INTRODUCTION

Tuberculosis remains one of the leading infectious causes of death globally \(^1,2\). The greatest impact of this disease is currently centred in Sub-Saharan Africa\(^3\). Before 1985 case notification rates had been falling by 1.6% per year, but rose at a rate of 7.7% per year in subsequent years \(^3\). This has translated into a 2 to 4 fold increase in the incidence of TB in many African Countries \(^2\). Okot Nwang M et al in a review of TB records in Uganda were able to demonstrate a doubling of cases of TB between 1985-89 \(^4\).

The HIV epidemic has contributed greatly to the deteriorating TB burden mentioned above. By the end of 2000, about 11.5 million HIV-infected people worldwide were coinfected with tuberculosis. Seventy percent of the coinfected were in sub-Saharan Africa \(^5\).

Several studies have shown that the yield of sputum microscopy in diagnosis of TB in HIV has been diminished significantly \(^3,6,7,8,9,10\). For example Johnson J L, Okwera et al in a study done in Uganda found 7% of culture positive cases had negative and scanty AFB smears among HIV co-infected compared to 2% of culture positive non HIV cases\(^6\). A similar study in Dakar, Senegal by Samb et al found that 57% of HIV positive patients had positive sputum smears compared to 76% of HIV negative patients among culture positive PTB cases \(^9\). Bruchfeld et al in a study done in Ethiopia found that direct smear was significantly less sensitive and specific in HIV positive patients \(^11\). Worodria W et al in a study on smear negative HIV patients found a significant proportion (21.7%) of these patients had culture positive TB \(^12\). Thus the diagnostic potential of direct smears is very low in HIV. One of the explanations for this increasing rate of smear negative disease is the tendency towards non-cavitary disease in HIV/TB co-infection. Relying on LJ culture for diagnosis of smear negative cases takes too long to be used for decisions on beginning TB treatment.
It is inevitable that, smear microscopy needs to be improved and/or other methods for diagnosis of smear negative disease be designed. Methods being tried include:

- Improving the selection of patients with TB among smear negative suspects by using management algorithms. For example, Samb B et al in a study on smear negative TB suspects found 4 symptoms were associated with culture positive TB: cough>21 days, chest pain>15 days, absence of expectoration and absence of dyspnœa. Failure to respond to a trial of antibiotics may be an additional indicator of smear negative TB and is included in some algorithms. However, non-response to antibiotics may only provide additional evidence, but a symptomatic clinical response may not exclude TB diagnosis.

- Improving the exclusion of patients with other conditions such as PCP, nocardiosis and salmonella bacteraemia by use of clinical predictors of these diseases (syndromic approach). However, overlap of symptoms of these respiratory diseases reduces the sensitivity of this syndromic approach.

- Improving specimen collection, especially for patients who cannot produce adequate sputum by simple chest physiotherapy, use of Bronchoscopy plus BAL or Sputum Induction.

- Improving laboratory diagnosis. Standard light microscopy may be improved by digestion and concentration of sputum. Various methods have been described (such as NALC + 2% NAOH, 4% NAOH, and NAOCL) and are generally followed by centrifugation. Use of bleach produced better recovery of AAFBs than did digestion with other methods (such as NAOH) with significant increases in sensitivity. Thus, Gebre N et al found that bleach digestion improved sensitivity from 30.8% to 60.2% in studies done in Ethiopia and India. P Farnia et al in a study comparing chitin, bleach and NALC found that bleach concentration method increased sensitivity from 46% to 78% while NALC concentration method increased sensitivity from 46% to 83% and Gebre-Selassie found that bleach
sedimentation increased sensitivity by more than 3 fold. However, Van Deun A et al in Bangladesh found that bleach concentration method increases the diagnostic yield but only to a minor extent if all factors (i.e. competent technician, proper staining and correct specimen) have been optimised.

The bleach and NALC concentration methods appear to have a promising diagnostic potential for PTB from previous studies. However, they have not been tried for diagnosis of smear negatives with paucibacillary TB who can only be diagnosed definitively by culture at the moment.
LITERATURE REVIEW

TUBERCULOSIS

Tuberculosis is an infectious disease caused by mycobacterium Tuberculosis and occasionally by mycobacterium Bovis and Africanum, which are organisms that belong to the mycobacterium tuberculosis complex group. Pulmonary TB is the most common and infectious form of MTB, occurring in over 80% of cases.

HIV co-infection may reduce the efficacy of diagnostic procedures like sputum microscopy. This is believed to be due to inability of TB patients with advanced immunosuppression to form cavities.

DIAGNOSIS OF TUBERCULOSIS

A diagnosis of TB may be suspected on the basis of clinical and radiological findings. Thus, a person with cough for more than 2 to 3 weeks with or without other respiratory symptoms should be suspected for PTB. However, the final diagnosis requires the isolation and identification of mycobacterium tuberculosis, the etiologic agent. Direct smear for AAFB is inexpensive, rapid and easy to perform, but has a low sensitivity. The efficacy of the direct smear has been further reduced in HIV co-infected TB suspects. Several approaches for improving the diagnosis of TB, especially in those cases that cannot be detected by direct smear have been tried as detailed below.

Use of diagnostic Algorithms

Samb B et al in a study of smear negative PTB suspects in Burundi and Tanzania, found four symptoms (that could be included in diagnostic algorithms) were associated with positive TB cultures; cough > 21 days, chest pain > 15 days, absence of expectoration and absence of dyspnoea. They reported that presence of any two of these symptoms diagnosed TB with 85%
sensitivity and 65% specificity. Inclusion of these symptoms in management algorithms would optimise the number of patients correctly treated as smear negative TB.

**Improving specimen collection**

Bronchoscopy plus BAL and sputum induction have been tried to improve specimen collection especially for patients who cannot produce adequate sputum samples. Parry et al in a study of 82 smear negative patients found 30 (36.6%) of these patients’ induced sputum samples were culture positive for mycobacteria. Eighteen (60%) of the culture positive induced sputum samples were AAFB positive after ZN staining. They concluded that sputum induction could be used for obtaining better specimens from smear negative PTB suspects. Worodria et al in a study of smear negative PTB suspects in Mulago hospital found 9 (50%) of 18 culture positive BAL specimens had positive ZN stains for AAFBs. However, Bronchoscopy and sputum induction methods are not readily available for routine diagnosis in developing countries.

**Syndromic approach**

A syndromic approach has been tried to improve exclusion of other conditions such as PCP, norcardiosis and PKS that may present like PTB. In Zimbabwe, Malin et al found the following clinical indicators to be predictive of PCP; respiratory rate > 40/minute, fine reticulonodular shadowing on chest radiography and severe hypoxia. However, many features of these pulmonary syndromes overlap thus reducing their sensitivities and specificities.

**Laboratory diagnosis**

**Smear examination**

**Direct smear**

Direct smear microscopy using acid-fast stains is a simple, inexpensive, and fast method, which detects infectious cases of TB. However, it is generally a diagnostic method with low sensitivity, with sensitivities ranging from 25 to 65% when compared to
culture 25. Several studies have shown that this sensitivity is further reduced in HIV patients with TB 3, 6, 7, 8, 9. Smear sensitivity also depends on the type of lesion, the quality of sputum specimen, the mycobacterium species, staining technique of the microscopist and the concentration of AFBs in the sputum.

**Fluorescent microscopy**

Fluorescent microscopy improves the sensitivity of direct smear microscopy because it enables use of a low objective, resulting in observation of a larger field. This significantly reduces the time required for examination of smears. It results in a slightly higher sensitivity than ordinary microscopy examination. It was found to have a sensitivity of 67.1% compared to 66.1% for ordinary microscopy in one series 7. However, the high costs of this equipment and running cost of the fluorescent microscope make its use prohibitive in practice 2, 18.

**Concentration methods**

Sensitivity of Standard Light Microscopy can be improved by digestion and concentration of sputum. Various digesting agents have been described such as, N-acetyl-L-cystein (NALC) + 2% Sodium hydroxide (NAOH), 4% NAOH, Dithiothreitol plus 2% NAOH, Sodium Hypochlorite (NAOCL) 8, 17, 18, 19, Ziphiram-Trisodium Phosphate (Z-TSP) and Oxalic Acid 27. Digestion is generally followed by concentration by centrifugation 8, 17. The sensitivity of these techniques depends on the toxicity of the digestant-decontaminant solution, heat build up in the centrifuge and the relative centrifugal force (RCF). The latter factor (i.e. RCF) is the most important. Adequate sedimentation efficiency (i.e. 95%) can be achieved by centrifugation at 3000 x g for 15 minutes 8.

**i) Household bleach (NAOCL) concentration method:**

- Improved recovery of mycobacteria with NAOCL is attributed to change in the surface properties of mycobacteria (i.e. charge) and or denaturation of sputum constituents leading to freeing of AAFBS and subsequent increase of sedimentation rate of AAFBs 19.
• Several studies have shown that digestion of sputum with NAOCL followed by centrifugation increases the sensitivity of sputum smear microscopy and decreases infection transmission. A systematic review of studies that had used the bleach concentration method identified 19 studies. In this review, Angeby et al found that in 15 of the 19 studies, there was a statistically significant improvement in proportion of positive tests or sensitivity ranging from 7 to 253%. This increased sensitivity is attributed to a clearer microscopic field after NAOCL digestion and higher bacillary concentrations per slide thus reducing the time for microscopy.

• NAOCL digestion followed by overnight sedimentation instead of centrifugation results in similar improvement in sensitivity. The RCF needed to pellet out mycobacteria is lower with NAOCL than other methods, giving NAOCL method a higher sensitivity than other digestion methods.

• However, some studies have shown minimal improvement with use of household Bleach or no increase at all in the sensitivity of microscopy.

• Other advantages of NAOCL are, it is cheap and readily available as household Bleach. Household bleach is marketed in various concentrations such as 3.5%, 3.7%, 4.0% and 5.0%. In Uganda it is readily available in a concentration of 3.5%.

ii) N-Acetyl-L-Cystein plus 2% NAOH concentration method:

• NALC is a mucolytic agent that causes rapid digestion of sputum and enables the decontaminant agent (NAOH) to be used at a lower final concentration of 1%. Sodium citrate is included in the digestant mixture to bind the heavy metal ions in the specimen, that may inactivate the NALC.

• Approximately 30% of tubercle bacilli may be killed by this method. NALC loses activity rapidly in solution thus necessitating daily preparation of fresh digestant.
• Farnia et al in a study of 430 samples from PTB suspects in Iran found NALC concentration method to have a sensitivity of 83% and specificity of 97% compared to sensitivity and specificity of 46% and 90% respectively, for direct smear 19.

• The method requires special equipment, thus making it less applicable in majority of laboratories in developing countries 27.

iii) Chitin concentration method 19

• Chitin is a polysaccharide formed by repeating units of N-acetyl-D-glucosamine.

• Both chitin and NALC have an acetyl amine group in their structure, which is responsible for the mucolytic effect of these compounds.

• Sputum digestion with chitin takes a considerably shorter time than NAOCL and NALC.

• Farnia et al found a sensitivity and specificity of chitin for diagnosis of PTB suspects to be 80% and 96% respectively compared to 46% and 90% for direct smear respectively.

Culture:

• Culture is more sensitive i.e. detects as few as 10 AFBS/ml of digested concentrated specimen and allows definitive diagnosis of MTB 27, 32.

• In an attempt to evaluate the utility of other specimens for diagnosis of PTB in smear negatives, Ssekasanvu et al found 15 (30%) of 50 smear negative patients had sputa that were culture positive for mycobacteria, 2 (4%) had positive blood cultures for mycobacteria and 2 (4%) had bone marrow aspirates that were culture positive for mycobacteria. They concluded that sputum culture was still the best specimen for diagnoses of smear negative PTB 33.

• There are three main groups of culture media for TB 27: 1. Egg based media e.g. Lowenstein-Jensen slope (LJ); 2. Agar based e.g. Middlebrook 7H-10, 7H-11; 3. Liquid media e.g. Herman Kirchner medium.
LJ is the most widely used for TB culture because it is easy to prepare, the least expensive, supports good growth of MTB and the Malachite green added suppresses growth of non-Mycobacterial organisms. Its main disadvantages are; it may take as long as eight weeks before cultures become positive and if contamination involves the total surface of the medium, culture is usually lost.\(^{27}\)

**Tuberculin skin test (TST)** \(^{32}\)

TST is based on the fact that MTB produces sensitivity to certain antigenic components of the organism resulting in delayed type hypersensitivity in a person with previous natural infection with MTB, infection with Mycobacteria Other than Tuberculosis (MOTT) or has had BCG vaccination. TST may be administered by the intracutanous route (mantoux test) or the multiple puncture method (i.e.Heaf test). Mantoux method is preferred for diagnostic purposes. Interpretation of results is very controversial because of influence of several factors such as, HIV, malnutrition, infection with MOTT and BCG.

**Newer rapid diagnostic technologies**

**BACTEC and other culture systems**

BACTEC is a radiometric method that facilitates early detection of mycobacterial growth. The method employs \(^{14}\)C-Palmitic acid (a radio-labelled substrate) in agar-based media such as Middle Brook 7H12. Growth of mycobacteria utilises \(^{14}\)C-palmitic acid resulting in release of \(^{14}\)CO.\(^{27, 34}\) Sampling of the airspace above the medium automatically by the BACTEC machine results in estimation and recording of the amount of radiolabelled gas. Growth of mycobacteria may be detected within 5-7 days. Other culture methods include the mycobacterium growth indicator tube (MGIT), which has a liquid culture medium. With MGIT system, growth is detected visually as positive cultures exhibit a bright orange fluorescence on exposure to a long-wave UV lamp. The MGIT requires a slightly longer time to detect a positive culture.
Genetic probes

Molecular techniques like the genetic probe, most commonly the single stranded radio labelled DNA probe, can be used for identification of mycobacteria grown in pure culture. Probes specific for the mycobacterium tuberculosis complex group (i.e. M.Bovis, M.TB, M.Avium, M.Africanum and M.Microti) are now available \textsuperscript{32, 34}. Amplification techniques targeting mycobacterial DNA or RNA have been developed, but currently, most experience is available from the direct detection of MTB complex in respiratory specimens. However, it is unlikely that most of these high tech methods will soon be applicable for routine patient care in developing countries because of their high costs \textsuperscript{34}.

High performance liquid chromatography (HPLC)

HPLC relies on identification of species-specific mycolic acids produced by the MTB complex by chromatographic methods. This method is used on primary cultures for identification of species within 6-18 hours rather than 2-6 weeks by Biochemical methods. Pilot studies have shown promise for direct detection of specific mycobacteria by HPLC in strongly smear positive sputum \textsuperscript{34}.

Micobacteriophage typing e.g. FASTPLAQUE TB assays \textsuperscript{35}.

This method uses phage amplification technology in which Bacteriophage D29 is used as an indicator to detect the presence of viable Bacilli in sputum samples. MTB present in decontaminated sputum is infected with the phage (Actiphage). A virucide (virusol) is then added to facilitate destruction of extra cellular phage. The phage replication in infected cells results in cell lysis and release of the progeny. Addition of a rapid growing mycobacterium strain, M.Smeggatis results in amplification of the progeny, which can be visualised as zones of clearing (plaques in a lawn of sensor cells). Results are available in 48 hours.
Chest Radiography.

Radiographic abnormalities may be important in estimating the probability of a given patient suffering from TB, especially smear negative TB. However, no radiographic pattern is diagnostic of TB. Following primary infection most persons have a normal chest x-ray. A minority of persons are left with apical or bi-apical fibronodular shadowing, others with minor isolated small fibrocalcific lesions (Ghon Focus) and thickening of apical pleura. Typical features of post-primary TB include: fibronodular, irregular shadowing with variable coalescence and cavitations, especially in the apical and/or posterior segment of the lungs. Rarely, patients may present with normal radiographs especially patients with HIV or endobronchial TB. TB in HIV may have radiographic features of primary disease. Thus x-rays can be very helpful in localising lesions in the lungs but the aetiology of such lesions can only be proved by bacteriology.