Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review


CRD summary
This review concluded that fluorescence microscopy is more sensitive than conventional microscopy with similar specificity. The authors' conclusions are likely to be reliable, but should be interpreted with some caution because of limitations in the literature search and the failure to adequately consider study quality in the analysis.

Authors' objectives
To compare the accuracy of fluorescence microscopy with conventional microscopy for the diagnosis of tuberculosis (TB).

Searching
PubMed (1950 to 2005), BIOSIS Previews (1969 to 2004), EMBASE (1974 to 2004) and Web of Science (1945 to 2004) were searched. The search terms were reported and included a diagnostic filter. Two TB journals were handsearched, experts in the field were contacted, and the reference lists of primary studies, reviews, textbook chapters and dissertations were screened for additional studies. Only studies published in the English language were eligible for inclusion.

Study selection
Study designs of evaluations included in the review
No inclusion criteria relating to the study design were applied: both prospective and retrospective studies were included. Studies that mainly addressed economic issues, reviews and case reports were excluded.

Specific interventions included in the review
Studies that compared fluorescence microscopy (FM) with conventional microscopy (CM) and included duplicate slides, one slide using a carbolfuchsin stain and the other using a fluorochrome stain, were eligible for inclusion. Studies in which the slide was first screened with a fluorochrome stain then confirmed with a carbolfuchsin stain were excluded. Only studies that evaluated sputum specimens were included. Studies that used microscopy methods specifically to detect non-tuberculous mycobacteria, or that used sputum smears to monitor response to anti-TB therapy, were excluded. Where specified, the stains used in the included studies were auramine, auramine 0 and auramine-rhodamine for FM and Ziehl-Neelsen or Kinyoun stain for CM. Thresholds required for positivity of both CM and FM were >0, >2 or >9 acid fast bacilli (AFB). Some studies used sputum processing methods such as acetyl-cysteine alkali, sodium hydroxide, N-acetyl-L-cysteine-sodium hydroxide followed by centrifugation (speed 3,000 g or not reported), shaker or trisodium phosphate.

Reference standard test against which the new test was compared
Studies that used culture as a reference standard and those that did not use a reference standard were included.

Participants included in the review
No inclusion criteria relating to the participants were applied: studies of both treated and untreated patients were included.

Outcomes assessed in the review
No inclusion criteria relating to the outcome were specified.

How were decisions on the relevance of primary studies made?
Two reviewers independently assessed studies for inclusion.
Assessment of study quality
The studies were assessed for the following methodological criteria: comparison of index test with independent reference standard; interpretation of the FM result without knowledge of the CM result and vice versa; microscopy carried out without knowledge of culture result; and prospective enrolment of consecutive patient with suspected pulmonary TB. Two reviewers independently assessed study quality; any disagreements were resolved through consensus.

Data extraction
Two reviewers independently extracted the data; any disagreements were resolved through consensus.

For studies that included a reference standard (culture), the sensitivity and specificity with 95% confidence intervals (CIs) were calculated separately for FM and CM; for calculation of these measures, most studies excluded any contaminated culture results. Differences in estimates of sensitivity and specificity between FM and CM were calculated. For studies that did not use a reference standard, data on the incremental yield (with 95% CIs) were extracted. This referred to the proportion of positive smears by FM minus the proportion of positive smears by CM.

When data were available for both Mycobacterium tuberculosis and non-TB mycobacteria, the sensitivity and specificity were calculated based on cultures positive for Mycobacterium tuberculosis alone. If resolved data were presented based on discrepant analysis then unresolved data were extracted where possible. The authors of the primary studies were contacted for further information where necessary.

Methods of synthesis
How were the studies combined?
Pooled incremental yields and differences in sensitivity and specificity between FM and CM were calculated using simple averages without applying any weightings. Summary receiver operating characteristic (SROC) curves were estimated using the Moses-Littenberg method. These were used to investigate the effects of variability in threshold on study results. Q* (the point where sensitivity equals specificity) and the area under the SROC curve (AUC) were calculated as global measures of accuracy.

How were differences between studies investigated?
No formal methods of investigating heterogeneity were reported. The studies were grouped by type of stain, number of AFB per smear required for positivity, and presence of a reference standard. Five studies were excluded from the analysis: two were considered outliers, two measured detection rates of AFB in sputa samples preserved with cetylpyridinium chloride, and one was a multicentre study that used an atypical study design. Pooled incremental yields were calculated separately for studies performed in high-burden and low-burden countries.

Results of the review
Forty-five studies reported in 30 publications were included in the review. The mean sample size was 1,907 patients or specimens with a median of 493 and a range of 12 to 23,427 (total number of samples/patient was 50,015).

Almost half of the studies used a reference standard (mycobacterial culture). Descriptive information on microscopy characteristics was commonly not reported.

Sensitivity of FM compared with CM (14 publications reporting 18 studies; 12 studies also allowed calculation of specificity).

The sensitivity of CM ranged from 32 to 94% and the sensitivity of FM from 52 to 97%. The simple pooled mean differences showed that sensitivity was 10% (95% CI: 5, 15) higher for FM than for CM. The sensitivity of FM was higher than that of CM in 16 studies, lower in one and equivalent in the other. The specificity of both CM and FM ranged from 94 to 100%. There was no difference in specificity between the two techniques based on simple pooled differences in specificity (0%, 95% CI: -0.9, +0.2). The AUC was 0.96 for FM compared with 0.94 for CM. Q* was 0.87 for CM and 0.92 for FM.
Difference in positivity rates (15 publications reporting 23 studies).

The difference in FM and CM positivity rates ranged from 0 to 33%, with all studies showing that the positivity rate of FM was either equivalent or greater to that of CM. On average, the positivity rate was 9% (95% CI: 5, 13) greater for FM compared with CM.

The use of sputum processing methods and different stains had little impact on the results. Two studies compared FM and CM in patients with human immunodeficiency virus (HIV) infection. The one that used a reference standard found that FM had a sensitivity of 73%, compared with 36% for CM; specificity was 100% for both techniques. The other study reported an incremental yield of 26% for FM compared with CM.

Authors’ conclusions
FM was more sensitive than CM, but specificity was similar for the two techniques. There was insufficient data to determine the value of FM in patients with HIV infection.

CRD commentary
This was a reasonably well-conducted and clearly reported review. The objective was focused and supported by defined inclusion criteria. The literature search was reasonably thorough, but a diagnostic filter was used in the electronic searches and only studies published in English were included. Relevant studies might therefore have been missed and the review might be subject to language bias. Details of the review process were reported and included appropriate steps to minimise bias. Although a formal quality assessment was undertaken, this was not incorporated into the synthesis of the results and the results were only available as a web appendix.

The methods used to synthesise the results were basic. However, a more sophisticated analysis, based on SROC curves, was also undertaken and this supported the findings of the more basic analysis. The authors’ conclusions appear reliable, but should be interpreted with some degree of caution because of limitations in the search and the failure to consider study quality in the meta-analysis.

Implications of the review for practice and research
Practice: The authors stated that the successful and widespread implementation of FM in TB-endemic countries might improve TB case-finding through an expected increase in direct smear sensitivity and expected decrease in time spent on microscopic examination.

Research: The authors stated that future studies should be prospective, blinded, have a reference standard and follow an adequate research protocol. The protocol should ideally encompass a 3-specimen set from the same patient, randomised for processing by different techniques (to avoid timing effects). In addition, the impact of FM in HIV-positive patients needs to be investigated further.

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Other publications of related interest
This additional published commentary may also be of interest. Fluorescence microscopy for tuberculosis diagnosis [correspondence]. Lancet Infect Dis 2007;7:236-40.

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This is a critical abstract of a systematic review that meets the criteria for inclusion on DARE. Each critical abstract contains a brief summary of the review methods, results and conclusions followed by a detailed critical assessment on the reliability of the review and the conclusions drawn.