

# The value of two versus three smears in identifying culture positive tuberculosis patients in Karonga district.

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## Abstract

As pulmonary tuberculosis (PTB) cases put an increasing burden on overstretched TB control programmes, it is crucial to explore ways to maximise the efficiency of ascertainment and diagnosis without compromising specificity or sensitivity, particularly in the spectrum of disease associated with HIV infection. There have been proposals to change the recommendation for collection of sputum specimens from PTB suspects from three to two to achieve this goal. We examined laboratory results from the Karonga Prevention Study from a 5 year period, to assess the ability of two smears to correctly identify smear or culture positive TB cases. A total of 1,992 PTB suspects with three sputum smears and at least one culture result were studied. Smears were auramine stained and examined with fluorescence microscopy and positives confirmed with Ziehl-Neelsen staining and light microscopy. Cultures were set up on Lowenstein-Jensen media. True negative and positive status was defined on the basis of culture. The sensitivity, specificity and positive and negative predictive values of two and three smears were compared.

Compared to culture the sensitivity, specificity and positive and negative predictive values of three smears were 70%, 98%, 92% and 92% respectively. Of those detected as smear-positive using three smears, at least 97% would have been detected by two. Among those with HIV serology results available, the sensitivity of two smears for detecting culture-positive tuberculosis was identical to that using three. In this setting, using fluorescence and light microscopy, collecting two rather than three smears would only marginally reduce sensitivity and would slightly improve the specificity of diagnosis of PTB; this is unaffected by HIV status.

## Introduction

Tuberculosis is a major cause of morbidity and mortality in Malawi, mainly due to the HIV epidemic. Numbers of people presenting with chronic cough have risen, with a major impact on overstretched laboratory services. The current recommendations for diagnosis of pulmonary tuberculosis (PTB) are to take three sputum specimens from each suspect over a two day period.<sup>1</sup> These three specimens may not be examined well due to time constraints. Were the recommended number of sputum samples to be reduced to two smears, there would be significant savings on sputum containers, slides and reagents, and time.<sup>2,3</sup> In Malawi, thousands of sputum samples and smears are processed each day, and it is important to assess whether a screening programme using fewer than three sputum smears is acceptably sensitive and specific.<sup>4</sup> Thorough examination of two slides may even be more sensitive and specific than rushed examination of three.

In Ntcheu district, changing policy from three to two

smears did not affect the proportion of suspects found to be smear positive or other programme outcome indicators.<sup>2</sup> In other studies it is estimated that between 77 and 83% of smear positive cases (on three smears) would have been found on the first smear and between 93 and 96% on the first two smears.<sup>5-9</sup> These figures have not changed over time, suggesting that the proportions are not significantly affected by HIV, although there are reports that those who are HIV positive are less likely than those who are HIV negative to be smear positive and less likely to have high grade smears.<sup>10-12</sup>

Where sets of sputum from each patient are identifiable in the laboratory, reading may not be done independently and retrospective review of recorded results can be misleading; overestimating the sensitivity and specificity of using just two smears if the results from the first two smears were compared with the results overall. It is not clear from published studies if slides were read blind to the other results from that patient.<sup>3,5,6,8</sup> As part of the Karonga Prevention Study (KPS) we have compared the sensitivity and specificity of two versus three sputum smears for the diagnosis of tuberculosis. The KPS is responsible for the diagnosis of tuberculosis in Karonga District, covering a population of around 200,000 people. Due to its organisation as a research project, the KPS is able to provide a quality laboratory service with rigorous examination and audit methods, where the prevalence of tuberculosis amongst suspects and the nature of clinical disease are similar to elsewhere in the country. The HIV prevalence amongst patients with laboratory confirmed tuberculosis in Karonga from 1996-1999 was 63%. The HIV prevalence among women seen in the antenatal clinics in 1999 was 10.2% and the prevalence amongst tuberculosis "suspects" is likely to be higher as they are older and tuberculosis-like symptoms are common among those with HIV infection whether or not they have tuberculosis.

## Methods

Sputum is collected from PTB suspects, ie those with a cough of at least 3 weeks duration. In Karonga district an enhanced passive surveillance system for TB is in operation. Individuals may self refer or be found at health centres, among hospital inpatients admitted for other reasons, or in the field in the course of other studies. Three sputum specimens are collected from each suspect, one on-the-spot, a second early morning specimen and a third on-the-spot specimen collected at the time of delivery of the second specimen.

Smears are prepared and first stained with auramine and examined under a fluorescence microscope (at x 40 magnification, with a minimum of 100 fields to be examined before assigning a negative result). All smears are examined independently in randomised order, and the two independent microscopists are not aware which combination of sputa make up a "set". Positives are destained and restained with Ziehl-Neelsen (ZN) stain and examined with a light microscope. Only those confirmed by ZN are recorded as positive. All suspect sputa are set up for culture on Lowenstein-Jensen media and incubated for 10 weeks. Positive and negative controls are used each time cultures are prepared. The data set presented here covers a period from the last week of November 1996 to the end of December 1999. A PTB suspect was included if they had three specimens collected within 7 days and if the three specimens were not all collected on the same day. Each person

appears only once; the first time they became eligible. A true negative case is defined here as someone with only negative cultures for *M tuberculosis* complex from sputa collected within one month of the set. A true positive case is someone with one or more positive cultures for *M tuberculosis* complex. Smears with any degree of smear positivity were counted as positive. Analyses compare sensitivity and specificity of diagnosis based on three smears and two smears against those diagnosed by culture. We have also compared the sensitivity of two smears for detecting those found to be smear positive on the basis of three smears. We have assumed that the order in which sputa were collected was important as early morning specimens have been shown to have a higher yield than on-the-spot specimens.<sup>7,13</sup> The order of specimens is therefore taken into account in the analysis.

## Results

All reported analyses are based on 1,992 sets of suspect sputa that met the criteria. The proportions smear and culture positive are shown in table 1.

No. of positive	Total sets	Percentage	Culture positive
3	289	14.5	273 (94.5)
2	35	1.8	30 (85.7)
1	14	0.7	7 (50.0)
0	1654	83.0	135 (8.2)
Total	992	100.0	445 (22.3)

Of the 445 classified as culture positive, 443 grew *M tuberculosis* and 2 grew *M bovis*. Among those classified as culture negative were 88 patients who had at least one specimen confirmed as growing an environmental mycobacterium. A total of 1524 sets (76.5%) were collected over a two day period as recommended. For 148 sets (7.4%), the first two specimens were collected on the same day, with the third specimen 1-4 days later. For the remainder, the second and third specimens were collected on a different day to the first specimen, for 273 individuals (13.7%) the 3 smears were obtained over a three to four day period, and for 47 individuals (2.4%) over a five to seven day period.

The sensitivity, specificity and positive predictive value of sputum smears compared to culture are shown in table 2. In 8 of the sets it was not possible to determine whether the first two specimens should be included as positive or negative as the first specimen was negative and the latter two specimens had the same date and only one was positive. The results have been calculated both assuming that they would have appeared negative on two smears ("worst case") and that they would have appeared positive ("best case"). Of these 8 sets 5 were culture positive (*M tuberculosis*) and 3 negative (of which one grew an environmental mycobacterium). The sensitivity and specificity of two and three smears are similar. From a public health aspect, there may be more interest in identifying smear positive cases because they constitute a greater transmission hazard than culture positive smear-negative cases. Table 2 also shows the sensitivity of two smears for detecting those who were positive on at least one of 3 smears. (By definition, specificity and positive predictive value in this comparison are 100%.) Of the 9 smear positive cases "missed" on 2 smears (in the worst case scenario), 5 were later confirmed by culture.

HIV results were available on 289 patients with culture positive PTB: 165 (57.1%) were HIV positive. The

Table 2: Comparison of diagnosis of tuberculosis using two or three smears

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Compared to culture positivity <sup>a</sup>				
Smear positive on (1 of:				
Three smears	69.7% (310/445)	98.2% (1519/1547)	91.7% (310/338)	91.9% (1519/1654)
Two smears (worst case)	68.5% (305/445)	98.4% (1523/1547)	92.7% (305/329)	91.6% (1523/1663)
Two smears (best case)	69.7% (310/445)	98.3% (1520/1547)	92.0% (310/337)	91.8% (1520/1655)
Detecting smear positivity <sup>b</sup> (ie at least one of 3 smears positive)				
Smear positive on (1 of:				
Two smears (worst case)	97.3% (329/338)	-	-	99.5% (1654/1663)
Two smears (best case)	99.7% (337/338)	-	-	99.9% (1654/1655)

a) gold standard based on culture status; b) gold standard based upon three smears

sensitivity of three sputum smears for detecting culture positive PTB was lower in HIV positives than HIV negatives (109/165, 66.1% versus 110/124, 88.7%,  $p < 0.001$ ) but among the patients with HIV results the sensitivity of two smears for detecting culture positive tuberculosis was identical to that of three for both HIV positive and HIV negative individuals. Among those who were smear positive on three smears, all of the 114 HIV negative individuals and all but one of 119 HIV positive individuals were detected on the first two smears (worst case scenarios), giving sensitivities of 100% and 99.2% respectively.

## Discussion

In this study, of 338 patients detected as smear positive on the basis of three specimens, at least 329 (97%) would have been detected if only two specimens had been examined. Using two smears rather than three, the sensitivity for detecting culture positive patients was only very marginally reduced, and the specificity was marginally improved. The high yield of two smears is consistent with other studies.<sup>3,5,7,8</sup> Since the slides were read blind it was not artificially high.

The high sensitivity in this study may be partly attributed to the use of fluorescence microscopy, which has been shown to be more sensitive than ZN-microscopy.<sup>9</sup> In addition, smears were read in duplicate by two technicians from 1998 which could improve both the sensitivity and specificity of smear reading. However results from 1997 (when one technician was used) and 1999 (after the change to two technicians) were very similar.

The potential for improving specificity by reducing the number of smears should not be forgotten. The more smears that are examined the higher the chance that one will be positive, but the proportion of false positives also increases.<sup>14,15</sup> In this study the proportion of patients with culture-confirmed tuberculosis was much lower in those with only one positive smear than in those with two or three (table 1). This proportion will be even lower if the single smear has only scanty bacilli.<sup>16</sup> Although it is important to diagnose as many tuberculosis cases as possible, particularly smear positive cases, the costs of misdiagnosis can be high. These include the financial and time costs to the health service and patients, and also the poor outcome in those apparently misdiagnosed as having tuberculosis.

In this population we have shown that mortality was four times as high in smear positive patients diagnosed on the basis of a single scanty smear as in those with culture confirmation.<sup>17</sup>

The potential for improving specificity is increased if the time saved by only examining two rather than three smears is considered, so that further effort can be spent on getting correct results from those smears examined. Our results, and those of others, suggest that this can be achieved with only minor loss of sensitivity. In practice, the time saving might also improve sensitivity in overloaded laboratories. Also, if smear negative, symptomatic tuberculosis suspects are further investigated with X-rays, trials of antibiotic therapy, and further sputum specimens if there is no improvement, then the number of tuberculosis patients missed should be minimised.<sup>3</sup>

There are reports that smears are less sensitive for detecting PTB in HIV positive patients than HIV negative patients,<sup>10-12</sup> but others have found similar proportions of HIV positive and HIV negative PTB patients to be smear positive.<sup>18,19</sup> Cavities are less common in conjunction with HIV, but HIV positive patients with no or minor X-ray changes may be smear positive.<sup>18</sup> In our study, although HIV positive patients with culture-proven PTB were less likely than HIV negative patients to be smear positive, the proportion of smear positive patients detected on the first two specimens was similar in HIV positive and negative patients. This is reassuring, as it is in communities with a high prevalence of HIV that the need for improving the efficiency of TB diagnosis is greatest.

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