



## Diagnosing pulmonary tuberculosis by pooling induced sputum

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### ABSTRACT

**Introduction:** Early diagnosis and treatment of pulmonary tuberculosis (PTB) remains fundamental in reducing transmissions and death. Sputum induction is recommended for the diagnosis of pulmonary tuberculosis (PTB) in patients who are unable to expectorate or smear negative.

**Objective:** The aim of this study was to evaluate the diagnostic accuracy of pooling two induced sputum specimens into one microbiological test over a single day for the diagnosis of PTB.

**Methods:** We prospectively enrolled consecutive hospitalized adults with suspected PTB from 2009–2016. Two induced sputum specimens were obtained on the same day and pooled together for AFB smear, culture and Xpert MTB/RIF testing. The final diagnosis of PTB was based on a positive culture from any respiratory specimen. All patients were followed up for 3 months.

**Results:** Of 420 patients, 86(20.5%) were diagnosed with PTB based on a positive respiratory culture. The sensitivity, specificity, positive and negative predictive values for pooled induced sputum were 98.8% (CI 93.7–100%), 100% (CI 98.9–100%) and 100% (94.6–100%) and 99.7% (CI 98.1–100%) respectively. Xpert MTB/RIF in pooled induced sputum was positive in 88.4% of the PTB patients.

**Conclusion:** In the diagnosis of PTB, testing two induced sputum specimens which were pooled together for one microbiological testing process may be comparable to repeat testing.

### 1. Introduction

Tuberculosis (TB) remains a global epidemic in this millennium causing significant mortality and morbidity. TB remains one of the top ten causes of death worldwide. In 2017, there were an estimated 10 million newly diagnosed TB cases and 1.6 million deaths from TB. The incidence and mortality rate of PTB in Singapore, an intermediate burden country was 47 and 0.99 cases per 100,000 population in 2017 [1]. The World Health Organisation (WHO) End TB strategy in 2015 aimed to end the global TB epidemic by 2030. One of the key components in End TB strategy was for effective TB control of which early diagnosis and initiation of treatment remained critical in reducing public transmission and deaths. There was significant progress in reducing TB cases and deaths in the last decade. However, there are still persistent gaps in detection and treatment with diagnostic uncertainties.

The diagnostic yield of the laboratory methods in diagnosis of pulmonary tuberculosis (PTB) is dependent on the quality of the sputum. Sputum induction had been demonstrated to be more effective in diagnosing pulmonary tuberculosis compared to spontaneous sputum

and gastric aspirates [2–6]. Brown et al. found no difference in the diagnostic yield of three sputum inductions done in one day compared to three separate days [7]. Sputum induction was also shown to be more cost effective compared to bronchoscopy [8–10]. Sputum induction was recommended for patients suspected of PTB who cannot expectorate or had initial smear negative by the Infectious Disease Society of America (IDSA) clinical practice guidelines [11]. A recent systematic review by Datta et al. demonstrated that PTB diagnosis was increased by pooling of sputum and provision of instructions for sputum collection which would be useful in areas with resource constraints [12]. The Xpert MTB/RIF assay had enabled rapid diagnosis of PTB and rifampicin resistance with variable clinical impact in different settings [13–16]. There was a paucity of data studying the effects of pooling induced sputum into one specimen with the addition of Xpert MTB/RIF to diagnose PTB on the same day.

We performed a prospective study in hospitalized patients to evaluate the diagnostic accuracy of pooling two induced sputum specimens into one microbiological test with the addition of Xpert MTB/RIF over a single day for the diagnosis of PTB. We hypothesize that this novel way of testing induced sputum could potentially reduce the workload of

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laboratories without compromising on the diagnostic yield of PTB.

## 2. Methods

The study was conducted at the National University Hospital (NUH), a tertiary hospital in Singapore. We prospectively enrolled consecutive hospitalized adult patients admitted to the isolation facilities with suspected PTB from April 2009–March 2016. The patients were enrolled by the respiratory physicians in the hospital experienced in the evaluation of patients with active PTB. The clinical probability of PTB was determined by two respiratory physicians (MYC and JN) based on the review of clinical and radiological records blinded by microbiological results and the patients were classified into three groups: low, moderate and high. A high clinical probability included symptoms of prolonged cough for more than 2 weeks, any of the systemic symptoms such as loss of weight, loss of appetite, night sweats, fever or hemoptysis and presence of any of the following on the chest radiograph: cavities, opacities, nodules or military pattern, pleural effusion and lymphadenopathy.

Patients were instructed to provide two spot sputum for acid fast bacilli (AFB) smear and culture testing. Sputum induction was performed in patients who were unable to expectorate or at the recommendation of the attending respiratory physicians. We excluded patients who had contraindications to sputum induction which included poorly controlled asthma with recurrent exacerbations, chronic obstructive pulmonary disease with a forced expiratory volume in 1 s (FEV1) of less than 50% predicted, hypoxia on room air (defined as pulse oximetry reading of less than 90%), hemodynamic instability, cardiac complications such as arrhythmias and acute myocardial infarct, hemoptysis, cognitive impairment and if informed consent was not obtained.

Sputum induction was performed in a negative pressure isolation room using hypertonic saline via an ultrasonic nebulizer. The process would be terminated when at least 5–10 ml of sputum was collected or if the patient showed signs of respiratory distress [17]. Two induced sputum specimens were obtained at least two hours apart and pooled together for one laboratory testing process for AFB smear, culture and Xpert MTB/RIF testing on the same day. These specimens were sent to the NUH microbiology laboratory for processing on the same day after collection. The NUH microbiology laboratory was one of the two designated laboratory in Singapore for AFB smear and culture processing. These specimens were decontaminated according to standard methods using N-acetyl-L-cysteine-sodium hydroxide (NALC–NaOH). Direct smears of the respiratory specimens were prepared using Auramine staining, followed by confirmatory Ziehl-Neelsen (ZN) staining. These respiratory specimens were cultured for *Mycobacterium tuberculosis* using BD MGIT™ BACTEC™ (BD, Sparks, MD, USA) and Lowenstein-Jensen medium. The turnaround time (TAT) for the AFB smear result was within 24 h of the specimens reaching the laboratory and the AFB culture result was reported after 8 weeks of incubation. The Xpert test was performed according to the standard protocol of the manufacturer.

Additional diagnostic procedures for example computed tomography (CT) of the thorax and flexible bronchoscopy were performed for evaluation of the patients where appropriate according to their treating respiratory physicians. Tuberculosis treatment was given at the discretion of the treating physician. The final diagnosis of confirmed PTB was based on a positive culture growing *Mycobacterium tuberculosis* from any respiratory specimen. Probable PTB was defined as patients who received tuberculosis treatment but were culture negative. The patients who did not have PTB were culture negative, did not receive tuberculosis treatment and had alternative diagnosis established by 3 months. All patients were followed up for 3 months after enrolment [18]. Ethics approval was granted by the institutional review board in Singapore (DSRB-B-09/083).

## 3. Statistical analysis

We determined our sample size based on the calculations made by Flahault et al. We estimated the prevalence of PTB to be 20%, the expected test sensitivity and specificity to be 95% and require the lower 95% confidence limit to be more than 0.8 with 0.95 probability. The estimated sample size was derived to be 250 [19]. All data were collected and entered into the database. The categorical data were expressed as percentages and continuous data as mean and standard deviation (SD). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of AFB smear, culture and Xpert MTB/RIF were calculated in relation to a positive culture for *Mycobacterium tuberculosis* in any respiratory specimen and the 95% confidence intervals (CI) were estimated.

## 4. Results

From April 2009 to March 2016, we enrolled 423 patients into the study. The final analysis was performed on 420 patients as 2 patients had failed sputum inductions and 1 patient had incomplete data collection. Out of the 420 patients, 266 patients had only pooled induced sputum tested. There were 36 patients who had initial testing of spontaneous sputum followed by pooled induced sputum, 83 patients who had pooled induced sputum followed by a single induced sputum and 35 patients who had initial testing of spontaneous sputum followed by pooled induced sputum and a single induced sputum (Fig. 1).

The baseline characteristics and clinical probability of the patients were shown in Table 1. The mean age of the patients was 58 years (SD: 19.1) and 63.3% were male. There were 86 (prevalence 20.5%) patients diagnosed with confirmed PTB of which 48 (55.8%) were smear positive and 38 (44.2%) were smear negative. There were 4 patients with probable PTB and 330 patients with no PTB (Table 2).

The pooled induced sputum detected 85 patients with culture positive PTB. The sensitivity, specificity, PPV and NPV of pooled induced sputum for detection of culture positive PTB were 98.8% (CI 93.7–100%), 100% (CI 98.9–100%) and 100% (94.6–100%) and 99.7% (CI 98.1–100%) (Table 3). Spontaneous spot sputum failed to detect 7 patients with PTB which were subsequently detected by pooled induced sputum. The single non-pooled induced sputum failed to detect 3 patients with PTB. Bronchoscopy was performed in 33 patients and bronchoalveolar lavage (BAL) specimens were obtained. Among the 33 patients undergoing bronchoscopy, 3 patients were AFB smear positive and culture positive for MTB. The other 30 patients had negative yield in their cultures. The BAL specimens did not yield additional cases of culture positive PTB. Xpert RIF/MTB in pooled induced sputum detected 88.3% of the PTB cases.

## 5. Discussion

We demonstrated in our study that pooled induced sputum had a relatively high accuracy with a sensitivity of 98.8% and specificity of 100% in diagnosing culture positive PTB. The results were comparable to repeat testing of induced sputum on consecutive days or the same day [7,20]. Al Zahrani et al. had shown that the yield for acid fast bacilli smear and mycobacterial culture was increased with repeat testing of induced specimens, 64% and 70% for one, 81% and 91% for two, 91% and 99% for three and 98% and 100% for four induced samples respectively [20]. Bronchoscopy in our study did not detect any additional cases of PTB beyond those diagnosed by pooled induced sputum. This was consistent with results from previous studies [7–9]. Bronchoscopy was an invasive and resource intense procedure compared to sputum induction which could be performed in resource limited settings. Our findings support the recommendations made by the Infectious Disease Society of America (IDSA) clinical practice guidelines which induced sputum was recommended as the initial sampling method instead of bronchoscopy [11].

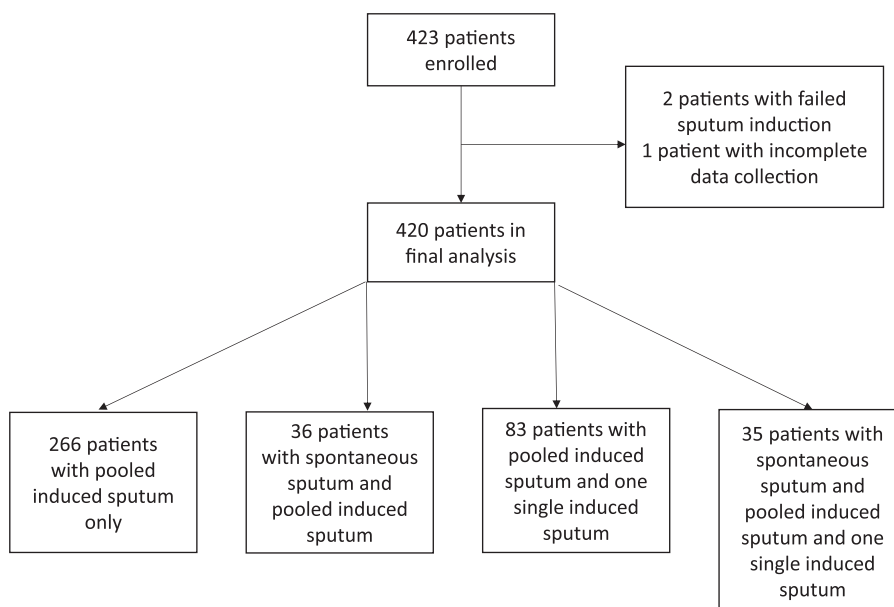


Fig. 1. . Study cohort.

**Table 1**  
Baseline patient characteristics and clinical probability.

Age	Mean (SD) 58 (19.1)
<b>Sex</b>	<b>n (%)</b>
Male	266 (63.3)
Female	154 (36.7)
<b>Symptoms</b>	<b>n (%)</b>
Cough > 2 weeks	182 (43.3)
Hemoptysis	47 (11.2)
Fever	223 (53.0)
Loss of weight	111 (26.4)
Loss of appetite	90 (21.5)
<b>Radiology</b>	<b>n (%)</b>
Cavities	31 (7.8)
Consolidation	186 (44.2)
Nodules	17 (4.0)
Pleural effusion	69 (16.4)
Others (which would include collapse and presence of more than 1 feature on radiology eg mass, pleural effusion and nodules)	117 (27.9)
<b>Clinical probability</b>	<b>n (%)</b>
Low	13 (3.1)
Moderate	294 (73.5)
High	113 (26.9)

SD: standard deviation.

**Table 2**  
Final diagnosis of the patients.

Diagnosis	n (%)
PTB	86 (20.5)
Community acquired pneumonia	172 (41)
Malignancy of the lung (primary and secondary)	42 (10)
Non-tuberculous mycobacteria (NTM)	10 (2.4)
TB pleuritis	20 (4.8)
Bronchiectasis, asthma and COPD	26 (6.2)
Parapneumonic effusion, empyema and lung abscess and others	60 (14.3)
Probable PTB (given empirical anti-tuberculous treatment but culture negative)	4 (0.95)

Our study was adequately powered to demonstrate a high diagnostic accuracy in pooling induced sputum for the diagnosis of PTB. The prevalence of PTB in our study population was comparable to other

studies in this area [8–10]. Only a small percentage of patients (0.7%) did not complete the study. We chose to have two induced sputum to be pooled together on the same day. This was based on the results of the systematic review by Mase et al. which concluded that 85.8% of the cases were detected with the first sputum specimen and subsequent incremental yield with the second and third sputum specimen was 11.9% and 3.1% respectively [21]. The World Health Organisation (WHO) had also recommended that the number of sputum specimens to be tested be reduced from three to two and had advocated that two spot spontaneous sputum could be collected and tested on the same day [22–24]. Our reference standard for diagnosis of PTB was a positive culture from all respiratory specimens. There was a three month follow-up period for all cases to ensure alternative diagnoses were found if cultures were negative for PTB. Although there were patients that were treated empirically for culture negative PTB, this remained to be a small proportion (0.95%). The methodology of this study was easily reproducible and could be implemented in real-world practice if resource was available for sputum induction. Laboratory processing for AFB smear and cultures could potentially be reduced by half with pooled induced sputum instead of separate day spot sputum specimens.

Previous studies had evaluated the role of pooling spontaneous sputum in PTB diagnosis. Mpagama et al. demonstrated in 50 patients that overnight pooled sputum had reduced median time to culture positivity compared to combination of both spot and early morning sputum (96 h (IQR 87–131) vs 110.5 h (IQR 137–180);  $p < 0.001$ ) [25]. The role of sputum pooling was also evaluated for Xpert testing in laboratory based studies of variable designs which demonstrated that accuracy of detection of MTB was maintained despite pooling and may save costs [26–28]. We were not aware of any study that studied the effects of pooling two induced sputum into one microbiological test on the same day. In an earlier study performed by Chew et al. demonstrated a high diagnostic accuracy of Xpert in induced sputum and may facilitate treatment in up to a quarter of PTB patients [29]. We demonstrated that Xpert testing in pooled induced sputum had similar accuracy.

Our study had several limitations. This was a single centre, non-randomized study without a standard arm which might lead to potential bias. There was no pre-defined criteria on when to proceed with further investigations such as bronchoscopy which was at the attending physicians' discretion. It was conducted in acutely ill hospitalized patients where there could be a higher pre-test probability and disease

**Table 3**  
Diagnostic accuracy of pooled induced sputum.

Test (number of PTB cases in pooled induced sputum/total number of PTB in cohort)	Sensitivity (%; 95% CI)	Specificity (%; 95% CI)	PPV (%; 95% CI)	NPV (%; 95% CI)
Culture (85/86)	98.8 (93.7–100)	100 (98.9–100)	100 (94.6–100)	99.7 (97.9–100)
Smear Microscopy (45/86)	52.3 (41.3–63.2)	98.8 (96.8–99.6)	91.8 (79.5–97.4)	91.9 (85.2–91.9)
Xpert MTB/RIF (76/86)	88.4 (79.7–94.3)	100 (98.9–100)	100 (94.0–100)	97.0 (94.9–98.4)

Data are presented as percentages (%) unless otherwise stated. 95% CI: 95% confidence interval; PPV: positive predictive value; NPV: negative predictive value.

burden. There was also a greater urgency to establish diagnosis and initiate treatment. Same day diagnosis of PTB could be achieved in large proportion of the patients with smear positivity thus avoiding delays in diagnosis. The results might not be applicable in patients suspected of PTB in the community or for immigration screening as the pre-test risk may be lower with less advanced disease. A recent study comparing spontaneous sputum obtained by health care worker instruction and induced sputum showed no significant difference in the same day diagnosis, initiation of treatment and had lower cost incurred for the instructed arm [30]. Sputum induction was a readily available service in our hospital unlike healthcare worker supervision of sputum collection. Our study did not evaluate the impact of this diagnostic test on treatment initiation and cost effectiveness which might differ in various healthcare systems.

In conclusion, pooling two induced sputum into one on the same day was a novel and efficient method to diagnose PTB with a high diagnostic accuracy in the intermediate burden setting. This process was safe, well tolerated and could potentially reduce the workload of laboratories which might be critical in resource poor, high burden countries. Future randomized studies are required to evaluate the optimal method in PTB diagnosis and impact on clinical outcomes.

### Ethics statement

The ethics approval was granted by the institution review board.

### Conflict of interest

The authors declare no conflict of interest.

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