

CHAPTER 1

1.0 Background

World Health Organization (WHO) has declared tuberculosis (TB) a disease of public health importance¹. One third of the world population is infected and every year over two million people die of TB worldwide². In Zambia the TB mortality rate is 102 per 100 000 population and case notification for sputum smear positive cases is almost twice that of the world, i.e. 120 per 100 000 population and 62 per 100 000 population respectively³.

Passive case detection of infectious persons and subsequent rendering them non infectious through chemotherapy is the WHO's recommended strategy of curbing the TB scourge¹. The mainstay of diagnosing pulmonary tuberculosis (PTB) in countries with limited resources remains sputum smear microscopy⁴. This method involves detecting acid fast bacilli (AFB) in sputum smears (or other biological specimens) that have been stained with a special dye, using a microscope. It is relatively inexpensive, easy and quick therefore allowing wide coverage with a high positive predictive value (PPV)⁵.

The sensitivity rate for smear microscopy is however low¹. For smear microscopy to be positive, a milliliter of sputum should contain between 5 000 to 10 000 bacilli¹. Additionally the test is inadequate for detecting PTB in patients co-infected with Human Immunodeficiency virus (HIV)⁶. However, studies have shown that the smear positivity yield can be improved by increasing the number of specimens examined per patient suspected of PTB and three sputum specimens have been established as the optimal number of samples required to diagnose PTB in suspected patients⁷. Three smears are helpful for case finding in suspects where the first and second smears are negative⁸. Therefore, the technical guidelines, by WHO and International Union against Tuberculosis and Lung Diseases (IUATLD) recommend that PTB suspected patients should submit three sputum samples as 'spot', 'morning' and another 'spot'⁹.

The guidelines have also recommended that a minimum of 100 high power fields (HPF) of each of the three smears should be meticulously examined by the laboratory technician for a maximum yield to be achieved and every positive slide must be graded as per WHO scale⁹.

A case of PTB is defined as follows; two smears positive for AFB or one smear positive with clinical and radiological findings or one smear positive with a positive culture¹⁰. From each sample collection to microscopic examination the process takes just above an hour¹¹. In recent years some TB experts have criticized the three smears policy for diagnosis of pulmonary tuberculosis. Critics to these guidelines are questioning the value of the third sputum sample in the diagnosis of pulmonary tuberculosis¹². As early as 1998, Nelson et al were able to establish that the overwhelming majority of culture-proven PTB cases are diagnosed from the first or second sputum specimen submitted to the laboratory and that only rarely is a third specimen of diagnostic value¹³.

The argument is that in most poor nations of sub-Saharan Africa, tuberculosis has increased dramatically over the past ten years mainly due to HIV pandemic therefore the number of people examined as tuberculosis suspects has also risen, stretching the capacities of laboratory services¹⁴. The heavy workload is exacerbated by poor staffing in most laboratories and compounded with unsustainable supplies of reagents and consumables. But studies that have weighed the cost of conducting the tests against disease control have cautioned against compromise on early detection of cases as eventual costs could be higher than the perceived costs of examining three samples⁴. Although some are unclear, other arguments advanced by those calling for reduction in the number of sputum samples are that the guidelines to examine three sputum samples for diagnosing PTB are based on old studies and reducing the number of samples would also reduce the repeated visits the patient has to make to the clinic¹⁴. But Van Cleef and Shumaila, have re-evaluated the value for three sputum specimens and reported considerable incremental yields of PTB cases detectable by the third sputum sample^{15,5}. Shumaila (2007) continues to argue that submission of three sputum samples does not increase the number of visits a patient makes to the clinic since under current guidelines all the three samples are collected within 24 hours as 'spot' on the first day and 'morning and another 'spot' on the next day⁵. In the Zambian setting, although examination of three sputum samples per suspected PTB patient equally does not cause greater inconvenience to the patient than two because they are done on two consecutive days (spot, morning, spot), some health facilities do not have secluded areas where patients can cough out sputum samples in privacy.

The International Standards for Tuberculosis Care (ISTC) states that *‘all patients (adults, adolescents, and children who are capable of producing sputum) suspected of having PTB should have at least two and preferably three, sputum specimens obtained for microscopic examination. When possible at least one early morning specimen should be obtained. In some settings because of the practicality and logistics, a third specimen may be helpful’*¹⁶.

This considerable debate has stimulated WHO to encourage extensive studies in various settings to evaluate the proposed examination of two sputum samples for microscopic diagnosis of PTB.

1.1 Statement of the problem

In Zambia, the National Tuberculosis Control Program still recommends the examination of three sputum smears for the diagnosis of PTB in symptomatic patients, collected at various intervals namely; spot, morning and another spot¹⁷. This is in agreement with current internationally recommended guidelines.

It is a well known fact that the smear positivity value increases with the increase in the number of sputum samples examined per pulmonary tuberculosis suspect¹². In the 1980s Wu et al established that examination of three sputum specimens is the optimal number required to diagnose PTB in suspected patients as less than three would arise in loss of cases while more are not rewarding (cost effective)⁷. This premise is being contested by some experts^{8,10,14}. In recent years many studies have shown that the third smear does not add significantly to case detection efficiency^{18,19,22}. A multicenter study by Rieder et al (2005) reported that the incremental yield for TB diagnosis from third smears ranged from 0.7 to 7.2%²³. On the other hand Shumaila recently (2007) illustrated the value of examining the third sputum sample for PTB diagnosis as it contributed a 9.8% yield in his study⁵. The other argument forwarded by those questioning examination of three sputum samples for diagnosis of PTB is that the large number of smears prepared compromises the quality of results because of less time for processing samples and examining the smears¹⁴. Critics of examining three smears for diagnosis of TB have also associated it with increased cost, although Walker et al in 2000 warned that the eventual cost of missing cases would be even higher than the cost associated with examination⁴.

WHO has responded cautiously to the debate of reducing the number of samples required for diagnosing PTB. It has encouraged conducting country specific studies to assess the operational effectiveness of reducing the number of sputum samples to two as suggested by some experts or retain the current three smear policy²⁴.

This study therefore compared the diagnostic performance of two smears (as suggested by some experts) with the conventional three smears for the diagnosis of PTB in urban health center settings of Lusaka District.

1.2. Study justification

Making the process of seeking smear microscopy more convenient and at the same time effective for patients in low income settings is one of the focuses for Foundation for Innovate New Diagnostics (FIND)¹¹. Recently there have been calls to reduce the number of sputum samples required to be examined for diagnosis of pulmonary tuberculosis from three to two, in HIV prevalent settings²³. As a precaution, WHO has recommended evaluation studies on these proposed guidelines to be undertaken by individual countries before adoption. This is to ensure that the proposed guidelines are not adopted at the expense of effective case detection. This study therefore analyzed the comparability of the conventional three smears with the suggested two smears for the purpose of diagnosing PTB in urban setting of Zambia.

1.3 Research question

Does examination of two sputum smears per symptomatic patient yield a lesser diagnosis of PTB cases than three smears?

1.4.0. Study objectives

1.4.1 Main objective.

To compare the diagnostic performance of two versus three smears for the diagnosis of PTB in selected health centers, in Lusaka urban.

1.4.2 Specific objectives.

- To define true tuberculosis cases using Lowenstein Jensen cultures as the gold standard.
- To establish the incremental yield of first, second, and third sputum smears examined for the positive cases.
- To determine and compare the sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), Positive Likelihood Ratio (LR+), Negative Likelihood Ratio (LR-) and test efficiency of ZN smear microscopy at two smears and at three smears.

CHAPTER 2

2.0 Literature review

Sputum is the main specimen for bacteriological diagnosis of PTB. It is a variable material in quantity, quality and bacterial content and these characteristics influence the sensitivity and specificity of techniques used for diagnosing PTB. Therefore the performances of methods used for bacteriological diagnosis of PTB vary under different settings. National and International guidelines of tuberculosis control recommend that microscopy examination of three sputum samples is necessary when examining patients suspected of PTB²⁵. A considerable debate exists on reconsidering the three sputum smears because more than two samples is considered not rewarding¹³. On the other hand, other studies have shown that as much as some settings would require only two sputum samples for diagnosis of PTB, others still require three¹⁶. This can be observed from the contrast in results reported by various studies^{5,7,12,15} which have evaluated the incremental yield of the third sputum sample.

2.1 Global perspective

In the 1980's, the guidelines for performing smear microscopy in the Shandong province of China were that five spontaneous sputum samples should be submitted for examination by patients suspected of PTB. Wu et al (2000) evaluated the diagnostic yield of this practice by reviewing smear results for patients who submitted five sputum samples between 1985 and 1991 in nine smear microscopy laboratories in Shandong province. During this period, 9 302 patients had submitted five sputum samples and out of this number, 6437 (69.2%) were smear positive. Upon establishing an incremental yield of each sample examined for the positives cases, the results were as follows; first smear detected 5439 (84.5%), second smear contributed 785 (12.2%), third smear added on 206 (3.2%), fourth smear 7 (0.1%) and fifth smear 0%. Wu et al concluded that there is no need for a fourth or fifth sputum smear examination and for efficiency, two sputum smears are adequate⁷.

Yilmaz et al¹² conducted a study at a Center for Chest Diseases in Turkey. They retrospectively reviewed records for patients with definite PTB during the year 2002. A total of 1 027 patients with culture proven PTB were identified, out of which 74% patients had at least one smear positive on microscopy while 26% were negative on smear results. Of the 760 smear positive

patients, 82.3% were identified on the first smear and 12.6% were detected on the second smear. Over ninety percent (94%) of the patients were diagnosed with the first two smears. The third smear examination provided 4.2% additional diagnostic yield. They concluded that the majority of the PTB cases can be diagnosed with the first two sputum specimens. Three or more sputum samples submitted to laboratories obtain small additional diagnostic yield. Reducing the number of samples to two would reduce work load of lab staff and costs.

Another study by Ozkutuk et al in Turkey analyzed smear results at a University Hospital for 2002 to 2006. The findings showed that 97% of sputum smear positive (SS+) patients were detected on the first smear. The second smear detected the remaining 3% of the patients while the third sputum did not add any diagnostic yield. This study therefore demonstrated that two sputum samples are sufficient for early detection of TB in our laboratories ¹⁰.

Contrary to the findings reported by the two studies above where the contributions by the third sputum samples were minimal (4.2% and 0% respectively), a similar study in Pakistan reported a much higher value. In 2006 a total of 2,222 TB suspects submitted three sputum samples on two consecutive days (spot, early morning, spot) at King Edward Medical University Hospital in Lahore. A total of 438 (19.7%) suspects were smear positive; of these 290 (66.2%) were positive in the first smear, 105 (24%) were negative in the first smear but positive in the second and 43 (9.8%) were positive in the third smear after two negatives. This study concluded by highlighting the high diagnostic value of 9.8% in third smear as being indicative of the importance of examining three smears for microscopy⁵.

2.2 Regional perspective

The University of Zimbabwe conducted an evaluation study which comprised of sputum smear results of laboratories from four countries (Uganda, Zimbabwe, Mongolia and Moldova). The study objective was to determine the frequency of single positive sputum results and its impact on the surveillance definition of sputum smear positive tuberculosis. The current definition of sputum smear positive pulmonary tuberculosis requires two positive smears or one positive smear plus one complex (e.g. culture) confirmatory evidence. The results showed that a quarter of the laboratory cases had no confirmatory results, almost entirely attributable to not examining

another sample. The study concluded that the empirical evidence challenges the need for confirmatory smears and recommended that accepting a single positive smear as sufficient for case definition would greatly increase the sensitivity of the surveillance definition without sacrificing its specificity⁸.

In Malawi, Crampin and others (2001) analyzed the results of two smears versus three smears examined using microscopy. They observed that the first two smears were able to detect 97% of smear positive cases while the third sputum added the remaining 3%. Using culture as gold standard, the sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) of the three smears were 69.7%, 98.2%, 91.7% and 91.9% respectively. Restriction to the first two samples gave similar results (68.5% for sensitivity, 98.4% specificity, 92.7% PPV and 91.6% NPV). Of those detected as smear positive using three smears; at least 97% would have been detected by two. This study concluded that using florescent microscopy, collecting two sputa rather than three would only marginally reduce sensitivity and would slightly improve specificity of diagnosing pulmonary tuberculosis. They postulated that in practice both sensitivity and specificity may be increased due to the time saved by examining two rather than three smears. They also highlighted the importance of improving specificity considering the cost of misdiagnosis¹⁴.

While an incremental yield of the third sputum of 3% was established by Crampin and others in Malawi, a similar study in Kenya by Van Cleef et al (2003) observed a much higher incremental yield from the third smear (8%). Tuberculosis (TB) suspects from Rhodes Chest Clinic, Nairobi, Kenya, were subjected to three sputum smear microscopy (ZN) examinations and a chest X-ray (CXR). Results were compared with Lowenstein-Jensen culture as the gold standard. The study objective was to establish the efficiency of the routine diagnostic processes. All laboratory tests and the CXR were available for 993 (71%) of the 1398 enrolled suspects. Of these, 554 (56%) were culture-positive. The sensitivity of ZN microscopy based on three sputum smears was 60% (95% CI: 57%–63%). This proportion was considered as optimal under routine circumstances, and is comparable with other reports (e.g. 69.7% sensitivity was reported by the Malawi study). The majority of smear positive patients (53%) were detected by the first spot smear examination, while the second smear yielded 40% of patients and the third spot smear contributed the

incremental yield of the remaining 8%. The fact that the screening processes miss out culture-positive cases is a concern but inherent. Unfortunately subjecting all TB suspects to culture which is the gold standard would not be feasible¹⁵.

2.3 National perspective

A similar incremental yield of 8% contributed by the third sputum observed in Kenya was also reported in an article in Zambia by Walker et al (2000). Researchers from London School of Hygiene and Tropical Medicine studied the cost effectiveness of diagnosing PTB with three smears in Katete District of Zambia. About 3 in 4 (77.1%) of PTB patients were diagnosed on the first smear while 15% and 7.9% were diagnosed on the second and third smears respectively. Laboratory supplies represented 61% of the average cost of performing a smear microscopy test and laboratory staff time represented 27%. The incremental cost per each case diagnosed rose sharply with each subsequent smear. The conclusion from this study was that a change in policy, from three smears to two, would quite alright detect over 90% of cases and would also release funds for use in other areas of TB control. However the researchers cautioned that if the change in policy means that some infectious patients will not be identified at an early stage, then eventual costs for this change could be quite high⁴.

CHAPTER 3

3.0 Methodologies

3.1 Study design

This was a prospective cross sectional study that took place from January 2011 to May 2012. The study reviewed routine ZN smear results for patients suspected of PTB and submitted three sputum samples (as per current policy) in four urban health center laboratories in Lusaka district. At the same time laboratory staff working in these laboratories stored the sputum samples for these patients following the routine use. These samples were then transported to the University Teaching Hospital (UTH) TB laboratory for culture on Lowenstein Jensen slopes which served as gold standard for this study. We used the culture proven routine ZN smear positive cases from health center laboratories to establish incremental yield for first, second and third smears. Relative to LJ culture results we also used the routine smear results to compute the sensitivity, specificity, PPV, NPV, test efficiency, and likelihood ratios for ZN smear microscopy at three smears (three smears strategy). By restricting our analysis to only the first two smears patients submitted, we again determined the sensitivity, specificity, PPV, NPV, test efficiency and likelihood ratios for two smears (two smears strategy). We then compared the performances of the two strategies based on the above variables. This study analyzed ZN and culture results for 1030 tuberculosis suspected patients.

3.2 Study settings

This study was conducted at the University Teaching Hospital (UTH) TB laboratory. UTH is a tertiary care teaching hospital located in the Zambian capital city of Lusaka. It consists of specialized laboratories (including a TB laboratory). TB laboratory offers a tertiary level test profile which ranges from microscopy, culture to molecular techniques. For External Quality Assessment, TB laboratory is enrolled with Chest Diseases laboratory (CDL), a national reference laboratory and Medical Research Council (MRC) of South Africa. It is mandated to supervise the quality of smear microscopy in all the diagnostic centers located in Lusaka, Western and Eastern Provinces of Zambia and serves as a reference laboratory for culture and drug susceptibility testing.

3.3 Study population

Lusaka district has twelve government health centers with laboratories performing smear microscopy. This study analyzed routine ZN smear results and sputum samples collected from four health center laboratories. These laboratories were chosen on the basis of their proximity to UTH namely; Kamwala, Kabwata, Kanyama and Chilenje health center laboratories. These laboratories participate in an ongoing TB microscopy External Quality Assessment (EQA) program conducted by UTH. The average smear positivity rate among these laboratories is 14% according to TB laboratory registers of 2009. Participants for this study were patients suspected of pulmonary tuberculosis and sent to the four study laboratories for clinical sputum examination.

3.4 Eligibility criteria:

Health center laboratories in Lusaka district performing smear microscopy using a standard TB laboratory register and participating in an external quality assessment program were eligible. Only patients referred to the study laboratories by clinicians for diagnosis of PTB and managed to submit three sputum specimens as per current NTP guidelines on sputum examination were included in the analysis for this study.

Exclusion criteria:

Sputum samples and records for patients already on anti-tuberculosis treatment were not reviewed for the purpose of this study. Sputum specimens and records for in-patients were also excluded as laboratory staff could not follow patients in the wards for consent.

3.5. Sample size

We employed the sample size formula for infinite population, Bill Golden, January 2004.

$$SS = \frac{Z^2 \times (p) \times (1 - p)}{C^2}$$

Basing on test Sensitivity.

SS= Sample size

Z = Z value (1.96 for 95% confidence level)

P = Estimated test sensitivity (70%)

C = Confidence interval (0.05)

$$SS = \frac{1.96^2 \times 0.7 \times 0.3}{0.05^2}$$

$$SS = \frac{3.84 \times 0.7 \times 0.3}{0.0025}$$

$$SS = \frac{0.8064}{0.0025}$$

$$SS = 322.56$$

Basing on specificity.

SS= Sample size

Z = Z value (1.96 for 95% confidence level)

P = Estimated test Specificity (98%)

C = Confidence interval (0.05)

$$SS = \frac{1.96^2 \times 0.98 \times 0.02}{0.05^2}$$

$$SS = \frac{3.84 \times 0.98 \times 0.02}{0.0025}$$

$$SS = \frac{0.075264}{0.0025}$$

$$SS = 30.1056$$

This study adopted a sample size of 350 participants culture positive cases (based on sensitivity calculation).

3.6. Sampling

We enrolled participants among patients suspected of PTB by clinicians and sent to submit sputum to the four study laboratories for routine ZN smear examination. We reviewed routine smear results and stored sputum samples for all patients who met our study criteria and consented participation in the study as they became available without randomization.

3.7. Specimen collection, storage and transportation

Patients were requested to submit three sputum samples as ‘spot’ ‘morning’ ‘spot’, over a period of twenty four hours (as per current NTP guidelines) at their local clinic for routine clinical diagnosis. The first ‘spot’ and the third ‘spot’ were collected at the health centers while the second specimen was an early morning sample coughed at home¹⁷. After performing routine AFB microscopy, health center laboratories stored sputum samples in the refrigerators (2.0°C to 8.0°C) for two days awaiting transportation to UTH Tuberculosis laboratory. Samples were collected from health centers three times a week using a transportation cooler box.

3.8.0. Data collection techniques

We reviewed routine ZN smear microscopy records for suspected PTB patients in the study health center laboratories and cultured their sputum using Lowenstein Jensen slopes at the University Teaching Hospital TB laboratory.

3.8.1. Review of records

This study collected routine ZN smear microscopy results recorded in TB laboratory registers in the health center laboratories under study. These results were used to establish the incremental yield for positive cases. We also used these routine microscopy results to determine the diagnostic performance of ZN microscopy at three smears and at two smears relative to culture, prior to comparisons.

3.8.2. Smear microscopy

Three smears (spot, morning, spot) were prepared and tested sequentially per each patient.

Smears were routinely prepared, stained and examined using Zielh Neelsen (ZN) Technique at local health center laboratories. National Tuberculosis control guidelines on ZN method were followed, i.e. 0.3% carbol fuchsin, 0.3% methylene blue and 3% hydrochloric acid, a decolorizer²⁶ (M.O.H Standard Operating Procedure, 2010). A positive and negative control slides were included with each run of staining batch to verify the correct performance of the procedures as well as the staining intensity of the acid fast bacilli (AFB). At least 100 fields were examined before a smear was declared negative. In the case of a positive smear, the number of tubercle bacilli was graded using WHO/IUATLD guidelines²⁷ as illustrated below.

Table.1. Grading of Zielh Neelsen microscopy smears.

Examinations	Results	Grading	No. of fields
No AFB seen	negative		100
1 – 9 AFB seen	Positive	Record actual figure.	100
10 – 99 AFB seen	Positive	1+	100
1 – 10 AFB seen	Positive	2+	50
>10 AFB seen	Positive	3+	20

3.8.3. Culture

Sputum samples were digested and homogenized at the University Teaching Hospital TB laboratory using a decontaminating solution composed of 4% sodium hydroxide solution, 2.0% sodium citrate and 2.9% N-acetyl L-cystein (a mucolytic agent). Two (2) to three (3) drops of sediments were then inoculated in two separate Lowenstein Jensen (LJ) slopes containing glycerol and pyruvate. Inoculated slopes were incubated at 37^oC in an incubator. Slopes were examined weekly up to 8 weeks in accordance with the Standard Operating procedure²⁸ (MoH, 2010). Colonies isolated from the cultures were examined microscopically (using ZN method) for confirmation of AFB. A control strain of *Mycobacterium tuberculosis* H37Rv was employed for quality control.

3.9. Data analysis

Using an online statistical tool ‘GraphPad’ available at; <http://graghpad.com/quickcalcs/contingency2.cfm>, this study employed Yates’ corrected Chi-square to determine the significance in the differences among the variables we used to compare the two strategies. Yates corrected Chi-square provides a stronger evidence for a significant result as it adjusts for continuity correction. A result yielding a p value of $P < 0.05$ was statistically significant. To compute confidence intervals, this study used formula 1 below and counter checked with an online probability and statistics tool available at; <http://ncalculators.com/statistics/confidence-interval-calculator.htm>.

Formula 1: Confidence intervals (CI)

$$p \pm 1.96 \times \sqrt{\frac{p(1-p)}{n}}$$

For computation of test sensitivity, specificity, PPV, NPV, test efficiency, LR+ and LR-, this study used formulae 2, 3, 4, 5, 6, 7 and 8 below (available online http://en.wikipedia.org/wiki/systematic_review) and complemented with a clinical calculator available online at; <http://vassarstats.net/clin1.html>.

Formula.2:

$$\text{Sensitivity} = \frac{\text{True positives by screening test}}{\text{Total positives by confirmatory test}} \times 100$$

Formula 3:

$$\text{Specificity} = \frac{\text{True negative by screening test}}{\text{Total negatives by confirmatory test}} \times 100$$

Formula 4:

$$\text{PPV} = \frac{\text{number of True Positives}}{\text{number of True Positives} + \text{number of False Positives}} \times 100$$

Formula 5:

$$\text{NPV} = \frac{\text{number of True Negatives}}{\text{number of True Negatives} + \text{number of False negatives}} \times 100$$

Formula 6:

$$\text{Test efficiency} = \frac{\text{number of True Positives} + \text{number of True Negatives}}{\text{Total Tests}} \times 100$$

Formula 7

$$\text{LR+} = \frac{\text{probability of an individual **with** the condition having a positive test}}{\text{probability of an individual **without** the condition having a positive test}}$$

Formula 8

$$\text{LR-} = \frac{\text{probability of an individual **with** the condition having a negative test}}{\text{probability of an individual **without** the condition having a negative test}}$$

3.10. Quality assurance

Normally all the microscopically examined slides in health center laboratories are stored and subjected to blinded re-checking by the University Teaching Hospital (TB laboratory) for External Quality Assessment (EQA). Quality control measures for culture included monitoring of equipment, reagents and performance indicators such as contamination rates.

3.11. Ethical considerations

This study obtained ethical approval from the University of Zambia Biomedical Research Ethics Committee (Assurance No.FWA00000338 IRB00001131 of IORG0000774). Permission was also obtained from Lusaka District Health Office to allow the study to be carried out in the selected health centers and the University Teaching Hospital Department of Pathology and Microbiology. Despite the fact that all patients received standard care, this study requested consent from patients for use of their samples beyond clinical purpose. The general benefit from this study is that it has contributed necessary knowledge required to review the optimal number of sputum samples sufficient for diagnosing pulmonary tuberculosis in Lusaka urban health center laboratories.

CHAPTER 4

4.0 RESULTS

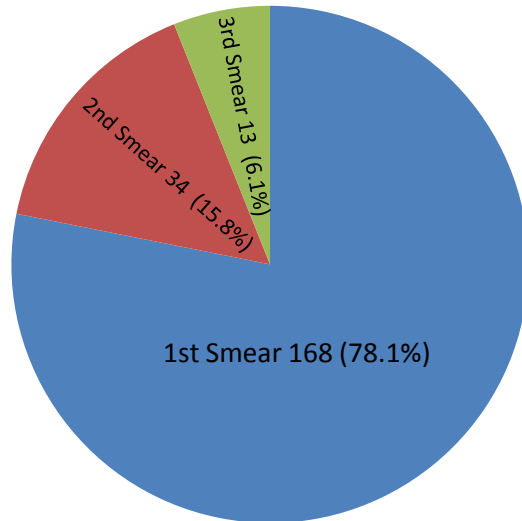
This study analyzed sputum samples from 1030 patients suspected of PTB. Out of this number, LJ sputum cultures were positive in 350 suspects, providing 34.0% (95% CI: 31.1%, 36.9%) positivity rate. ZN microscopy using conventional three smears detected 215 cases, providing a positivity rate of 20.9% (95% CI: 18.4%, 23.4%) but could not detect 135 cases that were positive by culture. These results are shown in table 2.

Table 2: Illustrating results for LJ culture and smear microscopy

Total participants	Culture positives	Culture negative	Total smear positives	True smear positives	False Smear positives	Total Smear negatives	True smear negatives	False smear negatives
1030	350	680	228	215	13	802	667	135

For the 215 cases that were ZN smear positive, this study established an incremental yield by proportioning them into cases identified on the first smear, second and third smears respectively. A total of 13 (6.1%) cases were identified on the third smear after being missed by the first and second smears (Figure 1).

Figure 1: Showing proportions of smear positive cases detected by 1st, 2nd, and 3rd, smears.



Next, this study stratified samples to form two strategies, namely; ‘three smear strategy’ and ‘two smear strategy’. For ‘three smear strategy’, we considered all the three samples submitted by the patient in the analysis (Table 3). Whereas for ‘two smear strategy’, we restricted the analysis to the first two samples the patient submitted (Table 4). Relative to culture we analyzed the diagnostic performance of each strategy basing on the following variables; sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), test efficiency, positive likelihood ratio (LR+) and negative likelihood ratio (LR-). Using values obtained for each strategy, we then made comparisons (Table 5).

Table 3: Standard 2 x 2 Table analyzing diagnostic performance of three smear strategy against LJ culture.

Definitive test

Screening test		Positives	Negatives	Totals
	Positives	True positives	False positives	
	Negative	False negatives	True negatives	
	Totals			

LJ culture

ZN three smears Strategy		Positive	Negative	Total
	Positive	215	13	228
	Negative	135	667	802
	Total	350	680	1030

$$\text{Sensitivity} = \frac{\text{True positives by screening test}}{\text{Total positives by definitive test}} \times 100$$

$$\text{Sensitivity} = \frac{215}{350} \times 100 = \mathbf{61.4\%} \text{ (95\% CI: 56.1\%, 66.5\%).}$$

$$\text{Specificity} = \frac{\text{True negatives by screening test}}{\text{Total negatives by definitive test}} \times 100$$

$$\text{Specificity} = \frac{667}{680} \times 100 = \mathbf{98.1\%} \text{ (95\% CI: 97.1\%, 98.9\%).}$$

$$\text{Positive predictive value} = \frac{\text{True positives of the screening test}}{\text{Total positives of the screening test}} \times 100$$

$$\text{Positive predictive value} = \frac{215}{228} \times 100 = \mathbf{94.3\%} \text{ (95\% CI: 90.2\%, 96.8\%).}$$

$$\text{Negative predictive value} = \frac{\text{True negatives of the screening test}}{\text{Total negatives of the screening test}} \times 100$$

$$\text{Negative predictive value} = \frac{667}{802} \times 100 = \mathbf{83.2\%} \text{ (95\% CI: 80.3\%, 85.7\%).}$$

$$\text{Test efficiency} = \frac{\text{True positives of the screening test} + \text{True negatives of the screening test}}{\text{Total number of participants}} \times 100$$

$$\text{Test efficiency} = \frac{215 + 667}{1030} \times 100 = \frac{882}{1030} \times 100 = \mathbf{85.6\%} \text{ (95\% CI: 83.4\%, 87.7\%).}$$

LR+ = Probability of an individual **with** the condition having a positive test
 Probability of an individual **without** the condition having a positive test

$$\text{LR+} = \frac{(215/350) \times 100}{(13/680) \times 100} = \frac{61.4\%}{1.9\%} = \mathbf{32.3} \text{ (95\% CI: 18.6, 55.4)}$$

probability of an individual **with** the condition having a negative test
 LR- = probability of an individual **without** the condition having a negative test

$$LR- = \frac{(135/350) \times 100}{(667/680) \times 100} = \frac{38.6\%}{98.1\%} = \mathbf{0.39} \text{ (95\%CI: 0.34, 0.44)}$$

(‘VassarStats’ an online statistical tool print out report is included in the appendices).

We constructed another 2 x 2 table to gauge the test performance of ‘two smear strategy’ in relation to culture.

Table 4: Standard 2 x 2 Table analyzing diagnostic performance of two smear strategy relative to LJ culture

Definitive test

Screening test		Positives	Negatives	Totals
	Positives	True positives	False positives	
	Negative	False negatives	True negatives	
	Totals			

LJ culture

ZN (two smear) Strategy		Positive	Negative	Total
	Positive	202	13	215
	Negative	148	667	815
	Total	350	680	1030

Basing on this table (two smear strategy), again we computed the sensitivity, specificity, PPV, NPV, test efficiency, LR+ and LR-.

$$\text{Sensitivity} = \frac{\text{True positives by screening test} \times 100}{\text{Total positives by definitive test}}$$

$$\text{Sensitivity} = \frac{202}{350} \times 100 = \mathbf{57.7\%} \text{ (95\% CI: 52.3\%, 62.9\%).}$$

$$\text{Specificity} = \frac{\text{True negatives by screening test}}{\text{Total negatives by definitive test}} \times 100$$

$$\text{Specificity} = \frac{667}{680} \times 100 = \mathbf{98.1\%} \text{ (95\% CI: 96.7\%, 98.9\%).}$$

$$\text{Positive predictive value} = \frac{\text{True positives of the screening test}}{\text{Total positives of the screening test}} \times 100$$

$$\text{Positive Predictive Value} = \frac{202}{215} \times 100 = \mathbf{93.9\%} \text{ (95\% CI: 89.6\%, 99.6\%).}$$

$$\text{Negative predictive value} = \frac{\text{True negatives of the screening test}}{\text{Total negatives of the screening test}} \times 100$$

$$\text{Negative Predictive Value} = \frac{667}{815} \times 100 = \mathbf{81.8\%} \text{ (95\% CI: 79.1\%, 84.4\%).}$$

$$\text{Test Efficiency} = \frac{\text{True positives of the screening test} + \text{True negatives of the screening test}}{\text{Total number of participants}} \times 100$$

$$\text{Test Efficiency} = \frac{202 + 667}{1030} \times 100 = \frac{869}{1030} \times 100 = \mathbf{84.4\%} \text{ (95\% CI: 82.2\%, 86.6\%).}$$

$$\text{LR+} = \frac{\text{Probability of an individual **with** the condition having a positive test}}{\text{Probability of an individual **without** the condition having a positive test}}$$

$$LR+ = \frac{(202/350) \times 100}{(13/680) \times 100} = \frac{57.7\%}{1.9\%} = \mathbf{30.3} \text{ (95\% CI: 17.5, 52.1)}$$

LR- = probability of an individual **with** the condition having a negative test
probability of an individual **without** the condition having a negative test

$$LR- = \frac{(148/350) \times 100}{(667/680) \times 100} = \frac{42.3\%}{98.1\%} = \mathbf{0.43} \text{ (95\% CI: 0.38, 0.48)}$$

(‘VassarStats’ an online statistical tool print out report is included in the appendices).

This study then compared the values for sensitivity, specificity, PPV, NPV, test efficiency, LR+ and LR- for the two strategies from the above computations.

Table 5: Comparing the sensitivity, specificity, PPV, NPV, test efficiency, LR+ and LR- for ‘three smear and ‘two smear strategies’

Variable	Three smear strategy	Two smear strategy	P-value
sensitivity	61.4%	57.7%	0.355
specificity	98.1%	98.1%	1
PPV	94.3%	93.9%	0.877
NPV	83.2%	81.8%	0.525
Test Efficiency	85.6%	84.4%	0.459
LR+	32.3	30.3	0.96
LR-	0.39	0.43	0.882

(Statistical print out report for each variable is included in the appendices). The p-values for all the variables tested showed no significant differences between the two strategies.

Chapter 5

Discussion

This study demonstrates that it is not valuable to examine more than two sputum smears per patient for diagnosis of pulmonary tuberculosis in urban health centers in Lusaka. We observed that from the 215 patients detected as sputum smear positive cases using three smears, 202 (94%) would still have been identified if only two sputum samples were examined as the third smear only contributed 13 (6.1%). Furthermore, this study compared the diagnostic performance of two smears against three smears on the basis of sensitivity, specificity, positive predictive value, negative predictive value, test efficiency, positive likelihood ratio and negative likelihood ratio which were more or less similar. The results of this study show that there were no differences between the two strategies.

Our results are in agreement with a study conducted at St Francis Hospital in Katete, a rural district in Zambia by Walker et al in 2000, who found that 77% of patients were detected on the first smear; a further 15% were diagnosed on the second smear and 7.9% on the third. Walker et al also established that the incremental cost per tuberculosis case diagnosed rose steeply with each subsequent smear. Their economic analysis showed that the cost of performing a third test, having already done two, increases rapidly with only a small gain in terms of additional cases of tuberculosis detected. They concluded that examining three samples for diagnosis of pulmonary tuberculosis was not cost effective as two would identify over 90% of cases⁴. In Tanzania, Ipuge et al (1996) analyzed routine results of direct sputum smear microscopy for acid-fast bacilli from 34 rural laboratories. They evaluated 61,580 tuberculosis suspects with the aid of 141,371 smears. The average positivity rate of cases found among suspects was 18.9% and an incremental yield of 83.4% with the first, 12.2% with the second, and 4.4% with the third smear was estimated. They concluded that under routine conditions the incremental yield from a third smear examination after two negative examinations is relatively small²⁹.

In neighboring Malawi, a similar study¹⁴ to ours compared the sensitivity of two versus three smears in identifying culture positive PTB patients and reported a sensitivity of 70%, specificity of 98%, PPV of 92% and NPV of 92% for three smears and found a slight reduction in sensitivity, when they considered two smears while the rest of the variables were unaffected.

In their conclusion they advocated for change in policy from examining three to two sputum samples when diagnosing pulmonary tuberculosis as this would save on time and ultimately improve the quality of smear microscopy. Similarly, we applied the variables used in the Malawian study to compare the diagnostic performance of two smears to three smears and our study equally could not establish a significant difference between the two strategies. Although the sensitivity rate for three smears (61.4%) in our study was lower than that obtained in the Malawian study (70%), it is worth noting that generally, the sensitivity for AFB microscopy is relatively low and variable, ranging between 20% – 60%^{30, 31}. Mfinanga et al (2007) in their study conducted in Tanzania reported a sensitivity value of smear microscopy as low as 36.9%³². Most recently in Nigeria, Onubogu et al (2012) reported even much lower sensitivity rate of 21.5% in HIV positive population⁶. Therefore increasing the numbers of smears beyond two does still not add much value in terms of gain in sensitivity.

Other studies in the region which have compared the optimal number of sputum samples for diagnosing pulmonary tuberculosis using different methodologies have reported similar findings. A study by Mabaera et al (2006) at the University of Zimbabwe determined the number of slides required to identify one additional case of sputum smear positive from the third smear. The study hypothesis was that not more than 100 and 75 slides, in Mongolia and Zimbabwe respectively, need to be examined to find one additional case of tuberculosis with a third serial diagnostic sputum smear examination. Their results showed that in Mongolia they were expected to examine 1153 and in Zimbabwe 132 slides in order to detect one additional case using the third smear. These figures were higher than what they had hypothesized. The researchers concluded that the current requirement of routine examination of three smears per patient suspected of pulmonary tuberculosis need to be reviewed in both Mongolia and Zimbabwe³³.

On the other hand, high incremental rates of the third smear have been reported in Pakistan. Shumaila et al (2007) conducted a descriptive study at King Edward Medical University/Mayo Hospital, Lahore Pakistan. Patients with respiratory symptoms and or abnormal chest X-rays provided three sputum samples each for acid-fast bacilli smear microscopy. Smears were prepared and stained by Ziehl-Neelsen staining method. Out of 2,222 tuberculosis suspects who submitted three sputum samples a total number of 438 (19.7%) suspects had at least one positive

smear; of these 290 (66.2%) were positive in first smear, 105 (24%) were negative in first smear but positive in second and 43 (9.8%) were positive in third smear after two negative smears⁴. Since Shumaila did not employ a comparative (definitive) method in his study, it is possible that some of the cases comprised in the 9.8% incremental yield of the third smear he observed were false positives.

A multi center study by Rieder et al (2005) analyzed data from 42 laboratories in four high TB burden countries and the results showed that incremental yield from the third smear ranged from 0.7 to 7.2%²³. This is one of the evidences which WHO cited in its rationale when it proposed revision of three smears policy in 2006. The diagnostic incremental yield of 6.1% for the third smear which our study has established in the Lusaka setting lies within this range.

Most of our laboratories are under staffed and challenged with high workloads. With the advent of HIV/AIDS, tuberculosis cases have risen in most Sub-Saharan nations in the last two decades and the number of people requiring smear microscopy has also increased¹⁴. Besides performing AFB smears, our technicians are also engaged in other laboratory works like reading malaria slides and conducting tests that support management of patients on anti retroviral therapy. Under WHO's proposed policy of 2006 based on two smears, countries with human resource crisis have been advice to reduce the number of specimens examined for screening of tuberculosis patients from three to two. If two smears are negative, then the suspect should follow the algorithm for sputum negative cases.

Therefore reducing the number of smears to be examined per tuberculosis suspect from three to two as proposed by WHO will reduce the strain on laboratory staff and consequently lead to improved quality of smear microscopy as staff will have ample time to read smears. In addition examining more than two smears per patient suspected of tuberculosis demands for more resources to allow procurement of enough sputum containers, microscope slides and other laboratory consumables. If the number of smears were reduced, the 'surplus' resources being used to accommodate the third smear would be utilized in a more cost effective manner. The stock outs of laboratory reagents and sputum containers would minimize since requirements per patient will reduce. Some of the saved resources can be redirected towards sustaining quality assurance programs. In Lusaka province EQA program for AFB smear microscopy was initiated

in 2003 by JICA-HIV/TB project. Since then the program has been strengthened by various cooperating partners who include CDC, ZAMBART and CIDRZ. A functional EQA system is one of the requirements demanded by the new WHO proposed policy which states that; *‘reduction of the number of specimens examined should only be considered in countries and settings with a well established laboratory network and a fully functional EQA programme for smear microscopy including Onsite evaluation with a feedback mechanism. Countries who do not want to introduce the two smears policy or with no functional EQA system can use the actual three smear policy²⁴*. Reducing the number of sputum samples to two would also be convenient to patients seeking sputum examination as they will be expected to submit fewer specimens than current. In a resource limited setting like ours, retaining the current policy which requires examination of three specimens per patient suspected of pulmonary tuberculosis is detrimental in the sense that some cases will continue to go undetected either due to stock outs which usually lead to interrupted services, or missed diagnosis arising from overwhelmed technicians who hurriedly read smears.

Study limitations

- This study was unable to ascertain the HIV status for the study participants.
- Our study was also unable to collect demographic data because it was incomplete in the laboratory registers we reviewed.

Future directions

Sensitivity for smear microscopy is generally low as observed in this study and indeed others, therefore there is need to invest in new diagnostic technologies like Gene Xpert and LAMP (Loop Mediated Amplification Method) which have higher sensitivity than smear microscopy.

Conclusion

This study has demonstrated that there is no significant difference in terms of diagnostic capacity between examining two smears and three smears for the purpose of diagnosing pulmonary tuberculosis in the health center laboratories in Lusaka. The results have shown that examining two smears per patient suspected of pulmonary tuberculosis would detect 94% of culture proven

cases therefore making the third smear less valuable. This study therefore concludes that the two smear strategy for diagnosis of pulmonary tuberculosis is adopted for Zambia.

6.0 References

1. WHO., (2008): **Global tuberculosis control, planning and financing**. Geneva, WHO.
2. Raviglione, M.C., Snider, D.E., Kochic, A., (1995): **Global epidemic of tuberculosis: Morbidity and mortality of a worldwide epidemic**. JAMA. 273: 220 - 226.
3. USAID ZAMBIA., (2009): **Tuberculosis profile**. (<http://www.usaid.gov> date accessed 10th March, 2010)
4. Walker, D., McNerney, R., Mwembo, M.K., (2000): **An incremental cost effectiveness analysis of the first, second and third sputum examination in the diagnosis of pulmonary tuberculosis**. Int J Tuberc Lung Dis. 4: 246 - 251.
5. Shumaila, S., Iffat, S., Rizwan, I., Saulat, V., (2007): **Value of three sputum smear microscopy in diagnosis of pulmonary tuberculosis**. Park J Med Res. 46: (4).
6. Onubogu, C. C., Nwokoye, N.N., Kunle-Ope, C.N., Raheem, T.Y., Igbasi, U.T., Tochukwu, N., Ejezie, C.O., Onyejebu, N., Omoloye, R., Onwujekwe, D., Idigbe, E.O., (2012): **Sensitivity of direct smear microscopy for the diagnosis of TB in high HIV prevalent population**. SRE. 7(5): 593 – 597.
7. Wu, Z.L., Wang, A.Q., (2000): **Diagnostic yield of repeated smear microscopy examinations among patients suspected of pulmonary tuberculosis in Shandong province of Chiana**, Int J Tuberc Lung Dis. 4: 1086 – 1087.
8. Mahebaera, B., Lauritsen, J.M., Katamba, A., Laticevschi, D., Naranbat, N., Rieder, H.L., (2007): **Sputum smear positive tuberculosis: empirical evidence challenges the need for confirmatory smears**. Int J Tuberc Lung Dis. 11 (9): 959 - 964.
9. Enarson, D.A., Reider, H.L., Anadottir, T., Trebuig, A., (2000): **Managemnt of tuberculosis: A guide for low income countries**, IUATLD, Paris.

10. Ozkutuk, A., Terek, G., Coban, H., Esen N., (2007): **Is it valuable to examine more than one sputum smear per patient for the diagnosis of pulmonary tuberculosis?** Jpn. J. Infect. Dis. 60: 73 - 75.
11. Smart, T., (2007): **TB diagnosis: Reducing the number of smears and clinic visits needed.** (Accessed online, <http://www.aidsmap.com/TB-diagnosis-Reducing-the-number-of-smears-and-clinic-visits-needed/page/1426641/>, 13th February, 2011).
12. Yilmaz, A., Bayram, Selvi U., Damadoglu, E., Gungor, S., (2008): **Diagnostic value of repeated sputum examination in pulmonary tuberculosis: How many sputum samples are adequate?** Tuberkuloz ve Toroks Dergis. 56 (2): 158 - 162.
13. Nelson, S.M., Deike, M.A., Cartwright, C.P., (1998): **Value of examining multiple sputum specimens in the diagnosis of pulmonary tuberculosis.** J Clin Microbiol. 6: 467 – 469.
14. Crampin, A.C., Floyd, S., Mwaungulu, F., Ndhlovu, R., Mwaiyeghele, E., Glynn, J., Wardorff, D.K., Fine, M., (2001): **Comparison of two versus three smears in identifying culture positive tuberculosis patients in a rural African setting with a high HIV prevalence,** Int J Tuberc Lung Dis. 5: 994-999.
15. Van Cleeff, M.R.A., Kivihya, N.L., Githui, W., (2003): **A comprehensive study of the efficiency of the routine pulmonary tuberculosis diagnostic process in Nairobi.** Int J Tuberc Lung Dis. 7: 186 – 189.
16. TCTA.,(2006): **International Standards for Tuberculosis Control (ISTC).** The Hague: **Tuberculosis Coalition for Technical Assistance,** (Accessed online, <http://www.who.int>, 20th June, 2012).
17. MoH., (2005): **National Guidelines for Quality Assurance of sputum Smear Microscopy in Tuberculosis Control.** Lusaka, MoH.

18. Yassin, M.A., Cuevas, LE., (2003): **How many sputum smears are necessary for case finding in pulmonary tuberculosis?** Trop Med Intern Health. 8: 927- 932.
19. Gopi, P.G., Subramani R., Selvakumar, N., (2004): **Smear examination of two specimens for diagnosis of Pulmonary Tuberculosis in Tiruvallur District, South India.** Int J Tuberc Dis. 8: 824 – 828.
20. Stone, B, L., Burman, W., Hildred, M.V., (1997): **The diagnostic yield of acid fast bacillus smear positive sputum specimens.** J Clin Microbiol. 35: 1030 - 101.
21. Finch, D., Beat, C.D., (1997): **The utility of single sputum specimen in the diagnosis of tuberculosis. Comparison between HIV infected and non infected patients,** Chest. 111: 1174 - 1179.
22. Leonard, M.K., Osterholt, D., Kourbatova, E.V., (2005): **How many sputum specimens are necessary to diagnose pulmonary tuberculosis?** Am J infect Control. 33: 994 - 999.
23. Rieder, H.L., Chiang, C.Y., Rusan, I.D., (2005): **A method to determine the utility of the third diagnostic and the second follow up sputum smear examination to diagnose tuberculosis cases and failures.** Int J Tuberc Lung Dis. 9: 384 – 391.
24. WHO., (2006): **Improving the diagnosis and treatment of smear negative pulmonary and extra pulmonary tuberculosis among adults and adolescents. Recommendations for HIV-prevalent and resource constrained settings.** (Accessed online www.who.int/entity/tb/publications/2006/tbhiv_recommendations, 2nd January, 2010).
25. WHO., (2003): **Treatment of tuberculosis, guidelines for national programmes.** Geneva, WHO.

26. MoH., (2010): **Standard Operating Procedures for TB Microscopy Centers.** Lusaka, MoH.
27. WHO., (1998): **Services in tuberculosis control.** Geneva, WHO.
28. MoH., (2010): **Standard Operating Procedures for TB culture facilities.** Lusaka, MOH
29. Ipunge, A.I., Rieder, H.L., Enarson, D.A., (1996): **The yield of acid fast bacilli from serial smears in routine laboratories in rural Tanzania.** Transaction of the Royal Society of Tropical Medicine and Hygiene, 90: 238 – 261
30. Urbanczik, R., (1985): **Present position of microscopy of culture in diagnostic mycobacteriology.** Zentralbl. Bakteriologie Mikrobiologie Hygiene A. 260 (1): 81- 87.
31. Aber, V.R., Allen, B.W., Mitchison, D.A., Ayuma, P., Edwards, E.A., Keyes A.B., (1980): **Quality control in tuberculosis bacteriology. 1. Laboratory studies on isolated positive cultures and the efficiency of direct smear examination.** Tubercle. 6; 1(3): 123-133.
32. Mfinanga, G.S., Ngadaya, E., Mtandu, R., Mutayoba, B., Basra, D., Kimaro, G., (2007): **The quality of sputum smears microscopy diagnosis of pulmonary tuberculosis in Dar es Salaam, Tanzania.** Tanzan Health Res Bull. 9 (3): 164 -168.
33. Mabaera, B., Naranba,t N., Dhliwayo, P., Rieder, H.L., (2006): **Efficiency of serial smear examinations in excluding sputum smear-positive tuberculosis.** Int J Tuberc Lung Dis. 10 (9):1030 –1035

APPENDICES

Participant information sheet

A study comparing diagnostic performance of two smears versus three smears for the diagnosis of tuberculosis in selected health centers of Lusaka District.

Dear patient,

I am a student at the University of Zambia, School of Medicine, studying for a Masters degree in Public Health. I hereby invite you to take part in my academic study.

About the study

This study wishes to compare two ways of testing for pulmonary tuberculosis. i.e. between the conventional method of collecting and testing three sputum samples and that of testing two sputa only.

Study purpose

Although this study is academic, its purpose is to assess the performance of WHO's newly suggested policy of examining two sputa instead of three for diagnosis of tuberculosis.

Study procedure

As per current policy, you will be requested to submit three sputum samples for smear microscopy as requested by your Doctor at this clinic and the requested tests (smear microscopy) will be done at this clinic too. This study thereafter wishes to subject one of your samples to a further test known as culture at the University Teaching Hospital (UTH) TB laboratory.

Benefits and risks

There are no direct risks by participating in this study as you are only expected to submit three sputum samples (standard care) as requested by your clinician. Results for culture positive sputum samples from UTH will be sent to the local clinics of origin. This could benefit some individual patients who were negative on the initial smear examination. The general benefit of this study is that it will contribute necessary knowledge required to evaluate the convenient and

optimal number of sputum samples needed to diagnose pulmonary tuberculosis for policy analysis.

Confidentiality

To ensure confidentiality this study will use clinic codes plus laboratory numbers to identify samples while names will be restricted for clinical use at this health center only.

Costs

Participants will not be requested to pay for any of the tests required in the study procedure.

Contact addresses

Principal investigator: Eddie Samuneti Solo, University of Zambia, School of Medicine, Department of Community Medicine, P.O. Box 50110, Lusaka, Zambia.

Cell- 0977 786951.

The Chairperson, University of Zambia Biomedical Research Ethics Committee, Ridgeway Campus, P.O. Box 50110, Lusaka, Zambia.

Telephone: 256067.

Consent form

I..... have read/ study details have been explained to me and been requested to take part in this study which requires my sputum samples I have submitted for clinical diagnosis be further used for study purposes.

I therefore agree that my sputum samples could be subjected to further testing (culture) and results be used for the purpose of research analysis besides clinical diagnosis. By signing this document I permit my specimens to be used.

Participant sign.....Date...../Thumb print.....

Research assistant sign.....Date.....

Information sheet: Bemba translated version.

ISAMBILILO LYA KUPALANYA UKUPIMA UBULWELE BWA TUBERCOLOSIS (TB)
UKUBOMFYA IFIKOOLA FIBILI NA IFIKOOLA FITATU MUFIPATALA IFISALILWE
MU LUSAKA DISTRICT.

Mwebalwele besu,

Ine ndi mwana wesukulu pe sukulu likalamba ilya University of Zambia. Ndemilombako ukusangwamo muli ilisambililo.

Ngefyo tusosele pakubala, ili isambililo lilepalanya inshila shibili iyshakupiminamo ubulwele bwa TB. Tulefwaya ukweshwa ukumona imibombele yakupima ifikoola fibili atemwa ne nshila iya ishibikwa iyakupima ifikoola fitatu.

Ili sambililo lishintile pakumona nga ukuboonfya ifikoola fibili pa kupima TB, ngefyo icilonganano cikalamba icipanga amafunde pa bumi ica World Health Organization(WHO) cilefwaya ukweshwa, nga kuti fya boomba muno muchalo chesu.

Lelo muli ilisambililo twalamulombako, ukuboomfya ifikoola fyenu mwalaleta mukupimisha kuno ku laboratory. Panuma yakupwisha ukupma pano pa clinic, isambililo lyesu lile mulomba ukusendapo cimo no kutwala kuchipala cha University Teaching Hospital (UTH) no kuya bomfya icipimo icikalamba.

Muli ili isambililo tamuli ubusanso iyo, pantu icikoola twalasenda panuma yakupima icipimo be pwishe ba shingan'ga benu.

Kuli imwe ubunonshi bwesambililo ili ni bwakuti, ubulwele nga tabu moneke pano paclinic limbi kuti bwamoneka ku cipimo echo twalapimina ku UTH. Bumbi ubononshi bwa ili isambililo nibwakuti, twala ishiba nga cakuti ifikoola fibili kuti fyalinga ukupiminako ubulwele bwa TB.

Abalweele abo abakasangwamo muli ilisambililo tabafwile ukulipila nangu cimo iyo.

Adilesi ukomwingepusha amepusho ni aya.

Mr. Eddie Samuneti Solo, University of Zambia, School of Medicine, Department of Community Medicine, P.O Box 50110, Lusaka, Zambia.

The Chairman,
University of Zambia Biomedical Research Ethics Committee,
Ridgeway Campus,
P.O. Box 50110,
Lusaka,
Zambia.

Fomu Yakusuminishanyapo

Ine,.....nabelenga/ nabanondolwela noku njipusha ukusangwamo mwisambililo ili, ililefwaya ukuboomfya ifikoola ndetele/nkaleta. Pakusaina ici icipepala ndi uwaipelesha ukuti ifikoola fyandi kuti ba fiboomfye mwi sambililo panuma yakupima pano pa clinic.

Signature.....date...../Kufwatika(Thumb print).....

Witness.....date.....

BUDGET

Activities/ item description	QTy	Unit cost (K)	Total cost (K)
Proposal development/report writing			
Reams of paper	15	30 000	450 000
Ink Cartridges	2	150 000	300 000
binding	30	10 000	300 000
Permission seeking			
UNZAREC fee	2	200 000	400 000
Transport to LDHMT	4	50 000	200 000
Laboratory supplies			
Microscope slides	20x50	95 000	475 000
Sodium hydroxide	1x500g	250 000	250 000
Sodium citrate	1x500g	200 000	200 000
N-acetyl L-cystein	1x10g	140 000	140 000
Potassium dihydrogen phosphate	1x500g	700 000	700 000
Disodium hydrogen phosphate	1x500g	6500 000	650 000
Potassium phosphate	1x25g	260 000	260 000
Magnesium sulphate	1x25g	200 000	200 000
Magnesium citrate	1x25g	205 000	205 000
Pyruvic acid	1x50g	200 000	200 000
Glycerol	2.5L	125 000	125 000
Malachite green	1x25g	300 000	300 000
Eggs	3 trays	25 000	90 000
Pipettes	1x1000	500 000	500 000
Examination gloves	4x100	200 000	800 000
Methanol	5L	150 000	150 000
Hydrochloric acid	2L	90 000	90 000
Carbol fuchsin powder	25g	135 000	135 000
Phenol crystals	1kg	300 000	300 000
Methylene blue	25g	230 00	230 000
Allowances (research assistants)	4x12mths	125 000	6 000 000
Sample transportation from health Centers to UTH	96 trips	45 000	4 320 000
Total			18 000 000

