Diagnosis of tuberculosis in developing countries in the era of high HIV transmission; alternative approaches
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Citation for published version (APA):
Yassin, M. A. (2005). Diagnosis of tuberculosis in developing countries in the era of high HIV transmission; alternative approaches Amsterdam: Rozenberg

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Chapter 1

General Introduction
General Introduction

Tuberculosis, a disease of great antiquity, was a major health problem in western countries during the Industrial Revolution of the 19th century when crowded urban communities were created and facilitated the spread of infection. However, increased standards of living and probably effective immunity acquired by successive generations resulted in a falling incidence before Koch discovered the causative agent *Mycobacterium tuberculosis* in 1882 and the discovery of Streptomycin in 1947. There is still open debate about the evolutionary origins of tuberculosis (TB) in human, but recent molecular studies have confirmed the presence of *M. tuberculosis* in ancient Egyptian skeletal tissues dating back to at least 5000 years ago 1. Although debate continues regarding the presence of TB in sub-Saharan Africa before the arrival of Europeans and the role of trade and travel in the dissemination of infection 24, the current epidemiological situation is indisputably dire. Because of the relentless spread of tuberculosis throughout the world, the World Health Organisation (WHO), in 1993, took the unprecedented step of declaring TB a global emergency 3.

The Global Epidemiology of Tuberculosis

Tuberculosis is usually caused by *M. tuberculosis* and occasionally by other mycobacteria such as *M. bovis, M. africanum* and *M. kansasii*. The former is transmitted by inhaling small droplets of aerosols containing tubercle bacilli, about 5μ in diameter and *M. bovis* can be acquired by drinking infected milk. It is estimated that one third of the world's population is infected, 20 million of which have active disease at any given time 4. There were 8.8 million new cases of TB of which 3.9 million were smear positive pulmonary tuberculosis (PTB) and more than 2 million deaths due to TB in 2002 5. TB is responsible for 7% of all and 25% of preventable adult deaths 6. The incidence of TB continues to rise especially in developing countries where more than 90% of the global TB cases and deaths occur 7. The spread of HIV infection has become the most important factor fuelling the number of TB cases in some regions of the world such as Africa in the last 2 decades and it seems that these dual infections will continue to be formidable scourges for the future, at least, in the developing countries. Poverty, displacement, war, famine and malnutrition should not be overlooked as these factors contribute to the spread of TB. TB has been considered as a disease of poverty 1011, Zumla et al, stated that “it must never be forgotten that this disease has always been, and still is, associated with poverty and deprivation. Thus, the eventual eradication of this ancient scourge will surely go hand in hand
with the creation of a caring global society and the ending of the present gross inequity that scandalizes the human race" 12.

It is currently accepted that a reduction in the incidence of *M. tuberculosis* infection can be achieved by as swift as possible identification of potential transmitters of tubercle bacilli, i.e., the identification of persons with TB of the respiratory tract. Amongst these the most infectious are the patients with high bacillary loads (5000–10,000 per ml of sputum) that allow the identification of the bacilli using sputum smear microscopy 13,14. WHO set a global target to achieve a case finding of 70% of smear positive pulmonary TB by the year 2005. This target however seems impractical as the current case detection rate is less than 50% (37% in 2002) and it is estimated that another 8 years (2013) are required to achieve it 15. This situation highlights the urgent need for new methods which are more sensitive, easily applicable and robust and that are inline with the expected increase in the number of TB cases in developing countries. Early diagnosis however is not entirely dependent on more sensitive diagnostic tools as their application is determined by the health care seeking behaviour of TB patients and accessibility of health facilities. The stigma associated with TB, which has been heightened by the population's perceived association of TB with HIV16 may deter patients from seeking health care from the nearby health facilities.

The goals of TB treatment are to ensure cure, avoid relapse, prevent death, stop transmission and to prevent the emergence of drug resistance. The directly observed treatment, short course (DOTS) is a strategy recommended by the WHO since 1995 and adopted by more than 180 countries in the world as the most effective and efficient strategy to control TB 7. The strategy has five components: political commitment, diagnosis primarily by sputum-smear microscopy among patients attending health facilities, short-course treatment with effective case management (i.e., direct observation of treatment at least for the initial phase), regular drug supply, and proper recording and reporting which could enable monitoring outcomes of every patient started on treatment. Standard short-course regimens can cure more that 95% of cases of new, drug-susceptible TB and the WHO target for TB control for 2005 is to treat 85% of detected cases successfully. The treatment success rate for the global cohort registered in 2001 was 82%; however it was substantially below average in the WHO African Region (71%) and in Eastern Europe (70%) which could be attributed, in part, to the complications of HIV co-infection and rapid spread of drug resistant TB7. Equally important, is the failure of National TB Control Programmes
to monitor the treatment outcome for all patients. Unfortunately, the DOTS strategy is not yet widely implemented and only 37% of all estimated smear-positive TB cases were treated under a DOTS programme in 2002. Moreover, the DOT component of the DOTS strategy burdens both health workers and those patients who have to travel long distances to reach a health centre and such inconvenience contributes to poor treatment compliance. Health facility based treatment is more expensive than community based treatment. Several studies achieved better compliance and overall treatment success rate using different treatment observers at the community level including community volunteers, village doctors and community health agents. The decentralisation of the DOTS strategy to the peripheral health units and to the community should be considered to achieve a reasonable coverage and better compliance with convenience and a minimum cost incurred to the patient.

**Tuberculosis and HIV infection**

The prevalence of TB started to decline in most developed countries before the advent of effective drugs. These changes were observed with improved socio-economic status and housing conditions, resulting in less attention given to the disease in wealthy nations. However, in the early 1980’s the interest in TB increased following small but definite upsurge of the disease in New York after decades of decline and the occurrence of outbreaks of HIV-related multiple drug resistant (MDR) TB. TB however has remained a common health problem in most developing countries even before the spread of HIV. Poverty, overcrowding, poor housing, natural and manmade disasters were the main reasons for the sustained transmission of TB before the 1980’s. However, the recent exponential increase in the number of TB cases in most sub-Saharan African countries is primarily due to the fast spread of HIV infection. *M. tuberculosis* and HIV-1 are virulent intracellular pathogens that invade and multiply within macrophages. The containment of the tubercle bacilli in HIV uninfected individuals relies on the presence of a functional immune system and especially an intact cell mediated immunity. Unfortunately, co-infection with HIV leads to immune disruptions giving an opportunity for latent TB infections to flare up and cause overt disease. In the other hand, *M. tuberculosis* infection both up-regulate HIV-1 infection and replication within macrophages and increase the efficiency of virus transmission from infected macrophages to T-cells. It has been documented that TB is accompanied by impaired IFN-gamma production and sustained production of type 2 cytokines (TNF-a, IL-5 and IL-10) which increases in vitro replication of HIV, speeding up the destruction of immune cells and ultimate pro-
gression to AIDS. In persons co-infected with the tubercle bacilli and HIV, the overall annual risk of developing active TB rises from about 0.4% to 8% - that is, 20 times the risk for TB infection without HIV. Although TB disease could occur at any stage of HIV infection, the risk depends, however, on the degree of immunesuppression – the risk of a patient with AIDS developing TB is 170 times higher than a non-immunosuppressed person. For these reasons, infection with HIV and *M. tuberculosis* have been dubbed “the cursed duet”.

The epidemiological overlap of TB and HIV is striking: in 2000, 11% of all new TB cases worldwide were infected with HIV, while in Africa, a continent that is hit hard by the dual infections, it was estimated that 37% of adult TB cases were attributable to HIV. The majority of these cases represent reactivation of prior TB infection, and the largest increase in numbers of TB cases throughout the region is in young adults. A study in 20 sub-Saharan African countries showed that TB incidence rates had risen approximately twice as fast in countries with high versus low or intermediate HIV seroprevalence between 1985 and 1995 and approximately one-third of TB cases in sub-Saharan Africa after 1985 would not have occurred had there been no HIV. This region has the highest incidence rate of TB per capita in the world, rising from 259/100,000 in 1997 to 290/100,000 in 2000 and HIV is responsible for an annual increase of 6% of TB cases. TB is the major killer of HIV infected individuals. It was estimated that the overall case fatality rate for HIV-related TB (including undiagnosed cases) to be over 50% in many developing countries. Globally, 11% of AIDS deaths are primarily due to TB although deaths from 29% to 50% were documented from autopsy studies in some high prevalence urban areas.

The HIV co-infection alters not only the epidemiology of TB, but also the clinical presentation, laboratory findings, response to treatment and overall prognosis of the patients and ultimately the performance of the TB Control Programmes. HIV infected TB patients usually have fast progression of the infection with severe clinical presentations. HIV co-infected TB patients tend to produce fewer bacilli and smear examinations usually yield scanty bacilli or negative as they rarely form cavitary disease and have higher rates of extrapulmonary TB (EPTB) than HIV uninfected individuals. Unlike the typical post-primary involvement of the upper lobes and visualisation of cavities on chest x-rays, in HIV co-infected individuals, chest x-rays often look normal or show atypical presentations with lymph-node and lower lobe field involvement with diffuse infiltrations whereas cavities are rarely seen. The tuberculin skin test is usually negative or shows lower readings, adverse drug reactions...
are frequent and compliance to ant-TB treatment is usually poor compared to uninfected patients. Re-infections and relapses after a course of treatment are common. The risk of development of multiple drug resistant (MDR) TB is higher for HIV co-infected individuals. Malabsorption of anti-mycobacterial drugs (Rifampicin and Ethambutol) in HIV infected patients, associated with acquired drug resistance leading to treatment failure has been reported. More TB patients die during or after treatment if they are co-infected with HIV, probably due to severe TB disease or other concomitant opportunistic infections. The “cursed duet” of infection with both HIV and *M. tuberculosis* is generating a threat to human health of unparalleled proportions which, if not taken seriously by health workers and policy makers, could become totally unmanageable. The control of the dual infections therefore needs a concerted effort of the national and international communities and collaboration of TB and HIV Control Programmes.

**Diagnosis of tuberculosis**

Early presentation, prompt diagnosis and adequate chemotherapy are cornerstones of TB control. TB Control Programmes in resource poor countries use a passive case-finding approach for the diagnosis of TB i.e., they screen TB among patients who visit health facilities for their illness by their own initiative. This means that the diagnosis of TB is primarily determined by whether the patient comes or not and when, after the onset of their illness, they visit health facilities. Early presentation of the patients is determined by multiple factors including accessibility to the health facilities, patient’s knowledge and perception about the severity of the disease and where and whom to consult first, their socio-economic status, gender, culture and belief. Not all individuals who are ill seek health care, neither all those presenting to the health facilities are diagnosed or treated. A simulation model of case-finding and treatment in TB control programmes developed by Piot in 1967 (figure based on 19) explains the process well. The model begins with the percentage of the whole population that is infected, of whom a certain percentage is symptomatic and yet a smaller percentage seeks care. Of those who seek care, some, but not all, are diagnosed appropriately. Furthermore, not all diagnosed patients actually begin treatment and an even smaller percentage goes on to complete it. The proportion of people cured thus represents a small fraction of the total number infected. Late presentation and diagnosis—sometimes referred to as “diagnostic delay”—are vital aspects to TB control; the longer people remain undiagnosed, the higher the burden of disease. The diagnostic delay could occur anywhere between the onset of symptoms of TB to the
final diagnosis and initiation of appropriate treatment. Hence, diagnostic delay could be either due to delay in presentation of patients ("patient delay") or a health facility fails to diagnose as early as possible when patients consult ("provider delay") or usually due to both. According to studies in Africa, median patients delay range from 3 weeks in Botswana to 17 weeks in Tanzania, and provider delay from 1 week in Ethiopia and South Africa to 11 weeks in the Gambia. Several studies have confirmed the failure of health services to properly investigate patients with symptoms suggestive of TB: in Ghana, there was an almost perfect correlation ($r=0.99$) between provider delay and failure to perform sputum microscopy. In Ethiopia, long health service delays were associated with a shortage of laboratory technicians. Both of these studies support the conclusion reached in Vietnam that delay to diagnosis of TB is due more to inability among health care providers to detect TB than to under-utilization of health care services and in Malawi, just over 50% of patients with chronic cough attending out-patient services were actually tested.

![Figure 1: The “Piot” model of case finding and treatment in TB control](image-url)
GENERAL INTRODUCTION

Diagnosis of Pulmonary TB

Sputum smear microscopy

According to the recommendations of the International Union Against Tuberculosis and Lung Disease (IUATLD)\textsuperscript{68}, a patient who presents to a health facility with symptoms of TB; persistent cough for more than 3 weeks, haemoptysis, fever, night sweats, weight loss and loss of appetite\textsuperscript{69} should submit 3 sputum samples in 2 days as spot-morning-spot samples. The first specimen is submitted at the first visit to the laboratory, and then the patient will be provided a sputum cup to submit the second specimen. This specimen should be collected on the next day early in the morning and the third specimen will be collected on the spot when the patient comes to the health facility with the morning specimen\textsuperscript{68}. Smears are prepared from the specimens on new glass slides, air dried, heat fixed and stained using the Ziehl-Neelsen (ZN) technique. The ZN technique provides consistent results and needs no special equipment\textsuperscript{70}. Several grading scales have been introduced, but the most popular is the scale recommended by WHO and IUATLD where smears are graded in to 5 categories based on the number of acid-fast bacilli (AFB) seen under microscope (negative, scanty [1-9 AFB/100 fields], +, ++ or ++++)\textsuperscript{70}. The American Thoracic Society (ATS) recommends to include patients with 1-9 AFB/100 fields as +, and a recent study in Bangladesh revealed that lower cut-off as lower as 1 AFB/100 fields would increase the sensitivity with only 1.5% false positive at most\textsuperscript{71}. Some National Tuberculosis Control Programmes (NTCP) revised these grading to suit the situation in their respective countries. For example in Ethiopia, smears are graded as negative, scanty (1 or 2 AFB/100 fields) or positive (≥3 AFB/100 fields) to reduce the time spent in counting bacilli for grading as the technicians are usually overburdened by performing other tests and further grading would not lead to change in the management of patients\textsuperscript{72}.

Direct smear microscopy remains as the main tool of case-finding for TB especially in developing countries, where the routine use of other methods is not practical due to the limited resources available. Even though the century old ZN smear microscopy of sputum is believed to be sensitive to identify the most infectious patients, who are in turn the main source of infection to the community, the technique is not sensitive for the diagnosis of TB\textsuperscript{73}, reaching a sensitivity of less than 60% in most African laboratories\textsuperscript{74,75}. The technique misses a substantial number of culture positive cases who are potentially infectious to others as smear-negative culture-positive disease accounts for 15-20% of \textit{M. tuberculosis} transmission\textsuperscript{76}. TB sus-
pects are also asked to visit health facilities repeatedly to submit 3 sputum samples and collect results. Unfortunately, not all individuals who submit the first on-the-spot sputum samples complete the diagnostic process and fail to return for several reasons. Studies in Malawi and Malaysia documented high drop out rates after submitting the first specimens; 37% and 33% respectively\textsuperscript{77,78}. The requirement for 2 further visits to the hospitals to submit the remaining samples and collect results was suggested as the main reason for the drop out. The need for repeated visits to complete the diagnostic process, which was on average 7 visits in Zambia \textsuperscript{79} and 4 visits in Malawi \textsuperscript{80} incur extra costs to the patient, including loss of income from work, transport and accommodation expenses. The economic burden on the patient to reach a diagnosis is significant and contributes to delay or drop out and ultimate deterioration of the patient’s condition and continued disease transmission. In addition to examining AFB smears for TB, the same laboratory technicians are responsible to process other specimens such as blood films for malaria, faecal examination for ova or parasites, urine and blood tests. The large number of TB cases in sub-Saharan Africa have overburdened control programmes, and the heavy workload of smear examinations poses a serious problem in these areas \textsuperscript{81}. Given their workload, laboratory technicians can not afford to expend the recommended 5-10 minutes to examine each smear, and forced to rush to complete their activities. This staff shortages can compromise the quality of their work \textsuperscript{82} and impedes refresher training programmes to maintain the quality of smear microscopy \textsuperscript{83,84}. Moreover, processing sputum specimens on the open bench is common and could increase the risk of aerosol transmission in the laboratories \textsuperscript{85,87} although this risk is minimal for direct smear preparation.

\textbf{The value of multiple sputum samples for the diagnosis of TB}

Pending a better test, smear examination remains the only practical method of bacteriologic confirmation in the developing world \textsuperscript{88} and operational research is now focused on decreasing the burden of smear microscopy without compromising its effectiveness as a case-finding strategy \textsuperscript{89}. An obvious way to decrease the burden would be to decrease the number of smears performed per patient. The additional case yield per additional sputum specimen provides an indication of how many cases would be detected using fewer than the currently recommended three-sample approach. Earlier studies from India tried to demonstrate the incremental yields of repeated specimens by collecting up to 8 samples from each TB suspect; 4 on-the-spot and 4 morning specimens were examined by direct smear microscopy and culture. Nair et al, 1976 collected sputum specimens from 1652 TB suspects (3% of
these suspects were smear positive) and documented 87% positivity from the first specimen, 97% from the first 2 specimens and 100% positivity from the first 3 specimens among the culture positive cases. The remaining 5 smears did not yield more positive patients as all the positives specimens were detected by the first 3. The second study however observed a progressive increase in the yield of sequential smears and 100% was attained only by examining the 8th sample. The first 2 smears identified 85% of the smear positive cases (89% of culture positive) and the first 3, 87% of the smear positive cases. Recent studies from Africa, China and India have identified positive smears from the first specimens in 77% to 92% of the cases, 93% to 99% from the first 2 specimens and 100% from 3 consecutive samples. Almost all the above studies suggest that the majority of smear positive cases could be identified by the first 2 specimens and the overall advantage of collecting the third specimen is negligible. Reducing the number of specimens submitted by each TB suspect has multiple advantages: the workload of laboratory technicians and laboratory consumables including reagents, glass slides and sputum containers will be reduced by one third, which could improve the quality of performance of the laboratory technicians and save expenses for the NTCP especially in countries with limited resources where consumables are usually scarce. Unfortunately, submitting 2 specimens (usually spot and next day morning) will not address the main issue of making sputum microscopy as convenient as possible for the patient since this approach will not have any advantage for the patients as they still need to visit health facilities repeatedly. Hence, techniques which could limit the number of visits to health facilities should be explored to optimise TB case finding without compromising the sensitivity of smear microscopy. Collecting and examining 2 specimens on the same day could be considered as an option to minimize the number of health facility visits and hence the cost to the patients.

**Alternative sputum processing techniques**

*The value of household bleach (NaOCl) in the diagnosis of tuberculosis*

Acknowledging the poor performance of direct smear microscopy for diagnosis of TB, different techniques have been tried to increase the yield of smear microscopy. The oldest of these techniques is the NaOCl sputum concentration technique described by Ellermann and Erlandsen in 1908, which was re-evaluated and compared with various other sputum digestants including Tegritol (sodium octyl sulphate) and Clorax (commercial alkaline sodium hypochlorite solution). The digestive action of tegritol and clorax on sputum, using isotonic solution as control was tried
and it was concluded from this early work that NaOCl alone caused a definite decrease in the volume of bacillary sediment. This resulted in concentrated low volume sediment, separated from an opalescent supernatant fluid. Hence, it increased the ease and efficiency of recovering *M. tuberculosis* from sputum specimens. These findings were not widely known and used for years. However, after almost half a century, many researchers revisited the bleach technique in the last decade and suggested that the performance of sputum smear microscopy can be significantly improved if sputum is liquefied with household bleach and then concentrated by centrifugation or overnight sedimentation. Most of these studies investigated the yield of bleach concentration after centrifugation. For this technique, sputum is mixed with an equal amount of about 5% NaOCl, and the mixture is incubated at room temperature for 10-15 minutes. Distilled water is added and the sample is concentrated by centrifugation. The increased yields obtained from this technique however have been variable. Wilkinson and Sturm in South Africa documented no additional yield from the direct smears of 166 TB suspects by using concentration by centrifugation. In this study, the bleach method detected 12 smears which were missed by the direct smears but missed 13 smears which were positive by the direct smears. The authors acknowledged that either liquefaction was incomplete (They used a centrifuge with relative centrifugal force (RCF) of 1500 g) or that the AFB were disrupted by the process. Studies from Ethiopia, Zambia and Honduras however documented a statistically significant improvement of the sensitivity with the bleach concentration method ranging from 16-125% compared to the direct smears, with the same specificity when culture was used as gold standard. The variation in the yield could partly be explained by the difference in the RCF used by the different studies and the duration of centrifugation. The optimum RCF and concentration time combination was suggested to be 4000 g and 15 minutes. However, increasing the RCF beyond 4000 g did not yield a proportional increase in the sensitivity. The extent of the care taken in labelling slides before staining also determines its reliability as smears prepared after bleach liquefaction are difficult to visualise the stained side by naked eyes resulting in false negative readings. The main concern with the bleach concentration using centrifugation is the requirement of high speed centrifuge and electricity which is often not available in most rural areas in developing countries and the requirement of extra time for the procedure. According to a recent survey among 84 key respondents from 69 countries (NTCP and reference laboratory managers), the main potential obstacles for the implementation of the bleach centrifugation technique were the requirement for a centrifuge, additional reagents and training of the laboratory staff. Alternatively, bleach concentration...
with overnight sedimentation (without a centrifuge) was also reported to be more sensitive than the direct smear and important as centrifugation. However, the need for one additional day for the overnight sedimentation limits the use of this technique. In areas where the health facilities are not accessible, the overall increase in the number of TB cases diagnosed from overnight sedimentation could be negligible as patients tend to drop out from the diagnostic process as the number of visits increases. Therefore, techniques which could address the key issues with the sputum concentration methods, such as centrifuge, electricity, additional reagents and time, are critical for NTCP which are usually overburdened with increasing number of TB cases. We have investigated more feasible approaches which could entertain most of the problems raised above. The uses of short-term (30-45 minutes) bleach digestion (without centrifuge or electricity) prior to ZN staining are described in detail in this thesis.

Other diagnostic methods

Radiography
Chest radiography has been used as supportive diagnostic tool for PTB, especially among patients who are smear negative and young children who could not produce sputum. Radiology provides essential information for the management and follow-up of TB patients and is valuable for monitoring complications. However, chest radiographic findings are not specific for TB and atypical presentations are common especially in HIV co-infected individuals and reliable radiographic readings need experience and expertise although the agreement even among experienced readers is usually poor. Radiological facilities are available only in hospital in most areas limiting its use in a wider range.

Culture
Mycobacterial culture has been considered as a gold standard for diagnosis of TB. The oldest of this method is the solid egg-based Löwenstein-Jensen (LJ) media and agar-based Middlebroke 7H11. Although mycobacterial culture on solid media is more sensitive and specific than direct smear microscopy, it takes weeks (3-8 weeks) to get the results and the requirement for bio-safety level III laboratories limits its use as a routine diagnostic method in resource poor countries. The recently introduced liquid based culture media like BACTEC MGIT 960 and BACTEC 460TB could yield more sensitive results in a few days. These methods are expensive and
could not be available for routine purposes. Nevertheless, mycobacterial culture is useful for surveillance of drug sensitivity and for other research purposes in reference laboratories.

**Others**

Sputum staining with Auramine phenol and examination under fluorescent microscope could increase the yield compared to the direct ZN smear and potentially advantageous for those laboratories that handle large number of specimens, and newer molecular techniques such as DNA amplifications and polymerase chain reaction (PCR) are rapid, but more of experimental use. Most of these techniques are expensive and are not feasible in areas where the majority of TB patients live.

**Diagnosis of extra-pulmonary tuberculosis**

Diagnosis of EPTB is more difficult as TB could affect any organ and some of which are not easily accessible to take specimen from and confirm the diagnosis. TB lymphadenitis accounts for the majority of the EPTB cases and cervical lymph-nodes are commonly affected. Although there are large geographic variations, *M. tuberculosis* is the commonest cause of lymph-node TB and *M. bovis* is responsible for less than 20% of the cases in countries where pasteurization of milk is not routine. The proportion of EPTB among all diagnosed TB is reported to be rising in most places primarily due to HIV co-infection and becomes a diagnostic challenge. The diagnosis of TB lymphadenitis could be confirmed by performing whole lymph-node biopsy followed by macroscopical and histo-pathological examination, culture of the biopsy material and/or PCR application. These procedures however require expertise and are costly. Fine needle aspiration (FNA) of enlarged lymph-nodes and preparation of smears for staining by ZN for AFB and Hematoxylin-Eosin for cytological assessment is considered as a simple, inexpensive and rapid method for diagnosis of lymph-node TB. FNA method eliminates the need for biopsies and is considerably less risky to the health personnel performing it. AFB could be demonstrated from the aspirate material in 20%-40% of TB patients and should be considered as the initial diagnostic procedure. In this thesis, we explored the feasibility of using FNA for diagnosis of EPTB in rural areas of Ethiopia.
Description of the Study

Study site:
All except the validation studies were conducted in the Southern Region of Ethiopia. The data for the “scanty” and bleach-validation studies were collected from 8 hospitals and all the tests were done at Zankli Medical Centre in Abuja, Nigeria.

Ethiopia is an ancient independent country located in eastern horn of Africa. The country covers an area of 1.13 million sq km. The climate is tropical monsoon with wide topographic-induced variations. The country has a high plateau with a central mountain range divided by the Great Rift Valley. Ethiopia is the second most populated country in Africa with an estimated population of 70.7 million (UN, 2003 estimate). Addis Ababa is the capital and Amharic is the official language, English is a major foreign language taught in Schools. The country has a population growth rate of 1.89%, total fertility rate of 5.44, one of the highest infant mortality rates in the world with 102 deaths/1000 live births and a life expectancy at birth of 45 years for male and 46 years for female (UN, The world Fact book, 2004). The estimated adult HIV prevalence of 6.6% (2003) and Ethiopia has the 7th highest number of new cases of TB in the world with an incidence of all TB cases of 370/100,000 people.

The country is divided into 9 ethnic-based regional states and 2 city councils. The studies included in this thesis were conducted in the Southern Nations, Nationalities and Peoples Region (SNNPR) which is located in the southwest of the country. SNNPR is the third biggest region covering 10% of the area of the country. The population of the region is estimated to be about 14 million and 93% live in the rural part. The region is divided into 13 zones and 104 districts (Woredas). In the Southern Region, as its long name indicates that there are more than 45 ethnic groups, each with their own language. Awassa is the regional capital and is situated in the Rift Valley 270 Km south of Addis Ababa. In the region, there are 14 hospital, 114 Health centres and more than 400 clinics. The health coverage of the region is about 50%. Malaria and TB are the main health problems among adults and the adult HIV prevalence was estimated to be 3.7%118. The TB Control Programme was started with the WHO DOTS strategy in 1995 and currently more than 80% of the health facilities are covered by the programme (Unpublished report, SNNPR Health Bureau, 2004).
Objectives and outlines of the studies

TB remains as the biggest health problem in adults in developing countries where health coverage is low, diagnostic facilities are inaccessible, resources are scarce and where HIV is fuelling the number of TB cases. The overall aim of this thesis is to develop simple, feasible, cheap and more sensitive approaches for the diagnosis of TB in developing countries.

Specific objectives include:
To analyse the incremental yield of repeated sputum samples collection for the diagnosis of TB (chapter 2).
To assess the efficacy and safety of short-term bleach (NaOCl) digestion of sputum prior to ZN staining for case-finding for PTB (Chapter 3).
To determine the value of bleach digested smears for the diagnosis of TB in HIV infected individuals (Chapter 4).
To validate the bleach digestion technique for the diagnosis of TB against the gold standard; sputum culture (Chapter 5).
To validate the bleach digestion technique for the diagnosis of TB against the gold standard among HIV infected TB patients (chapter 6).
To verify if sputum smears graded as “scanty” are false positive (Chapter 7).
To describe factors associated with delayed presentation to health facilities by patients with symptoms of TB (chapter 8).
To determine the feasibility of one-day sputum submission method for the diagnosis of TB (chapter 9).
To explore simple procedures for the diagnosis of EPTB in rural areas (chapter 10).
To determine the prevalence of HIV infection among TB patients in rural settings of Ethiopia (chapter 11).
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Ethiopia Study sites

Ethiopia – Southern Nations, Nationalities and Peoples Region
Nigeria - Abuja