

MAJOR ARTICLE

Bleach Sedimentation: An Opportunity to Optimize Smear Microscopy for Tuberculosis Diagnosis in Settings of High Prevalence of HIV

Maryline Bonnet,¹ Andrew Ramsay,^{3,a} Willie Githui,⁴ Laramie Gagnidze,¹ Francis Varaine,² and Philippe J. Guerin¹

¹Epicentre and ²Médecins Sans Frontières, Paris, France; ³Liverpool School of Tropical Medicine, Liverpool, United Kingdom; and ⁴Centre for Respiratory Diseases Research, Kenya Medical Research Institute, Nairobi, Kenya

Background. The purpose of the study was to evaluate the performance and feasibility of tuberculosis diagnosis by sputum microscopy after bleach sedimentation, compared with by conventional direct smear microscopy, in a setting of high prevalence of HIV.

Methods. In a community-based study in Kenya (a population in which 50% of individuals with tuberculosis are infected with HIV), individuals with suspected pulmonary tuberculosis submitted 3 sputum specimens during 2 consecutive days, which were examined by blind evaluation. Ziehl-Neelsen–stained smears were made of fresh specimens and of specimens that were processed with 3.5% household bleach followed by overnight sedimentation. Two different cutoffs for acid-fast bacilli (AFB) per 100 high-power fields (HPF) were used to define a positive smear: >10 AFB/100 HPF and 1 AFB/100 HPF. Four smear-positive case definitions, based on 1 or 2 positive smears with the 1 AFB or 10 AFB cutoff, were used.

Results. Of 1879 specimens from 644 patients, 363 (19.3%) and 460 (24.5%) were positive by bleach sedimentation microscopy, compared with 301 (16.0%) and 374 (19.9%) by direct smear microscopy, with use of the 10 AFB/100 HPF ($P < .001$) and 1 AFB/100 HPF ($P < .001$) cutoffs, respectively. Regardless of the case definition used, bleach sedimentation microscopy detected significantly more positive cases than did direct smear microscopy: 26.7% (172 of 644) versus 21.7% (140 of 644), respectively, with the case definition of 1 positive smear and the 1 AFB/100 HPF cutoff ($P < .001$), and 21.4% (138 of 644) versus 18.6% (120 of 644), respectively, with the case definition of 1 positive smear and the 10 AFB/100 HPF cutoff ($P < .001$). Inter- and intrareader reproducibility were favorable, with κ coefficients of 0.83 and 0.91, respectively. Bleach sedimentation was relatively inexpensive and was not time consuming.

Conclusions. Bleach sedimentation microscopy is an effective, simple method to improve the yield of smear microscopy in a setting of high prevalence of HIV. Further evaluation of this method, under operational conditions, is urgently needed to determine its potential as a tool for tuberculosis control.

Direct sputum smear microscopy, despite its low sensitivity, remains the cornerstone of tuberculosis (TB) diagnosis [1]. More-sensitive diagnostics are urgently needed to replace microscopy in peripheral laboratories and to allow diagnosis of tuberculosis at health care

centers without laboratory facilities. Although several promising diagnostic tests are in development, few are likely to be suitable replacements for microscopy or to bring tuberculosis diagnosis closer to communities in the short term [2].

Recent series of systematic reviews have indicated that microscopy for tuberculosis diagnosis may be optimized, with considerable benefits for patients and health care services [3–7]. The proposed approaches include the digestion of sputum with bleach, followed by a specimen-concentration step, before the smear preparation [3, 8].

Evaluations of diagnostics for infectious diseases are frequently of poor quality [9]. Systematic reviews of tuberculosis diagnostics, including the series on optimizing smear microscopy referred to above, report that

Received 16 September 2007; accepted 8 January 2008; electronically published 29 April 2008.

^a Present affiliation: UNICEF-UNDP-World Bank-World Health Organization Special Programme for Research and Training for Tropical Diseases, Geneva, Switzerland.

Reprints or correspondence: Maryline Bonnet, Médecins Sans Frontières, Rue de Lausanne 78, CH-1211 Geneva 21, Switzerland (maryline.bonnet@geneva.msf.org).

Clinical Infectious Diseases 2008;46:1710–6

© 2008 by the Infectious Diseases Society of America. All rights reserved.

1058-4838/2008/4611-0010\$15.00

DOI: 10.1086/587891

poorly designed and poorly implemented evaluations are common and result in highly variable results [3–5, 10, 11]. The flaws include failure to conduct evaluation among the most-appropriate patient populations (i.e., individuals with suspected tuberculosis who attend outpatient facilities), lack of blinding of the specimen evaluation, lack of quality control of microscopy, and lack of attention to bleach quality and stability. The use of different criteria to define a suspected tuberculosis case or different thresholds to define a positive smear or a smear-positive case could all introduce variability [12]. Also, studies of bleach microscopy have failed to answer 3 important operational questions about microscopy for tuberculosis diagnosis: (1) can it benefit peripheral clinics, where the majority of patients seek care; (2) can it improve tuberculosis diagnosis in HIV-infected patients, for whom the need for improved detection of paucibacillary disease is critical; and (3) what is its benefit when a very sensitive acid-fast bacilli (AFB) cutoff is used to define a positive smear and a smear-positive case [4].

The bleach microscopy methods that have been evaluated include those that employ different bleach concentrations and different concentration methods (i.e., centrifugation, flotation, or sedimentation). Furthermore, some workers described the addition of distilled water to the sputum-bleach mixture before the concentration step, whereas others did not. This shows that there is an inadequate quantity of evidence about any particular bleach method. Of the various proposed methods, those that use locally obtained domestic bleach and overnight sedimentation at room temperature appear to be inexpensive and suitable for peripheral laboratories in low-income countries. We set out to evaluate such a method among patients with suspected tuberculosis in a peripheral health care setting with a high prevalence of HIV. Unfortunately, because culture for *Mycobacterium tuberculosis* was not available in our clinic, as is the case in many developing countries, we do not report culture data.

METHODS

Setting and patients. Participants were drawn from the urban outpatient clinic supported by the Kenyan Ministry of Health and Médecins Sans Frontières (Doctors Without Borders), which provides HIV and tuberculosis treatment to the community living in the slum of Mathare (Nairobi, Kenya). In 2001, the HIV prevalence in the adult population of Nairobi was 15%, and 50% of patients with tuberculosis were infected with HIV [13]. In Mathare, 76% of patients with smear-negative suspected tuberculosis were HIV infected (M. Bonnet, personal communication). Consecutive patients aged >15 years who presented with a cough for >2 weeks were eligible for the study [14]. Intake of antituberculosis drugs or quinolone in the 4 weeks before the screening was an exclusion criterion. De-

mographic information, treatment history, and clinical characteristics were recorded.

Specimen collection and processing. Patients submitted 3 sputum specimens during 2 consecutive days, after they were given instructions on how to produce a good quality specimen [15]. The first specimen was collected in the clinic at the initial consultation, the second at home early the next day, and the third in the clinic when the patient brought the morning specimen [16]. A minimum quantity of 1 mL of specimen was required. Smears were made from each specimen for direct smear microscopy. Each smear was heat fixed and was stained using the hot Ziehl-Neelsen method (1% filtered carbol-fuchsin and 0.1% methylene blue). The remainder of the specimen was transferred to a 15-mL disposable plastic conical tube with an equal volume of neat commercial bleach (3.5% NaOCl). The mixture was agitated using a vortex mixer and was placed vertically on the bench, away from drafts, at room temperature for overnight sedimentation (15–18 h). After sedimentation, the supernatant was poured off, and the sediment was mixed with the remaining fluid. One or 2 drops were transferred with a sterile glass pipette to a slide. A bleach smear was made, was air dried, was heat fixed, and was stained by the same Ziehl-Neelsen method.

The direct smear and bleach smear specimens were examined by bright-field microscopy (magnification, $\times 1000$) on site by the same laboratory technician who was blind to the result of previous smears from the same patient. Staff turnover of the 2 study technicians prevented the same technician from reading all smears from the same patient. The exact number of AFB observed in 100 high-power microscopic fields (HPF) of each smear was recorded on unique, separate laboratory forms and in the laboratory register by the laboratory supervisor. For both direct smear and bleach sedimentation, a positive smear was defined using 2 different grading scales: the World Health Organization–International Union Against Tuberculosis and Lung Disease scale, requiring ≥ 10 AFB/100 HPF, and the scale recommended by the American Thoracic Society, requiring ≥ 1 AFB/100 HPF [16–18]. The microscopic appearance of a direct smear was defined as “good” if blue cellular elements were present without debris, as “too thick” if cells were lying on top of each other, as “too thin” in cases of insufficient background elements, as “under decolorized” if background was purple or red, and as “overheated” if red crystals were seen. Similar assessment of bleach smears was not conducted because of the digestion of the cellular elements in the smears.

As part of internal quality management, 100% of positive and 10% of negative bleach smears and direct smears were blindly reexamined monthly by the laboratory supervisor. External quality assessment consisted of 100 randomly selected direct smears blindly reexamined at the end of the study by

the tuberculosis laboratory of Kenya Medical Research Institute (KEMRI) (Nairobi, Kenya).

NaOCl solution. The 3.5% domestic bleach available on the Kenyan market (Jik Bleach Regular; Reckitt Benckiser East Africa; active ingredient, sodium hypochlorite at 3.5% mol/vol when packed) was used without the addition of distilled water [19]. The same batch of bleach was used during the entire 10-month study duration. To reduce the risk of oxidation, the solution was stored in 750-mL dark bottles, with minimum head-space in a dark area [20]. The room temperature was monitored. Each week, a 250-mL aliquot of bleach “working solution” was collected to process the specimens for that week. All remaining working solution was discarded at the end of the week. The date and hour of aliquoting of the working solution were recorded on the weekly container. Before use, the presence of free chlorine was assessed using a swimming pool tester with diethyl-p-phenylene diamine 1 (pocket type; chlorine range, 0.1–6.0 mg/L) after prior dilution at $d = 2.10^4$. The quality of NaOCl was considered adequate if the concentration was in the range 1.5–2 mg/L, in accordance with manufacturer’s recommendations.

Reproducibility assessment. Random selections of direct smears and bleach smears were read a second time on the same day by a different technician and were read by the same technician 24 h after the first reading, to assess inter- and intrareader reproducibility, respectively. The laboratory supervisor masked the identification of the slide to ensure that the reading was blind.

Operational aspects. A list of parameters was measured to evaluate the feasibility of bleach sedimentation microscopy compared with that of direct smear microscopy: (1) the du-

ration of bleaching (i.e., the time spent adding the bleach to the specimen in the tube and shaking the tube), (2) sedimentation, (3) the smearing (including the drying of the slide) and staining of a daily batch of specimens, and (4) the duration of the reading of individual slides. The temperature at the start and end of sedimentation was monitored. The cost of reagents and consumables to perform bleach sedimentation microscopy was assessed on the basis of Kenyan market price.

Sample size. The minimum sample size to detect at least a 10% difference in the percentage of smear-positive cases found by bleach sedimentation microscopy compared with direct smear microscopy was calculated [8]. With an average of 20% smear-positive cases detected routinely by direct smear microscopy in the study clinic, a risk of 0.05, a power of 80%, and a 10% dropout rate, the sample size was 690 patients. With a positive-smear detection rate of 20%, an expected κ coefficient >0.8 , and a precision of 10%, the minimum sample size required to assess the test reproducibility was 220 smears [21].

Data analysis. Data were double entered using Epidata 3.1 (EpiData Association) and were analyzed using SPSS 11.0 for Windows (SPSS). Patient, specimen, and smear characteristics were described. Positive smear specimens and patient detection rates were compared between bleach sedimentation microscopy and direct smear microscopy, with use of McNemar’s test for matched data. Four definitions of a sputum smear-positive case were used:

1. AFB on 2 of 3 smears examined, 1 of which had ≥ 10 AFB/100 HPF detected [22],
2. ≥ 1 AFB/100 HPF in 2 of 3 smears [14],
3. ≥ 10 AFB/100 HPF in 1 of 3 smears, and
4. ≥ 1 AFB/100 HPF in 1 of 3 smears.

The gain of bleach smears was calculated as the increase in positive smears by bleach sedimentation microscopy, compared with direct smear microscopy, divided by the total number of positive smears detected by direct smear microscopy. Likewise, the gain of direct smears was calculated as the increase in positive smears by direct smear microscopy, compared with bleach sedimentation microscopy, divided by the total number of positive smears detected by bleach sedimentation microscopy. The same calculation was made for smear-positive cases. The positive-smear-detection yield of bleach sedimentation microscopy performed on the first specimen was compared with that of direct smear microscopy performed on the first 2 specimens and on all 3 specimens, with use of case definitions 3 and 4 described above. Inter- and intrareader reproducibility were assessed by the calculation of the κ coefficient, which measures the agreement between 2 readings. A κ coefficient ≥ 0.80 signifies almost perfect agreement.

The National Ethical Review Committee of KEMRI and the Comité de Protection des Personnes (Saint Germain en Laye,

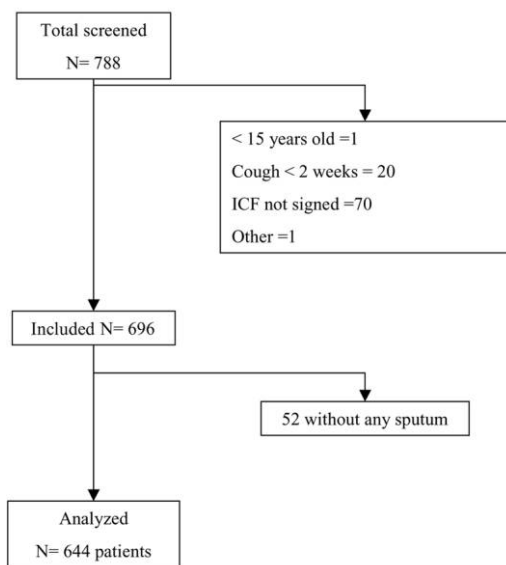


Figure 1. Study flow chart. ICF, informed consent form

Table 1. Positive-smear detection among 1879 specimens, with a threshold of 10 or 1 acid-fast bacilli (AFB) per 100 high-power fields (HPF).

Threshold	Direct smear microscopy		Bleach sedimentation microscopy		P
	No. of positive specimens	Percentage positive (95% CI)	No. of positive specimens	Percentage positive (95% CI)	
10 AFB/100 HPF	301	16.0 (14.4–17.6)	363	19.3 (17.6–21.2)	<.001
1 AFB/100 HPF	374	19.9 (18.1–21.8)	460	24.5 (22.5–26.5)	<.001

France) approved the study. Written informed consent was obtained from the participants.

RESULTS

A total of 788 patients with suspected pulmonary tuberculosis were screened, and 644 (81.7%) were recruited for the study (figure 1). The mean age was 32 years (SD, 10.3), the male:female ratio was 0.8 (287:356; data was missing for 1 patient), 121 (18.8%) of the patients had a history of tuberculosis, 37 (5.7%) received a course of broad-spectrum antibiotics in the 2 weeks before recruitment, and 12 (1.9%) had a chest radiograph. All patients had sputum production, 593 (92.1%) had a fever in the past week, 596 (92.5%) had chest pain, 109 (16.9%) had hemoptysis, 550 (85.4%) had night sweats, 245 (38.0%) had weight loss, and 484 (75.2%) had appetite loss.

Among the 644 recruited patients, 614 (95.3%) provided 3 specimens, 7 (1.1%) provided 2 specimens, and 23 (3.6%) provided 1 specimen. A total of 1879 specimens were collected and processed. Of these specimens, 1401 (74.6%) were purulent, 414 (22.0%) were mucoid, 56 (3.0%) were blood stained, and 8 (0.4%) were salivary. Of 1879 direct smears, 1855 (98.7%) were good, 5 (0.3%) were too thick, and 19 (1.0%) were too thin.

The results of smear microscopy are presented in tables 1 and 2. In 36 (1.9%) of the 1879 specimens, bleach smears were unreadable. Regardless of the threshold used to define a positive smear, bleach sedimentation microscopy yielded significantly

more positive smears than did direct smear microscopy. With the threshold of 10 AFB/100 HPF or 1 AFB/100 HPF, 62 (20.6%) of 301 positive smears and 86 (23.2%) of 371 positive smears, respectively, were detected by bleach sedimentation, compared with 10 (2.8%) of 363 positive smears and 2 (0.4%) of 460 positive smears, respectively, detected by direct smear microscopy. Significantly fewer positive smears were missed by bleach sedimentation microscopy with the threshold of 1 AFB/100 HPF than with the threshold of 10 AFB/100 HPF ($P < .01$). Of 363 positive smears (with the threshold of 10 AFB/100 HPF) detected by bleach sedimentation microscopy, 289 (79.6%) were detected as positive by direct smear microscopy. Of the remaining 74 positive smears detected only by bleach sedimentation microscopy, 51 (68.9%) showed scanty results (1–9 AFB/100 HPF) and 23 (31.1%) were negative by direct smear microscopy. The benefit of the bleach sedimentation method was greater for the mucoid specimens than for the purulent specimens.

The results of smear-positive case detection are presented in table 3. Regardless of case definition, bleach sedimentation microscopy detected significantly more smear-positive cases than did direct smear microscopy. Compared with direct smear microscopy performed on 2 or 3 specimens, bleach sedimentation microscopy performed on the first specimen detected as many smear-positive cases with case definition 3 and detected significantly more smear-positive cases with case definition 4 (table 4). Both direct smear microscopy and bleach sedimentation

Table 2. Positive-smear detection among 1879 specimens by macroscopic aspect, with a threshold of 10 or 1 acid-fast bacilli (AFB) per 100 high-power fields (HPF).

Specimen aspect	Threshold of 10 AFB/100 HPF				Threshold of 1 AFB/100 HPF			
	Direct smear microscopy	Bleach sedimentation microscopy	Gain ^a	P	Direct smear microscopy	Bleach sedimentation microscopy	Gain ^a	P
Purulent (n = 1401)	257 (18.3)	305 (21.8)	48 (18.7)	.02	312 (22.3)	374 (26.7)	62 (19.9)	.001
Mucoid (n = 414)	34 (8.2)	46 (11.1)	12 (35.3)		49 (11.8)	69 (16.7)	20 (40.8)	
Blood stained (n = 56)	10 (17.9)	12 (21.4)	2 (20.0)		13 (23.2)	17 (30.4)	4 (30.8)	
Salivary (n = 8)	0 (0)	0 (0)	...		0 (0)	0 (0)	...	

NOTE. Data are no. (%) positive specimens, unless otherwise indicated.

^a Gain of bleach sedimentation microscopy, given as the increase in positive smears detected (% of total number of positive smears detected).

Table 3. Smear-positive case detection with use of different case definitions.

Case definition	Direct smear microscopy		Bleach sedimentation microscopy		<i>P</i>	Gain of bleach sedimentation microscopy ^a	Gain of direct smear microscopy ^a
	No. of positive specimens	Percentage positive (95% CI)	No. of positive specimens	Percentage positive (95% CI)			
1 (<i>n</i> = 621)	116	18.7 (15.7–22.0)	136	21.9 (18.7–21.4)	<.001	20/116 (17.2)	1/136 (0.7)
2 (<i>n</i> = 621)	126	20.3 (17.2–23.7)	155	25.0 (21.6–28.6)	<.001	29/126 (23.0)	0
3 (<i>n</i> = 644)	120	18.6 (16.0–21.9)	138	21.4 (18.3–24.8)	<.001	18/120 (15.0)	1/138 (0.7)
4 (<i>n</i> = 644)	140	21.7 (18.6–25.1)	172	26.7 (23.3–30.2)	<.001	32/140 (22.9)	1/172 (0.6)

NOTE. Case definitions are as follows: (1) acid-fast bacilli (AFB) on 2 of 3 smears examined, in 1 of which the result is ≥ 10 AFB/100 high-power fields (HPF); (2) ≥ 1 AFB/100 HPF in 2 of 3 smears; (3) ≥ 10 AFB/100 HPF in 1 of 3 smears; and (4) ≥ 1 AFB/100 HPF in 1 of 3 smears.

^a Gain data are given as the increase in positive smears detected/total number of positive smears detected (%).

microscopy performed on the first specimen detected only 2 more cases than did bleach sedimentation microscopy alone.

Inter- and intrareader reproducibility of both methods showed κ coefficients ≥ 0.8 (table 5). Quality control reported a 95%–100% agreement rate between the technician's results and the supervisor's results for the monthly internal control and a 99% rate for the external control.

Considering the operational aspects, a median of 12 specimens (interquartile range [IQR], 8.5–15) were processed daily. The mean \pm SD duration of bleaching was 18.6 ± 7.6 min, and the mean \pm SD duration of sedimentation was 16.8 ± 0.8 h. The median temperature at the start (late afternoon) and the end (early morning) of sedimentation was 30.0°C (IQR, 27.2–32.7) and 26.0°C (IQR, 23.7–28.0), respectively. Monitoring of the presence of free chlorine in the bleach solution showed that levels were adequate, with NaOCl concentrations in the range of 1.5–2 mg/L at weekly controls. The mean \pm SD duration of smear preparation was longer for processed specimens (52.9 ± 25.6 min) than for fresh specimens (21.4 ± 8.3 min; $P < .001$), because of the longer drying time for slides made from processed specimens. The mean \pm SD duration of staining was similar for both methods: 45 ± 10.4

min for bleach sedimentation microscopy and 47.1 ± 10.6 min for direct smear microscopy ($P = .12$). No statistically significant difference was observed in the mean \pm SD duration for reading a positive smear by bleach sedimentation microscopy (3.1 ± 0.6 min) compared with by direct smear microscopy (3.0 ± 0.6 min). The additional cost of specimen processing was €0.20/specimen, which included the cost of bleach and of disposable products (2-mL syringes, 15-mL plastic conical tubes, and plastic pipettes).

DISCUSSION

We report a significant increase in the number of positive smears and the number of affected patients detected using bleach sedimentation microscopy compared with conventional direct smear microscopy, regardless of the smear-positive case definition and AFB threshold used. One digested smear performed as well as 3 direct smears, which is consistent with recent results from a hospital-based study in Nigeria with a similar prevalence of concordant HIV infection and tuberculosis [23].

This study avoided the limitations associated with previous

Table 4. Smear-positive case detection with use of bleach sedimentation microscopy and/or direct smear microscopy and case definitions 3 and 4.

Case definition	Bleach sedimentation microscopy on first specimen		Direct smear and bleach sedimentation microscopy on first specimen		Direct smear microscopy on first 2 specimens		Direct smear microscopy on 3 specimens	
	No. of positive specimens	Percentage positive (95% CI)	No. of positive specimens	Percentage positive (95% CI)	No. of positive specimens	Percentage positive (95% CI)	No. of positive specimens	Percentage positive (95% CI)
3 (<i>n</i> = 644)	119	18.5 (15.5–21.7)	121	18.8 (15.8–22.0)	117	18.2 (15.3–21.4)	120	18.6 (16.0–21.9)
4 (<i>n</i> = 644)	150	23.3 (20.1–26.7)	152	23.6 (20.4–27.1)	135	21.0 (17.9–24.3)	140	21.7 (18.6–25.1)

NOTE. Case definitions are as follows: (3) ≥ 10 acid-fast bacilli (AFB) per 100 high-power fields (HPF) in 1 of 3 smears and (4) ≥ 1 AFB/100 HPF in 1 of 3 smears. With use of case definition 3, bleach sedimentation microscopy performed on the first specimen had $P = .84$ for comparison with direct smear microscopy performed on 2 specimens and $P = 1.00$ for comparison with direct smear microscopy performed on 3 specimens. $P = .001$ and $P = .03$ for the same comparisons with use of case definition 4. With use of case definition 3, direct smear microscopy and bleach sedimentation microscopy both performed on the first specimen had $P = .52$ for comparison with direct smear microscopy performed on 2 specimens and $P = 1.00$ for comparison with direct smear microscopy performed on 3 specimens. $P < .001$ and $P = .004$ for the same comparisons with use of case definition 4.

Table 5. Results of inter- and intrareader reproducibility studies.

Reproducibility	Direct smear microscopy		Bleach sedimentation microscopy	
	No. of readings	κ (95% CI)	No. of readings	κ (95% CI)
Interreader	180	0.83 (0.76–0.86)	219	0.86 (0.84–0.88)
Intrareader	187	0.91 (0.88–0.94)	190	0.93 (0.89–0.95)

studies and reported in a meta-analysis of sputum-processing methods [3, 10]. First, it was a prospective, community-based study in a setting of high prevalence of HIV. The criteria to define a suspected case of tuberculosis were based on the World Health Organization guidelines for smear-microscopy screening in settings of high HIV prevalence [14]. The rate of smear-positive cases among suspected cases of tuberculosis is in the expected range of 5%–20% for countries in which tuberculosis is one of the most frequent causes of chronic cough [16]. Second, the quality of the smear microscopy was high, which was the condition in which the lowest benefit of the specimen-processing method would be expected [24]. Indeed, we report a significant benefit associated with the use of bleach sedimentation microscopy in a setting of care that already uses optimized direct smear microscopy and collects high-quality specimens. Third, the results were reported per specimen and per patient, by use of different standards for the AFB threshold and case definition. Fourth, our bleach method was standardized as much as possible, and different practical aspects were monitored. Unlike methods used in previous studies, the processing method did not require distilled water, which simplified the procedure and presumably avoided contamination of smears with saprophytic AFB from tap water, which is used routinely in conditions in which distilled water is not available [24]. Finally, the study was strengthened by its methodology based on recruitment of consecutive individuals with suspected tuberculosis and a blind evaluation.

The bleach sedimentation method is simple and does not require additional expertise beyond that required for conventional direct smear microscopy. Materials and reagents are affordable and are available locally in countries where tuberculosis is endemic. Specimen preparation does not require significantly extra workload, and the longer time required for the drying of slides (after smearing) involves no extra labor time. We did not experience faster or easier reading (because of AFB being more readily seen in smears with less debris) by bleach sedimentation microscopy, as was reported in previous studies [8]. Instead, technicians reported difficulties in focusing, because of the lack of background material in the smears, and complained of eye fatigue.

Bleach sedimentation can result in fragile smears [24]. The 36 (1.9%) unreadable bleach smears were mainly caused by the

smear washing out. Indeed, the smear can wash off during slide staining, and care is required to avoid this problem. Overheating of slides may result in the formation of crystals of hydroxide, which might compromise readings. Another drawback of bleach sedimentation is the poor stability of bleach when stored in suboptimal conditions [3, 8]. In our study, we monitored the presence of free chlorine in the bleach solution, using a pool tester. This testing required previous dilution of the solution, because the highest concentration for a simple test to detect free chlorine is 250 mg/L (Color Comparator; Wagtech), and higher concentrations require spectrophotometric titration. This monitoring requires additional work, which can introduce dilution errors and may be difficult to perform in routine conditions. Nevertheless, our experience and previous reports show that bleach solutions at concentrations of <6% available chlorine have an acceptable shelf life of at least 6 months if stored under suitable conditions (i.e., a cool place in opaque, nonreactive bottles with airtight caps) [20]. The 1-day delay of the overnight bleach sedimentation is a limitation, which may be overcome by use of a shorter sedimentation time [3].

The percentage of smears with AFB that were missed by bleach sedimentation microscopy, compared with by direct smear microscopy, was relatively low (0.4% with the 1 AFB/100 HPF threshold and 2.7% with the 10 AFB/100 HPF threshold). Despite the absence of culture (a limitation of this study), these bleach sedimentation–negative smears were likely to be false-negative results, perhaps explained by the aforementioned difficulty in focusing, the potential dispersion of AFB throughout the specimen, the breaking up of AFB clumps after homogenization, or smear fragility [24, 25]. Because of the same limitation of no culture, the proportion of positive smears caused by *Mycobacterium* species other than *M. tuberculosis* could not be reported. A recent survey conducted in a district of Nairobi reported that the isolates in 8.2% of 85 positive specimens were identified as *Mycobacterium* species other than *M. tuberculosis* [26].

In conclusion, this study suggests that the bleach sedimentation method can significantly improve the yield of microscopy for tuberculosis diagnosis when used in a peripheral clinic in a setting of a high prevalence of HIV. Its benefit remains significant even when a sensitive AFB threshold is used. The

method is simple, affordable, and appropriate for laboratories that already perform microscopy. Additional evaluation, under operational conditions, is urgently required to determine its potential as a tool for tuberculosis control. Cost-effectiveness analyses of strategies that combine direct smear microscopy and bleach sedimentation microscopy are necessary to optimize services and to balance the need for increased sensitivity with the need to prevent patient dropout during diagnostic process.

Acknowledgments

We thank Médecins Sans Frontières France, which sponsored the study, and the Médecins Sans Frontières teams in Mathare and Nairobi, which were very supportive. A special thanks to the study technicians Thomas Agendo, Purity Muthoni Ndwiga, Andrew Mwangi Chege, and Ali Abdullahi and to Stéphanie Charrondièrè, who coordinated the study preparation in Mathare. We gratefully acknowledge the personnel of the tuberculosis laboratory of KEMRI, which performed the external quality control of the sputum smear microscopy. We thank also Laurence Bonte, for her technical assistance, and Oliver Yun, for his excellent support in the editing of the article.

Financial support. Médecins Sans Frontières.

Potential conflicts of interest. All authors: no conflicts.

References

1. Apers L, Wijarajah J, Mutsvangwa J, Chigara N, Mason P, van der Stuyft P. Accuracy of routine diagnosis of pulmonary tuberculosis in an area of high HIV prevalence. *Int J Tuberc Lung Dis* **2004**; *8*:945–51.
2. Guillemin M, Usdin M, Arkininstall J. Tuberculosis diagnosis and drug susceptibility testing: an overview of the current diagnostic pipeline. **2006**. Available at: http://www.accessmed-msf.org/fileadmin/user_upload/diseases/tuberculosis/Diagnostics%20Pipeline%20Report.pdf. Accessed 26 June 2007.
3. Steingart KR, Ng V, Henry M, et al. Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review. *Lancet Infect Dis* **2006**; *6*:664–74.
4. Steingart KR, Ramsay A, Pai M. Optimizing sputum smear microscopy for the diagnosis of pulmonary tuberculosis. *Expert Rev Anti Infect Ther* **2007**; *5*:327–31.
5. Steingart K, Henry M, Ng V, et al. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. *Lancet Infect Dis* **2006**; *6*:570–81.
6. Mase SR, Ramsay A, Ng V, et al. Yield of serial sputum specimen examinations in the diagnosis of pulmonary tuberculosis: a systematic review. *Int J Tuberc Lung Dis* **2007**; *11*:485–95.
7. Keeler E, Perkins MD, Small P, et al. Reducing the global burden of tuberculosis: the contribution of improved diagnostics. *Nature* **2006**; *444*(Suppl 1):49–57.
8. Angeby KA, Hoffner SE, Diwan VK. Should the “bleach microscopy method” be recommended for improved case detection of tuberculosis? Literature review and key person analysis. *Int J Tuberc Lung Dis* **2004**; *8*: 806–15.
9. UNICEF-UNDP-World Bank-WHO Special Programme for Research and Training in Tropical Diseases. Available at: <http://www.who.int/tdr>. Accessed 26 June 2007.
10. Pai M, O'Brien R. Tuberculosis diagnostics trials: do they lack methodological rigor? *Expert Rev Mol Diagn* **2006**; *6*:509–14.
11. Ramsay A, Squire SB, Siddiqi K, Cunningham J, Perkins MD. The bleach microscopy method and case detection for tuberculosis control. *Int J Tuberc Lung Dis* **2006**; *10*:256–8.
12. Bonnet M, Ramsay A, Gagnidze L, Githui W, Guerin PJ, Varaine F. Reducing the number of sputum samples examined and thresholds for positivity: an opportunity to optimise smear microscopy. *Int J Tuberc Lung Dis* **2007**; *11*:953–8.
13. World Health Organization. Global tuberculosis control. Report WHO/HTM/TB/2004.331. Geneva, Switzerland: WHO, **2004**.
14. World Health Organization. TB/HIV: a clinical manual. 2nd ed. Report WHO/HTM/TB/2004.329. Geneva, Switzerland: WHO, **2004**.
15. Khan MS, Dar O, Sismanidis C, Shah K, Godfrey-Faussett. Improvement of tuberculosis case detection and reduction of discrepancies between men and women by simple sputum-submission instructions: a pragmatic randomised controlled trial. *Lancet* **2007**; *369*:1955–60.
16. World Health Organization. Laboratory service in tuberculosis control. Microscopy part I. Report WHO/TB/98.258. Geneva, Switzerland: WHO, **1998**.
17. Rieder HL, Van Deun A, Kam KM, et al. Priorities for tuberculosis bacteriology services in low-income countries. 2nd ed. Paris: International Union Against Tuberculosis and Lung Disease, **2007**. Available at: <http://www.tbrieder.org/>. Accessed 26 June 2007.
18. American Thoracic Society. Diagnostic standards and classification of tuberculosis in adults and children. *Am J Respir Crit Care Med* **2000**; *161*:1376–95.
19. Githui WA, Matu SW, Tunge N, Juma E. Biocidal effect of bleach on *Mycobacterium tuberculosis*: a safety measure. *Int J Tuberc Lung Dis* **2007**; *11*:798–802.
20. Fraiss S, Ng YL, Gulabivala K. Some factors affecting the concentration of available chlorine in commercial sources of sodium hypochlorite. *Int Endod J* **2001**; *34*:206–15.
21. Mackinnon A. A spreadsheet for the calculation of comprehensive statistics for the assessment of diagnostic tests and inter-rater agreement. *Comput Biol Med* **2000**; *30*:127–34.
22. Enarson DA, Rieder HL, Arnadottir T, Trébuq A. Management of tuberculosis: a guide for low income countries. 5th ed. Paris: International Union Against Tuberculosis and Lung Disease, **2000**.
23. Lawson L, Yassin MA, Ramsay A, et al. Short-term bleach digestion of sputum in the diagnosis of pulmonary tuberculosis in patients co-infected with HIV. *Tuberculosis (Edinb)* **2007**; *87*:368–72.
24. Van Deun A, Maug AK, Cooreman E, et al. Bleach sedimentation method for increased sensitivity of sputum smear microscopy: does it work? *Int J Tuberc Lung Dis* **2000**; *4*:371–6.
25. Wilkinson D, Sturm AW. Diagnosing tuberculosis in a resource-poor setting: the value of sputum concentration. *Trans R Soc Trop Med Hyg* **1997**; *91*:420–1.
26. Buziba NG, Chebore SJ, Akoru CN, Ndinya JO, Siteni JK. Prevalence of *M. tuberculosis* and MOTT among HIV+ and HIV– patients attending the chest clinic in a district hospital, Nairobi, Kenya. *Int J Tuberc Lung Dis* **2007**; *11*:S87.