



## ORIGINAL RESEARCH PAPER

## Pulmonary Medicine

**SINGLE-DAY APPROACH FOR DIAGNOSIS AND TREATMENT OF PULMONARY TUBERCULOSIS : IMPLICATIONS IN END TB STRATEGY .**
**KEY WORDS:** Pulmonary tuberculosis , Mycobacterium tuberculosis, Sputum microscopy, Acid fast bacillus, Ziehl-Neelsen stain .

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**ABSTRACT**
**Background:** According to Revised National Tuberculosis Control Program (RNTCP) diagnosis of pulmonary tuberculosis in India requires examination of 2 sputum samples collected over two days i.e. 'spot' and next day 'morning' samples.

**Objective:** To assess the feasibility of diagnosing pulmonary tuberculosis by examining 2 spot sputum samples in one day and to compare this approach with current RNTCP protocol.

**Methods:** A total of 375 subjects having cough >2wks were enrolled into the study. 3 sputum samples were collected from each of the study participant; first spot(S1), second extra-spot(S2) sample 1hr after collection of first sample, third morning(M) sample collected next day morning. These specimen were subjected to standard sputum smear microscopy for acid fast bacilli as per RNTCP guidelines. For one-day protocol results of "S1 & S2 "samples and for two-day protocol results of "S1 & M " samples were considered.

**Results:** Number of sputum positive pulmonary tuberculosis cases diagnosed with standard two-day protocol was 119 whereas the experimental one-day protocol diagnosed 120 cases(P value 0.7) . Comparing with standard 2-day protocol this new 1-day protocol had sensitivity 98.32% , specificity 100%, PPV 100%, NPV 99.17% .

**Conclusion:** Single day method can be adopted as the standard diagnostic approach for pulmonary tuberculosis after large scale multi-centre randomized controlled trials.

**Introduction:**

Diagnosis of pulmonary tuberculosis in low income high burden countries often relies on direct sputum smear microscopy for acid fast bacillus(AFB). Since the policy change of RNTCP in April'2009, diagnosis of pulmonary tuberculosis in India requires smear microscopy examination of two sputum samples; one spot and another morning sample, switching from earlier three sputum sample collection over two days<sup>(1)</sup>. From tuberculosis program point of view this approach saves time and cost while providing similar diagnostic efficacy<sup>(2-5)</sup>. However the patients still have to come to health centre on two consecutive days; the time and economic savings for the patient appears negligible<sup>(6)</sup>. WHO in its policy statement of May '2011 has advocated for implementation of single day approach for diagnosis of pulmonary tuberculosis in countries which have successfully implemented the two sputum specimen case finding strategy, especially in settings where patients are likely to default from diagnostic process<sup>(7)</sup>. This study was conducted with an objective to assess the feasibility of diagnosing pulmonary tuberculosis by examining 2 spot sputum samples in one day and to compare this approach with current RNTCP protocol.

**Materials and Methods:**

This study was conducted in the Department of Pulmonary Medicine, SCB Medical college between Septemeber'2010 to August'2012. It was a cross-sectional study. Patients attending the Outpatient department with cough for more than two weeks were recruited into the study. The study participants included referred patients from different primary health centres as well as patients coming directly to this hospital. Accordingly duration of cough varied from minimum requisite for this study 2 weeks to maximum 40 weeks. They were informed about the nature of the study in their own language and a verbal consent was taken. Those who had previously taken anti-tubercular therapy for more than one month and those who did not give consent were excluded from the study. A detailed clinical history was taken followed by thorough clinical examination for each study participant. Routine blood investigations and chest x-rays were done to study the clinico-radiological-microbiological associations. Subsequently they were asked to collect and submit three sputum samples ; first a spot sample (S1), second an extra-spot sample (S2) collected one hour after the first sample and the third sample (M) collected on

the next day early morning. To ensure optimum sample quality the method of sputum collection was demonstrated to the study participants by a trained personnel with the aid of visual display board and written instructions whenever necessary. Spot samples were collected under the direct supervision of the laboratory technicians. When the patients coughed up only saliva or did not produce at least 2ml of sputum, they were encouraged to give another better specimen. The smear preparation, Ziehl-Neelsen (ZN) staining and microscopy were done according to RNTCP guidelines, but in a blinded manner by the RNTCP trained technician. The three sputum samples collected from each study participant were given a random code. The microscopy technician as well as the researchers were unaware which sample belonged to which subject. Any one sample positive for acid fast bacillus(AFB) was considered as a case of pulmonary tuberculosis. The performance of 1-day protocol was assessed by results of spot (S1) and extra-spot (S2) samples and the 2-day protocol was assessed with results of spot(S1) and morning (M) samples. Data were entered and analyzed in Epi-info version 2007. Categorical variables were expressed regarding numbers and proportions. Chi-square test was used for showing an association . P< 0.05 was considered significant.

**Results:**

Total number of study participants were 375. Majority(74.7%) were males (M:F=2.95:1). Maximum number of the patients (40.6%) belonged to age-group 21-40. 60.8% of the subjects belonged to BPL (Below poverty line) category. Total number of spot and extra-spot samples collected were 375 each. 18 of these 375 patients defaulted in submission of next day morning samples i.e. the total number of morning sputum samples collected were 357. Flow of patients and study protocol depicted in the flow chart (Fig. 1). Number of sputum AFB positive samples were; 120 in spot (S1), 110 in extra-spot(S2) and 119 in morning(M) sample. Total number of smear positive pulmonary tuberculosis cases in the study were 123 considering the results of all three samples. Number smear positive pulmonary tuberculosis patients diagnosed on 1-day (S1 & S2) protocol were 120. Number of new smear positive cases detected in morning samples which were smear negative in 1-day protocol were only 2 i.e. 1-day protocol missed 1.6% of cases. Whereas 3 out of the 18 dropped out patients were sputum positive for AFB in first spot sample. The

conventional 2-day protocol diagnosed 119 (96.75%) while the experimental 1-day protocol diagnosed 120 (97.56%) of all smear positive tuberculosis patients (Table 1). Chi-Square test was applied for comparison between the two protocols (Table 2). P-value was 0.7, which implies there is no statistical difference between these two diagnostic protocols. Gold standard investigation for diagnosis of pulmonary tuberculosis is sputum culture, but in program setting 2-day spot-morning method is considered as standard approach. When the new 1-day method was compared against 2-day method as gold standard (Table 3); it had sensitivity 98.32%, specificity 100%, positive predictive value 100%, negative predictive value 99.17%.

**Discussion :**

Till date WHO recommends sputum microscopy for diagnosis of pulmonary tuberculosis. Due to low sensitivity of this investigation, number of sputum samples required to be tested to achieve satisfactory diagnostic yield was a matter of intense research<sup>(8)</sup>. Many countries adopted a spot-morning-spot sputum sample strategies in national TB control programmes. After many studies including those from India it was stated in WHO 2007 document that approximately 85.8% patients were diagnosed with first sputum sample. Incremental yield of second sample was 11.9% and that of third sample was only 3.1%<sup>(8,9)</sup>. WHO recommended examination of two sputum samples may suffice in countries with established quality control measures for laboratories. The diagnostic yield of morning sputum sample was higher compared to spot samples and hence RNTCP, India adopted Spot-Morning sputum sample strategy<sup>(8)</sup>. The advantages and disadvantages of three schemes i.e. " Spot-Morning-Spot", "Spot-Morning" and "Spot-ExtraSpot" is discussed in a tabular form in Table-4. The "END TB Strategy" proposed by WHO with a goal to end the 'global tuberculosis epidemic' by the year 2035, has set the targets of reduction of TB deaths by 95% and reduction of TB incidence rate by 90% compared to 2015. Financial protection to TB affected families is a new target of 'End TB strategy' so that "No TB affected families should face catastrophic financial loss due to TB" by 2020<sup>(10)</sup>. With the current RNTCP protocol patients have to come to the health facility on two consecutive days for sputum sample submission. Almost always the patients are accompanied by an adult attendant during these hospital visits. The cost of the diagnostic process for the patient includes transport, accommodation, food for at least two persons and the clinic cost whenever applicable which may include laboratory investigations, X-rays etc.. According to a study conducted in Nepal and Yemen this cost accounts for approximately one week's per capita income in these countries<sup>(11)</sup>. Excluding the clinic cost, rest expenditure gets doubled for a 2-day protocol compared to 1-day protocol. More importantly there is contributory hidden cost related to loss of income due to these hospital visits. These are expected to be the major factors responsible for patients dropping out of the diagnostic process<sup>(12-14)</sup>. Anticipated number of TB cases worldwide in 2014 were 9.6 million, but only 6 million cases were reported to WHO i.e. only 63 % of the anticipated number of cases were reported; 37% of cases were either undiagnosed or not reported<sup>(15)</sup>. This reflects a gap in reporting as well as access to health care. Ten countries account for 74% or 2.4 millions missed tuberculosis cases globally and India tops the list contributing to 27% of these cases<sup>(16)</sup>. The longer it takes to diagnose and start treatment, an undiagnosed sputum positive pulmonary tuberculosis patient keeps on transmitting the infective organism to others during transport, repeated hospital visits, during the stay in various places of accommodation. If the diagnostic process can be completed in one day and treatment started on the same day itself, these issues of providing financial protection to patients, reducing the drop-out rates from diagnostic process or finding the missed cases of tuberculosis, breaking the chain of infection transmission can be addressed.

In this study the single day approach was found to have similar diagnostic accuracy as the current RNTCP 2-day protocol for diagnosis of tuberculosis. Similar studies done in the past with their primary results are given in Table 5<sup>(17-25)</sup>. Seven out of nine studies had results similar to our study and recommended 1-day approach for diagnosis of pulmonary tuberculosis.

**Conclusion:**

1-day Protocol for diagnosis of pulmonary tuberculosis has equal diagnostic efficacy as standard 2-day protocol and it can be adopted as the standard diagnostic approach under RNTCP after multi-centric large scale studies.

**Limitations of the study :**

1. Sample size was small.
2. It was not a multi-centric study.
3. Smear positive cases could not be confirmed by culture.

**Table 1- Comparison of diagnostic efficacy of 1-day Vs 2-day protocols .**

Protocol	Subjects (N')	Smear Negative	Smear Positive (n)	Total smear positives in Study(N)	% of cases diagnosed (n/N)
2-day Protocol (D2)	357	238	119	123	96.75
1-day Protocol (D1)	375	255	120		97.56

N'-Total number of subjects in the protocol, n- number of smear positive pulmonary tuberculosis cases diagnosed by the protocol, N-Total number of smear positive pulmonary tuberculosis cases in the study.

**Table 2- Comparison of 1-day Vs 2-day Protocol.**

Protocol	Positive	Negative	P-Value
2-day Protocol (D2)	119	4	0.7
1-day Protocol (D1)	120	3	

**Table 3- Diagnostic validity of 1-day protocol against standard 2-day protocol**

Proto col	2-day Protocol (D2)			Total	Sensi tivity (%)	Speci ficity (%)	PPV (%)	NPV (%)
	Positive	Negative						
					98.32	100	100	99.17
1-day Proto col (D1)	Posit ive	117	0	117				
	Nega tive	2	238	240				
<b>Total</b>		119	238	357				

PPV=Positive Predictive Value, NPV=Negative Predictive Value

**Table 4- Comparison of different diagnostic strategies**

Schemes	Advantages	Disadvantages
<b>Spot-Morning - Spot</b>	High Diagnostic yield	High laboratory Cost -2 day hospital visits -Financial loss to patient -Patient drop out -Delay in starting treatment
<b>Spot-Morning</b>	-No significant loss in diagnostic yield -Saves Laboratory Cost	Still 2 day hospital visit -Financial loss to patient -Patient drop out -Delay in starting treatment
<b>Spot-Extra Spot</b>	-No significant loss in diagnostic yield (As proven in this study also) -Saves Laboratory Cost -Only 1 day hospital visit -No Financial loss to patient -No Patient drop out -Treatment starts same day	-Morning sample has better diagnostic yield than extra-spot sample.

When adjusted for the patients dropping out of submitting morning sputum samples there is no significant difference in number TB patients detected between morning and extra spot sample .

**Table 5- Findings of earlier studies on 1-day smear microscopy method.**

Author	Place of study	Year	N	Results
Anyim M et al <sup>(20)</sup>	Nigeria	2006	752	There was no significant difference in the diagnostic values between the 2-day 3 sputum sample AFB microscopy and 1-day 2sputum samples afb microscopy
Cambanis A et al <sup>(19)</sup>	Ethiopia	2006	243	There is no statistical difference between the same day method and standard 2-day three sputum method for diagnosis of tuberculosis.
Hirao S et al <sup>(18)</sup>	Nigeria	2007	224	It could be possible to diagnose TB in a single day by examining two spot specimen.
Rawat J et al <sup>(17)</sup>	India	2010	513	2-day protocol did not show statistically significant difference in performance compared with 1-day protocol.
Myneedu VP et al <sup>(25)</sup>	India	2011	330	Sensitivity of the standard method and same day method were 58.25 % and 40.07% respectively whereas specificity were similar in both i.e. 99.55%.
Miremba P et al <sup>(21)</sup>	Uganda	2012	229	Sensitivity of both the frontloading and standard schemes was 91.1% while their specificities were 86.2% and 91.7% respectively. There was excellent agreement between the diagnostic capacity of the two methods.(P value 0.47)
Nayak P et al <sup>(24)</sup>	India	2013	255	Same day microscopy method missed 17% smear positive cases and did not increase the proportion of suspects providing second sample.
Chandra TJ et al <sup>(22)</sup>	India	2014	153	Diagnosis of lung tuberculosis is possible with two spot sputum samples with modified ZN staining.
Chandra TJ et al <sup>(23)</sup>	India	2016	318	Sputum smear positivity was similar for both standard and same day method for diagnosis of pulmonary tuberculosis.
This study	India	2016	375	1-day method has same diagnostic efficacy as standard 2-day method.(P-Value 0.7)

N- Number of study participants .

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