3.0. Materials and Methods
3.0. Materials and Methods

3.1. Materials

3.1.1. Data sources and mode of ascertainment

Secondary data of all pulmonary tuberculosis cases registered under the Revised National Tuberculosis Control Programme (RNTCP) in the Pune Municipal Corporation (PMC) area was collected. Data was collected for a four year period between July 1997 to June 2001. Data was collected from tuberculosis registers and quarterly reports from a major Tuberculosis Unit (TU) in the Pune PMC area. Data accessed from quarterly report is shown in appendix A.

Data obtained from the quarterly reports was cross checked by verification of cases from tuberculosis registers for the respective years.

3.1.2. Data entry and variables

Data was entered into Microsoft Excel worksheet (version 2000). Data of a four year period (between July 1997 to June 2001) was collected for the following:

a. Total number of chest symptomatics registered in the PMC area under the RNTCP between July 1997 to June 2001.

b. Total number of tuberculosis cases diagnosed in the PMC area on the basis of smear microscopy i.e. (smear positive cases of pulmonary tuberculosis) between July 1997 to June 2001.
c. Total number of cases identified on the basis of radiological examination and other clinical criteria (smear negative cases of pulmonary tuberculosis) between July 1997 to June 2001. Data on the total number of chest symptomatics screened, and total number of smear positive and smear negative cases diagnosed as pulmonary tuberculosis cases under the RNTCP over a four year period (between July 1997 to June 2001) was compiled.

3.1.3. Analysis of data

Diagnostic and screening efficiency of current routinely used diagnostics was determined through this analysis. Screening efficiency was defined as the number of pulmonary tuberculosis cases diagnosed using a given diagnostic from amongst all symptomatics. Diagnostic efficiency was defined as the number of tuberculosis cases diagnosed on the basis of positive smear results or other diagnostics in the diagnostic paradigm amongst all pulmonary tuberculosis cases.

3.1.4. Bacterial culture media

**Löwenstein Jensen (LJ) medium (Himedia # M-162):**

Per liter

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Löwenstein Jensen base</td>
<td>23.31 g</td>
</tr>
<tr>
<td>Monopotassium phosphate</td>
<td>2.4 g</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.24 g</td>
</tr>
<tr>
<td>Magnesium citrate</td>
<td>0.60 g</td>
</tr>
<tr>
<td>L - asparagine</td>
<td>3.6 g</td>
</tr>
</tbody>
</table>
3.0 Materials and Methods

Potato flour 30 g
Malachite green 0.40 g
Glycerol 15.62 ml
Eggs 625 ml

3.1.5. Staining of slides

Ziehl Neelsens Carbol Fuchsin Method

1% Carbol Fuchsin
20% Sulfuric acid.
0.1% Methylene Blue

3.1.6. Processing of sputum samples and isolation of DNA

Sodium Hydroxide (4% NaOH)
0.002% Phenol red indicator
8% HCl
Proteinase K buffer for lysis of Mycobacteria
10mg/ml Proteinase K
10% SDS
TE (10mM Tris.HCl pH 8.0, 1mM EDTA pH 8.0) stored at -20°C

3.1.7. Amplification of DNA isolated from sputum samples

Buffer

10X Taq DNA polymerase buffer (100mM TAPS pH 8.8, 500mMKCl, 15mM MgCl₂)
2.5mM dNTP mix (deoxy Nucleotide Tri-Phosphate)
Taq (Thermophilus aquaticus) DNA polymerase
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All reagents from Bangalore Genei Pvt. Ltd., Bangalore.

**Primers** (Bangalore Genei Pvt. Ltd., Bangalore)

Primers for MPB64 gene

FP -- 5' TTGGCGAGCATCTACATGCT 3'

RP -- 5' CTAAATGTGTCCAGAAGCCAT 3'

3.1.8. Agarose Gel electrophoresis

2% Agarose (Bangalore Genei, India #AG-1)

Tris Borate EDTA Buffer (TBE 5X) – 54 gm Tris base, 27.5gm boric acid, 20 ml EDTA.

Gel loading buffer (6X) - 0.25% bromophenol blue, 40% (w/v) sucrose in water, Stored at 4°C.

3.1.9. Staining of gels

10 mg/ml ethidium bromide. Stored in dark at 4°C.

3.1.10. Standards

Mc Farlands standard

1% solution of anhydrous barium chloride (0.1 ml)

1% cold sulfuric acid (9.9 ml)

(Kept in the refrigerator until a fine white precipitate of barium sulfate became visible after vigorous shaking)

3.2. Methods

3.2.1. Ethical considerations

Prior to testing of clinical samples brainstorming sessions with Chief Medical Officer (Tuberculosis), Chief Pathologist, and Radiologists of the
Tuberculosis Unit were organized. Necessary permission from the PMC authorities was taken to test the sample after it had been processed for all investigations at the Tuberculosis Unit. Diagnostic and treatment decisions were made by site physicians according to the Revised National Tuberculosis Control Program (RNTCP) guidelines and results of PCR were not to influence diagnostic decision making.

3.2.2. Collection of samples

Sputum samples were collected in disposable sputum cups which were sealed appropriately to prevent leakages and transported in appropriate sample carriers. 144 respiratory samples (sputum with or without saliva) from 144 individuals were collected and tested by sputum smear microscopy, culture and PCR. Of these, 123 samples were from 123 individuals suspected of having pulmonary tuberculosis. The remaining 21 samples which formed the control group, were from hospital patients with diseases other than tuberculosis.

i. Collection of samples from Tuberculosis Unit

Ninety seven samples (single sample) were taken from 97 highly probable tuberculosis suspects who were referred to or who presented at a tuberculosis clinic in the PMC area. Early morning sample was collected in screw capped sputum containers. Sample was opened in sterile conditions under laminar flow at Tuberculosis Unit and used for sputum smear microscopy. The same sample was then stored in refrigerator not more than 24 hours and then transported to University laboratory for further processing.

ii. Collection of samples during morbidity survey from a slum
Thirty Materials and Methods

Twenty six samples were collected from 26 suspects identified during a morbidity survey from a slum community. A cross sectional survey of households was carried in an urban slum. Interviews were conducted by a team comprising of a field supervisor and eighteen post graduate (Health Sciences) students using a pre-structured questionnaire (Appendix C). The interview respondents were adult members of the households studied. Through this survey active case finding of those with a prolonged cough was carried out (affirmative response when asked if they had a cough). A list of symptoms like cough for greater than three weeks with or without sputum, low grade fever in evening, weight loss, chest pain were used to classify symptomatics. All respondents who gave an affirmative response to one or more than one of the symptoms associated with pulmonary tuberculosis were referred to a government dispensary nearby.

Sputum samples were collected by holding health camps (n=12), referring symptomatics to nearby health centers (n=1) and collection of samples by health workers (n=13). Smears were processed using the Ziehl-Neelsen method performed at the Municipal Laboratory as prescribed under RNTCP. The same samples were used for culture on Löwenstein Jensen culture media and PCR using primers specific for MPB64.

iii. Collection of samples during from hospital controls

Twenty one samples from 21 hospital patients with a disease other than tuberculosis were examined by all three diagnostics described above.

These data were compared with available clinical (n=144) and radiological (n=61) data. The study was conducted blind. Technicians performing
smear microscopy and radiologist/clinician reading X-ray films were blind to results of PCR. All samples were coded and taken to the laboratory for culture and PCR. Culture was performed at the laboratory by the researcher, and the culture bottles were then transported to the Tuberculosis Unit and interpreted by pathologists at the Unit to avoid bias.

3.2.3. Isolation of DNA

i. Preparation of positive control

Isolation of DNA from respiratory samples was carried out in a class II biosafety cabinet in a designated biosafety laboratory, following universal biosafety precautions. Biohazardous waste was disposed appropriately in separately maintained beakers filled with hypochlorite, autoclaved at 15 lb pressure in an isolated biohazard autoclave and then incinerated.

a) Two to three colonies of *M. tuberculosis* of H37 Ra were suspended in 30μl of TE buffer and vortexed vigorously to resuspend the cells. The suspension was heated in a water bath at 85°C for sixty minutes in order to kill the bacilli. Then 30μl of the bacterial suspension of was treated with proteinase K lysis buffer at 37°C for sixty minutes. The lysate was then treated at 100°C for ten minutes to inactivate proteinase K. 10 μl of lysate was used for PCR.

b) A single colony of *M. tuberculosis* H37 Ra was picked up with a sterile needle, and suspended into 30μl of sterile distilled water. The suspension was vortexed, and boiled at 100°C for ten minutes to lyse the bacilli.

c) McFarland's standard with a density corresponding to approximately 3 x 10⁸ mycobacteria/ml of suspension was prepared. Solution of the *M. tuberculosis*
positive control H37 Ra corresponding to McFarland No. 1 standard was prepared by sequentially diluting the sample ten-fold. Concentrations of *M. tuberculosis* from $10^8$ to $10^1$ bacilli/ml were obtained. DNA extraction was performed by boiling 1 ml of H37 Ra *M. tuberculosis* dilution for 10 minutes and centrifuging the samples for 10 minutes at 10,000 rpm. The resulting supernatant was used for PCR.

**ii. Isolation of DNA from clinical samples**

Sputum samples were decontaminated and concentrated by a modified Petroff’s method. Equal amount of 4% NaOH was added to the sample, mixed and incubated in a water bath at 37°C, for less than twenty minutes for digestion. The solution was centrifuged at 10,000 rpm, for ten minutes at room temperature. The supernatant was decanted, a drop of phenol red indicator was added to the pellet and vortexed. The sample was then neutralized with 0.8% HCl and then centrifuged at 10,000 rpm for ten minutes at room temperature. The supernatant was removed and the pellet was washed with 1ml distilled water, vortexed and transferred to micro-centrifuge tubes. The resuspended samples were used for culture by streaking on LJ medium and for PCR as described below. The resuspended samples were centrifuged at 10,000 rpm for ten minutes in a microfuge, and washed once more with sterile water. The resulting pellet was resuspended in proteinase K buffer having a final concentration of 2% SDS and 1 mg/ml Proteinase K for lysis of Mycobacteria (described in Materials) and incubated overnight in a water bath at 37°C. Proteinase K was inactivated by heating at 100°C for ten minutes.
3.2.4. Amplification of DNA from respiratory samples

PCR was performed in three different areas physically separated from each other. DNA was extracted and added to the reaction mix in the biosafety cabinet as described above, the post amplification identification was done in another part of the laboratory and the reaction mix (without template) was made in a sterile laminar flow hood.

Amplification reaction was typically performed in 50μl volume reaction mix containing 0.25nmole of forward and reverse primer, 2mM final concentration of dNTP, 1.5 U of Taq Polymerase in 1X Taq Polymerase buffer and 10μl of extracted DNA. Primers specific for M. tuberculosis complex specific gene MPB64, having a molecular weight of 22,400 were used. The reagents were mixed thoroughly and amplified on an MJ Research Mini Cycler using the following conditions:

PCR cycles: 40
Initial denaturation 95°C for 5 minutes
i) Denaturation: At 95°C for one minute
ii) Annealing: At 55°C for one minute
iii) Extension: At 72°C for one minute
Final extension 72°C for 7 minutes

The general precautions used in all these PCR reactions were as follows:

- All the reagents were stored at -20 degrees Celsius.
- Positive displacement pipettes were used.
- Fresh tips and PCR tubes were used every time.
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- The pre PCR, actual PCR and post PCR work was carried out in separate areas.
- All the reagents and the PCR mix were centrifuged briefly prior to amplification.

3.2.5. Agarose gel electrophoresis

Agarose gel 1.5 % (1.5 g in 100ml) was prepared in 0.5X TBE. The amplified product was loaded on the gel after mixing with gel loading buffer to a working concentration of 1X. 0.5X TBE was used as running buffer. The products were electrophoresed at a constant voltage of 5 V/cm of gel at room temperature. 100 bp molecular weight marker was resolved simultaneously.

3.2.6. Staining of gel

Agarose gels were stained for 10-15 minutes with ethidium bromide (10 mg/ml) used to the working concentration of 0.5 µg/ml. Alternatively DNA samples were loaded on a 1.5% gel with 0.5µg/ml of ethidium bromide in it. The stained gel was visualized on a UV transilluminator (Vilber Lourmat, France).

Alternatively gels were visualized under ultraviolet light and the image was stored in the gel documentation system (Biorad, Quantity One Quantitation Software, Version 4).

3.2.7. Strains and culture of Mycobacteria

Non-pathogenic strain, *Mycobacterium tuberculosis* H37Ra was obtained from National Tuberculosis Research Institute, Bangalore and used as a positive control. The strain was then cultured on Löwenstein Jensen's egg based medium. The medium was prepared as follows according to the manufacturers
3.0 Materials and Methods

3.73 grams of LJ base (Hi Media, India) was added to 60ml of distilled water and 1.2 ml of glycerol was added to it. The solution was boiled to dissolve the base completely and autoclaved. The surface of the eggs were washed with soap and water followed by distilled water and swabbed with ethanol. Eggs were kept under ultra violet light for twenty minutes. Eggs were broken in the laminar flow and 100 ml of egg solution was mixed with the autoclaved base, with constant stirring. The medium was poured in glass culture bottles and kept for inspissation at a slanted position in the water bath at 85°C for forty-five minutes. Medium was allowed to cool to room temperature before being used for culture of *M. tuberculosis* 37Ra. Culture of bacilli from suspected sputum samples was done by streaking 100 µl of concentrated sample. Culture was put up at the University laboratory and bottles were then maintained at the municipal laboratory.

Culture bottles were maintained at 37°C in an incubator. Culture results were monitored at one, two and four weeks and reported positive if growth was found after five to six weeks. Positive cultures were confirmed by microscopy for AFB. Cultures were declared negative if there was no growth by twelve weeks. Characterization of Mycobacteria was done at the Corporation laboratory by primary differential tests for atypical Mycobacteria. Results were interpreted by chief pathologist at the Municipal laboratories, to eliminate bias.

Other strains of Mycobacteria, *M. bovis*, *M. microti*, *M. intracellulare*, *M. bovis BCG*, *M. kansasii* were obtained from National Institute of Leprosy And Other Mycobacterial Diseases, Jalma. Strains were maintained and sub cultured at 37°C.
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3.2.8. Acid fast staining

Acid fast staining was done with the Ziehl Neelsons Carbol Fuchsin method as follows. A smear was made using 10 μl of the *M. tuberculosis* suspension, air dried and heat fixed. The slide was flooded with 1% carbol fuchsin for 5 minutes and flamed (using a low flame) for another 5-10 minutes. Care was taken to ensure that the slide did not dry. The slide was then washed and decolourised with 20% sulfuric acid for 1 minute, washed again and counterstained with 0.1% methylene blue for 30 seconds, air dried and observed. Acid fast bacilli were counted using a light microscope and recorded.

3.2.9. Calculation of efficiency

As no single gold standard was available for comparison of the performance of the individual tests, analysis of results was done using a variety of standards. Efficiency of microscopy, culture and PCR in terms of sensitivity, specificity, positive predictive value and negative predictive value was done using the gold standard of culture for the culture positive samples and smear microscopy, combined microbiological data, chest radiographic findings and clinical follow up data for culture negative samples.

Description of parameters used

i. **Culture:** part of the sample brought to the laboratory was put up for culture in screw capped bottles at 37 degrees celcius, on LJ medium. The culture bottles were monitored weekly. Any mycobacterial growth within 4-6 weeks was considered positive. All vials were monitored for growth for a minimum of 8-12 weeks before being considered negative. Primary differential tests were done for
3.0 Materials and Methods

atypical mycobacteria wherever required. To eliminate bias culture results were interpreted by chief pathologist at PMC laboratory.

**ii. Smear status:** scoring of slides was done as prescribed under RNTCP. Data on type of sample, gradation, and date of the smear examination recorded as per RNTCP guidelines (Appendix B) were noted down after the interpretation of PCR results to eliminate bias.

**iii. PCR result:** the PCR result was noted as positive, and negative.

**iv. Clinical probability:** To determine the clinical probability of the cases various details were recorded from the available clinical data maintained at the Tuberculosis Unit. These included signs and symptoms, duration of first symptoms, previous history/family history, reports of all the tests done like smear status, radiological profile, tuberculin response, ESR, clinical records, response to broad spectrum antibiotics (Appendix D), and response to antituberculous drugs in case of patients (Appendix E). These and consultation with the chest physician at the clinic were used to determine the clinical probability of the cases.

All symptomatics who were positive by PCR only were followed up by health worker and medical doctor within a year of first presenting to the clinic and medical evaluation was done (Appendix F). Those cases who reported to have taken ATT later were considered as cases of tuberculosis.

**v. ATT response:** The ATT response of the patients was noted done at 2, 4, and 6 months. Parameters used to determine the positive response to ATT were weight gain, and conversion of smear status from positive to negative at end of 2, 4, and
6 months in case of smear positives, and radiological profile of the patient at the end of treatment in case of smear negatives (Appendix E).

All these parameters were considered to calculate the sensitivity, specificity, positive and negative predictive value using the formulae outlined below.

**Table 9: Table for calculation of sensitivity, specificity, efficiency, positive and negative predictive value**

<table>
<thead>
<tr>
<th>TEST</th>
<th>Disease</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Positive</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Negative</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>Total</td>
<td>A+C</td>
<td>B+D</td>
</tr>
</tbody>
</table>

**Sensitivity** = \( \frac{a}{a+c} \)

**Specificity** = \( \frac{d}{b+d} \)

**Positive predictive value** = \( \frac{a}{a+b} \)

**Negative predictive value** = \( \frac{d}{c+d} \)

**Efficiency** = \( \frac{a+d}{a+b+c+d} \)

### 3.2.10. Cost effective analysis of PCR to routine diagnostics

Cost comparison of PCR to three routinely used diagnostic tools for diagnosing *M. tuberculosis* i.e. sputum smear microscopy, radiological examination by X-ray, and culture was done as per the methodology used by Roos *et al.*, and Cook *et al.* The cost components included material costs media bases,
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chemicals, reagents, glassware and plasticware, labour costs, costs of equipment, and drug costs. Material and equipment costs for routine diagnostics were provided by the RNTCP. Information on quantities were as prescribed by the programme (Appendix G). Data on materials and equipment needed for PCR was based on the materials and equipment needed for the test standardized in the laboratory. Staff salaries (labour costs) required for routine diagnostics were provided by the programme and for culture and PCR were calculated taking into consideration the set up of a research laboratory of the University.

Prices on drugs are based on report by Muniyandi et al. Costs related to treatment of false positives were divided into drug costs. The number of false positives was derived from estimates and literature. In this analysis a lower percentage was used. The sensitivity and specificity of PCR are calculated based on results obtained in the laboratory. The cost of false negatives i.e. debilitating effect on patients and transmission of disease was beyond the scope of this study. Costs related to the costs of running of the clinic and the laboratory such as cost of buildings, training, supervision and overheads were not included in the study.

The unit costs including equipment costs, material costs, and labour costs were calculated taking into consideration the number of samples per year which were based on an average for total symptomatics screened in the Pune PMC area for tuberculosis (based on retrospective analysis of four year data in Pune PMC described in results). The number of samples per day were calculated as total samples per year/ number of working days. Number of working days were
calculated as 269 days i.e. 365 – (52 Sundays + 24 working holidays + 20 days leave).

3.2.11. Calculation of equipment, labour and material costs

i. Equipment costs

Equipment required for diagnosis of tuberculosis using sputum smear microscopy and radiological examination by X-ray were listed on basis of methodology used in the programme and that for culture and PCR based on the set up of the research laboratory. Equipment cost per sample was calculated using the formula (cost of equipment x rate of depreciation) per total number of samples per year. The rate of depreciation for all equipment was taken as 7.07% (as per schedule XIV of the Company's Act straight line single shift method).

ii. Labour costs

The direct labour cost for performing a given test was determined from the yearly salary of the technician based on the methods used by Cook et al. The labour cost was defined as cost per hour with the assumption that the technician worked for eight hours per day for 269 days. The number of labour minutes required for the performance of each test was multiplied by the derived cost per minute of technologist time to determine the exact labour cost. Calculation of direct labour cost required an estimation of the minutes required for test performance.

Test performance was divided into preanalytical, analytical and post analytical activities. Preanalytical activities were defined as those activities associated with preparation of test like the preparation and labeling of reagents,
and preparation of work area. Analytical activities were defined as the actual test performance and interpretation of results. Post analytical activities involved were report making and documentation of the results. Labour costs per hour were calculated using the formula: Annual salary/number of working days and then costed as per the time required to process a batch of average number of samples per day.

\textit{iii. Material costs}

Comparative material costs for sputum smear microscopy, X-ray, culture, and PCR were calculated as per the methodology used by Roos \textit{et al.} Costs for each of the diagnostic techniques were calculated per sample/examination. Unit costs were calculated for a single smear examination, for culture it was calculated for 10 ml of Löwenstein Jensen medium. Material costs for radiological examination by X-ray were calculated for a single X-ray plate (12 x 15) as used in the programme in Pune PMC. Costs for PCR were calculated based on an in-house PCR test using MPB64 primers for a 50 μl amplification mix.

Indirect material costs of reusable glassware and plasticware for each diagnostic for a year were divided by the total number of samples in year to derive the cost per sample.

\textbf{3.2.12. Cost effective analysis: routine and anticipated procedure}

The cost effective analysis compared the routine diagnostic procedure currently in use under the Revised National Tuberculosis Control Program with a theoretical anticipated PCR procedure. In the current routine
method chest symptomatics (especially, those having cough for three weeks or more) are offered sputum examinations. Three samples are collected: a spot specimen on the first day, and early next morning and a spot specimen on the second day. Patients with two or three positive smears are immediately started on treatment. Symptomatics with one positive smear undergo further investigations with broad spectrum antibiotics repeat sputum examination and radiological examination by X-ray. Those with no positive smears are prescribed a course of antibiotic treatment and re-examined with three sputum examinations. Those positive are started on ATT the non responders further undergo chest X-ray and are re-evaluated. Patients are categorized into appropriate groups and are referred for treatment.

The anticipated PCR procedure assumes that when a person with tuberculosis symptoms presents at the clinic a single sample is collected on the spot. The sample is then subjected to PCR examination. The results will be available on the same or the next day. Persons with negative results are assumed to have no tuberculosis and may be referred for further examination or treatment of their symptoms. The assumption made is that use of X-ray for diagnosis of tuberculosis will not be necessary.

The model for the routine diagnostic procedure is based on the set up at a Tuberculosis Unit under the RNTCP in the Pune PMC area. The model for the anticipated PCR technique is based on the set up of a research laboratory of an University. The baseline values for sensitivity and specificity of microscopy are obtained from literature and that for PCR were based on results from test
standardized in laboratory. Number of false positive were assumed to be the same by both procedures i.e. patients with routine procedure 10% and with PCR 10%. It was assumed that 100% of patients with a negative smear were put on broad spectrum antibiotics and examined by repeat sputum examinations of which 10% were diagnosed by repeat sputum examinations and the remaining were subjected to radiological examination by X-ray.

Muniyandi et al have reported that the unit cost of treating a smear positive case is Rs 392 and that of a smear negative case is Rs 277. Thus based on this report the cost of treating a case of tuberculosis is considered as an average of the two i.e. Rs 335. The cost effectiveness was calculated as (cost of diagnosis of all cases) + cost of treating false positives / correctly diagnosed cases.

Steps in diagnosis and caseload using routine procedure and anticipated PCR procedure were calculated as shown in the Table 10a.b.

**Table 10a: Steps in diagnosis and caseload using routine procedure**

<table>
<thead>
<tr>
<th>Step code</th>
<th>Steps in diagnosis</th>
<th>Calculation formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>TB suspects</td>
<td>20% A</td>
</tr>
<tr>
<td>B</td>
<td>Total diagnosed TB cases</td>
<td>65% B</td>
</tr>
<tr>
<td>C</td>
<td>Smear +</td>
<td>A-C</td>
</tr>
<tr>
<td>D</td>
<td>Smear – suspects</td>
<td>10% B*</td>
</tr>
<tr>
<td>E</td>
<td>Smear + (repeat sputum)</td>
<td>100%(D - E)</td>
</tr>
<tr>
<td>F</td>
<td>X-rays / antibiotics</td>
<td>25% B</td>
</tr>
<tr>
<td>G</td>
<td>Smear - cases</td>
<td>A-B</td>
</tr>
<tr>
<td>H</td>
<td>Non TB cases</td>
<td>10% G</td>
</tr>
<tr>
<td>I</td>
<td>False positives (smear negative cases)</td>
<td>C+E+G-I</td>
</tr>
<tr>
<td>J</td>
<td>Correctly diagnosed TB cases</td>
<td>2 (C+E)</td>
</tr>
<tr>
<td>K</td>
<td>Real number of TB cases</td>
<td>K - J</td>
</tr>
<tr>
<td>L</td>
<td>Number of TB cases missed</td>
<td></td>
</tr>
</tbody>
</table>
### Table 10b: Steps in diagnosis and caseload using anticipated procedure

<table>
<thead>
<tr>
<th>Step code</th>
<th>Steps in diagnosis</th>
<th>Calculation formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>TB suspects</td>
<td>91.5% H*</td>
</tr>
<tr>
<td>B</td>
<td>Total diagnosed TB cases</td>
<td>B</td>
</tr>
<tr>
<td>C</td>
<td>PCR +</td>
<td>A - C</td>
</tr>
<tr>
<td>D</td>
<td>PCR -</td>
<td>10%B</td>
</tr>
<tr>
<td>E</td>
<td>Non Tb cases</td>
<td>B - F</td>
</tr>
<tr>
<td>F</td>
<td>False positives</td>
<td>2 (C)*</td>
</tr>
<tr>
<td>G</td>
<td>Correctly diagnosed TB cases</td>
<td>H - G</td>
</tr>
<tr>
<td>H</td>
<td>Real number of TB cases</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Number of TB cases missed</td>
<td></td>
</tr>
</tbody>
</table>

* Based on sensitivity data from previous study and literature search.

Various assumptions were made to determine the cost effectiveness. Based on results of PCR test standardized for MPB64 primers done in the laboratory sensitivity of microscopy was taken to be 51% and sensitivity and specificity of PCR was taken as 91.5% and 86% respectively. Number of false positive patients with routine procedure and with PCR were considered to be 10%. It was assumed that 100% of patients with a negative smear were put on antibiotic treatment and repeat sputum examination of which 10% were identified by repeat sputum examination. The remaining were then examined by X-ray.