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

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In low-income and middle-income countries, direct (unconcentrated) sputum smear microscopy is the primary method for diagnosing pulmonary tuberculosis. The method is fast, inexpensive, and specific for *Mycobacterium tuberculosis* in high incidence areas. The main limitations of direct microscopy are its relatively low sensitivity, especially in individuals co-infected with HIV, and variable quality of the test in programme conditions. Thus, there is a need to identify methods to improve the sensitivity of microscopy. Physical and chemical sputum processing methods, including centrifugation, sedimentation, and bleach, have been studied and found to show promise. We did a systematic review to assess the ability of different processing methods to improve the sensitivity of microscopy. By searching many sources, we identified 83 studies. Overall, by comparison with direct smears, the results suggested that centrifugation with any of several chemical methods (including bleach) is more sensitive, that overnight sedimentation preceded by chemical processing is more sensitive, and that specificity is similar. There were insufficient data to determine the value of sputum processing methods in patients with HIV infection. Operational studies are needed to determine whether the increased sensitivity provided by processing methods is sufficient to offset their increased cost, complexity, and potential biohazards, and to examine their feasibility.

Introduction

The burden of disease and death caused by tuberculosis is immense, with 8·8 million cases and nearly 2 million deaths estimated to have occurred in 2003 alone.[1] The HIV epidemic has had a huge impact, driving up incidence rates dramatically in sub-Saharan Africa.[1–4] In addition, tuberculosis is a major cause of death among people who are HIV infected, currently accounting for at least 11% of AIDS deaths worldwide.[5] An important barrier to global tuberculosis control is the low rate of case detection. Although the proportion of smear-positive cases identified is increasing, the proportion that were identified globally under directly observed therapy (short-course) programmes (the internationally recognised tuberculosis control strategy) was only 45% in 2003.[1] The World Health Assembly set a global target to detect 70% of new smear-positive cases (70% case detection rate) by 2005. This target was not met.[6] To prevent transmission of *Mycobacterium tuberculosis* and to provide appropriate care for patients, prompt and accurate diagnosis of tuberculosis is a matter of great urgency.[7,8]

Sputum microscopy is the most important test for the diagnosis of pulmonary tuberculosis in low-income and middle-income countries, where 95% of tuberculosis cases and 98% of deaths occur.[9] In these countries, most laboratories use smears of unconcentrated sputum (direct smears) with Ziehl-Neelsen staining. Microscopy is fast, simple, inexpensive, widely applicable, and highly specific for *M tuberculosis* in tuberculosis-endemic countries. In addition, microscopy identifies the most infectious patients.[10–12] Although microscopy has been reported to have greater than 80% sensitivity for identifying cases of pulmonary tuberculosis in some settings,[13,14] the sensitivity of the test has been low and variable in other reports (range 20–60%).[15] Smear-negative tuberculosis is disproportionately higher in HIV-positive than in HIV-negative individuals,[16,17] and has been linked to poor treatment outcomes, including death, especially in areas devastated by the HIV epidemic.[18,19] Microscopy contributes little to the diagnosis of paediatric pulmonary tuberculosis,[20] and does not, by definition, identify smear-negative pulmonary tuberculosis. Clearly, improvement in the sensitivity of microscopy would be of great potential value.

Reports describing newer sputum processing methods as well as calls for re-examination of existing methods have prompted interest in the assessment of chemical processing and sputum concentration to improve the sensitivity of microscopy.[11,21–23] Chemicals, such as sodium hydroxide (NaOH) and a solution of N-acetyl L-cysteine and sodium hydroxide (NaLC-NaOH) to liquefy sputum, together with centrifugation, are widely used in modern laboratories.[24,25] A recent review of studies using sodium hypochlorite (NaOCl; bleach) to treat sputum followed by centrifugation found a significant increase in sensitivity compared with the direct smear method.[21] This review, however, did not address gravity sedimentation and other physical or chemical methods that have been investigated.

We did a systematic review to assess the ability of various sputum processing methods to improve the accuracy of microscopy, compared with the direct (unconcentrated) method. We specifically addressed two questions: (1) Do sputum processing methods increase the sensitivity of microscopy in persons with and without HIV infection? (2) What is the influence on overall test accuracy of specific chemical treatments of sputum, of physical concentration methods, and of combinations of these methods?

Methods

Search strategy and selection criteria

Our initial searches were done in 2004 and updated in 2005. We searched the following databases for primary

studies: 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 bacilli", "sputum microscopy", "bacteriology", "sensitivity and specificity", "sputum concentration", and "direct microscopy". We hand searched 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 articles not already captured by the electronic searches. In addition, we contacted investigators and experts for ongoing and unpublished studies, and scanned reference lists from primary studies, review articles, and textbook chapters.

Our search strategy aimed to identify all studies published in English that reported results of chemical and physical methods to improve the accuracy of microscopy. The following types of studies were excluded: (1) investigations of specimens other than sputum; (2) studies that determined the sensitivity for processed smears that did not include a direct smear comparison; (3) studies that focused on non-tuberculous mycobacteria; (4) studies in which microscopy was used to monitor treatment response; (5) assessments of cost-effectiveness or other economic issues; and (6) case reports and reviews. No restrictions were made with respect to study design (eg, prospective or retrospective) or patient selection, on the understanding that some studies might include both untreated and treated patients. We included studies with culture as a reference standard and those without a reference standard. Two reviewers (VN and MH) independently screened the accumulated citations for eligible studies. A third reviewer (KS) independently assessed all full text articles.

Data extraction

Two reviewers (KS and VN) independently extracted data from eligible studies on the following characteristics: methodological quality, blinding, sampling design, sputum collection characteristics, smear preparation, stain used, chemical and physical methods for sputum processing, duration of sedimentation, and use of a reference standard (mycobacterial culture). For studies that used centrifugation, data were expressed either in units of gravity (g) for relative centrifugal force or revolutions per minute (rpm) for speed. Relative centrifugal force and speed are related by the radius of the centrifuge rotor, although few studies provided adequate data to allow us to state the same units for all studies. If data on centrifugation were not clearly reported, then the information was coded as "not reported". We also attempted to contact investigators for additional information. Interrater agreement of the reviewers on the accuracy of smear microscopy was 100%. Unresolved differences about the data were decided by consensus before finalising data extraction. If culture data were available for both *M tuberculosis* and non-tuberculous mycobacteria, then we calculated sensitivity and specificity on the basis of the numbers of cultures positive for *M tuberculosis* alone. Some investigators provided corrected or resolved data on accuracy, after doing discrepant analyses in which discordant results between the index test and reference standard were resolved, post hoc, by use of clinical or other laboratory data. Because discrepant analysis may be a potential source of bias,[26] we chose to include the unresolved data.

Assessment of study quality

Quality assessment included the following items:[27] (1) Was there an independent comparison of smear with mycobacterial culture? (2) Was there blinded interpretation of smear and culture results? (3) Was there blinded interpretation of direct and processed smear results? (4) Did the study prospectively recruit consecutive patients suspected of having pulmonary tuberculosis?

Data collation and meta-analysis

As studies were heterogeneous in many respects, particularly in sputum processing methods, we first grouped studies by type of stain used, chemical or physical sputum processing method, and presence of culture. We further stratified studies by use of centrifugation by force (speed): less than 2000 g (<2500 rpm) and 2000 g or

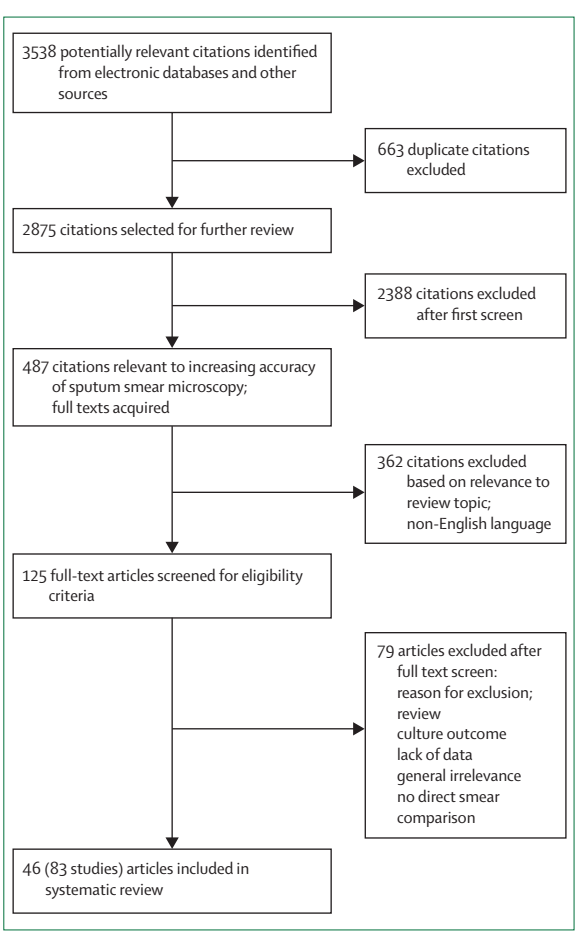

Figure 1: **Flow diagram for study selection**

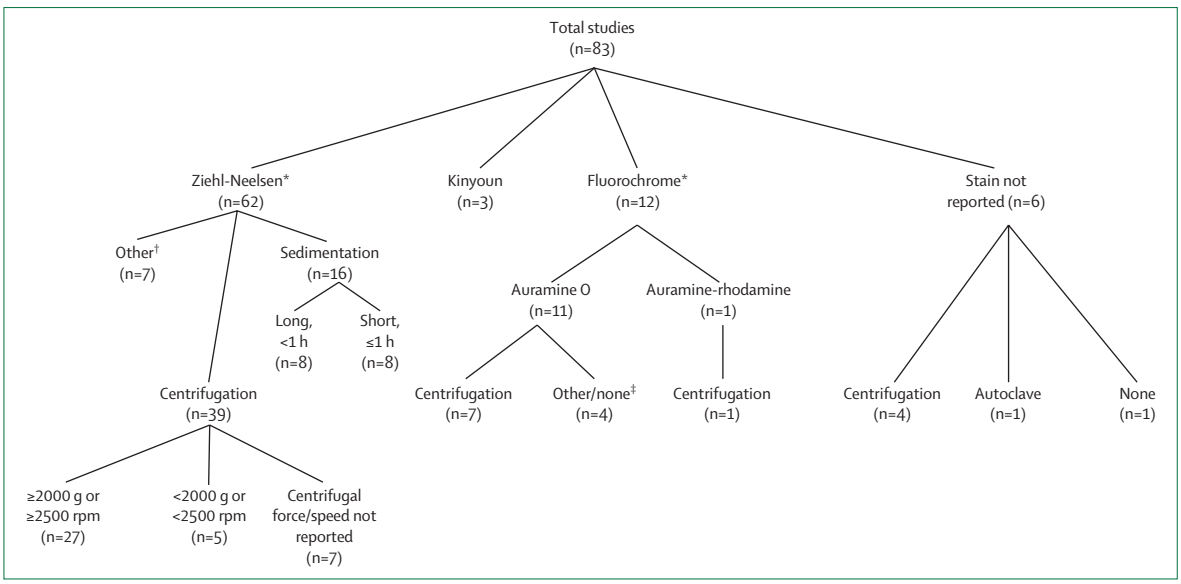

Figure 2: **Classification tree for subgroup analyses of sputum processing studies, by type of microscopy stain and physical method**
*Nine studies that used Ziehl-Neelsen staining and four studies that used fluorochrome staining were excluded from subgroup analyses. †Other (Ziehl-Neelsen stain): centrifugation/flocculation=1; flotation=5; glass beads=1. ‡Other/none (fluorochrome stain): flotation=1; polycarbonate membrane filter=2; none=1. rpm=revolutions per min.

greater (≥2500 rpm); and studies that used gravity sedimentation by duration: short (≤1 h) and long (>1 h). We then analysed data within specific subgroups of at least four studies to delineate the effect of various processing methods. With respect to accuracy, sensitivity refers to the proportion of culture-positive samples that are identified as positive by the smear method in question; specificity refers to the proportion of culture negative samples that are identified as negative by the smear method. For calculation of these measures, most studies excluded contaminated culture results. For studies that did not use cultures, we determined incremental yield. Incremental yield refers to the proportion of positive smears (smear positivity) by the processed smear minus the proportion of positive smears by the direct smear.

We used methods recommended for diagnostic meta-analyses.[27,28] Data were analysed using Meta-DiSc software (version 1·1·1).[29] Sensitivity, specificity, and positivity rates were calculated for processed and direct smears for each study, along with their 95% confidence intervals. We calculated the difference between processed smear and direct smear estimates and then pooled them across studies using simple averages, along with their 95% confidence intervals. No weighting was used. However we separately calculated the mean sensitivity for processed smear and direct smear for the four largest studies. True positive rates (sensitivity) and false positive rates (1 – specificity) from each study were summarised by summary receiver operating characteristic (SROC) curve analyses. Because true positive and false positive rates are usually correlated and vary with thresholds for test positivity, we analysed them as pairs. Unlike a traditional ROC plot, each datapoint in the SROC space represents the results of a separate study. The SROC curve was obtained by fitting a regression curve to pairs of true positive and false positive rates.[28] The SROC analysis provided two global measures of test accuracy: area under the curve (AUC) and the Q* index. An AUC of 1·0 (eg, 100%) indicates perfect discriminatory ability in the diagnostic test. The Q* index, defined by the point at which 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). Since Q* reflects the overall accuracy of the test, it is an appropriate measure when both high sensitivity and high specificity are desirable. The closer the Q* value is to 1·0, the more accurate the test.[28,30,31]

In diagnostic meta-analyses, heterogeneity refers to a high level of variability in sensitivity and specificity. Because of the differences in the processing methods and the anticipated variability in accuracy, we decided, a priori, to avoid the pooling of sensitivity and specificity. We addressed heterogeneity by use of pre-specified subgroup analyses.

Results

Description of included studies

Of the 3538 citations identified after literature searches, 46 articles (44 published,[23,32–74] [reference 73 as an abstract] and two unpublished [L Cuevas, Liverpool School of Tropical Medicine, Liverpool, UK, personal communication) consisting of 83 studies, met our eligibility criteria (figure 1). We considered most studies to be independent (reference 37 [study b] is a substudy). Therefore, no effort was made to account for lack of independence. Of the

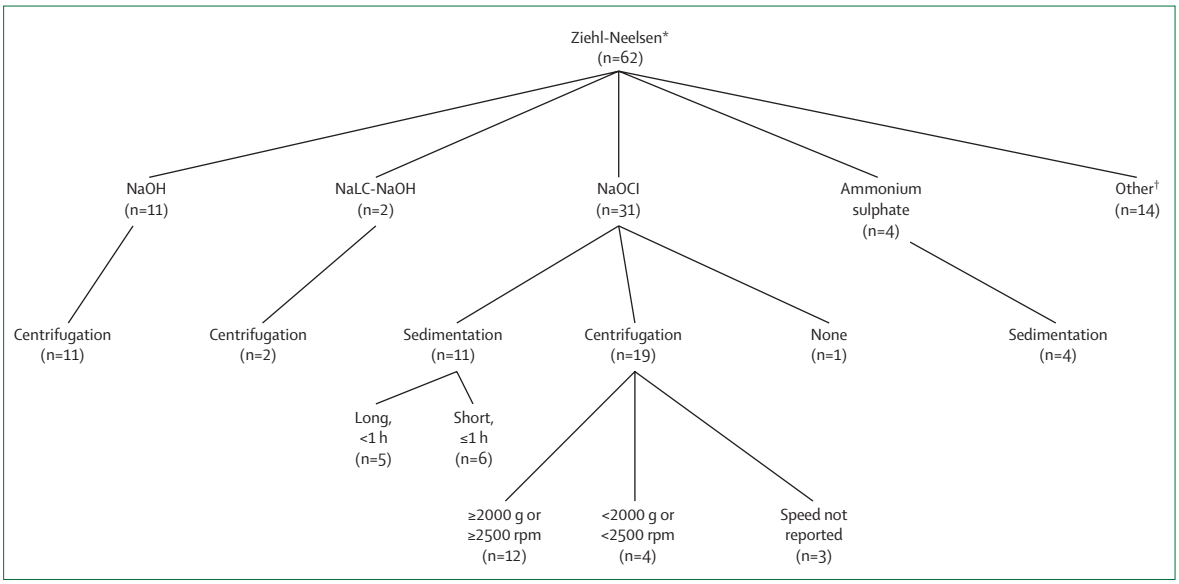

Figure 3: **Classification tree for subgroup analyses of sputum processing studies using Ziehl-Neelsen stain, by chemical and physical sputum processing method**
*Nine studies were excluded from subgroup analyses. †Other chemical: chitin=1; chlorhexidine gluconate-bleach (NaOCl)=1; dithiothreitol=2; dithiothreitol/sodium hydroxide (NaOH)=2; ferric chloride=1; NaOH/ferric chloride=1; NaOH/picric acid=1; sodium carbonate/carbolic acid=1; xylol/NaOCl with flotation=1; universal sample processing solution (USP; guanidinium hydrochloride, Tris-Cl, EDTA, Sarkosyl, β-mercaptoethanol)=1; no chemical, glass beads=1; centrifugation/autoclave without a chemical=1. NaLC-NaOH=N-acetyl-L-cysteine sodium-hydroxide solution; rpm=revolutions per minute.

83 studies, 65 (78%) used carbolfuchsin stain, 12 (14%) used fluorochrome stain, and in six (7%) studies the staining method was unspecified. Of the 65 studies using carbolfuchsin stain, 62 (95%) used Ziehl-Neelsen and three (5%) used Kinyoun stain. 36 (43%) of the 83 studies used culture as a reference standard. 26 (31%) studies used a blinded interpretation of direct and processed smear results, although no study explicitly reported blinding to culture. The median sample size of the 83 studies was 256 patients or specimens (range 8–3287; IQR 402). In 55 (66%) studies, the number of acid-fast bacilli (AFB) per smear for positivity was stated: 12 (22%) studies used more than nine AFB per smear; 14 (25%) studies used more than two AFB per smear; and 29 (53%) studies used more than

Study* (first author, year, country)	Study population	Number patients or specimens	Chemical processing method	Centrifugation force/speed	Centrifugation time (min)	Sensitivity (95% CI)		Difference in sensitivity (PS – DS)
						DS	PS	
Allwood, 1997, Malaysia[73]	PTS	173	NaOCl	1500 g	15	0·43 (0·29–0·58)	0·52 (0·38–0·66)	+9%
Ängeby (a), 2000, Honduras[32]	Known or PTS	303	NaOCl	3000 g	20	0·57 (0·41–0·71)	0·65 (0·50–0·79)	+8%
Ängeby (b), 2000, Honduras[32]	Routine sputum	303	NaOH	3000 g	15	0·57 (0·41–0·71)	0·76 (0·61–0·87)	+19%
Apers, 2003, Zimbabwe[33]	PTS	256	NaOH	2000–3000 g	15–20	0·68 (0·61–0·74)	0·87 (0·82–0·91)	+19%
Bruchfeld (a), 2000, Ethiopia[37]	PTS	509	NaOCl	3000 g	15	0·54 (0·46–0·62)	0·63 (0·55–0·70)	+9%
Bruchfeld (b), 2000, Ethiopia[37]	PTS	96	NaOCl	3000 g	15	0·39 (0·29–0·49)	0·50 (0·40–0·60)	+11%
Chakravorty, 2005, India[41]	PTS	571	USP	5000–6000 g	10–15	0·69 (0·63–0·74)	0·98 (0·96–0·99)	+29%
Farnia (a), 2002, Iran[44]	PTS	430	NaLC-NaOH	3000 g	15	0·50 (0·38–0·62)	0·89 (0·79–0·95)	+39%
Fodor, 1995, Iran[45]	Smear-positive patients	36	Chlorhexidine gluconate-NaOCl	2000 rpm	5	0·74 (0·57–0·88)	0·89 (0·73–0·97)	+15%
Gebre (a), 1995, Ethiopia[47]	PTS	100	NaOCl	800–3000 g	15–20	0·31 (0·19–0·45)	0·69 (0·55–0·81)	+38%
Naganathan, 1979, India[55]	PTS; abnormal chest radiograph	1499	NaOH	4000 rpm	20	0·80 (0·77–0·84)	0·77 (0·74–0·81)	–3%
Perera, 1999, Sri Lanka[57]	PTS	163	NaLC-NaOH	4000 g	15	0·63 (0·54–0·71)	0·92 (0·86–0·96)	+29%
Vasanthakumari (a), 1998, India[68]	Symptomatic patients	1000	NaOH	3000 rpm	15	0·57 (0·49–0·66)	0·91 (0·85–0·95)	+34%
Wilkinson, 1997, S Africa[69]	PTS	166	NaOCl	1500 g	15	0·43 (0·32–0·54)	0·44 (0·33–0·55)	+1%

DS=direct smear; NaLC-NaOH=N-acetyl-L-cysteine sodium-hydroxide solution; NaOCl=bleach; NaOH=sodium hydroxide; PS=processed smear; PTS=pulmonary tuberculosis suspects; rpm=revolutions per minute; USP=universal sample processing solution (guanidinium hydrochloride, Tris-Cl, EDTA, Sarkosyl, β-mercaptoethanol). *See webtable for further details on studies.

Table 1: **Studies comparing sensitivity for Ziehl-Neelsen-stained direct smears and sputum smears processed by centrifugation with a chemical**

zero AFB per smear. 67 (81%) studies used a single sputum specimen for preparing both the direct and processed smear, and eight (10%) studies indicated the amount of time dedicated to reading one slide. Figure 2 shows the distribution of the various physical sputum processing methods for all 83 studies by stain. Figure 3 shows the distribution of chemical and physical sputum processing methods for the subgroup of studies using Ziehl-Neelsen staining. The webtable gives additional information on study population characteristics, methods, processing methods, and quality for all studies in this review.

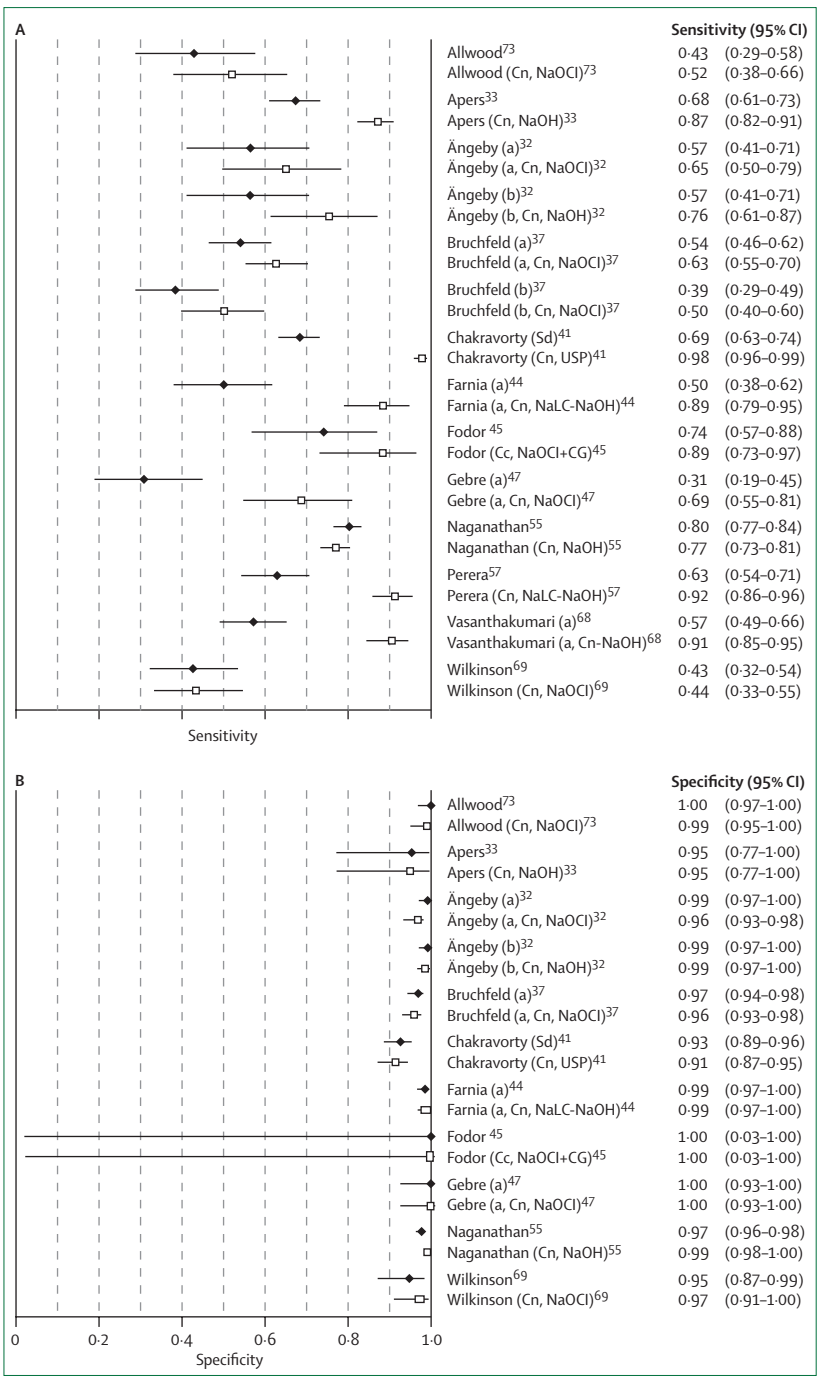

Figure 4: **Sensitivity and specificity estimates of direct smear and processed sputum smear microscopy, processed by centrifugation and any chemical**

(A) Sensitivity (14 studies). (B) Specificity (11 studies). Point estimates of sensitivity and specificity from each study are shown as solid diamonds for direct smears and as open squares for processed smears. The solid lines represent 95% CIs. Studies within a single article are denoted by lower-case letters. Cn=centrifugation; Sd=sedimentation; Cc=cytocentrifugation; NaOCl=bleach; NaOH=sodium hydroxide; NaLC-NaOH=N-acetyl-L-cysteine sodium-hydroxide solution; CG=chlorhexidine gluconate; USP=universal sample processing solution (guanidinium hydrochloride, Tris-Cl, EDTA, Sarkosyl, β-mercaptoethanol).

Sputum processing methods and sensitivity of microscopy in people with and without HIV infection

Unless specifically stated, the subgroup analyses below were done on studies using the Ziehl-Neelsen stain (62 studies). In this subgroup, nine (15%) studies[43,54,59] were excluded for the following reasons: results from two studies[43] were derived from only eight samples; in three studies,[59] incremental yield was determined by the number of sputum smears that were initially negative on direct examination, but found to be positive after sputum processing, rather than a comparison of direct and processed smears; and four studies[54] used dithiothreitol, a chemical not broadly applicable to tuberculosis programmes in low-income countries. For completeness, these nine studies are included in the webtable.

Centrifugation combined with any chemical method

We identified 32 studies that investigated the effect of centrifugation with a chemical (usually either bleach or NaOH) on microscopy. 14 studies with culture calculated sensitivity (table 1, figure 4) and 18 studies without culture determined incremental yield (table 2). In the subgroup of studies with culture comparison, sputum processing yielded a mean 18% (95% CI 11–26%) increase in sensitivity, with 13 studies showing an increase[32,33,37,41,44,45,47,57,68,69,73] and one study showing a decrease[55] (table 1). There were insufficient data to do subgroup analyses by either gravitational force or speed. We separately calculated the mean sensitivity of processed smears compared with direct smears for the four largest studies and found similar results.[37,41,55,68] For 18 studies without culture, the mean increase in incremental yield after processing was 7% (95% CI 3–11%), 15 studies[32,34,39,42,47,48,49,51,52,53,62] reporting an increase, two studies[67,74] reporting a decrease, and one study[36] reporting no difference (table 2).

17 studies compared direct and processed smears after sputum treatment with bleach and centrifugation; six studies[32,37,47,69,73] with culture calculated sensitivity (table 1, figure 5); 11 studies[32,34,39,42,47,48,51,53,62] without culture determined incremental yield (table 2). In studies with culture comparison, the mean increase in sensitivity after sputum processing was 13% (95% CI –1 to 26). In all six studies, sensitivity for processed smears was higher than that for direct smears. In studies without culture, the mean increase in incremental yield after sputum processing was 9% (95% CI 5–14%), with all studies[32,34,39,42,47,48,51,53,62] noting an increase.

Study* (first author, year, country)	Number patients or specimens	Chemical method	Centrifugation force/speed	Centrifugation time (min)	Positivity (95% CI)		Difference in positivity (PS – DS)
					DS	PS	
Ängeby (c), 2000, Honduras[32]	971	NaOCl	3000 g	20	0·08 (0·06–0·10)	0·10 (0·09–0·12)	+2%
Ängeby (d), 2000, Honduras[32]	1422	NaOCl	3000 g	20	0·02 (0·02–0·03)	0·03 (0·02–0·04)	+1%
Aung, 2001, Myanmar[34]	948	NaOCl	3000 g	15–20	0·26 (0·23–0·29)	0·31 (0·28–0·34)	+5%
Biswas (a), 1987, India[36]	102	NaOH	..	..	0·45 (0·35–0·55)	0·45 (0·35–0·55)	0%
Cameron (2a), 1945, USA[39]	211	NaOCl	3000 rpm	30	0·22 (0·17–0·29)	0·33 (0·27–0·40)	+11%
Cameron (2b), 1945, USA[39]	211	NaOH	3000 rpm	30	0·22 (0·17–0·29)	0·27 (0·21–0·34)	+5%
Cameron (2c), 1945, USA[39]	211	None	3000 rpm	30	0·22 (0·17–0·29)	0·29 (0·23–0·36)	+7%
Contijo Filho (b), 1979, Brazil[42]	122	NaOCl	1200 g	30	0·34 (0·26–0·44)	0·36 (0·28–0·45)	+2%
Gebre (b), 1995, Ethiopia[47]	500	NaOCl	800–3000 g	15–20	0·08 (0·06–0·11)	0·14 (0·11–0·18)	+6%
Gebre (c), 1995, India[47]	103	NaOCl	800–3000 g	15–20	0·16 (0·09–0·24)	0·34 (0·25–0·44)	+18%
Gebre-Selassie (b), 2003, Ethiopia[48]	200	NaOCl	3000 g	15	0·09 (0·05–0·13)	0·30 (0·24–0·37)	+21%
Gopinathan, 1984, India[49]	65	NaOH	3000 g	15	0·19 (0·10–0·30)	0·35 (0·24–0·48)	+16%
Habeenzu, 1998, Zambia[51]	488	NaOCl	3000 g	15–20	0·14 (0·12–0·17)	0·24 (0·20–0·28)	+10%
Harries, 1998, Malawi[74]	319	NaOH	3000 rpm	20	0·26 (0·21–0·31)	0·25 (0·21–0·31)	–1%
Kochhar (a), 2002, India[52]	1484	NaOH	3000 g	15	0·09 (0·08–0·11)	0·13 (0·11–0·14)	+4%
Miörner (a), 1996, Ethiopia[53]	545	NaOCl	3000 g	15	0·17 (0·14–0·21)	0·28 (0·25–0·32)	+11%
Saxena (a), 2001, India[62]	304	NaOCl	1500 rpm	15	0·17 (0·13–0·22)	0·32 (0·26–0·37)	+15%
Tech, 1965, Philippines[67]	581	NaOH	..	20	0·28 (0·24–0·32)	0·21 (0·17–0·24)	–7%

DS=direct smear; NaOCl=bleach; NaOH=sodium hydroxide; PS=processed smear; rpm=revolutions per minute; ..=not reported. *See webtable for further details on studies.

Table 2: **Studies comparing incremental yield of Ziehl-Neelsen-stained direct smears and sputum smears processed by centrifugation with a chemical**

See **Online** for webtable

Four studies[32,33,55,68] with culture compared sensitivity for direct and processed smears after sputum treatment with NaOH and centrifugation (table 1). The mean increase in sensitivity after sputum processing was 17% (95% CI –7 to 41), with three studies[32,33,68] reporting an increase. In six studies without culture that assessed the effect of sputum treatment with NaOH and centrifugation, the mean increase in incremental yield after sputum processing was 3% (95% CI –5 to 11), with three studies[39,49,52] reporting an increase, two studies[67,74] reporting a decrease, and one study[36] reporting no difference (table 2).

Gravity sedimentation combined with any chemical method

16 studies, eight of which used culture, investigated the effect of sedimentation with various chemical agents, usually either bleach or ammonium sulphate. Of eight studies with cultures, four used short sedimentation times of 30–45 min (L Cuevas, personal communication)[44,71] and four studies used overnight sedimentation[44,46,63,68] (table 3). In the subgroup of studies with culture, all four studies using overnight sedimentation found an increase in sensitivity, with a mean gain of 23% (95% CI –1 to 47), whereas the four studies with short sedimentation times found a mean increase in sensitivity of 9% (95% CI –19 to 38). In the subgroup of studies without culture, all five studies using overnight sedimentation[23,48,52,53] found an increase in incremental yield, with a mean gain of 5% (95% CI –3 to 14). An additional three studies with short sedimentation times (L Cuevas, personal communication)[42,70] without culture noted incremental yields of +5%, –4%, and +8%, respectively (table 4).

11 studies assessed sputum treatment with bleach and sedimentation. Of four studies (L Cuevas, personal communication)[44,71] that used culture, one study using overnight sedimentation noted a 33% increase in sensitivity, whereas the three studies with short sedimentation times reported no or little increase in sensitivity (table 3). Of seven studies without

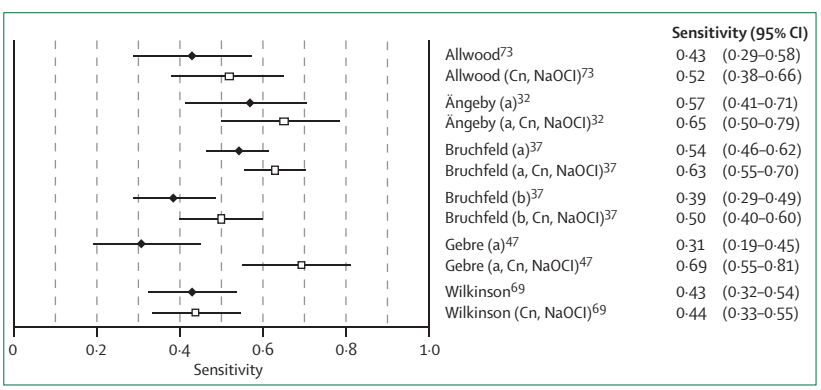

Figure 5: **Sensitivity estimates of direct smear and processed sputum smear microscopy, processed by bleach and centrifugation**

Point estimates of sensitivity from each study are shown as solid diamonds for direct smears and as open squares for processed smears. The solid lines represent the 95% CIs. Studies within a single article are denoted by lower-case letters. Cn=centrifugation; NaOCl=bleach.

Study* (first author, year, country)	Study population	Number patients or specimens	Chemical processing method	Sedimentation time	Sensitivity (95% CI)		Difference in sensitivity (PS – DS)
					DS	PS	
Cuevas (a), 2005, Nigeria†	PTS not on treatment	183	NaOCl	30–45 min	0·59 (0·52–0·66)	0·59 (0·52–0·67)	0%
Cuevas (b), 2005, Nigeria†	PTS not on treatment	230	NaOCl	30–45 min	0·48 (0·42–0·55)	0·48 (0·42–0·55)	0%
Farnia (b), 2002, Iran[44]	PTS	430	NaOCl	Overnight (12–15 h)	0·50 (0·38–0·62)	0·83 (0·73–0·91)	+33%
Farnia (c), 2002, Iran[44]	PTS	430	Chitin	30 min	0·50 (0·38–0·62)	0·86 (0·76–0·93)	+36%
Garay, 2000, Zimbabwe[46]	Symptomatic patients	242	$(NH_4)_2SO_4$-NaOH	Overnight (12 h)	0·58 (0·43–0·71)	0·81 (0·68–0·90)	+23%
Lawson, 2006, Nigeria[71]	PTS not on treatment	756	NaOCl	30–45 min	0·49 (0·44–0·54)	0·50 (0·45–0·54)	+1%
Selvakumar, 2002, India[63]	Symptomatic patients	2341	Phenol $(NH_4)_2SO_4$	Overnight	0·83 (0·80–0·86)	0·85 (0·82–0·88)	+2%
Vasanthakumari (b), 1988, India[68]	Symptomatic patients	1000	$(NH_4)_2SO_4$-NaOH	Overnight	0·57(0·49–0·66)	0·91 (0·85–0·95)	+34%

DS=direct smear; NaOCl=bleach; NaOH=sodium hydroxide; $(NH_4)_2SO_4$=ammonium sulphate; PS=processed smear; PTS=pulmonary tuberculosis suspects.*See webtable for further details on studies. †L Cuevas, Liverpool School of Tropical Medicine, Liverpool, UK, personal communication.

Table 3: Studies comparing sensitivity for Ziehl-Neelsen-stained direct smears and sputum smears processed by gravity sedimentation with a chemical

Study* (first author, year, country)	Number patients or specimens	Chemical processing method	Sedimentation time	Positivity (95% CI)		Difference in positivity (PS – DS)
				DS	PS	
Contijo Filho (a), 1979, Brazil[42]	122	NaOCl	30 min	0·34 (0·26–0·44)	0·30 (0·22–0·38)	–4%
Cuevas, 2005, Ethiopia†	198	NaOCl	30 min	0·26 (0·20–0·32)	0·31 (0·25–0·38)	+5%
Gebre-Selassie (a), 2003, Ethiopia[48]	200	NaOCl	Overnight (16 h)	0·09 (0·05–0·13)	0·26 (0·20–0·32)	+17%
Kochhar (b), 2002, India[52]	1484	Ammonium sulphate-NaOH	Overnight	0·09 (0·08–0·11)	0·13 (0·11–0·14)	+4%
Miörner (b), 1996, Ethiopia[53]	545	NaOCl	Overnight (15–18 h)	0·17 (0·14–0·21)	0·21 (0·17–0·24)	+4%
Van Deun (a), 2000, Bangladesh[23]	3287	NaOCl	Overnight	0·16 (0·14–0·17)	0·17 (0·16–0·18)	+1%
Van Deun (b), 2000, Bangladesh[23]	1568	NaOCl	Overnight	0·16 (0·14–0·18)	0·17 (0·15–0·19)	+1%
Yassin, 2003, Ethiopia[70]	200	NaOCl	30–45 min	0·18 (0·13–0·24)	0·26 (0·20–0·33)	+8%

DS=direct smear; NaOCl=bleach; NaOH=sodium hydroxide; PS=processed smear. *See webtable for further details on studies. †L Cuevas, Liverpool School of Tropical Medicine, Liverpool, UK, personal communication.

Table 4: **Studies comparing incremental yield for Ziehl-Neelsen-stained direct smears and sputum smears processed by gravity sedimentation with a chemical**

culture (L Cuevas, personal communication),[23,42,48,53,70] four studies using overnight sedimentation[23,48,53] showed a 6% mean increase in incremental yield (95% CI –6 to 18), and three studies (L Cuevas, personal communication)[42,70] with short sedimentation times noted above had an inconsistent effect on incremental yield (table 4).

Sputum processing methods for identifying AFB in HIV-infected patients

We identified only two studies that determined the sensitivity for direct and processed sputum smears in pulmonary tuberculosis suspects with HIV infection (L Cuevas, personal communication).[37] In one study (96 patients),[37] which assessed the effect of bleach and centrifugation, sensitivity increased 11% after sputum processing. A second study (230 patients; L Cuevas, personal communication) noted no change in sensitivity after sputum was treated with bleach and gravity sedimentation for 30–45 min.

See Online for webfigure

Sensitivity of direct and processed sputum smears in studies using fluorescence microscopy

Within the subgroup of studies using fluorescent staining, eight studies determined sensitivity for direct and processed smears compared with culture.[54,58,66] Results from four studies,[54] in which sputum was initially treated with dithiothreitol, were thought to be outliers and excluded. Of the remaining four studies, three determined the sensitivity of fluorescence microscopy after sputum treatment with NaLC-NaOH and centrifugation,[58,66] and one study after treatment with bleach and the use of a polycarbonate membrane filter.[66] All four studies showed an increase in sensitivity after sputum processing (mean +12%; 95% CI 1–22; webfigure).

Effect of physical or chemical sputum processing methods on specificity of sputum smear microscopy

The specificity of microscopy after processing with physical and chemical methods was similar to the direct smear method, with one exception.[57] In this study, which

investigated the effect of NaLC-NaOH and centrifugation, we speculate the finding of low specificity may be a consequence of contaminated culture media or inclusion of specimens from treated patients. After excluding results from this study, the mean specificity for direct smears was 0·98 (range 0·92–1·00) and for processed smears 0·98 (range 0·91–1·00; 22 studies, mean increase 0%, 95% CI –1 to 1, data not shown). A representative forest plot for a subgroup of 11 studies that compared specificity for direct smears with smears processed with a chemical agent and centrifugation is shown in figure 4.

Influence on overall test accuracy of specific methods

Figure 6 shows SROC curves for direct microscopy and for microscopy after processing with a chemical and centrifugation. Compared with the direct smears, processed smears showed an improvement in discriminatory ability and an increase in accuracy. These differences were, however, not statistically significant, based on overlapping confidence intervals for the AUCs.

Discussion

Our systematic review of 83 studies, most using Ziehl-Neelsen staining, that compared microscopy using processed sputum with the direct smear method, indicates the following: (1) sputum treated with bleach or NaOH and concentrated by centrifugation is, on average, more sensitive; (2) sputum subjected to overnight sedimentation preceded by treatment with ammonium sulphate or bleach, is, on average, more sensitive, based on a small number of studies; (3) the specificity for processed smears is similar to that for direct smears; and (4) there are insufficient data to indicate whether the gains in sensitivity described above will also apply in patients with HIV infection.

The benefits of sputum processing found in this review confirm findings of Ängeby and colleagues,[21] who reported that sputum treated with bleach followed by centrifugation increased the sensitivity of microscopy. However, we were unable to determine the relative importance of the chemical treatments versus physical processes such as centrifugation or sedimentation. We identified only one study that used centrifugation without chemical treatment.[39] This study (211 specimens), which did not include culture, reported a 7% increase in incremental yield after sputum processing with centrifugation of autoclaved sputum, compared with an 11% increase after sputum processing with bleach and centrifugation. Although other chemical methods (eg, chitin and NaLC-NaOH),[36,40,41,44–46,50,52,57–60,63,65,66,68,72] and other physical methods (eg, flotation and flocculation),[36,38,39,42,59,60,64–66,72] were also assessed in some studies, the number of such studies was too small to make meaningful inferences about their efficacy.

This systematic review had several strengths. First, the comprehensive search strategy enabled us to retrieve relevant studies dating as far back as 1919, as well as to access unpublished data and conference abstracts, thus limiting publication bias. Moreover, we followed a standard, two-stage protocol in which two reviewers independently screened and selected studies followed by data extraction.[27] Finally, we stratified data into pre-specified subgroups to account for heterogeneity.

This review also had limitations. Few of the studies defined the criteria for pulmonary tuberculosis suspects. Strict criteria for pulmonary tuberculosis suspects might result in a greater proportion of positive smear results. The proportion of positive smear results, therefore, varied

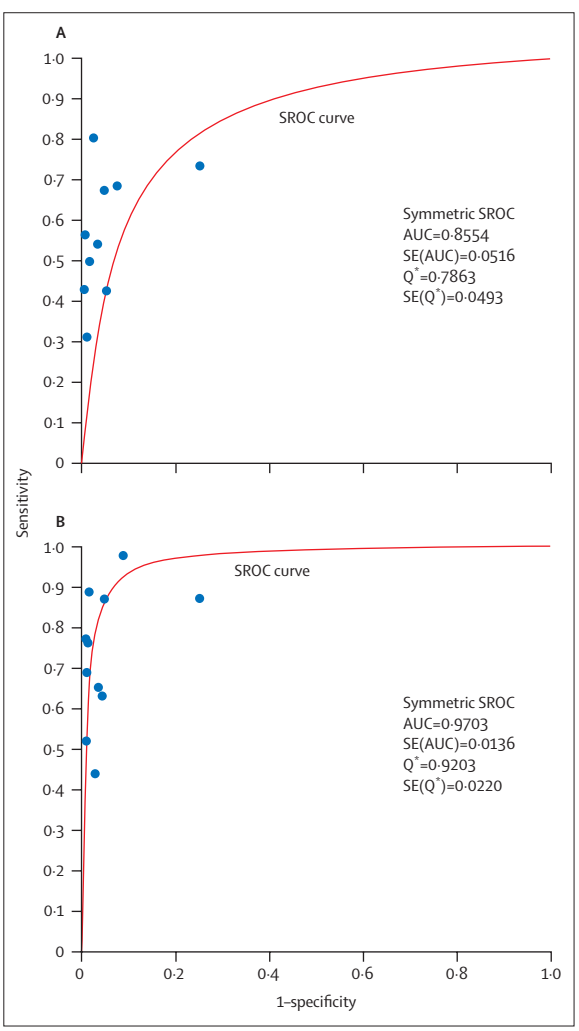

Figure 6: **Summary receiver operating characteristic (SROC) curves for studies using Ziehl-Neelsen stain**
(A) Direct smear microscopy without a processing method. (B) Processed smear microscopy, sputum processing with centrifugation and any chemical. Each solid circle represents a study in the analysis. 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. SROC curves are shown for studies that included both sensitivity and specificity estimates.

widely across studies. Moreover, although we excluded studies, a priori, specifically using smears to monitor response to anti-tuberculosis therapy, only 44 (53%) studies explicitly described the study population as tuberculosis patients who were not on treatment. Sputum specimens from patients receiving anti-tuberculosis therapy are likely to contain fewer AFB. Although false-positive smears from non-viable organisms in the sputum may at times be detected in treated patients,[58,66,75] this observation was not found to be an issue in these studies. Also, some of the studies included combinations of inpatients and outpatients. An inpatient population might be biased towards patients with more advanced disease. Ideally, it would have been preferable to have addressed these potential confounding factors independently, because differing criteria for patient selection and the clinical status of the study populations might have introduced significant heterogeneity.

In this review, only eight studies[59,61,67,68,72] indicated the amount of time dedicated to reading one slide. Clearly, the more time spent, the greater the likelihood of finding AFB. The time required for reading could also be an important variable in determining the possible benefits of the various processing methods. Only 36 (43%) of studies used a culture comparison, limiting the computation of sensitivity. In those studies that did use culture, few provided information about the quality of cultures used (eg, the proportion of contaminated culture results). If culture quality was not good in some studies, the accuracy estimates may have been biased.

Another set of problems involved shortcomings in study design. Only 18 (20%) 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, thus the sample unit differed and may have had an effect on the precision of the accuracy estimates. Few studies reported a blinded interpretation of direct and processed smears; this is a major limitation of currently available literature. Lack of blinding may have resulted in an overestimation of the sensitivity of the processed smear.[76]

Studies were done in different countries and settings, but primarily in universities and research centres. Several investigations were done in both research laboratories and peripheral locations,[32,47,58] and as might be expected, greater increases in sensitivity after sputum processing were found in the research laboratories. Therefore, it is not known how the sputum processing methods would perform in routine settings, particularly in peripheral health centres. An important issue that we did not address was laboratory quality management to improve the reliability of diagnostic laboratory services. Quality management includes training, standards, monitoring visits, and internal and external quality control.[77] In addition, we did not address issues such as cost-effectiveness, feasibility, and safety of implementing the processing methods. Although statistical tests and graphical methods are available to detect potential publication bias in meta-analyses of randomised trials, such techniques have not been adequately evaluated for diagnostic data.[78] Thus, it is difficult to rule out publication bias in our review. Finally, our search strategy may have missed some relevant studies by excluding non-English publications.

Determination of the appropriate use of sputum processing methods will be a multistep process. First, studies should be done to assess the effects of different sputum processing methods under controlled conditions and to identify methods optimised for timing, concentration, and ease of use. These studies should be followed by large, blinded, multicentre studies of one or two selected processing methods in comparison with direct smears in settings with high and low HIV prevalence. Studies should prospectively recruit consecutive patients and use a reference standard. Every effort should be made to ensure that direct and processed smears are read independently of each other, and that smear results are interpreted independently of culture results. Finally, demonstration projects should be done to assess operationally feasible sputum processing methods with respect to performance targets. These projects would confirm performance in settings of intended use (microscopy centres) and determine case-finding, cost, work flow, and programmatic impact. In designing studies, we recommend keeping the patient foremost in mind, so that the process for obtaining a diagnosis and communicating this information to the patient does not become longer and more complex. 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.[79] Research on the performance and implementation of sputum processing methods should follow internationally coordinated and standardised approaches, both to strengthen the country-specific evidence base and to permit comparison with data from elsewhere. Consensus guidelines, such as the Standards for Reporting of Diagnostic Accuracy statement,[80] can be used to improve the quality and reporting of future studies.

Conclusions and policy implications

The evidence in this review suggests that processing sputum by use of centrifugation and various chemicals, including bleach and NaOH, increases the sensitivity of microscopy compared with the direct smear method, and has similar specificity. Unfortunately, because of the design of the studies included, it was not possible to distinguish the unique contribution of the various chemical and physical methods. The review does not enable us to determine whether the methods studied here would yield similar results if carried out in peripheral laboratories in low-income countries, because of the

Search strategy and selection criteria

Details of the search strategy and selection criteria can be found in the Methods section.

following concerns: feasibility of centrifugation in settings with irregular power supply; limited human and financial resources; inadequate training capacity; and potential biohazard posed by centrifugation. Any new technologies or methods that are implemented should be accompanied by efforts to strengthen laboratory quality management systems. Finally, we were surprised by the generally weak evidence base that supports such a fundamental and important component of tuberculosis control as microscopy, and by the substantial design flaws that limit the information that can be derived from such a large number of studies. Perhaps our most important conclusion is that any study evaluating tuberculosis diagnostics should be designed carefully so as to avoid the shortcomings we have identified in the existing literature.

Conflicts of interest

We declare we have no conflicts of interest.

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

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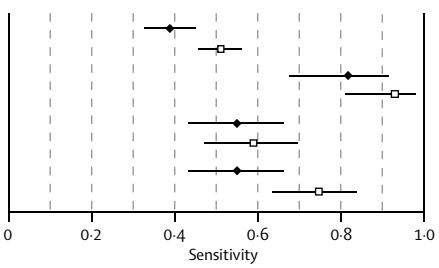

	Sensitivity (95% CI)	
Peterson (a)[58]	0·39	(0·33–0·45)
Peterson (a, Cn, NaLC-NaOH)[58]	0·51	(0·46–0·57)
Peterson (b)[58]	0·82	(0·68–0·92)
Peterson (b, Cn, NaLC-NaOH)[58]	0·93	(0·82–0·99)
Smithwick (a)[66]	0·55	(0·43–0·67)
Smithwick (a, Cn, NaLC-NaOH)[66]	0·59	(0·47–0·70)
Smithwick (b)[66]	0·55	(0·43–0·67)
Smithwick (b, PMF, NaOCl)[66]	0·75	(0·64–0·84)

WebFigure: **Fluorescence microscopy**
Sensitivity estimates of direct smear and processed sputum smears, processing with a chemical and physical method. Point estimates of sensitivity from each study are shown as solid diamonds for direct smears and as open squares for processed smears. The solid lines represent the 95% CIs. Studies within a single article are denoted by lower-case letters. NaLC-NaOH=N-acetyl-L-cysteine sodium-hydroxide solution; NaOCl=bleach; PMF=polycarbonate membrane filtration. Fluorochrome stains: Peterson et al[58] (a) auramine O, (b) auramine-rhodamine; Smithwick et al[66] (a) and (b) auramine.

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

Study (first author, year, country)	Stain	Study population	Number patients or specimens	Consecutive or random selection of patients or specimens	Blinded	Smear preparation	Processing method		Definition of smear positivity	Reference standard
							Chemical	Physical		
Allwood, 1998, Malaysia[73]	ZN	PTS	173	Yes	Yes	Single	NaOCl	Cn	IUATLD	Culture
Ängeby (a), 2000, Honduras[32]	ZN	PTS and PTP	303	..	Yes	Single	NaOCl	Cn	IUATLD	Culture
Ängeby (b), 2000, Honduras[32]	ZN	Routine sputum	303	..	Yes	Single	NaOH	Cn	IUATLD	Culture
Ängeby (c), 2000, Honduras[32]	ZN	PTS and PTP	971	..	No	Single	NaOCl	Cn	IUATLD	None
Ängeby (d), 2000, Honduras[32]	ZN	Routine sputum	1422	..	No	Single	NaOCl	Cn	IUATLD	None
Apers, 2003, Zimbabwe[33]	ZN	PTS	256	Yes	Yes	Single	NaOH	Cn	≥1 AFB	Culture
Aung, 2001, Myanmar[34]	ZN	New and re-treatment cases	948	..	..	Single	NaOCl	Cn	≥1 AFB	None
Biersack,1998, Nigeria[35]	..	AFB positive on concentrated smear	100	..	..	..	NaOH	Cn	..	None
Biswas (a), 1987, India[36]	ZN	PTS	102	..	..	Single	NaOH	Cn	..	None
Biswas (b), 1987, India[36]	ZN	PTS	102	..	..	Single	Xylol/NaOCl	Flotation	..	Culture
Bruchfeld (a), 2000, Ethiopia[37]	ZN	PTS	509	Yes	..	Pooled	NaOCl	Cn	≥1 AFB	Culture
Bruchfeld (b), 2000, Ethiopia[37]	ZN	PTS	96	Yes	..	Pooled	NaOCl	Cn	≥1 AFB	Culture
Cameron (1a), 1945, USA[38]	..	PTS and PTP on treatment	329	..	No	Single	NaOH	Not used	..	None
Cameron (1b), 1945, USA[38]	..	PTS and PTP on treatment	329	..	No	Single	Not used	Autoclave	..	None
Cameron (1c), 1945, USA[38]	..	PTS and PTP on treatment	329	..	No	Single	NaOCl	Cn	..	None
Cameron (2a), 1945, USA[39]	ZN	PTS and PTP on treatment	211	..	No	Single	NaOCl	Cn	..	None
Cameron (2b), 1945, USA[39]	ZN	PTS and PTP on treatment	211	..	No	Single	NaOH	Cn	..	None
Cameron (2c), 1945, USA[39]	ZN	PTS and PTP on treatment	211	..	No	Single	None	Autoclave/Cn	..	None
Cameron (a), 1946, USA[40]	KN	PTS and PTP on treatment	397	..	No	Single	NaOH/ potassium alum	Not used	≥3 AFB	None
Cameron (b), 1946, USA[40]	KN	PTS and PTP on treatment	397	..	No	Single	NaOCl	Not used	≥3 AFB	None
Chakravorty, 2005, India[41]	ZN	New PTS not on treatment	571	..	..	Single	USP	Cn	WHO	Culture
Contijo Filho (a), 1979, Brazil[42]	ZN	PTP on treatment	122	..	..	Single	NaOCl	Sd	WHO	None
Contijo Filho (b), 1979, Brazil[42]	ZN	PTP on treatment	122	..	..	Single	NaOCl	Cn	WHO	None
Contijo Filho (c), 1979, Brazil[42]	ZN	PTP on treatment	122	..	..	Single	NaOCl	Flotation (Soltys)	WHO	None
Corper (a), 1949, USA[43]	ZN	PTP	8	..	..	Single	NaOCl	Cn	≥1 AFB	None
Corper (b), 1949, USA[43]	ZN	PTP	8	..	..	Single	NaOCl	Cn/mechanical homogenisation	≥1 AFB	None
Cuevas, 2005, Ethiopia†	ZN	PTS	198	Yes	..	Other	NaOCl	Sd	≥1 AFB	None
Cuevas (a), 2005, Nigeria†	ZN	PTS not on treatment	183	..	Yes	Other	NaOCl	Sd	≥1 AFB	Culture
Cuevas (b), 2005, Nigeria†	ZN	PTS not on treatment	230	..	Yes	Other	NaOCl	Sd	≥1 AFB	Culture
Farnia (a), 2002, Iran[44]	ZN	PTS	430	..	Yes	Single	NaLC-NaOH	Cn	≥1 AFB	Culture
Farnia (b), 2002, Iran[44]	ZN	PTS	430	..	Yes	Single	NaOCl	Sd	≥1 AFB	Culture
Farnia (c), 2002, Iran[44]	ZN	PTS	430	..	No	Single	Chitin	Sd	≥1 AFB	Culture

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Fodor, 1995, Hungary[45]	ZN	AFB smear-positive patients	36	..	Yes	Single	Chlorhexidine gluconate/ NaOCl	CytoCn	..	Culture
Garay, 2000, Zimbabwe[46]	ZN	Symptomatic patients	242	Yes	..	Single	$(NH_4)_2SO_4$-NaOH	Sd	..	Culture
Gebre (a), 1995, Ethiopia[47]	ZN	PTS	100	..	Yes	Single	NaOCl	Cn	ALA	Culture
Gebre (b), 1995, Ethiopia[47]	ZN	PTS	500	..	Yes	Single	NaOCl	Cn	ALA	None
Gebre (c), 1995, India[47]	ZN	PTS	103	..	Yes	Single	NaOCl	Cn	ALA	None
Gebre-Selassie (a), 2003, Ethiopia[48]	ZN	PTS	200	Yes	..	Single	NaOCl	Sd	≥1 AFB	None
Gebre-Selassie (b), 2003, Ethiopia[48]	ZN	PTS	200	Yes	..	Single	NaOCl	Cn	≥1 AFB	None
Gopinathan, 1984, India[49]	ZN	PTP on treatment	65	..	..	Single	NaOH	Cn	≥3 AFB	None
Greenfield, 1919, ..[50]	..	PTS	20	..	No	Single	Sodium carbonate/ carbolic acid	Cn	≥1 AFB	None
Habeenzu, 1998, Zambia[51]	ZN	PTS	488	..	No	..	NaOCl	Cn	..	None
Harries, 1998, Malawi[74]	ZN	PTS	319	..	..	Single	NaOH	Cn	..	None
Kochhar (a), 2002, India[52]	ZN	Specimens submitted to microbiology lab	1484	..	..	Single	NaOH	Cn	IUATLD	None
Kochhar (b), 2002, India[52]	ZN	Specimens submitted to microbiology lab	1484	..	..	Single	$(NH_4)_2SO_4$-NaOH	Sd	IUATLD	None
Lawson, 2006, Nigeria[71]	ZN	PTS not on treatment	756	..	Yes	Other	NaOCl	Sd	≥1 AFB	Culture
Miörner (a), 1996, Ethiopia[53]	ZN	PTS	545	..	Yes	Single	NaOCl	Cn	..	None
Miörner (b), 1996, Ethiopia[53]	ZN	PTS	545	..	Yes	Single	NaOCl	Sd	..	None
Murray (a), 2003, UK[54]	ZN	PTS and known NTM	78	..	No	Single	Dithiothreitol	Not used	≥1 AFB	Culture
Murray (b), 2003, UK[54]	A	PTS and known NTM	78	..	No	Single	Dithiothreitol	Not used	≥1 AFB	Culture
Murray (c), 2003, UK[54]	ZN	PTS and known NTM	78	..	No	Single	Dithiothreitol	Cn	≥1 AFB	Culture
Murray (d), 2003, UK[54]	A	PTS and known NTM	78	..	No	Single	Dithiothreitol	Cn	≥1 AFB	Culture
Murray (e), 2003, UK[54]	ZN	PTS and known NTM	78	..	No	Single	Dithiothreitol/ NaOH	Cn	≥1 AFB	Culture
Murray (f), 2003, UK[54]	A	PTS and known NTM	78	..	No	Single	Dithiothreitol/ NaOH	Cn	≥1 AFB	Culture
Murray (g), 2003, UK[54]	ZN	PTS and known NTM	78	..	No	Single	Dithiothreitol/ NaOH	Cn 2×	≥1 AFB	Culture
Murray (h), 2003, UK[54]	A	PTS and known NTM	78	..	No	Single	Dithiothreitol/ NaOH	Cn 2×	≥1 AFB	Culture
Naganathan, 1979, India[55]	ZN	PTS with cough and abnormal chest radiograph	1499	..	Yes	Single	NaOH	Cn	≥10 AFB	Culture
Oliver, 1942, USA[56]	..	PTP	500	..	..	..	NaOCl	Cn	..	None
Perera, 1999, Sri Lanka[57]	ZN	PTS	163	Yes	No	Single	NaLC-NaOH	Cn	≥3 AFB	Culture
Peterson (a), 1999, USA[58]	AO, KNO	Sputum/culture request	1806	..	Yes	Single	NaLC-NaOH	Cn	≥3 AFB	Culture
Peterson (b), 1999, USA[58]	AR, ZNO	Sputum/culture request	887	..	Yes	Single	NaLC-NaOH	Cn	≥3 AFB	Culture
Petran (a), 1939, USA[59]	ZN	Specimens submitted to state public health lab	484	..	..	Single	NaOH	Cn	..	None
Petran (b), 1939, USA[59]	ZN	Specimens submitted to state public health lab	171	..	..	Single	Ferric chloride	Flocculation	..	None

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Petran (c), 1939, USA[59]	ZN	Specimens submitted to state public health lab	171	..	..	Single	NaOH+ferric chloride	Cn/flocculation	..	None
Pottenger, 1931, USA[72]	ZN	Specimens known to have rare bacilli	20	..	No	Single	NaOH/picric acid	Flotation	..	None
Rattan (a), 1994, India[60]	ZN	Specimens submitted to lab	100	Yes	Yes	Single	NaOCl+xylene flotation	Flotation	≥1 AFB	None
Rattan (b), 1994, India[60]	AO	Specimens submitted to lab	100	Yes	Yes	Single	NaOCl+xylene flotation	Flotation	≥1 AFB	None
Sacenau, 1993, USA[61]	KN	PTS	110	..	Yes	Single	NaOCl	CytoCn	..	Culture
Saxena (a), 2001, India[62]	ZN	PTS	304	..	..	Single	NaOCl	Cn	..	None
Saxena (b), 2001, India[62]	A	PTS	304	..	..	Single	NaOCl	Cn	..	None
Selvakumar, 2002, India[63]	ZN	Symptomatic patients	2341	..	Yes	Single	Phenol $(NH_4)_2SO_4$	Cn	≥1 AFB	Culture
Slosarek, 1977, eastern Europe* and Mongolia[64]	ZN	PTS	450	..	Yes	Single	Not used	Glass beads	..	None
Smithwick (a), 1979, USA[65]	AO	Specimens submitted to CDC	328	..	..	..	NaOCl	Polycarbonate membrane filter	>2 AFB	None
Smithwick (b), 1979, USA[65]	AO	Specimens submitted to CDC	328	..	..	..	NaLC-NaOH	Cn	>2 AFB	None
Smithwick (a), 1981, USA[66]	A	Specimens submitted to state lab	916	..	..	..	NaLC-NaOH	Cn	..	Culture
Smithwick (b), 1981, USA[66]	A	Specimens submitted to state lab	916	..	..	..	NaOCl	Polycarbonate membrane filter	..	Culture
Tech, 1965, Philippines[67]	ZN	Specimens from tuberculosis ward, private chest clinic	581	..	No	Single	NaOH	Cn	..	None
Van Deun (a), 2000, Bangladesh[23]	ZN	Specimens submitted to diagnostic and treatment centre	3287	Yes	Yes	..	NaOCl	Sd	≥1 AFB	None
Van Deun (b), 2000, Bangladesh[23]	ZN	Specimens submitted to diagnostic and treatment centre	1568	Yes	Yes	..	NaOCl	Sd	≥1 AFB	None
Vasanthakumari (a), 1998, India[68]	ZN	Symptomatic patients	1000	Yes	..	Single	NaOH	Cn 2×	≥3 AFB	Culture
Vasanthakumari (b), 1998, India[68]	ZN	Symptomatic patients	1000	Yes	..	Single	$(NH_4)_2SO_4$-NaOH	Sd	≥3 AFB	Culture
Wilkinson, 1997, S Africa[69]	ZN	PTS	166	Yes	..	Separate	NaOCl	Cn	..	Culture
Yassin, 2003, Ethiopia[70]	ZN	PTS	200	Yes	Yes	Single	NaOCl	Sd	ATS	None

Reference 23 (a) and (b) are independent studies using a variation in method: bleach then sedimentation (a), and sedimentation then bleach (b); reference 37 study (b) includes only HIV-infected patients and is a substudy of 37 (a); reference 47 studies (a), (b), and (c) are independent studies conducted at three different sites; Cuevas, 2005, studies (a) and (b) are independent studies conducted with HIV-negative and HIV-positive patients, respectively. *Participating countries: Bulgaria, Czechoslovakia, East Germany, Mongolia, Poland. †L Cuevas, Liverpool School of Tropical Medicine, Liverpool, UK, personal communication. A=auramine; AFB=acid-fast bacilli; ALA=American Lung Association; AO=auramine O; AR=auramine-rhodamine; CDC=Centers for Disease Control and Prevention; Cn=centrifugation; IUATLD=International Union Against Tuberculosis and Lung Disease; KN= Kinyoun; KNO=overstain with Kinyoun; NaLC-NaOH=N-acetyl-L-cysteine sodium-hydroxide solution; NaOCl=bleach; NaOH=sodium hydroxide; $(NH_4)_2SO_4$=ammonium sulphate; NTM=non-tuberculous mycobacteria; PTS=pulmonary tuberculosis suspects; PTP=pulmonary tuberculosis patients; rpm=revolutions per minute; Sd=sedimentation; USP=universal sample processing solution (guanidinium hydrochloride, Tris-Cl, EDTA, Sarkosyl, β-mercaptoethanol); WHO=World Health Organization; ZN=Ziehl-Neelsen; ZNO=overstain with Ziehl-Neelsen; ..=not reported.

***Webtable*: Characteristics and methodology of 83 studies comparing sputum smear microscopy of processed smears with the direct smear method**

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