INTRODUCTION

Tuberculosis remains the number one killer infectious disease affecting adults in developing countries. The 1990 WHO report on the Global Burden of disease ranked TB as the seventh most morbidity causing disease in the world and expected it to continue in the same position up to 2020. Each year, 8.74 million develop tuberculosis and nearly 2 million die. This means that someone somewhere contracts TB every four seconds and one of them dies every 10 seconds. WHO 2006 report on global Tuberculosis control published on March 24th, world TB Day, once again ranks India as world's most heavily affected country. It was estimated that there were 1.8 million new TB cases in India in 2004; that is, one in five of all cases worldwide. Roughly, 330000 people died with TB in 2004. Nearly 1000 people every day. These figures put India some way ahead of the second ranking country, China, which had about 1.3 million new episodes of TB in 2004. In 1993, WHO declared TB a global emergency and devised the directly observed treatment short course (DOTS) strategy and recommended that all countries adopt this strategy.

The strategy is built on five pillars, viz. political commitment and continued funding for TB control programmes, diagnosis by sputum smear examination, Uninterrupted supply of high quality anti-TB drugs, Intake under direct observation, and Accurate recording and reporting of all registered cases. The change in number of sputum from three to two under RNTCP was a retrograde step made by research support. In addition, the RNTCP recommends exa of two sputum smears in two days for diagnosis. This may not be practicable under all condition, especially in difficult areas. It further adds to the delay the diagnosis & initiation of treatment, increase spread of disease in community and causes inconvenience to patients as well as for the health system.

Pulmonary TB represents an important worldwide public health problem. Tuberculosis remains a leading cause of death
globally. In 2005 there were an estimated 8.8 million new cases of tuberculosis worldwide\textsuperscript{1}. Incidence of tuberculosis is greatest among those with conditions impairing immunity\textsuperscript{20}, such as diabetes. In India 18\% of patients with pulmonary tuberculosis have diabetes\textsuperscript{1}.

Tuberculosis remains a major cause of mortality in developing countries and in these countries, diabetes prevalence is increasing rapidly\textsuperscript{2}. At present an epidemic of DM is ongoing both in developed and developing countries. With recognition of this explosive increase in number of people diagnosed with DM all over the world, a whole new field of related interaction between DM and pulmonary tuberculosis has been thrown open\textsuperscript{3}. Consistent with this, a recent case control Study from India of risk factors for TB found a univariate odds ratio of 1.8 for previously diagnosed diabetes, which strengthened to 2.44 when controlled for other risk factors, including low socioeconomic status.

Studies have noted that the risk of developing TB was 11 to 18 times greater in Diabetics than in normal population\textsuperscript{2}. Increased reactivation of tuberculosis lesions has also been recorded in diabetics. At the same time, tuberculosis appears to aggravate hyperglycaemia, with patients requiring higher than before doses of insulin.

As much as 14.8\% of pulmonary tuberculosis and 20.2\% of smear-positive i.e. infectious tuberculosis may be directly linked to diabetes. Diabetes is also probably responsible for
the urban incidence of smear positive tuberculosis being 15.2% higher than that in rural areas\textsuperscript{3}.
**PROFORMA**

Name:                         Date of Admission:  
Age:                          Date of Discharge:    
Sex:                          Indoor/ Outdoor No:  

History:                     

C/C:                          

<table>
<thead>
<tr>
<th>COMPLAINTS</th>
<th>DURATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough with expectoration</td>
<td></td>
</tr>
<tr>
<td>Breathlessness</td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td></td>
</tr>
<tr>
<td>Weight loss</td>
<td></td>
</tr>
<tr>
<td>Hemoptysis</td>
<td></td>
</tr>
<tr>
<td>Anorexia</td>
<td></td>
</tr>
<tr>
<td>Weight loss</td>
<td></td>
</tr>
<tr>
<td>Chest pain</td>
<td></td>
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</tbody>
</table>

Past History:                     

History of Tuberculosis          

History of Diabetes              

Duration                           

Treatment -OHA
-Insulin

Immunization status of BCG

History of IHD/ HT

Family History:

History of Tuberculosis

History of Diabetes to any Family member

Personal History:

Diet: Veg. / Non Veg.

Appetite: decreased/ increased

Sleep: normal/ disturbed

Bowel /Bladder

Habit: Smoking

Alcohol

• GENERAL EXAMINATION:

• Vitals: T : Pallor

P : Clubbing

BP :

Lymphadenopathy

RR : Cyanosis

Icterus

Edema feet
- **Respiratory system examination**
  - **Inspection**
  - **Palpation**
  - **Percussion**
  - **Auscultation**

- **Other systems examination:**

- **INVESTIGATIONS:**

- **Blood Investigations**
  - CBC with ESR, LFT, RFT
  - RBS, FBS, PP2BS, HB1AC, S. lipid profile

- **Radiological Investigations:**
  - X-ray chest
  - USG Thorax, USG KUB (if Required)
  - CT Thorax (if required)

- **ECG**

- **Urine Routine**

- **Urine culture (if required)**

- **Sputum Examination:**
  - Sputum AFB - Mycobacteria
  - Culture AFB (if required)
- CBNAAT (if required)
- LPA (if required)
  - Sputum C/S (Pyogenic organism)
  - Mantoux test
  - Bronchoscopy (if required)
    - BAL AFB
**laboratory materials**

The following laboratory materials should always be available in the laboratory;

**Chemical & Reagents**
- Carbol fuchsin (1%)
- Sulphuric acid (25%)
- Methylene blue (0.1%)
- Synthetic immersion oil
- Methylated spirit
- 5% Phenol
- Silica gel

**Consumables**

- Glass slides for microscopy & slide boxes for storing slides
- Diamond markers
- Broom sticks
- Glass rods
- Staining racks
- Sputum containers
- Spirit lamp
- Foot operated bin
- Lens paper
Filter paper

**Stationery**

Laboratory forms for sputum examination

Laboratory Register

Estimated quantity of reagents & materials required for 1000 smears

Carbol fuchsin(1%)—5000ml

Methylene blue(0.1%)—3000

Sulphuric acid(25%)—6000

Immersion oil—50ml

Phenol 5%--200liter

Methylated spirit—1000ml

Filter paper(whatmann no-1 pack of 100)—1 pack

Filter paper—1 pack

Lens paper(book of leaves)—20sheets

Lint cloth or fine silk—5

Diamond marker—4

Grease pencils or marking pens—4

Sputum containers—1100

Broom sticks—1100

New glass slides—1100
Slides box (100 slides) — 11
Black/Red disposal bags of bio degradable material — 100
Silica gel — 100 gms
MORPHOLOGY OF M. Tuberculosis

- Slightly curved or straight bacilli.
- 0.2 to 0.6 by 1.0 to 10 µm size.
- Filamentous or mycelium like growth may occur.
- Cell wall with high lipid content that includes mycolic acid with long branched chain.
- The mycobacterial cell-wall contains mesodiaminopimelic acid (DAP) and outer lipid bilayer.
- The envelope consists of two distinct parts- The plasma membrane and around it the cell wall.
- Mycobacterial membrane has some distinctive components notably the lipopolysaccharides, lipoarabinomannan (LAM), lipomannan and phosphotydyl inositol
- Cell wall core is composed of three covalently attached macro molecules: peptidoglycan, arabinoglycan and mycolic acid.

Staining:

- Not readily stained by Gram’s Stain.
- Ziehl – Nielsen Stain is required to stain bacilli.
- Once bacilli have been stained they are not easily decolorized by acid or alcohol. This resistance to decolorization is called acid-fastness. Acid-fastness is due to mycolic acid.
• Fluorescence stain may be useful in some cases.
RESULTS

Name of DMC---------SMIMER--------------------------------------------------------------
Lab Serial No-------------------------------------------------------------------------

<table>
<thead>
<tr>
<th>Date of Exam.</th>
<th>Specimen</th>
<th>Visual Appearance (M,B,S)</th>
<th>Results Neg. or (pos)</th>
<th>Positive(grading)</th>
<th>3+</th>
<th>2+</th>
<th>1+</th>
<th>Scanty*</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c*</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

M=Mucopurulent, B=Blood stained, S=Saliva

AFB seen in 100 oil immersion fields

a=Spot sample

b=Morning sample

c*=Same day sputum after First sample "a" (1-day protocol)

Date--------------------------- Signature of Lab. Technician.
Results

Total no patients=440

No. of patients did not deposits Morning sample (2-Day sample)=16 ie=3.6%

No. of patients in which only morning sample are deposited=2

No. of patients in which All three samples are Negative=6

No of patients in which 1st sample spot ie(a) are Negative=12

No of patients in which 2nd spot sample ie (b) are Negative=5

No of patients in which All three sample with Scanty AFB present=24

<table>
<thead>
<tr>
<th></th>
<th>1st spot(a) 1-Day</th>
<th>2nd spot(b) 1-Day</th>
<th>Morning sample 2nd day (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of pts with Grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1+</td>
<td>140</td>
<td>155</td>
<td>112</td>
</tr>
<tr>
<td>2+</td>
<td>111</td>
<td>112</td>
<td>105</td>
</tr>
<tr>
<td>3+</td>
<td>131</td>
<td>133</td>
<td>162</td>
</tr>
<tr>
<td>Scanty AFB</td>
<td>37</td>
<td>25</td>
<td>36</td>
</tr>
<tr>
<td>Total</td>
<td>419</td>
<td>425</td>
<td>415</td>
</tr>
<tr>
<td>% of Total</td>
<td>95.2%</td>
<td>96.5%</td>
<td>94.3%</td>
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</table>

A Total of 440 cases were recruited in the study

Sixteen patients (3.6%) did not report to the laboratory on the second day.

The 2-day protocol ie (Morning sample) was capable of detecting 415 patients (94.3%) were smear positive.
In the 1-day 1\textsuperscript{st} spot sample ie (a) protocol 419 patients(95.2\%) were smear positive.

In the 1-day 2\textsuperscript{nd} spot sample ie (b) protocol, 425 patients (96.5\%) were smear positive.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
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</thead>
<tbody>
<tr>
<td>Valid</td>
<td>440</td>
<td>50.1</td>
<td>50.1</td>
<td>50.1</td>
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<tr>
<td>F</td>
<td>98</td>
<td>11.1</td>
<td>11.1</td>
<td>61.2</td>
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<tr>
<td>M</td>
<td>342</td>
<td>38.7</td>
<td>38.7</td>
<td>99.9</td>
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<tr>
<td>Total</td>
<td></td>
<td>100.0</td>
<td>100.0</td>
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</table>

Report

<table>
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<tr>
<th>Sex</th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
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<tbody>
<tr>
<td>F</td>
<td>29.7449</td>
<td>98</td>
<td>12.74113</td>
</tr>
<tr>
<td>M</td>
<td>36.7632</td>
<td>342</td>
<td>13.75165</td>
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<tr>
<td>Total</td>
<td>35.2000</td>
<td>440</td>
<td>13.83143</td>
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</table>

Statistics

<table>
<thead>
<tr>
<th>Age</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>440</td>
<td>35.2486</td>
<td>13.92763</td>
</tr>
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Discussion

In this paper

We have shown that an alternative 1-day protocol for sputum collection could be equally effective, compared to a 2-day protocol.

The advantages of adopting a 1-day protocol are manifold;

- Reduces the number of patients visits
- Decreases drop-out rates
- Reduces the diseases transmission among the contacts of these patients

Being a more patients-friendly protocol, the 1-day approach could help in achieving and improving the current target of RNTCP to detect 70% of new smear positive cases in the population.
CONCLUSION

The 1-day protocol of sputum collection was found to deliver statistically similar results compared to The currently recommended 2-day protocol.

Similar study needs to be conducted in a broader scale at community-based peripheral Health Institutes, to decide on the feasibility of its incorporation at a National level.
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