Review

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

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Most of the world's tuberculosis cases occur in low-income and middle-income countries, where sputum microscopy with a conventional light microscope is the primary method for diagnosing pulmonary tuberculosis. A major shortcoming of conventional microscopy is its relatively low sensitivity compared with culture, especially in patients co-infected with HIV. In high-income countries, fluorescence microscopy rather than conventional microscopy is the standard diagnostic method. Fluorescence microscopy is credited with increased sensitivity and lower work effort, but there is concern that specificity may be lower. We did a systematic review to summarise the accuracy of fluorescence microscopy compared with conventional microscopy. By searching many databases and contacting experts, we identified 45 relevant studies. Sensitivity, specificity, and incremental yield were the outcomes of interest. The results suggest that, overall, fluorescence microscopy is more sensitive than conventional microscopy, and has similar specificity. There is insufficient evidence to determine the value of fluorescence microscopy in HIV-infected individuals. The results of this review provide a point of reference, quantifying the potential benefit of fluorescence microscopy, with which the increased cost and technical complexity of the method can be compared to determine the possible value of the method under programme conditions.

Introduction

Sputum smear microscopy (henceforth referred to as microscopy) is currently recommended for the diagnosis of pulmonary tuberculosis in low-income and middle-income countries, where more than 90% of tuberculosis cases occur.[1–5] Microscopy is rapid, relatively simple, inexpensive, and highly specific in areas where there is a high prevalence of tuberculosis. In addition, microscopy identifies the most infectious patients and is widely applicable.[6,7] In some studies, microscopy has been reported to have greater than 80% sensitivity for identifying cases of pulmonary tuberculosis;[8,9] however, in other reports, the sensitivity of the test has been relatively low and variable (range 20–60%).[10,11] Microscopy has limited value for the diagnosis of tuberculosis in children and does not, by definition, identify smear-negative tuberculosis, which is more likely in HIV-positive than HIV-negative individuals.[1,3,12,13] Smear-negative tuberculosis has been associated with poor treatment outcomes, including death, especially in areas hit hard by the HIV epidemic.[14,15] For these reasons, methods to improve the sensitivity of microscopy are urgently needed, particularly in countries with a high HIV burden. One method, used most commonly in high-income countries and credited with improved sensitivity, is fluorescence microscopy.[16–18]

Introduced in the 1930s,[19] fluorescence microscopy uses an acid-fast fluorochrome dye (eg, auramine O or auramine-rhodamine) with an intense light source such as a halogen or high-pressure mercury vapour lamp. By comparison, conventional microscopy uses the carbolfuchsin Ziehl-Neelsen or Kinyoun acid-fast stains, and can be used with a conventional artificial light source or reflected sunlight.[2] The most important advantage of fluorescence microscopy is that it uses a lower power objective lens (typically 25×) than conventional microscopy (typically 100×), enabling the microscopist to assess the same area of a slide in less time.[20,21] Substantial savings in work effort have been reported with fluorescence microscopy, suggesting it may be cost-effective in some low-income countries.[20–22] Fluorescence microscopy has been credited with increased sensitivity,[22–26] and, for this reason, has been proposed by some experts for use in countries with a high prevalence of HIV infection.[1,22] Other advantages include the simplicity of the fluorochrome staining method compared with Ziehl-Neelsen methods.[27,28] A potential shortcoming of fluorescence microscopy is the possibility of false-positive results because inorganic objects may incorporate fluorochrome dyes.[29,30] In the 1970s, Kubica[25] assessed the relative value of fluorescence and conventional microscopy in a multicentre study of 61 163 Ziehl-Neelsen and 27 808 fluorescence microscopy specimens from nine countries. Kubica[25] found that fluorescence microscopy improved sensitivity, but left a lingering doubt about specificity.

We did a systematic review to summarise the evidence on the accuracy of fluorescence microscopy, according to the guidelines and methods proposed for diagnostic systematic reviews and meta-analyses.[31] We specifically addressed the following questions: (1) what is the sensitivity of fluorescence microscopy compared with conventional microscopy? (2) What is the specificity of fluorescence microscopy compared with conventional microscopy? (3) What is the impact of sputum processing on the sensitivity and specificity of fluorescence microscopy? (4) Is there a difference in sensitivity and specificity between auramine O and auramine-rhodamine stains? (5) Does the examination of smears with fluorescence microscopy take less time than with conventional microscopy?

Methods

Search strategy and selection criteria

We followed a standard protocol for doing systematic reviews of diagnostic test evaluations.[31] We searched electronic databases for primary studies and conference abstracts: PubMed (1950 to May, 2005), BIOSIS (1969 to November, 2004), Embase (1974 to 2004), and Web of Science (1945 to 2004). The search terms used included the following: "tuberculosis", "*Mycobacterium tuberculosis*", "acid-fast AND bacilli", "sputum AND microscopy", "bacteriology", "sensitivity AND specificity", "fluorescence", and "direct microscopy". We hand searched the indices of two journals devoted to tuberculosis, *The International Journal of Tuberculosis and Lung Disease* (1997 to 2005) and *The Indian Journal of Tuberculosis* (1953 to 2004), for relevant articles not already captured by the electronic database search. In addition, we identified additional studies by contacting experts in the field, and by searching reference lists from primary studies, review articles, text book chapters, and dissertations.

Our search strategy aimed to identify all available studies published in English that compared the results of fluorescence microscopy and conventional microscopy. The following studies were excluded: (1) procedures done on specimens other than sputa, (2) use of microscopy methods specifically to detect nontuberculous mycobacteria, (3) use of sputum smears specifically to monitor response to anti-tuberculosis therapy, (4) studies mainly on cost-effectiveness or other economic issues, (5) case reports, and (6) reviews. No restrictions were made with respect to study design (eg, prospective or retrospective), or selection of patients, on the basis that some studies might include both untreated and treated patients. We included only studies in which duplicate slides were prepared: one slide using a carbolfuchsin stain, the other with a fluorochrome stain. We excluded studies in which the slide was first screened with a fluorochrome stain and the same slide subsequently confirmed with a carbolfuchsin stain. We included studies with culture as a reference standard and those without a reference standard.

Initially, two reviewers (VN and MH) screened citations retrieved from all sources. To identify relevant studies pertaining specifically to fluorescence microscopy, a second screen was done (VN and MH) of full texts from citations found relevant in the first screen. A third reviewer (KS) did a final independent screen on all full text articles and bibliographies.

Data extraction

Two reviewers (KS and MH) independently extracted data from the full set of eligible studies on the following aspects: methodological quality, staining and microscope characteristics, outcome measures (ie, sensitivity, specificity, and incremental yield), and reference standard (defined as mycobacterial culture). Interrater agreement of the reviewers on outcome measures was 100%. When
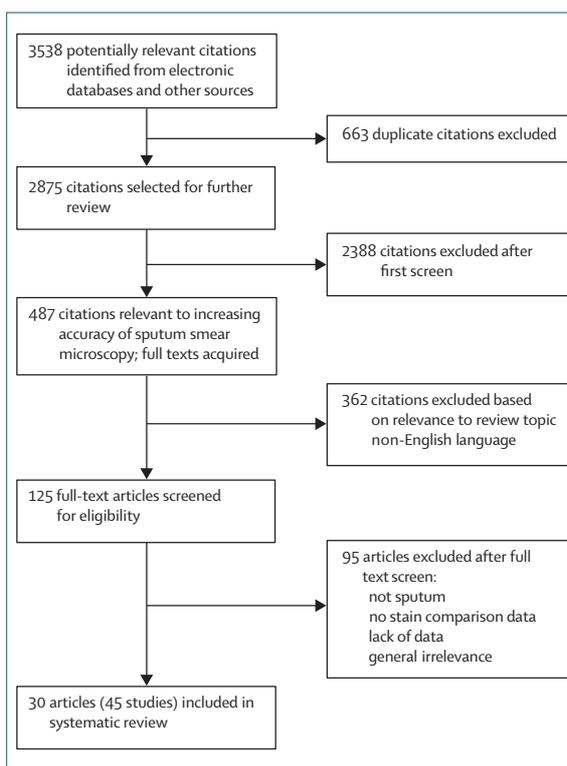

Figure 1: **Flow diagram for study selection**

data were not explicitly reported, reviewers coded the information as "not reported". Remaining disagreements were resolved by consensus before finalising data extraction.

When culture data were available for both *M tuberculosis* and nontuberculous mycobacteria, we calculated sensitivity and specificity based on cultures positive for *M tuberculosis* alone. Some authors provided corrected or resolved data on accuracy, after doing discrepant analyses. Since discrepant analysis (where discordant results between index test and reference standard are resolved, post hoc, using clinical or other laboratory data) may be a potential source of bias in diagnostic evaluations,[32] we preferentially included unresolved data where available.

Assessment of study quality

We assessed the quality of studies using the following criteria, suggested as important for diagnostic studies:[31] (1) was there a comparison of the index test with an independent reference standard? (2) Was the fluorescence microscopy result interpreted without knowledge (blinded) of the interpretation of the conventional microscopy result and vice versa? (3) Was microscopy done without knowledge of the culture result? (4) Did the study prospectively recruit consecutive patients suspected of having pulmonary tuberculosis? To overcome the problem of missing data, we attempted to contact investigators for additional information on study quality and results.

Study* (first author, year, country)	FM stain	Number patients or specimens	CM		FM		FM–CM sensitivity	FM–CM specificity
			Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)		
Bennedsen (a), 1966, Denmark[20]	AR	372	0·62 (0·57–0·67)	..	0·67 (0·61–0·71)	..	+5%	..
Bennedsen (b), 1966, Denmark[20]	AR	372	0·52 (0·47–0·57)	..	0·52 (0·46–0·56)	..	0%	..
Bennedsen (c), 1966, Denmark[20]	AR	23 427	0·55 (0·51–0·59)	1·00 (0·99–0·99)	0·63 (0·59–0·66)	1·00 (0·99–0·99)	+8%	0%
Bennedsen (d), 1966, Denmark[20]	AR	23 427	0·55 (0·51–0·58)	1·00 (0·99–0·99)	0·58 (0·54–0·62)	1·00 (0·99–0·99)	+3%	0%
Burdash, 1975, USA[41]	AR	250	0·94 (0·78–0·99)	1·00 (0·98–1·00)	0·97 (0·83–0·99)	0·99 (0·96–0·99)	+3%	−1%
Githui, 1993, Kenya[26]	A	1480	0·65 (0·61–0·67)	0·97 (0·95–0·98)	0·80 (0·77–0·82)	0·96 (0·93–0·97)	+15%	−1%
Holst, 1959, India[27]	A	1354	0·66 (0·62–0·69)	0·98 (0·96–0·98)	0·67 (0·63–0·70)	0·98 (0·96–0·98)	+1%	0%
Kivihya-Ndugga (a), 2003, Kenya[22]	..	993	0·32 (0·27–0·35)	1·00 (0·99–1·00)	0·67 (0·62–0·70)	0·99 (0·97–0·99)	+35%	−1%
Kivihya-Ndugga (b), 2003, Kenya[22]	..	993	0·60 (0·55–0·64)	0·98 (0·96–0·99)	0·78 (0·73–0·81)	0·98 (0·96–0·99)	+18%	0%
Kumar, 1979, India[46]	AO	574	0·89 (0·85–0·92)	0·96 (0·93–0·98)	0·94 (0·90–0·96)	0·97 (0·94–0·98)	+5%	+1%
Narain (a), 1971, India[46]	..	436	0·84 (0·80–0·87)	..	0·90 (0·86–0·92)	..	+6%	..
Narain (b), 1971, India[49]	..	436	0·71 (0·66–0·75)	..	0·84 (0·80–0·87)	..	+13%	..
Singh (b), 1998, Nepal[24]	A	205	0·48 (0·38–0·56)	0·94 (0·86–0·98)	0·57 (0·47–0·65)	0·94 (0·86–0·98)	+9%	0%
Somlo, 1969, USA[52]	AR	3000	0·52 (0·46–0·58)	0·99 (0·98–0·99)	0·71 (0·65–0·76)	0·98 (0·97–0·98)	+19%	−1%
Tansuphasiri, 2002, Thailand[53]	AO	392	0·69 (0·59–0·77)	0·97 (0·94–0·99)	0·60 (0·50–0·68)	0·98 (0·95–0·99)	−9%	+1%
Truant, 1962, USA[57]	AR	585	0·78 (0·65–0·87)	..	0·93 (0·83–0·98)	..	+15%	..
Ulukanligil, 2000, Turkey[56]	AO	40	0·70 (0·53–0·83)	..	0·93 (0·79–0·98)	..	+23%	..
Weiser, 1966, USA[28]	AO	345	0·52 (0·37–0·65)	0·99 (0·97–0·99)	0·63 (0·48–0·75)	0·97 (0·94–0·98)	+11%	−2%

*See webtable for further details on studies. Difference between fluorescence microscopy and conventional microscopy (CM) sensitivity estimates: mean +10% (95% CI 5, 15). Difference between FM and CM specificity estimates: mean 0% (95% CI −0·9, +0·2). A=auramine; AO=auramine O; AR=auramine-rhodamine; ..=not reported.

Table 1: Studies comparing sensitivity and specificity of conventional and fluorescence microscopy

See **Online** for webtable

Data collation and meta-analysis

We used standard methods recommended for meta-analyses of diagnostic test evaluations.[31,33] As studies were heterogeneous in many respects, including selection of patients, staining and counterstaining or oxidising procedures, type of microscope, magnifications used, and use of a reference standard (mycobacterial culture), we grouped studies by type of stain (ie, auramine O or auramine-rhodamine), number of acid-fast bacilli (AFB) per smear required for positivity, and presence of a reference standard. For purposes of analysis, studies with Kinyoun stains were initially analysed separately, but because this did not change the overall results, we included the Kinyoun studies with Ziehl-Neelsen studies. Studies that used auramine were grouped with studies that used auramine O; studies using auramine-rhodamine dye were grouped separately.

Four studies were excluded from subgroup analysis.[23,34,35] Results from the studies by Bell and Brown[23] and Damle and Kaundinya[34] were considered outliers, possibly because of a combination of untreated and treated patients in their study populations. Selvakumar and colleagues[35] measured detection rates of AFB in sputa samples preserved with cetylpyridium chloride. This study showed reduced detection of AFB using Ziehl-Neelsen smears preserved with cetylpyridium chloride.[35] Although excluded from the analyses, these four studies are included in the webtable. In addition, we did not include the results from Kubica's multicentre study[25] in the subgroup analyses because of the atypical study design, in which data were combined from many contributors rather than provided for separate investigations.

To calculate sensitivity and specificity of Ziehl-Neelsen-stained and fluorochrome-stained microscopy smears, we cross-tabulated each result against culture. Sensitivity refers to the proportion of culture-positive sputum samples that are identified as positive by the staining method in question; specificity refers to the proportion of culture-negative sputum samples that are identified as negative by the same smear method. For calculation of these measures, most studies excluded any contaminated culture results. For studies that did not use a reference standard, we calculated the incremental yield. Incremental yield refers to the proportion of positive smears (smear positivity rate) by fluorescence microscopy minus the proportion of positive smears by conventional microscopy.

Data were analysed using Meta-DiSc software (version 1.1.1).[36] Sensitivity, specificity, and positivity rates were calculated for FM and CM for each study, along with their 95% confidence intervals. We then estimated the difference between FM and CM estimates and then pooled them across studies using simple averages. No weighting was used. However, we

separately calculated the mean sensitivity for FM and CM for the four largest studies and separately for studies performed in high-burden and low-burden countries. In addition to the sensitivity and specificity estimates and forest plots generated for this review, true positive rates (sensitivity) and false positive rates (1–specificity) were summarised using a summary receiver operating characteristic (SROC) curve. Because true positive and false positive rates are correlated and vary with the thresholds (cut points for determining test positives) used in the original studies, we did not pool the sensitivity and specificity estimates separately; instead we analysed true positive and false positive rates as pairs, and explored the effect of variability in cut-points on study results. Unlike a traditional ROC plot that explores the effect of varying thresholds on sensitivity and specificity in a single study, each data point in the SROC space represents an individual study. As described by Littenberg and Moses,[33] the SROC curve is obtained by fitting a regression curve to pairs of true positive and false positive rates.

The SROC curve and the area under the curve (AUC) give an overall summary of test performance and show the trade-off between sensitivity and specificity. A symmetric, shoulder-like SROC curve suggests that variability in thresholds used could, in part, explain variability in study results. An AUC of 1·0 (eg, 100%) indicates perfect discriminatory ability in the diagnostic test. In addition, the Q* index is another useful global summary of the SROC curve and test performance. The Q* index, defined by the point where sensitivity equals specificity on the SROC curve, is the point on the SROC curve that is intersected by the anti-diagonal, the top-left corner of the SROC region. A Q* value of 1·0 indicates 100% accuracy (sensitivity and specificity of 1·0).[33,37,38]

In meta-analyses, heterogeneity refers to the degree of variability between study results. Such heterogeneity could be a result of variability in thresholds, disease spectrum, assay methods, and study quality between studies. In the presence of significant heterogeneity, pooled or summary estimates from meta-analyses are hard to interpret. Because of the heterogeneity in the methods used for microscopy and the anticipated variability in accuracy estimates, we decided a priori to avoid simple pooling of sensitivity and specificity. Also, as described previously, we addressed heterogeneity by using subgroup (stratified) analyses.

Results

Description of included studies

Of the 3538 citations identified after literature searches, 30 articles consisting of 45 studies met our eligibility criteria.[20,22–24,26–28,30,34,35,39–58] We considered most studies to be independent (references 24 [study b] and 50 [study b] are substudies). Therefore, no effort was made to account for lack of independence. Figure 1 shows the process for

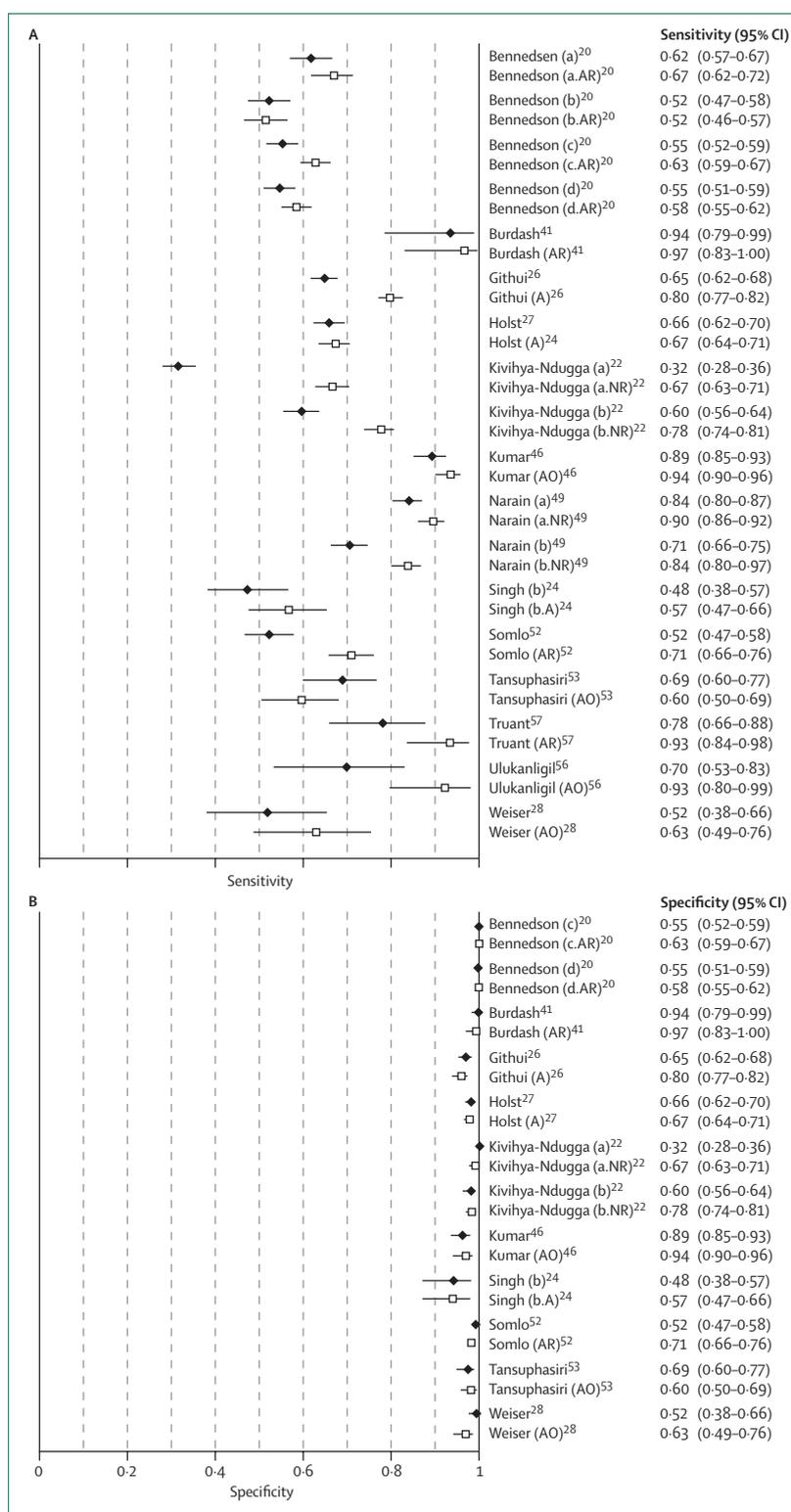*Figure 2:* **Forest plot of sensitivity and specificity estimates of conventional microscopy (CM) and fluorescence microscopy (FM)**
(A) Sensitivity (18 studies). (B) Specificity (12 studies). Point estimates of sensitivity and specificity from each study are shown as solid circles for CM and as open squares for FM. The solid lines represent 95% CIs. A=auramine; AO=auramine O; AR=auramine-rhodamine; NR=not reported.

Study* (first author, year, country)	FM stain	Number patients or specimens	Positivity rate (95% CI)		FM positivity– CM positivity
			CM	FM	
Ba (a), 1999, Senegal[39]	AO	2630	0·19 (0·13–0·20)	0·22 (0·25–0·23)	+3%
Ba (b), 1999, Senegal[39]	AO	2630	0·17 (0·15–0·18)	0·18 (0·12–0·19)	+1%
Ba (c), 1999, Senegal[39]	AO	2630	0·15 (0·17–0·16)	0·16 (0·12–0·17)	+1%
Bogen (a), 1941, USA[40]	AO	1000	0·37 (0·30–0·40)	0·45 (0·44–0·47)	+8%
Bogen (b), 1941, USA[40]	AO	250	0·00 (0·00–0·01)	0·12 (0·09–0·16)	+12%
Freiman (a), 1943, USA[42]	AO	192	0·66 (0·54–0·72)	0·68 (0·18–0·21)	+2%
Freiman (b), 1943, USA[42]	AO	461	0·21 (0·18–0·25)	0·21 (0·10–0·24)	0%
Gilkerson (a), 1963, USA[58]	AO	400	0·15 (0·16–0·18)	0·26 (0·20–0·30)	+11%
Gilkerson (b), 1963, USA[58]	AO	207	0·20 (0·16–0·25)	0·30 (0·22–0·37)	+10%
Habeenzu (a), 1998, Zambia[43]	A	488	0·14 (0·16–0·16)	0·31 (0·21–0·35)	+17%
Habeenzu (b), 1998, Zambia[43]	A	488	0·24 (0·21–0·27)	0·31 (0·21–0·35)	+7%
Jain, 2002, India[44]	AR	493	0·33 (0·21–0·37)	0·42 (0·32–0·46)	+9%
Koch, 1964, USA[45]	AO	427	0·19 (0·14–0·23)	0·27 (0·20–0·31)	+8%
Lempert, 1944, England[47]	A	300	0·14 (0·13–0·18)	0·15 (0·12–0·19)	+1%
Lind, 1941, USA[48]	AO	1123	0·10 (0·04–0·12)	0·11 (0·08–0·12)	+1%
Prasanthi (a), 2005, India[50]	AO	200	0·50 (0·43–0·57)	0·69 (0·62–0·75)	+19%
Prasanthi (b), 2005, India[50]	AO	31	0·32 (0·17–0·51)	0·58 (0·39–0·76)	+26%
Richards (a), 1941, USA[30]	A	12	0·67 (0·39–0·90)	1·00 (0·75–1·00)	+33%
Richards (b), 1941, USA[30]	A	12	0·83 (0·56–0·97)	1·00 (0·75–1·00)	+17%
Ritterhoff, 1945, USA[51]	AO	597	0·52 (0·45–0·55)	0·63 (0·55–0·67)	+11%
Singh (a), 1998, Nepal[24]	A	2600	0·38 (0·36–0·39)	0·42 (0·46–0·44)	+4%
Thompson, 1941, USA[54]	AO	1528	0·04 (0·00–0·05)	0·04 (0·01–0·05)	0%
Wilson, 1952, Australia[55]	AR	1098	0·13 (0·13–0·15)	0·14 (0·14–0·16)	+1%

*See webtable for further details on studies. Difference between fluorescence microscopy (FM) and conventional microscopy (CM) positivity rates: mean=+9% (95% CI 5, 13). A=auramine; AO=auramine O; AR=auramine-rhodamine.

Table 2: **Studies comparing incremental yield of conventional and fluorescence microscopy**

Study* (first author, year, country)	Number AFB required for positivity					
	Conventional microscopy			Fluorescence microscopy		
	>0	>2	>9	>0	>2	>9
Bennedsen (a), 1966, Denmark[20]	0·62 (0·57–0·67)	..	..	0·67 (0·61–0·71)	..	..
Bennedsen (b), 1966, Denmark[20]	..	..	0·52 (0·47–0·57)	..	..	0·52 (0·46–0·56)
Bennedsen (c), 1966, Denmark[20]	0·55 (0·51–0·59)	..	..	0·63 (0·59–0·66)	..	..
Bennedsen (d), 1966, Denmark[20]	..	..	0·55 (0·51–0·58)	..	..	0·58 (0·54–0·62)
Githui, 1993, Kenya[26]	..	0·65 (0·61–0·67)	..	..	0·80 (0·77–0·82)	..
Holst, 1959, India[27]	..	0·66 (0·62–0·69)	..	..	0·67 (0·63–0·70)	..
Narain (a) 1971, India[49]	0·84 (0·80–0·87)	..	..	0·90 (0·86–0·92)	..	..
Narain (b) 1971, India[49]	..	0·71 (0·66–0·75)	..	..	0·84 (0·80–0·87)	..
Singh (b), 1998, Nepal[24]	0·48 (0·38–0·56)	..	..	0·57 (0·47–0·65)	..	..
Tansuphasiri, 2002, Thailand[53]	..	0·69 (0·59–0·77)	..	..	0·60 (0·50–0·68)	..
Ulukanligil, 2000, Turkey[56]	0·70 (0·53–0·83)	..	..	0·93 (0·79–0·98)	..	..
All studies combined	0·64 (0·51–0·77)	0·68 (0·65–0·71)	0·54 (0·49–0·57)	0·74 (0·59–0·89)	0·73 (0·61–0·85)	0·55 (0·47–0·62)

*See webtable for further details on studies. Data are mean (95% CI) sensitivity. ..=not reported.

Table 3: **Studies comparing sensitivity of conventional and fluorescence microscopy at different thresholds for acid-fast bacilli (AFB) smear positivity**

study selection and exclusion. For conventional microscopy, 43 (96%) studies used Ziehl-Neelsen stain and two (4%) used Kinyoun stain. For fluorescence microscopy, 32 (71%) studies used auramine O or auramine stain, nine (20%) used auramine-rhodamine, and four (9%) used an unspecified fluorochrome dye.

Study* (first author, year, country)	FM stain	Number patients or specimens	Processing method	CM		FM		FM–CM sensitivity	FM–CM specificity
				Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)		
Burdash, 1975, USA[41]	AR	250	Acetyl-cysteine alkali, centrifugation 3000 g	0·94 (0·78–0·99)	1·00 (0·98–1·00)	0·97 (0·83–0·99)	0·99 (0·96–0·99)	+3%	−1%
Githui, 1993, Kenya[26]	A	1480	Shaker	0·65 (0·61–0·67)	0·97 (0·95–0·98)	0·80 (0·77–0·82)	0·96 (0·93–0·97)	+15%	−1%
Holst, 1959, India[27]	A	1354	NaOH, centrifugation speed not reported	0·66 (0·62–0·69)	0·98 (0·96–0·98)	0·67 (0·63–0·70)	0·98 (0·96–0·98)	+1%	0%
Kumar, 1979, India[46]	AO	574	NaOH, centrifugation 3000 g	0·89 (0·85–0·92)	0·96 (0·93–0·98)	0·94 (0·90–0·96)	0·97 (0·94–0·98)	+5%	+1%
Truant, 1962, USA[57]	AR	585	Trisodium phosphate	0·78 (0·65–0·87)	..	0·93 (0·83–0·98)	..	+15%	..
Ulukanligil, 2000, Turkey[56]	AO	40	NALC-NaOH, centrifugation 3000 g	0·70 (0·53–0·83)	..	0·93 (0·79–0·98)	..	+23%	..
Weiser, 1966, USA[28]	AO	345	NALC-NaOH, centrifugation speed not reported	0·52 (0·37–0·65)	0·99 (0·97–0·99)	0·63 (0·48–0·75)	0·97 (0·94–0·98)	+11%	−2%

*See webtable for further details on studies. Difference between fluorescence microscopy (FM) and convention microscopy (CM) sensitivity estimates: mean +10% (95% CI 3, 18). Difference between FM and CM specificity estimates: mean −1% (95% CI −2, +0·8). A=auramine; AO=auramine O; AR=auramine-rhodamine; NALC-NaOH=N-acetyl L-cysteine-sodium hydroxide; ..=not reported.

Table 4: Studies comparing sensitivity and specificity of conventional and fluorescence microscopy after a sputum processing method

22 (49%) studies used a reference standard, all of which were mycobacterial culture. The mean sample size was 1907 patients or specimens (median 493, range 12–23 427, SD 4874). Descriptive information on microscopy characteristics (eg, total magnification for conventional and fluorescent microscopes, time to read slide, and light source for the fluorescent microscope) was commonly not reported. Data about the quality of culture was largely unavailable. The webtable provides additional information on study population, microscopy characteristics, methods, and quality.

Sensitivity of fluorescence microscopy compared with conventional microscopy

Table 1 shows the studies that compared sensitivity and specificity of conventional and fluorescence microscopy. Figure 2 shows the corresponding sensitivity and specificity forest plots. Sensitivity of conventional microscopy ranged from 0·32 to 0·94, and sensitivity of fluorescence microscopy ranged from 0·52 to 0·97. Fluorescence microscopy was on average 10% more sensitive than conventional microscopy (p<0·001; 95% CI 5–15, Z test for difference between proportions). The sensitivity of fluorescence microscopy was higher than conventional microscopy in 16 studies,[20,22,24,26–28,41,46,49,52,56,57] lower in one study,[53] and equivalent in one study.[20] We separately calculated the mean sensitivity of fluorescence microscopy compared with conventional microscopy for the four largest studies,[20,26,52] and by countries with high versus low tuberculosis burden and found similar results (data not shown). The mean increase in incremental yield was 9% (95% CI 5–13). With respect to incremental yield, 21 studies reported fluorescence microscopy positivity rates that were higher than rates for conventional microscopy,[24,30,39,40,42–45,47,48,50,51,55,58] and two studies reported no difference in rates (table 2).[42,54]
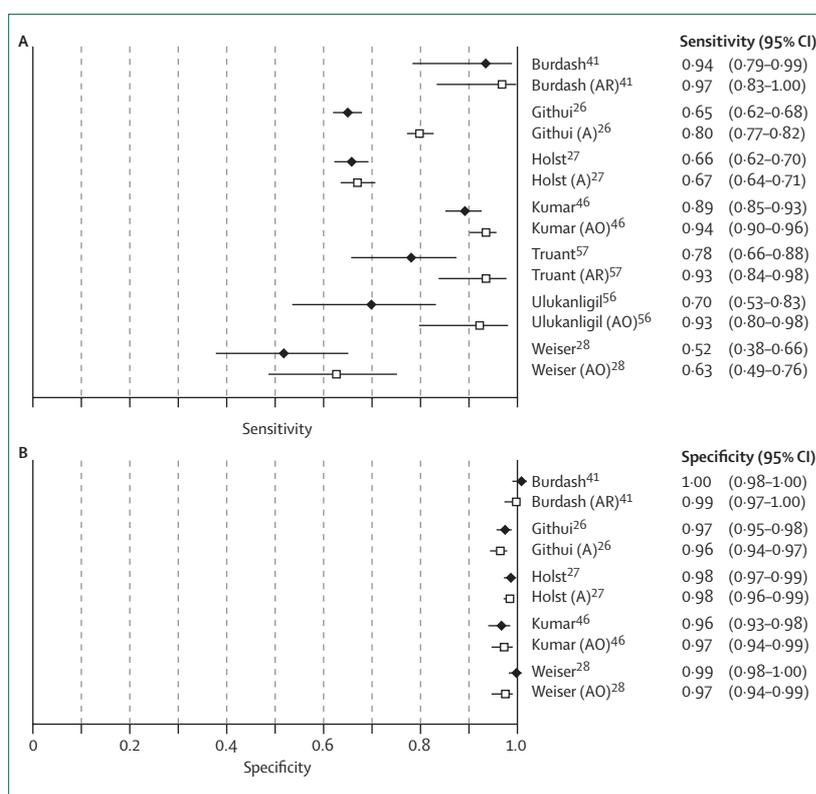

Figure 3: Forest plot of sensitivity and specificity estimates of conventional microscopy (CM) and fluorescence microscopy (FM), when a sputum processing method was used before smear preparation
(A) Sensitivity (7 studies). (B) Specificity (5 studies). Point estimates of sensitivity and specificity from each study are shown as solid circles for CM and as open squares for FM. The solid lines represent 95% CIs. A=auramine; AO=auramine O; AR=auramine-rhodamine.

Specificity of fluorescence microscopy compared with conventional microscopy

Specificity estimates spanned a similar range for conventional microscopy and fluorescence microscopy

Study* (first author, year, country)	FM stain	Number patients or specimens	CM		FM		FM–CM sensitivity	FM–CM specificity
			Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)		
Githui, 1993, Kenya[26]	A	1480	0·93 (0·88–0·96)	0·50 (0·15–0·84)	0·93 (0·88–0·96)	1·00 (0·63–1·00)	+15%	−1%
Holst, 1959, India[27]	A	1354	0·65 (0·61–0·67)	0·97 (0·95–0·98)	0·80 (0·77–0·82)	0·96 (0·93–0·97)	+1%	0%
Kumar, 1979, India[46]	AO	574	0·66 (0·62–0·69)	0·98 (0·96–0·98)	0·67 (0·63–0·70)	0·98 (0·96–0·98)	+4%	+1%
Singh (b), 1998, Nepal[24]	A	205	0·48 (0·38–0·56)	0·94 (0·86–0·98)	0·57 (0·47–0·65)	0·94 (0·86–0·98)	+9%	0%
Tansuphasiri, 2002, Thailand[53]	AO	392	0·69 (0·59–0·77)	0·97 (0·94–0·99)	0·60 (0·50–0·68)	0·98 (0·95–0·99)	−9%	+1%
Ulukanligil, 2000, Turkey[56]	AO	40	0·70 (0·53–0·83)	..	0·93 (0·79–0·98)	..	+23%	..
Weiser, 1966, USA[28]	AO	345	0·52 (0·37–0·65)	0·99 (0·97–0·99)	0·63 (0·48–0·75)	0·97 (0·94–0·98)	+11%	−2%

*See web table for further details on studies. Difference between fluorescence microscopy (FM) and conventional microscopy (CM) sensitivity estimates: mean +8% (95% CI −2, +17). Difference between FM and CM specificity estimates: mean 0% (95% CI −1, +1). A=auramine; AO=auramine O; ..=not reported.

Table 5: Studies comparing sensitivity and specificity of conventional microscopy and fluorescence microscopy with auramine O and auramine stains

Study* (first author, year, country)	Number patients or specimens	CM		FM		FM–CM sensitivity	FM–CM specificity
		Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)		
Bennedsen (a), 1966, Denmark[20]	372	0·62 (0·57–0·67)	..	0·67 (0·61–0·71)	..	+5%	..
Bennedsen (b), 1966, Denmark[20]	372	0·52 (0·47–0·57)	..	0·52 (0·46–0·56)	..	−1%	..
Bennedsen (c), 1966, Denmark[20]	23 427	0·55 (0·51–0·59)	1·00 (0·99–0·99)	0·63 (0·59–0·66)	1·00 (0·99–0·99)	+8%	0%
Bennedsen (d), 1966, Denmark[20]	23 427	0·55 (0·51–0·58)	1·00 (0·99–0·99)	0·58 (0·54–0·62)	1·00 (0·99–0·99)	+4%	0%
Burdash, 1975, USA[41]	250	0·94 (0·78–0·99)	1·00 (0·98–1·00)	0·97 (0·83–0·99)	0·99 (0·96–0·99)	+3%	−1%
Somlo, 1969, USA[52]	3000	0·52 (0·46–0·58)	0·99 (0·98–0·99)	0·71 (0·65–0·76)	0·98 (0·97–0·98)	+19%	−1%
Truant, 1962, USA[57]	585	0·78 (0·65–0·87)	..	0·93 (0·83–0·98)	..	+15%	..

*See webtable for further details on studies. Difference between fluorescence microscopy (FM) and conventional microscopy (CM) sensitivity estimates: mean +8% (95% CI 1, 14). Difference between FM and CM specificity estimates: mean −1% (95% CI −1, +0·4). ..=not reported.

Table 6: Studies comparing sensitivity and specificity of conventional microscopy and fluorescence microscopy with auramine-rhodamine stain

(specificity 0·94–1·00; table 1, figure 2). On average, the specificity of fluorescence microscopy was similar to of conventional microscopy (mean difference 0%; p=0·21; 95% CI −0·9, +0·2; Z test for difference between proportions).

Table 3 shows a comparison of conventional microscopy and fluorescence microscopy with three different thresholds as the definition of smear positivity (ie, >0, >2, and >9 AFB per smear). The sensitivity of conventional and fluorescence microscopy in these studies ranged from 0·48 to 0·93. By use of the most conservative threshold for positivity of more than nine AFB per smear, conventional microscopy detected on average 54% and fluorescence microscopy detected 55% of specimens found to be positive for *M tuberculosis* on culture. When more than two AFB per smear was used as the definition of a positive smear, the sensitivity of both fluorescence microscopy and conventional microscopy increased; however, the mean sensitivity estimate of fluorescence microscopy (0·73, 95% CI 0·61–0·85) was slightly greater than that of conventional

microscopy (0·68, 95% CI 0·65–0·71), suggesting an advantage of fluorescence microscopy in low-grade positives. The mean specificity estimates of fluorescence microscopy and conventional microscopy were similar (0·97, 0·97, and 1·00) at thresholds of more than zero, two, and nine AFB per smear, respectively (data not shown).

Impact of sputum processing on the sensitivity and specificity of fluorescence microscopy

Table 4 shows sensitivity (seven studies) and specificity (five studies) of conventional microscopy and fluorescence microscopy for studies that used various sputum processing methods, including processing with household bleach, before staining and reading of smears. Figure 3 shows the corresponding sensitivity and specificity forest plots. Sensitivity estimates of conventional microscopy ranged from 0·52 to 0·94, and from 0·63 to 0·97 for fluorescence microscopy. Fluorescence microscopy was 10% more sensitive than conventional microscopy (95% CI 3–18), with all studies

showing an increase (summary measures are not weighted for size of study).[26–28,41,46,56,57] Specificity of fluorescence microscopy was similar to conventional microscopy (mean difference –1%, 95% CI –2, +0.8).

Difference in sensitivity and specificity between auramine O and auramine-rhodamine stains

Studies using auramine O for comparison with conventional microscopy are shown in table 5. Sensitivity (seven studies) of conventional microscopy ranged from 0·48 to 0·93, and from 0·57 to 0·93 for fluorescence microscopy. Fluorescence microscopy was on average 8% more sensitive than conventional microscopy (95 % CI –2, +17). The sensitivity of fluorescence microscopy was higher than conventional microscopy in six studies,[24,26–28,46,56] and lower in one study.[53] There was no difference in the specificity of fluorescence microscopy and conventional microscopy (six studies; mean difference 0%, 95% CI –1, 1).

Studies using auramine-rhodamine for comparison with conventional microscopy are shown in table 6. Sensitivity (seven studies) of conventional microscopy ranged from 0·52 to 0·94, and from 0·52 to 0·97 for fluorescence microscopy. Fluorescence microscopy was on average 8% more sensitive than conventional microscopy (95% CI 1–14). The sensitivity of fluorescence microscopy was higher than conventional microscopy in six studies[20,41,52,57] and lower in one study.[20] Specificity of fluorescence and conventional microscopy was similar (four studies; mean difference –1%, 95% CI –1, +0·4).

Fluorescence microscopy in HIV infection

Only two studies assessed the accuracy of fluorescence microscopy in patients with documented HIV infection. In one study (339 patients), which used mycobacterial culture, fluorescence microscopy sensitivity was two times higher than that of conventional microscopy and specificity was similar (fluorescence microscopy: sensitivity 0·73; specificity 1·00; conventional microscopy: sensitivity 0·36; specificity 1·00).[22] A second study without a reference standard reported a 26% incremental yield of fluorescence microscopy compared with conventional microscopy in HIV-infected patients thought to have pulmonary tuberculosis on clinical and radiological examination.[50]

Time taken to examine smears by fluorescence and conventional microscopy

Ten studies provided information on the time required to read both carbolfuchsin and fluorochrome-stained slides.[20,39,42,57] However, only the study by Bennedsen and Larsen[20] used mycobacterial culture as a reference standard, allowing for the computation of sensitivity and specificity. This large double-blinded study (23 427 specimens; blinded to both smear and culture results), found that fluorescence microscopy, which took 1 min, had higher sensitivity and equivalent specificity
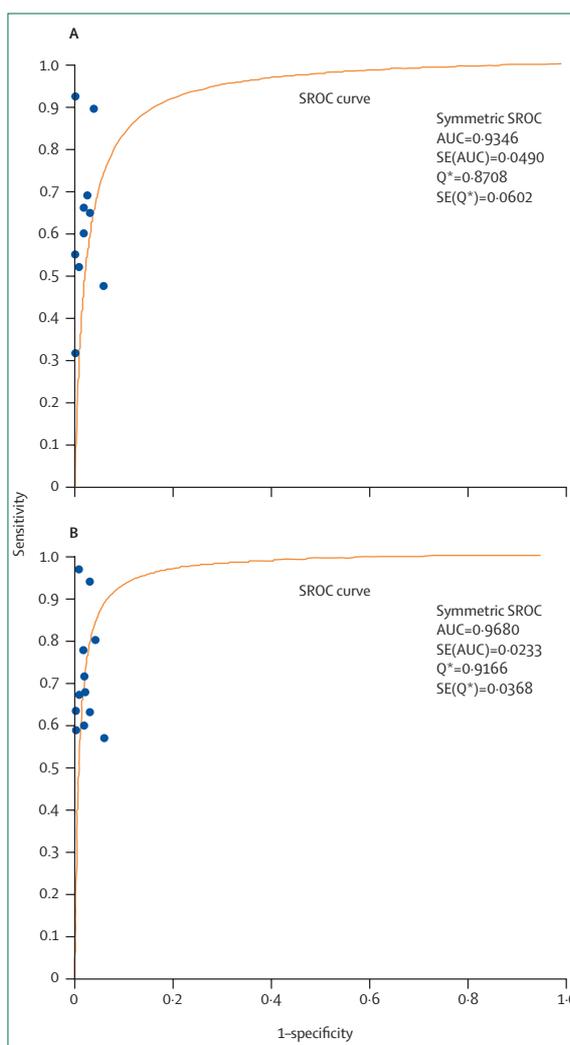

Figure 4: **Summary receiver operating characteristic (SROC) curves for studies that included both sensitivity and specificity estimates**
(A) Conventional microscopy studies. (B) Fluorescence microscopy studies. Each solid circle represents each study in the meta-analysis. The curve is the regression line that summarises the overall diagnostic accuracy. AUC=area under the curve; SE (AUC)=standard error of AUC; Q*=an index defined by the point on the SROC curve where the sensitivity and specificity are equal, which is the point closest to the top-left corner of the ROC space; SE (Q*)=standard error of Q* index.

compared to conventional microscopy, which took 4 min.

Impact of fluorescence microscopy on overall accuracy
Figure 4 shows SROC curves for conventional and fluorescence microscopy for studies that included both sensitivity and specificity estimates. Compared with conventional microscopy, fluorescence microscopy showed improved discriminatory ability of the test as well as higher accuracy (conventional microscopy AUC=0·94; fluorescence microscopy AUC=0·96), with a corresponding increase in the Q* index (conventional microscopy: Q*=0·87; fluorescence microscopy

Q*=0·92). These differences, however, were not statistically significant, based on overlapping confidence intervals for the AUCs.

Discussion

Our systematic review of 45 studies comparing fluorescence microscopy with conventional microscopy suggests that: (1) fluorescence microscopy is more sensitive for the diagnosis of pulmonary tuberculosis than conventional microscopy; (2) the specificity of fluorescence microscopy for detection of acid-fast organisms in sputum is similar to that of conventional microscopy; (3) the increased sensitivity of fluorescence microscopy is greater in low-grade positives; (4) fluorochrome-stained smears take less time to examine than smears stained with the Ziehl-Neelsen method; (5) when a variety of sputum-processing methods are used, the advantage of fluorescence microscopy remains, based on a small number of studies; (6) by comparing different fluorescent staining techniques, there seems to be little difference in sensitivity or specificity between smears stained with auramine O and auramine-rhodamine, based on a small number of studies; and (7) there are currently limited data on the use of fluorescence microscopy to diagnose tuberculosis in HIV co-infected patients. However, the available evidence suggests fluorescence microscopy may be promising in this population.

Our systematic review had several strengths. First, the comprehensive search strategy with various overlapping approaches allowed us to retrieve relevant studies dating as far back as 1941. Moreover, two reviewers independently and reproducibly completed screening, study selection, and data extraction. Finally, we analysed data within specific subgroups to lessen the effect of heterogeneity and highlight the impact of different thresholds for AFB smear positivity, the presence of a sputum processing method, and the type of fluorochrome stain on the accuracy of microscopy.

This review also had limitations. Few of the studies defined the criteria for suspected pulmonary tuberculosis. The more stringent the criteria for tuberculosis suspects, the more likely a study would identify higher smear positivity. The smear positivity rates, therefore, varied widely between studies. Another problem concerned limited data on the clinical status of patients and disease severity. Differing criteria for patient selection and clinical status of the study populations might have introduced significant variability in findings between studies.

Most studies did not provide information about the quality of cultures used (eg, the proportion of contaminated culture results). If culture quality was, in fact, not good in some studies, the accuracy estimates may have been biased. One source of false-negative results may be poor smear preparation and staining techniques, for example if the smear is too thick or too thin, or if counterstaining or oxidising is overdone so that the AFB are not visible.[59] Inorganic material that absorbs fluorochrome stains may

on occasion be mistakenly identified as AFB.[21,59] In addition, most studies did not provide information on the composition of the carbolfuchsin dye, a factor known to affect the sensitivity of microscopy.[60,61] Another factor that may contribute to variability in findings between studies is whether the Ziehl-Neelsen staining method is always done first, as might be required in a diagnostic setting. This approach could bias results in favour of the Ziehl-Neelsen smear, since the portions of sputum containing the most bacilli may have been used before the specimen is processed by the fluorochrome technique.

Other problems involved issues of study design and methods. Although, as previously stated, we analysed data within specific subgroups, the fact remains that the studies in this review formed a heterogeneous group, which presented challenges for analysis. Therefore, all the summary measures reported in this review should be interpreted with this limitation in mind. Also, only about one third of the studies recruited samples in a random or consecutive manner. Therefore, most studies lacked the sound probabilistic sampling framework possible in consecutive or random sampling designs. Some studies involved comparisons using individual patients and others used individual specimens, and thus the sample unit differed and may have had an impact on the precision of the accuracy estimates. If, for example, in a given patient one of three conventional microscopy smears was positive, whereas all three fluorescence microscopy smears were positive, the ability of conventional microscopy and fluorescence microscopy to detect a case of tuberculosis would be equal. However, with culture as a reference standard, if all three specimens were culture positive, the sensitivity of fluorescence microscopy would be 100%, and conventional microscopy, only 30%. Furthermore, many of the studies were not done in a blinded fashion. Studies used different thresholds to define a positive smear and only half of the studies used a reference standard, limiting the computation of sensitivity. An additional limitation was incomplete information on microscopy characteristics, such as method of examination of smears, magnification to confirm AFB, type of light source, and time required to read slides.

Studies were done in different countries under differing conditions and most often at universities and research centres. Therefore, it is not known how fluorescence microscopy will perform in general health services settings. Few studies reported on the specific training and experience of the technicians who actually read the sputum smears; differences in the expertise of microscopists could account in part for the variation in sensitivity reported in different studies. This review did not address operational or cost-effectiveness issues. However, capital costs associated with fluorescence microscopy have decreased in recent years, and simple and relatively inexpensive equipment for fluorescence microscopy are available (eg, fibre optic-based and light-

emitting diode-based systems), which may serve as alternatives to expensive fluorescent microscopes.

Finally, despite the comprehensive literature searches, we may have missed some relevant studies on this topic. Also, by limiting our language to English, we have not taken into account publications in other languages. Although statistical tests (eg, Begg and Egger tests) and graphical methods (eg, funnel plots) are available to detect potential publication bias in meta-analyses of randomised controlled trials, such techniques have not been adequately evaluated for diagnostic data.[62] It is, therefore, difficult to rule out publication bias in our review.

Ideally, studies that compare fluorescence microscopy with conventional microscopy should be done in a blinded, prospective fashion, with a reference standard, and follow a sound research protocol. One suggested protocol for future studies is, where resources and settings permit, specimens within a three-specimen set from the same patient should be randomised for processing by different techniques. This will ensure that bias associated with timing of specimen collection and the first technique used can be excluded. Finally, the impact of fluorescence microscopy needs to be studied more thoroughly in HIV-infected patients, a population in whom microscopy in general tends to produce a low yield.

Conclusions and policy implications

The evidence in this systematic review suggests that the successful and widespread implementation of fluorescence microscopy in tuberculosis endemic countries might be reasonably expected to improve tuberculosis case-finding through an expected increase in direct smear sensitivity and an expected decrease in time spent on microscopic examination. This would translate into quicker turn-around times for smear results and thereby potentially reduce patient drop-out from the diagnostic process. Through reducing the workload of over-burdened health laboratories in low-income countries, quality improvements in the performance of other essential medical laboratory services might also be expected from the implementation of fluorescence microscopy.

Current international policy recommendations on fluorescence microscopy focus on issues such as workload, specificity, and blinded re-checking.[59,63,64] The results of our review provide a point of reference for policy makers, quantifying the potential benefit of fluorescence microscopy, with which the increased cost and technical complexity of the method can be compared to determine the possible value of the method under programme conditions. 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: (1) feasibility and sustainability of fluorescence microscopy in settings with irregular electricity supply, limited human and financial resources, and inadequate training; (2) the lack of internationally agreed external quality

assessment methods for blinded rechecking of fluorescent smears; (3) uncertainty about the stability of fluorescence microscopy reagents under field conditions; and (4) uncertainty about the acceptability of enclosed dark rooms to microscopists in tropical settings. Implementation is, therefore, likely to be complex and models for implementation are not available. Barriers to implementation should be surmountable through well-conceived operational research. Funding support is urgently needed for such research initiatives, especially in populations with high HIV and tuberculosis burden.

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Errata

Steingart KR, Henry M, Ng V, et al. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis 2006; **6:** 570–81. In figure 2, the specificity and 95% CI were incorrectly displayed. The correct version is shown here. In Table 5, entries for Githui, 1993, Kenya[26] the sensitivity of conventional microscopy (CM) should read 0·65 (95% CI 0·62–0·68) and the specificity 0·97 (0·95–0·98). For fluorescence microscopy (FM), the sensitivity should read 0·80 (0·77–0·82) and the specificity 0·96 (0·94–0·97). For Holst, 1959, India[27] the sensitivity of CM should read 0·66 (0·62–0·70) and the specificity 0·98 (0·97–0·99). For FM, the sensitivity should read 0·67 (0·64–0·71) and the specificity 0·98 (0·96–0·99). For Kumar, 1979, India[46] the sensitivity of CM should read 0·89 (0·85–0·93) and the specificity 0·96 (0·93–0·98). For FM, the sensitivity should read 0·94 (0·90–0·96) and the specificity 0·97 (0·94–0·99). The difference between FM and CM sensitivity should read +5%.

Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 2005; **5:** 558–67. Reference 15 should read Brandão AB, Fuchs SC. Risk factors for hepatitis C virus infection among blood donors in southern Brazil: a case-control study. BMC Gastroenterol 2002; **2:** 18.
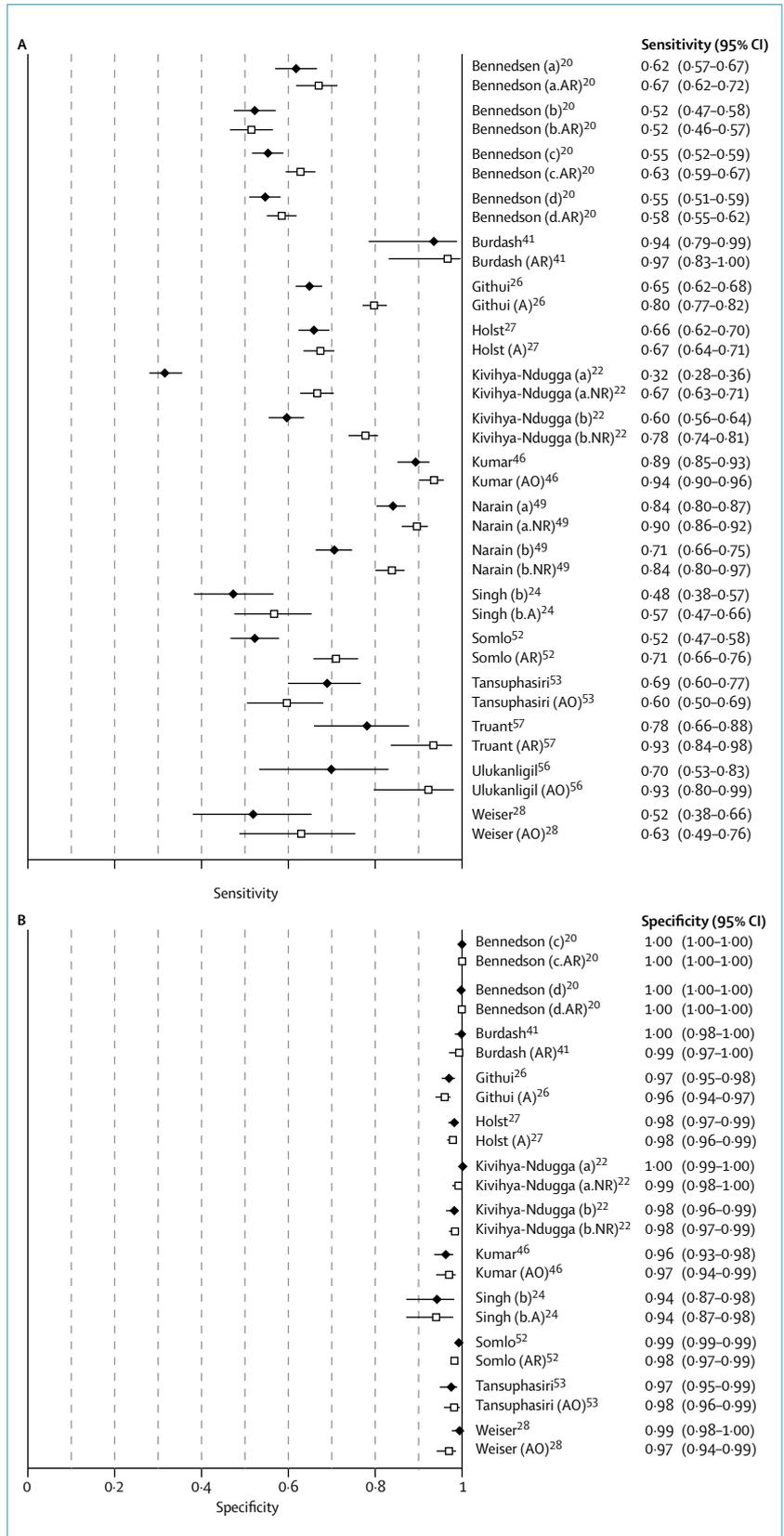

Figure 2: Forest plot of sensitivity and specificity estimates of conventional microscopy (CM) and fluorescence microscopy (FM)
(A) Sensitivity (18 studies). (B) Specificity (12 studies). Point estimates of sensitivity and specificity from each study are shown as solid circles for CM and as open squares for FM. The solid lines represent 95% CIs. A=auramine; AO=auramine O; AR=auramine-rhodamine; NR=not reported.

A — Sensitivity (95% CI)

Study	Sensitivity (95% CI)
Bennedsen (a)[20]	0·62 (0·57–0·67)
Bennedson (a.AR)[20]	0·67 (0·62–0·72)
Bennedson (b)[20]	0·52 (0·47–0·58)
Bennedson (b.AR)[20]	0·52 (0·46–0·57)
Bennedson (c)[20]	0·55 (0·52–0·59)
Bennedson (c.AR)[20]	0·63 (0·59–0·67)
Bennedson (d)[20]	0·55 (0·51–0·59)
Bennedson (d.AR)[20]	0·58 (0·55–0·62)
Burdash[41]	0·94 (0·79–0·99)
Burdash (AR)[41]	0·97 (0·83–1·00)
Githui[26]	0·65 (0·62–0·68)
Githui (A)[26]	0·80 (0·77–0·82)
Holst[27]	0·66 (0·62–0·70)
Holst (A)[27]	0·67 (0·64–0·71)
Kivihya-Ndugga (a)[22]	0·32 (0·28–0·36)
Kivihya-Ndugga (a.NR)[22]	0·67 (0·63–0·71)
Kivihya-Ndugga (b)[22]	0·60 (0·56–0·64)
Kivihya-Ndugga (b.NR)[22]	0·78 (0·74–0·81)
Kumar[46]	0·89 (0·85–0·93)
Kumar (AO)[46]	0·94 (0·90–0·96)
Narain (a)[49]	0·84 (0·80–0·87)
Narain (a.NR)[49]	0·90 (0·86–0·92)
Narain (b)[49]	0·71 (0·66–0·75)
Narain (b.NR)[49]	0·84 (0·80–0·97)
Singh (b)[24]	0·48 (0·38–0·57)
Singh (b.A)[24]	0·57 (0·47–0·66)
Somlo[52]	0·52 (0·47–0·58)
Somlo (AR)[52]	0·71 (0·66–0·76)
Tansuphasiri[53]	0·69 (0·60–0·77)
Tansuphasiri (AO)[53]	0·60 (0·50–0·69)
Truant[57]	0·78 (0·66–0·88)
Truant (AR)[57]	0·93 (0·84–0·98)
Ulukanligil[56]	0·70 (0·53–0·83)
Ulukanligil (AO)[56]	0·93 (0·80–0·99)
Weiser[28]	0·52 (0·38–0·66)
Weiser (AO)[28]	0·63 (0·49–0·76)

Sensitivity

B — Specificity (95% CI)

Study	Specificity (95% CI)
Bennedson (c)[20]	1·00 (1·00–1·00)
Bennedson (c.AR)[20]	1·00 (1·00–1·00)
Bennedson (d)[20]	1·00 (1·00–1·00)
Bennedson (d.AR)[20]	1·00 (1·00–1·00)
Burdash[41]	1·00 (0·98–1·00)
Burdash (AR)[41]	0·99 (0·97–1·00)
Githui[26]	0·97 (0·95–0·98)
Githui (A)[26]	0·96 (0·94–0·97)
Holst[27]	0·98 (0·97–0·99)
Holst (A)[27]	0·98 (0·96–0·99)
Kivihya-Ndugga (a)[22]	1·00 (0·99–1·00)
Kivihya-Ndugga (a.NR)[22]	0·99 (0·98–1·00)
Kivihya-Ndugga (b)[22]	0·98 (0·96–0·99)
Kivihya-Ndugga (b.NR)[22]	0·98 (0·97–0·99)
Kumar[46]	0·96 (0·93–0·98)
Kumar (AO)[46]	0·97 (0·94–0·99)
Singh (b)[24]	0·94 (0·87–0·98)
Singh (b.A)[24]	0·94 (0·87–0·98)
Somlo[52]	0·99 (0·99–0·99)
Somlo (AR)[52]	0·98 (0·97–0·99)
Tansuphasiri[53]	0·97 (0·95–0·99)
Tansuphasiri (AO)[53]	0·98 (0·96–0·99)
Weiser[28]	0·99 (0·98–1·00)
Weiser (AO)[28]	0·97 (0·94–0·99)

Specificity