Optimizing sputum smear microscopy for the diagnosis of pulmonary tuberculosis

‘Sputum smear microscopy has high specificity in tuberculosis-endemic countries, but modest sensitivity that varies among laboratories. However, currently used microscopy methods can be optimized to generate higher sensitivity and yield.’

Karen R Steingart, Andrew Ramsay and Madhukar Pai†
†Author for correspondence

Department of Epidemiology, Biostatistics & Occupational Health, McGill University, Respiratory Epidemiology & Clinical Research Unit, Montréal Chest Institute, 1020 Pine Avenue West, Montréal H3A 1A2, Canada
Tel.: +1 514 398 5422
Fax: +1 514 398 4503
madhukar.pai@mcgill.ca

The challenge of tuberculosis case detection

The global burden of disability and death due to tuberculosis (TB) is immense. In 2005 alone, an estimated 8.8 million people developed TB and almost 2 million died, including 195,000 HIV-infected individuals [1]. Although the incidence of TB is constant or falling in all regions of the world, there has been a continued increase in the total number of new TB cases in Africa, Southeast Asia and the Eastern Mediterranean region [1,2]. The expansion of DOTS, the international TB control strategy, has resulted in a new smear-positive case-detection rate of 60% globally, approaching the 2005 WHO target of 70% case detection [1]. However, the majority of DOTS programs in high TB-burden countries have fallen short of this target [1].

More than 90% of TB patients live in low- and middle-income countries [2], where the diagnosis of TB relies primarily on identification of acid-fast bacilli on sputum smears using a conventional light microscope. In these countries, most laboratories use smears of unconcentrated sputum (direct smears) with Ziehl–Neelsen (ZN) staining. The DOTS strategy focuses on passive case finding of sputum smear-positive patients [3]. Typically, a patient who presents to a local health center or national TB program facility with a cough lasting more than 2–3 weeks submits a minimum of three consecutive sputum specimens (spot, morning, spot) for examination by sputum smear microscopy (hereafter referred to as microscopy). Microscopy is relatively simple, inexpensive, widely applicable and highly specific for Mycobacterium tuberculosis in TB-endemic countries [3]. Approximately 25 smears can be prepared and examined by a microscopist in a single day. In addition, microscopy identifies the most infectious patients [4–6]. However, microscopy has several limitations. Although it has been reported to have more than 80% sensitivity compared with culture for identifying cases of pulmonary TB in some settings [7,8], the sensitivity of the test has been lower and variable in other reports (range 20–80%) [9,10]. Moreover, the sensitivity of microscopy is limited in paucibacillary disease (e.g., pediatric and HIV-associated TB) [11,12].

In addition to the problem of variable sensitivity, microscopy requires significant labor and training. These factors result in missed cases, reduced access to diagnostic services and heavy workloads to already overburdened health systems. Repeated visits by the patient over several days may be required to establish a diagnosis. Work in Malawi has shown that a significant proportion of smear-positive patients attending a district hospital drop out
of the diagnostic pathway before their results can be communicated to them and treatment started [13]. Thus, the modest sensitivity of microscopy and the complex diagnostic pathway contribute to delays in diagnosis, enabling the disease to progress and increasing the potential for transmission of *M. tuberculosis* [7].

A TB working group has estimated that a rapid and accessible test for TB with sensitivity for smear-positive and -negative cases greater than 85% and specificity of 97% could save approximately 400,000 lives a year [14]. Experts in TB diagnostics have called attention to the need to improve and possibly replace microscopy with a simpler test. Several new diagnostic tools are currently in the pipeline but will take time to develop and evaluate and, if found to be effective, to implement [11,15,16]. However, few of the diagnostic tools under development will be appropriate for the lower levels of health systems in developing countries where the majority of patients present. Therefore, in most resource-limited countries, microscopy will remain the primary means of microbiological diagnosis of TB for the foreseeable future. Thus, strategies that optimize microscopy services need to be explored urgently.

As part of a project commissioned by the WHO Special Programme for Research and Training in Tropical Diseases (TDR), a series of systematic reviews were performed to determine the strength of existing evidence, identify knowledge gaps and define a research agenda for microscopy. In particular, these reviews addressed sputum processing methods [10], fluorescence microscopy [17] and the yield of serial sputum specimen examinations [18]. Findings from the three reviews are summarized in Table 1 and described below.

### Table 1. Findings from systematic reviews on optimization of sputum smear microscopy.

<table>
<thead>
<tr>
<th>Systematic review</th>
<th>Total number of studies in the review</th>
<th>Median sample size (range)</th>
<th>Outcome measures*</th>
<th>Principal findings</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum processing</td>
<td>83</td>
<td>256 (8-3287)</td>
<td>Sensitivity, specificity and incremental yield of positive smears</td>
<td>Sputum processing (mainly with bleach or sodium hydroxide) yielded an average 18% increase in sensitivity. Sputum subjected to overnight sedimentation preceded by treatment with ammonium sulfate or bleach was, on average, 23% more sensitive. Specificity was unaffected by sputum processing</td>
<td>[10]</td>
</tr>
<tr>
<td>Fluorescence microscopy</td>
<td>45</td>
<td>493 (12-23,427)</td>
<td>Sensitivity, specificity and incremental yield of positive smears</td>
<td>Fluorescence microscopy was, on average, 10% more sensitive than conventional microscopy. Specificity of both fluorescence and conventional microscopy was similar</td>
<td>[17]</td>
</tr>
<tr>
<td>Serial sputum specimens</td>
<td>37</td>
<td>153 (5-11,650)†</td>
<td>Sensitivity and incremental yield of third sputum specimen</td>
<td>Mean incremental yield and/or increase in sensitivity from examination of third sputum specimen ranged between 2 and 5%</td>
<td>[18]</td>
</tr>
</tbody>
</table>

*Sensitivity is defined as the proportion of culture-positive samples found positive with the given microscopy method; specificity is defined as the proportion of culture-negative samples found negative with the given microscopy method.

†For 36 studies, including all smear-positive patients.
suggested that processing sputum by use of centrifugation and various chemicals, including bleach and sodium hydroxide, increases the sensitivity of microscopy compared with the direct smear method and has similar specificity. However, the review did not enable us to determine whether the methods studied here would yield similar results if carried out in peripheral laboratories in low-income countries owing to the following concerns: feasibility of centrifugation in settings with irregular power supply; limited human and financial resources; inadequate training capacity and the potential biohazard posed by centrifugation.

**Is there evidence that microscopy can be optimized using fluorescence microscopy?**

The review on fluorescence microscopy identified a total of 45 eligible studies [17]. The results (18 studies with culture as the reference standard) showed that sensitivity of conventional microscopy ranged from 32 to 94% and sensitivity of fluorescence microscopy ranged from 52 to 97%. Fluorescence microscopy was on average 10% more sensitive than conventional microscopy (95% CI: 5–15%). The average specificity of fluorescence microscopy was 98%, similar to that of conventional microscopy.

Two studies assessed the accuracy of fluorescence microscopy in patients with documented HIV infection. In one study (339 patients) that used mycobacterial culture, sensitivity of fluorescence microscopy was twice as high as that of conventional microscopy and specificity was similar (fluorescence microscopy: sensitivity 73%, specificity 100%; conventional microscopy: sensitivity 36%, specificity 100%) [21]. A second study reported a 26% increase in yield of fluorescence microscopy compared with conventional microscopy in HIV-infected patients thought to have pulmonary TB on clinical and radiological examination [22].

The finding of quicker examination times for smear results with fluorescence microscopy compared with light microscopy using ZN staining was substantiated in this review. Results from a large double-blinded study found that fluorescence microscopy, which took 1 min, had higher sensitivity and equivalent specificity compared with conventional microscopy, which took 4 min [23]. Although traditional fluorescent microscopes with mercury vapor lamps have been considered too expensive for use in resource-limited settings, newer, less-expensive fluorescent microscopes with light-emitting diodes (LEDs) are now available. Recently, Nguyen and colleagues found good agreement between fluorescence microscopy smear readings using LEDs and traditional high-pressure mercury vapor lamps [24]. This is a key area for further research [25].

In summary, the above-mentioned review demonstrated that, compared with conventional microscopy, fluorescence microscopy has higher sensitivity and comparable specificity, thus dispelling any lingering doubts regarding the loss of specificity because of fluorescing artifacts. The available evidence suggests that fluorescence microscopy may be promising in HIV-infected individuals. In addition, fluorochrome-stained smears take less time to examine than smears stained using the ZN method.

However, before changes in policy that support broad implementation of fluorescence microscopy can be considered, particularly in low-income countries, several issues need to be addressed:

- Feasibility and sustainability of fluorescence microscopy in settings with irregular electricity supply, limited human and financial resources and inadequate training
- Lack of internationally agreed external quality assessment methods for blinded rechecking of fluorescent smears
- Uncertainty regarding the stability of fluorescence microscopy reagents under field conditions
- Uncertainty regarding the general acceptability of fluorescence microscopy to laboratory workers in tropical settings

**Is there evidence that microscopy can be optimized by the examination of two (not three) sputum specimens?**

Current international TB guidelines recommend the microscopic examination of three sputum specimens for the evaluation of individuals suspected of having pulmonary TB. Mase and colleagues conducted a systematic review of studies that quantified the diagnostic yield of the third sputum specimen [18]. This review identified a total of 37 eligible studies that provided data on incremental (additional) yield in smear positivity and additional gain in sensitivity of the third specimen. Although heterogeneity in study methods and results presented challenges for data synthesis, the analysis found that the incremental yield in smear positivity and the sensitivity of the third specimen, without performing subgroup analyses, ranged from 0 to 11% depending on numerous variables, such as the use of a reference standard, study population, study design, microscopy stain used and processing method. Various subgroup analyses suggest that, regardless of the method of data stratification, the mean incremental yield in smear positivity and the mean sensitivity of the third specimen were between 2 and 5%.

Thus, the findings of this review have implications for policy in areas of high TB prevalence and limited resources, where microscopy is the main, or only, diagnostic tool available and laboratory services are being overwhelmed with requests for microscopy. It is possible that a two-specimen approach would have either a negligible adverse impact on case finding or actually improve case finding through improved quality of service, including a shortened time to diagnosis. Omitting the third specimen could alleviate the overwhelming workload of laboratories, particularly in countries with high demands for microscopy and human resource crises. This would allow time to be invested in more thorough examination of the two remaining...
specimens and reduce the number of smears requiring rechecking in external quality assessment schemes. In high-burden settings, laboratories performing microscopy are not only responsible for diagnosing TB but also for the diagnosis of other conditions, such as HIV, anemia, syphilis and malaria. Thus, the time saved from the inefficient examination of a third specimen may be applied toward improvements in testing for other diseases.

However, national TB programs will need to consider several issues carefully before adopting the two-specimen approach:

- Microscopy workload and human resources available
- Potential decrease in numbers of patients dropping out of the diagnostic pathway owing to loss to follow-up
- Savings in time and costs that could be potentially diverted to improve the quality of microscopic examination or specimen collection procedures
- Potential decrease in numbers of smears required for blinded rechecking in quality-assurance programs
- Potential for both decreases and increases in case detection
- Strategies for obtaining a third sputum specimen examination in the case of a single positive smear in order to satisfy the WHO definition of a smear-positive case
- Strategies for following-up those patients negative on two smears

Conclusions & policy implications

In conclusion, recent systematic reviews on sputum processing, fluorescence microscopy and serial sputum specimens suggest that currently used microscopy methods can be optimized to generate higher than usual yields. On the basis of the evidence in these reviews and expert opinion, the TDR has launched an initiative to support the development of diagnostic trial sites that will conduct research on methods to optimize microscopy for TB. This initiative will support the development of trial sites that will undertake studies on:

- Optimum timing and composition of sputum specimen sets for efficient diagnosis of sputum smear-positive TB
- Use of lower-cost fluorescence microscopy systems for the diagnosis of sputum smear-positive TB
- Sputum processing methods involving bleach digestion and a physical concentration step (centrifugation or gravity sedimentation) for the diagnosis of sputum smear-positive pulmonary TB
- Potential for reducing time to diagnosis and number of patient visits required by examining two specimens on the same day that the patient first presents

We hope that these initiatives will address the major gaps we identified in our reviews and generate quality evidence that will inform global policies on TB care and control.

Acknowledgements

The authors would like to thank Mohamed Abdel Aziz (Stop TB Department, WHO, Geneva, Switzerland), Jane Cunningham (UNICEF/UNDP/World Bank/WHO Special Programme for Research and TDR, Geneva, Switzerland), Megan Henry (County of Sacramento Department of Health and Human Services, CA, USA), Philip C Hopewell (University of California, CA, USA), Sundari R Mase (Santa Clara County Public Health Department, CA, USA), Vivienne Ng (Albany Medical College, NY, USA), Mark D Perkins (Foundation for Innovative New Diagnostics, Geneva, Switzerland) and Richard Urbanzczik (WHO Tuberculosis Laboratory Consultants Group, Schoenem, Germany) for their participation in conducting the systematic reviews on microscopy.

References

Optimizing sputum smear microscopy for the diagnosis of pulmonary TB


Website


Affiliations

- Karen R Steingart, MD, MPH
  Assistant Clinical Professor, Medical Advisor, Division of Pulmonary and Critical Care Medicine, San Francisco General Hospital, University of California, San Francisco, CA; Francis J Curry National Tuberculosis Center, 3180 18th Street, Suite 102, San Francisco, CA 94110-2028, USA
  Tel.: +1 415 502 4600
  Fax: +1 415 502 4620
  karenst@u.washington.edu

- Andrew Ramsay, MSc
  Tel.: +41 22 791 1545
  Fax: +41 22 791 4854
  ramsaya@who.int

- Madhukar Pai, MD, PhD
  Assistant Professor, Department of Epidemiology, Biostatistics & Occupational Health, McGill University, Respiratory Epidemiology & Clinical Research Unit, Montréal Chest Institute, 1020 Pine Avenue West, Montréal H3A 1A2, Canada
  Tel.: +1 514 398 5422
  Fax: +1 514 398 4503
  madhukar.pai@mcgill.ca