>
<td>6.9</td>
</tr>
<tr>
<td>7</td>
<td>Kumar et al.</td>
<td>2008</td>
<td>Delhi</td>
<td>393</td>
<td>5.1</td>
<td>4.2</td>
<td>31.3</td>
<td>hupB DNA</td>
<td>3.2</td>
</tr>
<tr>
<td>8</td>
<td>Suman et al.</td>
<td>2009</td>
<td>Ludhiana</td>
<td>60</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Khosravi et al.</td>
<td>2010</td>
<td>Iran</td>
<td>200</td>
<td>2.5</td>
<td>5</td>
<td>5</td>
<td>IS6110</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Kohli et al.</td>
<td>2011</td>
<td>Delhi</td>
<td>100</td>
<td>1</td>
<td>0</td>
<td>13</td>
<td>hupB DNA</td>
<td>4</td>
</tr>
<tr>
<td>11</td>
<td>Maurya et al.</td>
<td>2011</td>
<td>Lucknow</td>
<td>450</td>
<td>13.4</td>
<td>45</td>
<td>63</td>
<td>IS6110</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>Baveja et al.</td>
<td>2011</td>
<td>Delhi</td>
<td>30</td>
<td>3.3</td>
<td>6.7</td>
<td>23.3</td>
<td>MPB64</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>Malhotra et al.</td>
<td>2012</td>
<td>Jaipur</td>
<td>555</td>
<td>2.7</td>
<td>8.28</td>
<td>23.78</td>
<td>IS6110</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>Sankar et al.</td>
<td>2013</td>
<td>Delhi</td>
<td>158</td>
<td>2.53</td>
<td>15.18</td>
<td>85.44</td>
<td>-</td>
<td>5.06</td>
</tr>
<tr>
<td>15</td>
<td>Singh et al.</td>
<td>2013</td>
<td>Kolkata</td>
<td>117</td>
<td>41.6</td>
<td>55.4</td>
<td>67.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>Bhanothu et al.</td>
<td>2014</td>
<td>Hyderabad</td>
<td>202</td>
<td>21.78</td>
<td>42.07</td>
<td>42.57</td>
<td>MPB64</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>Goel et al.</td>
<td>2013</td>
<td>Delhi</td>
<td>546</td>
<td>0.18</td>
<td>1.83</td>
<td>22.2 (20/90)</td>
<td>65kDa gene</td>
<td>2.6 (13/494)</td>
</tr>
<tr>
<td>18</td>
<td>Shrivastava G et al.</td>
<td>2014</td>
<td>MP</td>
<td>227</td>
<td>4.84</td>
<td>3.08</td>
<td>50.66</td>
<td>IS6110</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>Shrivastava I et al.</td>
<td>2014</td>
<td>MP</td>
<td>225</td>
<td>0.45</td>
<td>5.5</td>
<td>38.5</td>
<td>IS6110</td>
<td>1.37</td>
</tr>
</tbody>
</table>
Comparison of Sensitivity and Specificity of Various Methods Against LJ Culture

The sensitivity and specificity of AFB smear microscopy were lower in comparison to culture based methods. AFB smear microscopy has shown 31.82% sensitivity and 100% specificity. The positive predictive value of ZN staining was found to be 100%, and the negative predictive value was 94.88%. The sensitivity of AFB smear can also vary with the amount of sample processed, as concentration occurs after processing with the petroffs method (Chowdhury et al., 2012). Bhanothu et al. (2014) have reported the sensitivity of AFB smear microscopy to be 21.78% and specificity to be 100%, with a PPV of 100%, and an NPV of 38.75%. Recently, Maurya et al. (2012) have reported 13.7% sensitivity and 100% specificity for AFB smear microscopy. In a report by Munir et al. (2008), the sensitivity of AFB smear microscopy was reported to be 20.84% and specificity to be 100%, with a PPV of 100%, and an NPV of 84%. These results are in concordance with our findings. There are various reports reporting that the sensitivity of ZN smear for extra-pulmonary specimen ranges from 0%-75% (Kamboj et al., 1994). This limitation has been reported to be due to the inadequacy and the paucibacillary nature of specimen (Tiwari et al., 2003; Yam et al., 2006). Sensitivity is further decreased if specimens are not of good quality, especially in children, and in HIV TB coinfection cases (Corbett et al., 2003; Getahun et al., 2007). In our study, 7/300 (2.33%) specimens were found to be positive by smear microscopy. All the smear-positive samples were positive by either LJ or MB liquid culture.

We report a sensitivity of 90.91% and specificity of 96.76%, with a PPV of 68.97%, and NPV of 99.26% for MB7H9 liquid culture. Bhargava et al. (2001) reported a sensitivity of 62.9% for MB7H9 broth culture when compared with LJ medium. In the present study, 29/300 (9.67%) samples were positive by MB liquid culture. Of these 29 samples, 4/29 (13.79%) isolates were identified as NTM by biochemical tests, and 25/29 (86.21%) were confirmed as Mycobacterium tuberculosis. 18/300 (6.00%) samples got contaminated and were therefore considered negative by culture. Out of the 29 positive samples, 20 were also positive by LJ culture. Therefore, the sensitivity of MB7H9 liquid culture was found to be relatively high when compared to the other techniques utilized in our study.
PCR tests that are based on amplification of the *IS6110* insertion sequence have exhibited a significant variation in specificity and sensitivity for the diagnosis of MTB infection in patient specimens (Myint *et al*., 2002; Tiwari *et al*., 2003; Bhattacharya *et al*., 2003). In our study, *IS6110* PCR exhibited 72.73% sensitivity and 73.74% specificity. The PPV and NPV for *IS6110* based PCR was found to be 17.98% and 97.16%, respectively. In a recent study by Makesh Kumar *et al*., (2014), a sensitivity of 66.66 % and a specificity of 74.41%, with an overall PPV and NPV of 8.33% and 98.46%, respectively, were reported for the *IS6110* PCR. Meanwhile, Gholoobi *et al*, (2014) reported a sensitivity of 58.33% and a specificity of 77.78%, with a PPV and NPV of 100% and 78.26%, respectively, for the *IS6110* PCR.

Other authors have reported the sensitivity and specificity of *IS6110* PCR as 87.5% and 100% (Chakravorty *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 EPTB samples. Zakham *et al*., (2012) reported a relatively high sensitivity of 92.4% and specificity of 98.0% for the *IS6110* PCR. In their study, the PPV and NPV were found to be 96.4% and 95.3%, respectively.

In our study, *MPB64* PCR exhibited 68.18% sensitivity and 78.78% specificity. The PPV and NPV of *MPB64* PCR were found to be 20.27% and 96.90%, respectively. In a recent study by Bhanuthu *et al*., (2014), *MPB64* PCR had shown 70.29% sensitivity and 100% specificity, while the PPV and NPV were found to be 100 % and 62.50%, respectively.

In our study, the sensitivity and specificity of *ESAT-6* PCR were observed to be 50.09% and 83.09%, respectively. The PPV and NPV values were 21.67% and 96.25%, respectively. A study by Rozati *et al*., (2006) found 96.40% sensitivity and 100% specificity, with an overall PPV and NPV value of 100% and 66.66%, respectively, by *ESAT-6* PCR.

Comparison of Sensitivity of PCR with Conventional Techniques

Several studies have reported using the *IS6110* based PCR, as opposed to traditional techniques, for the rapid detection of TB (Ani *et al*., 2009; Abraham *et
Discussion

In our study, 36 specimens that tested negative by conventional techniques yielded positive results by all three PCR (\textit{IS6110}, \textit{MPB64} and \textit{ESAT-6}) assays used.

In our sample set, 89/300 (29.67%), 74/300 (24.67%), and 60/300 (20%) were positive by \textit{IS6110}, \textit{MPB64}, and \textit{ESAT-6} based PCR, respectively. Of 22 samples that were positive by LJ culture, 6/22 (27.27%), 7/22 (31.12%), and 9/22 (40.91%) samples turned out to be negative by the respective PCR based assays. Out of these, 4 samples were identified as NTM by biochemical tests. We used primers that were specific for \textit{Mycobacterium tuberculosis}, so NTMs were not detected in our PCR assays. With the rising incidence of infections due to NTM, it is important to include primers that are genus specific.

The 2 samples which were not detected as positive by \textit{IS6110} PCR were detected by \textit{MPB64} PCR. The 3 samples which were missed by \textit{MPB64} were detected by \textit{IS6110}. The 5 samples which were missed by \textit{ESAT-6} were identified by either \textit{IS6110} or \textit{MPB64} PCR. Negative results with the PCR assay may be due to the presence of PCR inhibitors, or improper proportioning of the sample, or absence/fewer copies of the insertion sequence (\textit{IS6110}) in some MTB strains. These observations are similar to those reported by other authors, e.g. Negi \textit{et al.} (2005) and Malhotra \textit{et al.} (2012).

Das \textit{et al.} (1995) have reported that 40% of MTB from clinical isolates from South India have either a single or no copy of this target element. Absence or presence of fewer copies of the target sequence in some strains of \textit{M. tuberculosis} has already been reported (Fomukong \textit{et al.}, 1994; Dale \textit{et al.}, 1997; El Baghdadi \textit{et al.}, 1997; Aqasino \textit{et al.}, 1998). Thus, a combination of \textit{IS6110} and \textit{MPB64} PCR was found to have a better sensitivity than either of these PCRs alone, or PCR targeting other genes like \textit{ESAT-6}, as there were no additional samples that were detected by \textit{ESAT-6} based PCR.

Contamination was observed in 11/300 (3.67%) samples, and these were considered negative by culture; 3/11 (27.27%) of these were found to be positive by
either PCR assays. The sensitivity and specificity values obtained in the study indicate that the nucleic acid amplification technique offers many advantages over conventional methods, and seems to be a good diagnostic tool for detecting *M. tuberculosis* in clinical samples.

**Mean Turn Around Time (TAT) and Cost Effectiveness of the Methods**

AFB smear microscopy is an inexpensive and simple technique, and laboratory staff could be trained to perform it relatively quickly and easily. Moreover, the results by AFB smear microscopy are available within hours, but the major drawback is its poor sensitivity. Time taken for detection of *MTB* by PCR is <1 day as compared to conventional methods of culture which require 3–8 weeks for solid culture, and 10–14 days for liquid culture due to the long doubling time of *MTB* (Negi *et al.*, 2005; Oberoi *et al.*, 2007; Malhotra *et al.*, 2012). Additionally, culture methods require technically skilled staff, necessary precautions, good bio-safety practices, and equipments to prevent chances of infection to laboratory personnel, and are relatively expensive. Per test price of the PCR test is also high but its sensitivity and short turn-around time outweighs its cost. Though cost effectiveness is a significant factor especially in resource-limited settings, the cost of losses in terms of lives is far more expensive than any other cost.

In the present study, 22/300 (7.33%) specimens yielded growth on LJ slants, and 29/300 (9.67%) on MB7H9 broth. The rate of detection by culture was found to be higher in our study (7.33%) as compared to other studies, e.g. 0.66% by Ghaleb *et al.*, (2013), 1.83% by Goel *et al.* (2013), 3.08% by Shrivastava G *et al.* (2014), 3.2% by Bhanu *et al.* (2005), 5.5% by Shrivastava I *et al.* (2014), 3.3–10.6% by Manjunath *et al.* (1991), 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), and 48.5% by Negi *et al.* (2005). In the present study, LJ culture was positive in 7.33% samples whereas it was 1.83%, 4%, 4.6%, 5.6%, and 13.6% in recent Indian studies by Goel *et al.*, (2013), Khanna and Aggarwal (2011), Kumar *et al.* (2008), Thangappah *et al.* (2011), and Mani *et al.* (2003), respectively. Kohli *et al.* (2011) could not diagnose any patient on LJ culture. The use of liquid culture increased the rate of detection of *MTB* from these genital specimens. A 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 more advantageous than solid media for the detection of \textit{MTB} (Prasad et al., 2012; Goel et al., 2013).

In our study, PCR was found to be the most sensitive method for detection of Mycobacteria among all the three methods used. PCR was found to be around 10 fold more sensitive than AFB smear, 3 fold more sensitive than liquid culture and 4 fold more sensitive than solid culture. These results are in agreement with an earlier study by Malhotra et al. (2012) in which PCR was found to be 9 fold more sensitive than AFB smear, and 3 fold more sensitive than liquid culture. Bhanu et al. (2005) had reported a 14 fold higher sensitivity of the PCR technique in comparison to smear examination. The higher sensitivity of PCR as reported by Bhanu et al. (2005) may be attributed to the lower smear positivity and smaller sample size in their study. Moreover, positivity depends crucially on sample collection from the correct site, and the presence of TB infection in the tested patient’s samples.