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# The Mono-Prep system increases the detection rate of sputum smear microscopy for diagnosing tuberculosis

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## Abstract

**Objective:** Direct sputum smear microscopy (DSSM) has a low detection rate. This study investigated whether an alternative method called Mono-Prep smear microscopy (MPSM) can enhance the diagnosis of tuberculosis in tuberculosis laboratories that perform direct smear microscopy in China.

**Methods:** A total of 117 sputum samples were collected from outpatients who attended Beijing Chest Hospital. DSSM, MPSM, solid culture, and Xpert MTB/RIF were performed on the samples.

**Results:** The positive rates of DSSM, MPSM, solid culture, and Xpert MTB/RIF were 27.4% (32/117), 40.2% (47/117), 35.9% (42/117), and 52.1% (61/117), respectively. MPSM could detect 15 more cases of tuberculosis compared with DSSM (47 vs 32) among 117 sputum samples. This represented a significantly higher positive rate and sensitivity of MPSM compared with DSSM. However, MPSM appeared to have a lower specificity (81.3%) compared with DSSM (90.7%) with the solid culture used as a standard.

**Conclusion:** Use of MPSM can increase the number of positive sputum samples, but it still needs improvement.

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## Introduction

Tuberculosis remains a major worldwide public health problem. Tuberculosis is the ninth leading cause of death worldwide, and the leading cause of mortality from a single infectious agent.[1] Laboratory diagnosis of tuberculosis is an important auxiliary means for its control.[2] One of the commonly used detection methods in tuberculosis laboratories in China is direct sputum smear microscopy (DSSM) because it is simple, cheap, and rapid. However, the detection rate of current DSSM is low, and it cannot fully meet clinical needs. Only 57% of the pulmonary cases of tuberculosis reported to the World Health Organization in 2016 had bacteriological evidence globally, and this rate was even lower in China (31%).[1] Therefore, DSSM needs to be further developed to improve the positive detection rate of smears. Therefore, this study investigated whether Mono-Prep smear microscopy (MPSM), which is a dual membrane filtration and auto-smear method, can enhance the diagnosis of tuberculosis.

## Methods

### *Study setting and design*

Sputum samples were collected from outpatients who attended Beijing Chest Hospital, affiliated to Capital Medical University (Beijing, China), in July 2017. All qualified sputum specimens (not saliva) that were not less than 5 mL were included in the study. The volumes of specimens used in the four methods were as follows: 0.05 to 0.1 mL for DSSM, 2 mL for MPSM, 2 mL for solid culture, and 1 mL for the Xpert MTB/RIF test. If duplicate samples from the same patient satisfied the inclusion criteria, only one sample was included in the study.

The study was approved by the Ethics Committee of Beijing Chest Hospital affiliated to Capital Medical University. All of the participants provided verbal informed consent.

### *Laboratory testing*

Before processing, direct smears were prepared from each sample for staining with auramine O and observed using a fluorescence microscope (Leica Microsystems Wetzlar GmbH, Wetzlar, Germany).[3] These samples were also tested by MPSM (http://www.corebiotech.co.kr). In brief, 2 mL of the remaining sputum samples were treated with Mono-Prep Solution (Core Biotech Inc., Ltd. Gwangju, South Korea) at a ratio of 1:2 for 30 minutes. Subsequently, a microscope slide and a filter tank were placed in the corresponding position of the matching instrument. A volume of 1.5 mL of the sample mixture was transferred into the filter tank. The Mono-Prep system collected bacilli by the dual membrane filtration method (Figure 1), where the upper membrane intercepted the impurities and the lower membrane intercepted *Mycobacterium tuberculosis* (MTB). The smears were then automatically prepared by the negative pressure adsorption method for the following staining step with auramine O and observed under a fluorescence microscope.

For the Xpert MTB/RIF method, the sample reagent was added to the sputum sample at a ratio of 2:1, oscillated for

15 to 30 seconds, and incubated for 15 minutes at room temperature. A volume of 2 mL of the treated sample was transferred into a cartridge. The cartridge was loaded into the Xpert MTB/RIF instrument (GeneXpert; Cepheid Inc., Sunnyvale, CA, USA) and an automatic process completed the remaining assay steps. After the reaction ended, the results could be directly observed under the detection system window.[4]

The remaining sputum sample was then decontaminated and thinned by treating it with 2% sodium hydroxide and 0.5% N-acetyl-L-cysteine for 15 minutes. The sputum was then processed for culture by centrifugation following a standard laboratory protocol.

### *Cost-effectiveness of MPSM and DSSM*

This study analyzed the cost-effectiveness of MPSM and DSSM. Measurement of cost was based mainly on direct costs (i.e., the cost to be paid for examination of the patient). The indirect cost included the cost of taking a standard course of treatment (USD 50.8) after being misdiagnosed as pulmonary tuberculosis. Direct nonmedical costs, including food, accommodation, and home care required during hospitalization of the patient, were not calculated. The cost-effectiveness ratio (total cost/detection rate) was calculated to analyze the cost-effectiveness of the two methods.

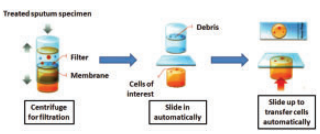


**Figure 1.** Dual membrane filtration method and auto-smear method (obtained from the instructions of the Mono-Prep system)

### *Statistical analysis*

Statistical comparisons were performed by the $\chi^2$ test using SPSS version 19.0 (IBM Corp., Armonk, NY, USA). A $P$ value $<0.05$ was considered significant.

## Results

All specimens were qualified sputum specimens, including 89 cases of mucopurulent sputum, 22 cases of cheese-like sputum, and six cases of bloody sputum.

Of the 117 specimens, 42 positive results and 75 negative results were reported using the solid Lowenstein–Jensen slant culture, 32 (27.4%) positive results were reported using DSSM, and 47 (40.2%) positive results were found using MPSM. The positive rate of MPSM was significantly higher than that of DSSM ($P < 0.01$). When the solid culture was used as the standard, the sensitivity of MPSM was significantly higher than that of DSSM ($P < 0.05$) (Table 1).

Among the 117 sputum specimens, 65 tested smear-negative using DSSM and MPSM. Of these 65 specimens, 13 tested

**Table 1.** Results of four different detection methods for *Mycobacterium tuberculosis*.

| Methods | Positive rate, % (n) | Sensitivity, % (n) | Specificity, % (n) |
|---|---|---|---|
| Solid L-J slant culture | 35.9 (42/117) | – | – |
| DSSM | 27.4 (32/117) | 59.5 (25/42) | 90.7 (68/75) |
| MPSM | 40.2 (47/117)** | 78.6 (33/42)* | 81.3 (61/75) |
| Xpert MTB/RIF | 52.1 (61/117) | 95.2 (40/42) | 72.0 (54/75) |

L-J: Lowenstein–Jensen; DSSM: direct sputum smear microscopy; MPSM: Mono-Prep smear microscopy. Calculation of sensitivity and specificity by culture was used as the standard. *$P < 0.05$ compared with DSSM; **$P < 0.01$ compared with DSSM

positive using the Xpert MTB/RIF method. Furthermore, 14 specimens with positive results of MPSM, but negative results of culture, were detected using the Xpert MTB/RIF method. In this test, 12 cases were positive and two were negative.

Table 2 shows all of the 52 positive specimens using both smear methods. Among the 52 smear-positive specimens, four tested negative using Xpert MTB/RIF. Of these, two tested positive using DSSM, but tested negative using MPSM. Additionally, two specimens tested negative using DSSM, but tested positive using MPSM (Table 2).

The cost of one DSSM was USD 2.3. Therefore, the cost-effectiveness ratio was USD $2.3 \times 117/32 =$ USD 8.4. The cost of one MPSM specimen was USD 4.5. Therefore, the cost-effectiveness ratio was USD $4.5 \times 117/47 =$ USD 11.2.

## Discussion

Sputum smear microscopy is widely used for diagnosing pulmonary tuberculosis globally.[5] New methods of detecting tuberculosis have been developed in recent years, but DSSM is still the primary method in low- and middle-income countries.[6] Although DSSM is an easy, inexpensive, rapid, and highly specific technique, its sensitivity is low, resulting in missed diagnosis.[7–9] The Mono-Prep system is a liquid-based cytology processor with patented dual membrane filters that are optimized for each specimen. The smears are then automatically prepared by the negative pressure adsorption method, effectively improving the positive rate of sputum smears.

**Table 2.** Smear-positive specimens confirmed using Xpert MTB/RIF.

| | Xpert MTB/RIF | | |
|---|---|---|---|
| Microscopy | + | – | Total |
| DSSM (+) and MPSM (–) | 3 | 2 | 5 |
| DSSM (–) and MPSM (+) | 18 | 2 | 20 |
| DSSM (+) and MPSM (+) | 27 | 0 | 27 |
| Total | 48 | 4 | 52 |

DSSM: direct sputum smear microscopy; MPSM: Mono-Prep smear microscopy

This study showed that MPSM had a higher tuberculosis smear-positive rate compared with DSSM. When culture was used as the standard, the sensitivity of MPSM was significantly higher than that of DSSM. However, MPSM appeared to have a lower specificity compared with DSSM. A total of 14 specimens with negative results of culture, but positive results of MPSM, were detected using Xpert MTB/RIF, of which 12 cases were positive and two were negative. Therefore, some patients might have been receiving anti-tuberculosis treatment, accounting for the lower specificity of MPSM.

Because the sensitivity of the Xpert MTB/RIF assay is high (up to 88%) in countries or regions with a high burden of tuberculosis,[10] it was used to further confirm the positivity of all of our specimens. MPSM showed higher consistency compared with DSSM because 18 of the 20 MPSM-positive/DSSM-negative specimens and only three of the five MPSM-negative/DSSM-positive specimens were confirmed as positive by the Xpert MTB/RIF assay. In total, four smear-positive cases were confirmed as negative by the Xpert MTB/RIF assay. These four cases were not confirmed as non-*Mycobacterium tuberculosis*, *Rhodococcus*, or *Nocardia*.[11–13]

Because the thickness and uniformity of smears affect the results of DSSM, the sputum volume used in this method was only 0.05 to 0.1 mL. MPSM can effectively liquefy sputum, kill MTB, and avoid laboratory pollution to guarantee the safety of operators. This method does not require centrifugation because the dual filter

membrane effectively intercepts MTB in the sputum and completely collects the bacteria, thus increasing the positive detection rate. This study also showed that after filtration, the sputum smear had a clean background and the operators could easily identify MTB. A disadvantage of MPSM was that in the process of the smears, some solution could not be filtered, which caused leakage of liquid. This might have been caused by impurities in the sputum specimens and an improper filter diameter.

MPSM and DSSM could not avoid the possibility of false positives. If a patient was misdiagnosed as tuberculosis, the patient had to pay an additional amount of USD 50.8. In this study, two smear-positive, but Xpert MTB/RIF negative, cases were detected by MPSM and DSSM, but they were not diagnosed as tuberculosis. Estimating other economic losses caused by misdiagnosis, including the cost of delayed diagnosis and treatment and the cost of progression of the disease, would be difficult.

In conclusion, MPSM is more expensive than DSSM. However, MPSM increases the number of positive sputum samples, increasing the number of cases of tuberculosis found. Further research and validation with more clinical samples will help confirm MPSM as a sensitive and effective method for tuberculosis. Further improvement can be expected for better use in the future.

## Authors' contributions

ZS and JD conceived the study. ZS and JW designed the study protocol. JW, CK, and FH carried out the MPSM assay. JW and YS performed the DSSM assay. JW and LZ carried out the culture. QL and YM carried out the Xpert MTB/RIF assay. JW and ZS performed analysis and interpretation of the data. JW drafted the manuscript. ZS critically revised the manuscript for intellectual content. All authors read and approved the final manuscript. ZS is the guarantor of the paper.

## Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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