Making the most of poor diagnostics: increasing access to tuberculosis treatment through optimized smear microscopy services
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Chapter 4

Optimizing sputum smear microscopy for the diagnosis of pulmonary tuberculosis.

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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.’

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.

### Is there evidence that microscopy can be optimized using chemical & physical sputum processing techniques?

The review on sputum processing methods identified a total of 83 studies [10]. Of these, 14 studies (culture used as the reference standard) investigated the impact of centrifugation with a chemical (usually either bleach or sodium hydroxide) on microscopy; 36 studies (including all smear-positive patients).

Sputum processing yielded a mean of 18% (95% confidence interval [CI]: 11–26%) increase in sensitivity with 13 studies showing an increase and one study showing a decrease. The review found moderate evidence for the use of bleach with centrifugation in sputum processing prior to microscopy (six studies using comparable methodologies and culture as the reference standard). The mean increase in sensitivity was 13% (95% CI: -1 to 26%). In all studies, sensitivity for processed smears was higher than for direct smears. In the only study (with mycobacterial culture) involving HIV-infected individuals (96 patients), sensitivity increased by 11% following processing with bleach and centrifugation [19]. A recent review conducted by Angelby and colleagues found similar results [20].

A total of eight studies (four using overnight sedimentation and four using short sedimentation times of 30–45 min) investigated the effect of sedimentation with a variety of chemical agents, usually either bleach or ammonium sulfate. All studies used culture as the reference standard. In studies using overnight sedimentation, the average increase in sensitivity was 23% (median 28%; range 2–34%), while in studies with short sedimentation times, the average increase was more modest at 9% (median 1%; range 0–36%).

The average specificity of microscopy following processing with physical and chemical methods was 98%, comparable with the direct smear method. The evidence in this review

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### 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 tuberculosis: a systematic review. Lancet Infect. Dis. 6(10), 664–674 (2006).

- 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.

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Website

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