Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review

CRD summary
This review concluded that centrifugation with any chemical modification increases the sensitivity of microscopy for the diagnosis of tuberculosis, with no impact on specificity, and that there was insufficient evidence to determine the value of sputum processing methods in patients with the human immunodeficiency virus. This was a well-conducted review and the results are likely to be reliable.

Authors’ objectives
To assess the ability of sputum processing methods to improve the accuracy of microscopy for the diagnosis of tuberculosis (TB).

Searching
PubMed, BIOSIS Previews, EMBASE and Web of Science were searched from inception to 2005 (PubMed) or 2004 (other databases); the search terms were reported. Two journals were handsearched, as were the bibliographies of included studies, reviews and textbook chapters. Experts in the field were also contacted. Only studies published in English were included.

Study selection
Study designs of evaluations included in the review
All study designs except case reports were eligible for inclusion.

Specific interventions included in the review
Studies evaluating chemical or physical methods to improve the diagnostic accuracy of microscopy, compared with the direct unconcentrated microscopic method, were eligible for inclusion. The included studies used carbolfuchsin or fluorochrome stain, or did not specify the stain used. Chemical modifiers included sodium hydroxide (with or without sodium lithocholate), sodium hypochlorite (bleach), ammonium sulphate, or other unspecified chemicals. Studies in which microscopy was used to monitor treatment response were excluded.

Reference standard test against which the new test was compared
Studies using culture as the ‘gold’ standard, or no reference standard, were included.

Participants included in the review
Studies of patients with suspected TB were eligible for inclusion. The studies included patients with suspected or with a confirmed diagnosis of pulmonary TB, patients undertaking routine sputum testing, or individuals with an abnormal chest X-ray.

Outcomes assessed in the review
There were no selection criteria relating to the outcomes. Sensitivity, specificity and positivity were reported.

How were decisions on the relevance of primary studies made?
Two reviewers independently screened titles and abstracts; a third reviewer screened full papers.

Assessment of study quality
Two reviewers independently assessed methodological quality in relation to the independence of smear testing, blinded interpretation of the test results, and the prospective recruitment of participants.

Data extraction
Two reviewers independently extracted the data for the review; any disagreements were resolved by consensus. Authors were contacted for additional information where required. The sensitivity, specificity and positivity, with 95% confidence intervals, were reported.
confidence intervals (CIs), were calculated for each study where reference standard results were reported. Where culture results were not reported, the incremental yield was extracted (number of positive smears from the improved method minus those from the unconcentrated microscopic method). Where studies had used discrepant analysis to resolve discordant results between the index test and reference standard, the unresolved data were extracted.

**Methods of synthesis**

How were the studies combined?

Differences between the sensitivity, specificity and positivity of processed and direct smears were calculated for each study and pooled across studies using no weighting. True-positive rates (sensitivity) and false-positive rates (1 minus the specificity) were plotted on summary receiver operating characteristic (sROC) curves; the area under the curve and the Q index (point where sensitivity equals specificity) were calculated as measures of test accuracy. The studies were grouped by the stain used, then the processing method, the presence of culture, and the centrifugation force (cut point 2,000 g/2,500 rpm) or gravity sedimentation duration (cut point 1 hour).

How were differences between studies investigated?

Subgroup analyses were undertaken to investigate heterogeneity. Forest plots and sROC curves were provided for the visual inspection of heterogeneity, study details were tabulated, and differences were discussed in the text.

**Results of the review**

Forty-six articles, reporting 83 studies, met the inclusion criteria.

Centrifugation with any chemical modification (32 studies).

Thirteen out of 14 studies that used culture for a comparison reported an increase in sensitivity with chemical modification; mean 18% (95% CI: 11, 26). Fifteen of 18 studies that did not use culture for a comparison reported an increase in incremental yield; mean 7% (95% CI: 3, 11). All 6 studies that compared direct and processed methods and used culture reported an increase in sensitivity with modified smears; mean 13% (95% CI: -1, 26). All 11 studies comparing direct and processed methods without culture reported an increase in incremental yield with modified smears; mean 9% (95% CI: 5, 14).

Subgroup analyses for studies using sodium hydroxide, studies using fluorescence microscopy, and studies in patients with the human immunodeficiency virus (HIV) were presented.

Gravity sedimentation combined with any chemical modification (16 studies).

All 4 studies with culture that used overnight sedimentation reported an increase in sensitivity; mean 23% (95% CI: -1, 47). The 4 studies with culture that used 30 to 45 minutes' sedimentation reported a mean increase in sensitivity of 9% (95% CI: -19, 38). All 5 studies without culture that used overnight sedimentation reported an increase in sensitivity; mean 5% (95% CI: -3, 14). The 3 studies without culture that used 30 to 45 minutes' sedimentation reported a mean change in sensitivity ranging from -4 to 8%.

Subgroup analyses for studies using bleach and studies in patients with HIV were presented.

None of the modifications had an effect on specificity. The sROC curves showed improvements for processed smears in discriminatory ability and increased accuracy, but these were not statistically significant.

**Authors’ conclusions**

Centrifugation with any chemical modification increased sensitivity, with no impact on specificity. There was insufficient evidence to determine the value of sputum processing methods in patients with HIV.

**CRD commentary**

The review addressed a clear research question. The authors undertook a comprehensive search, but there is the potential for language bias as only English language papers were included. Each stage of the review was conducted in duplicate, thereby reducing the potential for error and bias. The analysis was appropriate. This was a well-conducted review and the results are likely to be reliable.
Implications of the review for practice and research

Practice: The authors stated that any new methods employed should be accompanied by a strengthening of laboratory quality management systems.

Research: The authors suggested that studies are undertaken to assess the effects of different sputum processing methods under controlled conditions, and to identify optimal timing, concentrations and ease of use. This should be followed by comparisons of large, blinded, multicentre studies of one or two selected processing methods versus direct smears in populations with high and low prevalence of HIV. The authors also suggested that patients should be prospectively recruited, a reference standard used, test results are verified independently, and studies reported using the Standards for the Reporting of Diagnostic Accuracy (STARD) guidelines.

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